CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.201100541

Benzylidene Acetal Type Bridged Nucleic Acids: Changes in Properties Upon Cleavage of the Bridge Triggered by External Stimuli

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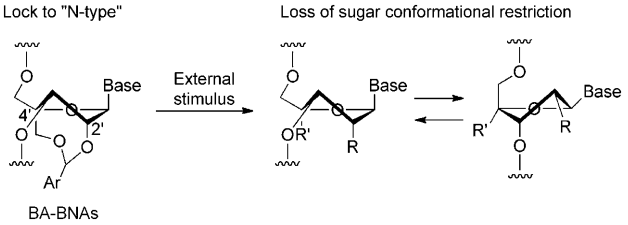
Abstract: Four classes of benzylidene acetal type bridged nucleic acids (BA-BNAs) were designed with 2′,4′-bridged structures that cleaved upon exposure to appropriate external stimuli. Cleavage of 6-nitroveratrylidene and 2-nitrobenzylidene acetal type BNA bridges occurred upon photoirradiation and subsequent treatment with thiol caused changes in secondary structure to afford 4′-*C*-hydroxymethyl RNA. Benzylidene and 4-nitrobenzylidene acetal type BNA responded to acids and reducing agents, respectively, resulting in hydrolysis of the acetal-bridged structure. Cleavage of the bridge removed sugar conformational restrictions and changed the duplex- and triplex-forming properties of the BNA-modified oligonucleotides. Moreover, oligonucleotides incorporating a single BA-BNA modification had considerably improved stability toward 3′-exonuclease, which was lost upon cleavage of the bridge. Thus, these new BNAs may be useful as therapeutic and detection tools by sensing various environments.

Keywords: DNA · nuclease resistance · nucleic acids · oligonucleotides · RNA

Introduction

The ability to respond to an external stimulus is an important requirement for molecular tools aimed at regulating biological phenomena or acting as sensing systems for nanodevices. The regulation of nucleic acid properties is a particularly attractive research area due to its diverse applications, such as gene regulation,[1] molecular diagnostics,[2] catalysts[3] or functional nanoscale materials.[4] Various external stimuli, such as light,[5] pH,[6] temperature,[7] change in redox potential,[8] or small molecules,[9] have been used for this purpose.

Previously, we[10a] and Wengel's[10b] group independently synthesized 2′-*O*,4′-*C*-methylene-bridged nucleic acid (2′,4′-BNA/LNA)[10] with an N-type locked sugar conformation imposed by the bridged structure, and demonstrated that oligonucleotides (ONs) containing 2′,4′-BNA/LNA have high affinity for complementary single-stranded RNA (ssRNA)[11] and double-stranded DNA (dsDNA).[12] Since these characteristics of 2′,4′-BNA/LNA are due to the fixed N-type sugar conformation, we were inspired to design benzylidene acetal type BNAs (BA-BNAs) that have a labile 2′,4′-bridged structure (Scheme 1). The sugar conformation of BA-BNAs is locked in the N-type conformation, but upon exposure to an appropriate external stimulus, the bridge is cleaved and the sugar conformational restriction is lost. This causes changes in the properties of the nucleic acid, including its ability to form duplexes or triplexes, and allows control of various molecular systems. We recently communicated the synthesis and properties of a light-responsive BNA that contained a photolabile 6-nitroveratryl group in the acetal-bridged structure (Scheme 2, 6-NV^B).[13] The bridged structure of 6-NV^B can be cleaved upon photoirradiation (form **II**) and subsequent treatment with glutathione (GSH) causes secondary structure changes to afford 4′-*C*-hydroxymethyl RNA (form **III**).[14] With these two steps required to change the structure of 6-NV^B, the binding affinity of ONs containing 6-NV^B for complementary ssRNA can be changed in two stages. Nucleic acids that respond to both light and GSH may have potential in therapeutics, diagnostics, or as detection agents by sensing GSH concentration.



Scheme 1. Cleavage of the bridged structure and loss of sugar conformational restriction triggered by external stimulus.

The introduction of other aryl groups into the bridged structure could provide BNAs that lose their locked sugar conformation in response to other external stimuli. Therefore, we designed three BA-BNAs containing either a 2-nitrobenzyl (2-NB^B), a benzyl (B^B), or a 4-nitrobenzyl (4-NB^B) group in the bridged structure (Scheme 2). 2-NB^B responds to light in the same manner as 6-NV^B, but the secon-

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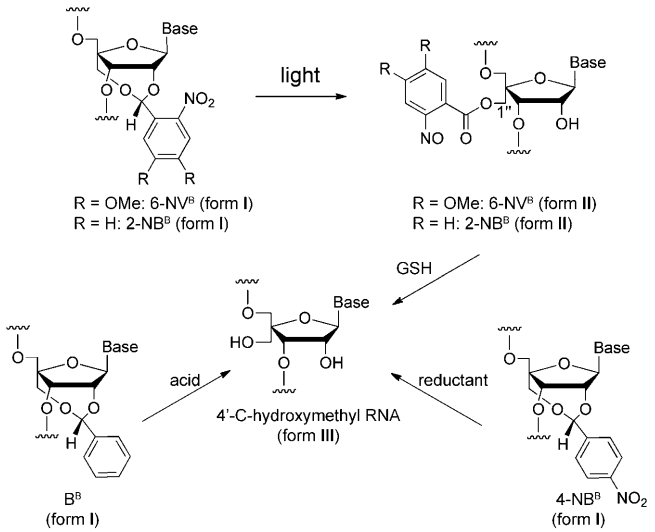
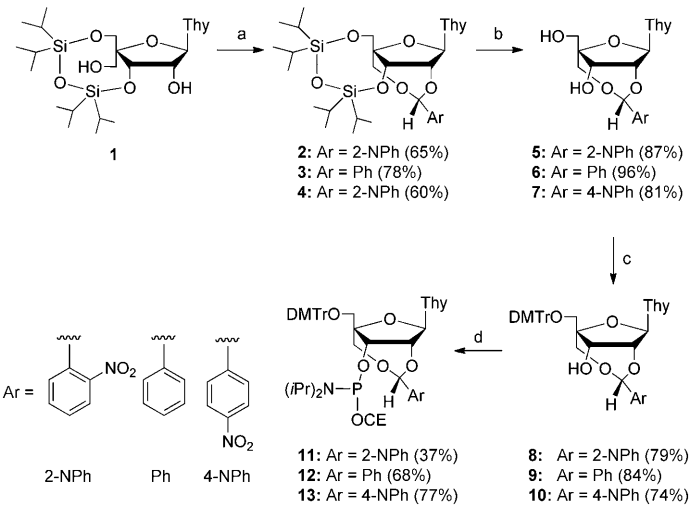
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201100541.



Scheme 2. Change in structure of BA-BNAs responding to various external stimuli.



Scheme 3. Synthesis of BA-BNA phosphoramidites. Reagents and conditions: a) 2-nitrobenzaldehyde, $ZnCl_2$, toluene, RT, 37 h (**2**); benzaldehyde, $ZnCl_2$, RT, 14 h (**3**); 4-nitrobenzaldehyde, $ZnCl_2$, HFIP, RT, 18 h (**4**); b) TBAF, THF, 0 °C; c) DMTrCl, pyridine, RT, 15 h (**8**); 11 h (**9**); 21 h (**10**); d) $(i\mathrm{Pr}_2\mathrm{N})_2\mathrm{PO}(\mathrm{CH}_2)_2\mathrm{CN}$, 4,5-dicyanoimidazole, CH_3CN, RT, 17 h (**11**); 40 h (**12**); 17 h (**13**). CE = cyanoethyl, DMTr = 4,4′-dimethoxytrityl, HFIP = 1,1,1,3,3,3-hexafluoroisopropanol, Thy = thymin-1-yl, TBAF = tetra-*n*-butylammonium fluoride.

dary reaction with GSH is predicted to proceed faster than that of 6-NV^B because of the increased electrophilicity of the 1″-*O*-carbonyl group. B^B has an electron-donating phenyl group at the bridged moiety, and the acetal structure is easily degraded under acidic conditions to form 4′-*C*-hydroxymethyl RNA. In contrast, 4-NB^B has a 4-nitrophenyl group at the bridged structure. The electron-withdrawing nitro group of 4-NB^B can be reduced to an electron-donating amino group by treatment with a reducing agent, upon which the acetal-bridged structure becomes very unstable and can be hydrolyzed even under neutral conditions. Thus, B^B has the potential to be a sensor of acidic conditions and 4-NB^B has the potential to be a sensor of reducing conditions. Herein, we report the synthesis of these new BA-BNAs and describe the changes in structure and properties of these BA-BNAs, including 6-NV^B.

Results

Synthesis of BA-BNAs phosphoramidites: BA-BNAs were synthesized from nucleoside derivative **1**,[13] as shown in Scheme 3.[15] When the 3′- and 5′-hydroxyl groups of the nucleoside are modified with the 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl (TIPDS) moiety, the sugar conformation is prelocked in the N-type conformation,[16] similar to that of 2′,4′-BNAs. Compound **1**, therefore, has advantages for the construction of the 2′,4′-bridged moiety. The 2′,4′-bridged structures were constructed by treating diol **1** with three types of aldehydes and ring-closed compounds **2**–**4** were obtained as single diastereomers. The configuration at the acetal carbon atom was determined from NOESY spectra (Figures S18 and S19 in Supporting Information and references [13] and [15]). The NOESY spectrum showed no correlation between acetal-H/1′-H or a correlation between acetal-H/TIPDS-H. It should not be possible to show such a NOESY spectrum for the other diastereomer. A plausible transition state model for this reaction is described in Supporting Information (Figure S21). Desilylation was carried out by using TBAF to afford the corresponding nucleoside analogues **5**–**7**. Tritylation at the primary hydroxyl group with DMTrCl and phosphitylation at the secondary hydroxyl group yielded phosphoramidites **11**–**13**. Phosphoramidites **11**–**13** and the 6-NV^B amidite building block were introduced into ONs **14**–**18** on an automated DNA synthesizer (Figure 1, details are given in the Supporting Information).

5′-d(GCGTT**X**TTTGCT)-3′ (**14a–d**)
5′-d(GCG**X**T**X**T**X**TGCT)-3′ (**15a–d**)
5′-d(TTTTT^{m}CT**X**T^{m}CT^{m}CT^{m}CT)-3′ (**16a–d**)
5′-d(TTTT**X**^{m}CT**X**T^{m}C**X**^{m}CT^{m}CT)-3′ (**17a–d**)
5′-d(TTTTTTTTT**X**T)-3′ (**18a–d**)

Figure 1. Sequences of ONs used in this study. **X**s indicate the modified residues. Series **a**, **b**, **c**, and **d** represent 6-NV^B-, 2-NB^B-, B^B- and 4-NB^B-modified ONs, respectively.

Change in structure of light-responsive BNA: We evaluated the cleavage of the 2-NB^B bridge upon photoirradiation. A UV-LED (365 nm) lamp was used as the light source. ON **14b** (form **I**), which contains one 2-NB^B unit, was photoirradiated at 365 nm for 5 s and the resulting products were analyzed by reverse-phase HPLC (RP-HPLC) and MALDI-TOF mass spectrometry (Figure 2B). The signals corresponding to the bridge-closed form (form **I**) disappeared and the bridge-opened form (form **II**) was generated. Furthermore, when ON **14b** was photoirradiated for 5 s and then treated with GSH for 60 min, 2-NB^B was transformed into 4′-*C*-hydroxymethyl RNA (form **III**; Figure 2C).

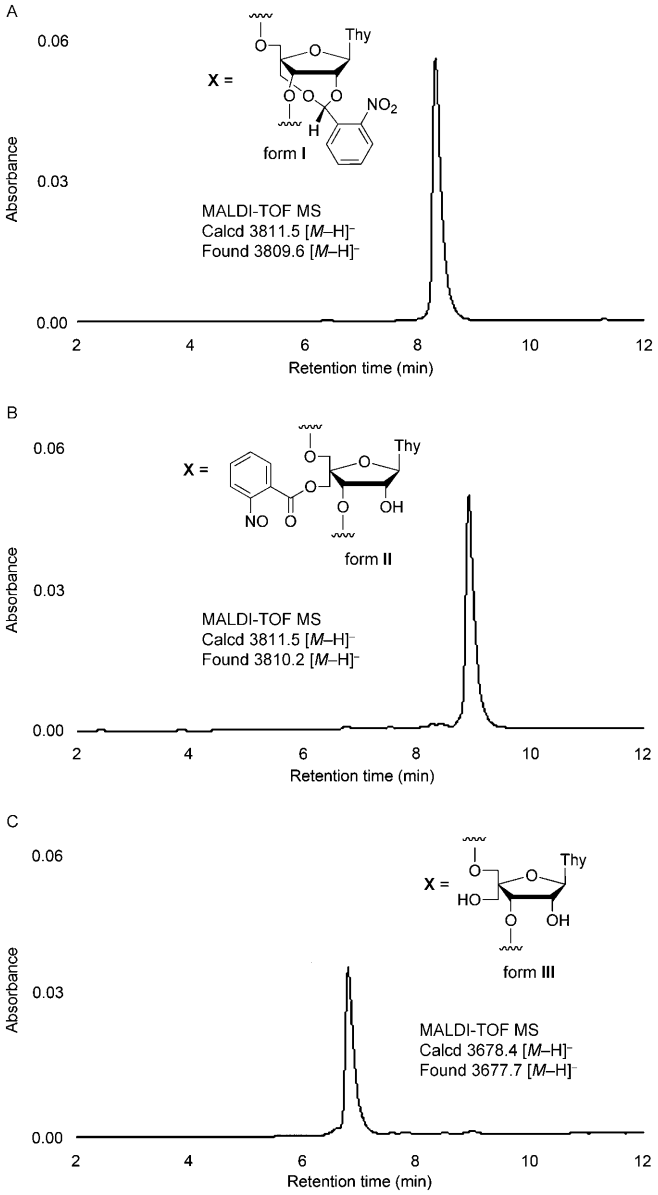


Figure 2. RP-HPLC analysis of ON **14a**. A) ON **14a** (form **I**) (10 μM) before photoirradiation. B) ON **14a** (10 μM) after irradiation at 365 nm for 5 s. C) ON **14a** (10 μM) after irradiation at 365 nm for 5 s and subsequent reaction with GSH (10 μM) for 60 min. The reaction mixture was analyzed by RP-HPLC by using an XTerra MS C18 column (4.6×50 mm) with a linear gradient of CH_3CN (6 to 24% over 15 min) in 0.1 M triethylammonium acetate (pH 7.0).

We compared the reaction efficiency of 2-NB^B with 6-NV^B against GSH. Following photoirradiation of ON **14a** and **14b** for 5 s (form **II**), the products were treated with GSH and the remaining intact ON was quantified by RP-HPLC (Figure 3). As expected, the reaction of 2-NB^B (form **II**) with GSH proceeded faster than that of 6-NV^B (form **II**) ($t_{1/2}$ = 6 vs. >200 min). The efficient reaction of 2-NB^B (form **II**) with GSH prompted us to evaluate the reactivity with glutamate (Glu), cysteine (Cys), and glycine (Gly), which are constituent amino acids of GSH. The results showed that 2-NB^B (form **II**) reacted only with Cys and was converted into 4′-*C*-hydroxymethyl RNA (form **III**). Interestingly, the reaction rate with Cys was faster than that with GSH (Figure 3; $t_{1/2}$ = <1 vs. 6 min), indicating that 2-NB^B (form **II**) could work not only as a good GSH senser, but also as a remarkable Cys sensor. These results indicate that the thiol group is important for the reaction of 6-NV^B and 2-NB^B (form **II**) with GSH.

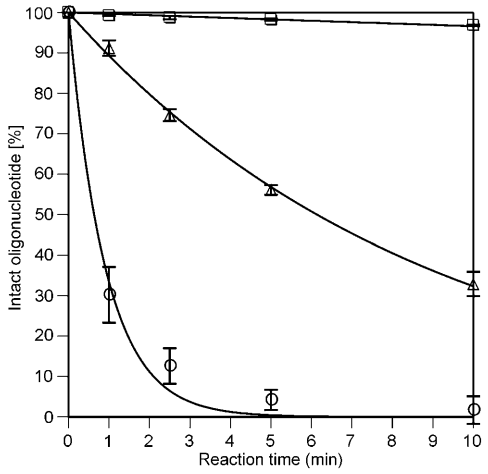


Figure 3. Time course of the reaction of ON **14a** (form **II**) with GSH (□; $t_{1/2}$ >200 min), ON **14b** (form **II**) with GSH △; $t_{1/2}$ = 6 min) and ON **14b** (form **II**) with Cys (○; $t_{1/2}$ < 1 min). Error bars indicate standard deviation (n = 3). Conditions: 25 mM sodium phosphate buffer (pH 7.2), 10 μM ON **14a** or ON **14b**, and 10 μM GSH or Cys; reaction temperature = 37 °C.

Change in structure of acid-responsive BNA: The change in the structure of B^B under acidic conditions was evaluated in a similar way. ON **14c** (form **I**), which contains one B^B unit, was placed in a solution of citrate phosphate buffer at pH 3.0 for 60 min and analyzed by RP-HPLC (Figure 4). The acetal-bridged structure was hydrolyzed and 4′-*C*-hydroxymethyl RNA (form **III**) was generated.

We next examined the efficiency of the hydrolysis of the acetal-bridged structure of B^B at pH 3.0–5.0 (Figure 5) and found that the hydrolysis rate decreased as the pH of the solution increased. This means that cleavage of the bridged structure of B^B is dependent on the pH under acidic conditions, suggesting that B^B holds promise for development as a pH sensor.

Change in structure of reductant-responsive BNA: The reaction of 4-NB^B with the reducing agents sodium ascorbate,[17] potassium ferrocyanide,[18] dithiothreitol,[19] GSH,[20] sodium thiosulfate,[21] and sodium dithionite[22] was examined. Of these reducing agents, only sodium dithionite could reduce the nitro group of 4-NB^B (Figure 6). The signal corresponding to the 4-NB^B-modified ON (form **I**) disappeared within 10 min, but 4′-*C*-hydroxymethyl RNA (form **III**) appeared somewhat later, indicating that reduction of the nitro group proceeded rapidly, but hydrolysis of the acetal-bridged structure proceeded at a slower rate. In contrast, the initial reduction of ON **14b** (form **I**) containing 2-NB^B occurs much more slowly and there are no traces of subse-

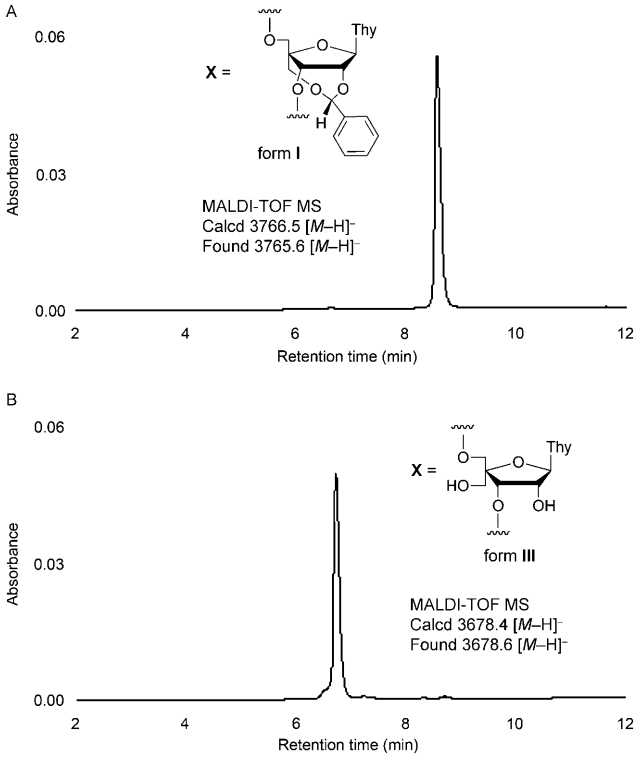


Figure 4. RP-HPLC analysis of ON **14b**. A) ON **14b** (10 μM) before treatment with acid. B) ON **14b** (10 μM) after reaction in a solution of citrate phosphate buffer at pH 3.0 for 60 min at 37 °C. The reaction mixture was analyzed by RP-HPLC by using an XTerra MS C18 column (4.6×50 mm) with a linear gradient of CH_3CN (6 to 24 % over 15 min) in 0.1 M triethylammonium acetate (pH 7.0).

quent hydrolysis even after 10 min (Figure S24 in the Supporting Information).

Duplex- and triplex-forming abilities: Next, the ability of the synthesized BA-BNAs to form duplexes and triplexes was evaluated. These BNAs reacted with each stimulus orthogonally and lost their sugar conformational restriction. These structural changes caused changes in the properties of the ONs, such as their duplex- and triplex-forming abilities. The duplex-forming abilities of BA-BNA-modified ONs were evaluated against complementary ssDNA/RNA by measuring T_m values (Table 1). The duplex-forming abilities of ONs **14a–d**, which contain one BA-BNA unit, changed only slightly upon bridge cleavage. The T_m values of duplexes formed between ONs containing three BA-BNA units and ssDNA or ssRNA were dramatically changed by external stimuli because the synergistic effects of bulky aryl moieties on the duplex stability[23] were canceled upon bridge cleavage. Figure 7 showed the ΔT_m values of each ON (form **II** and **III**) relative to that of form **I**. ONs **15a–d** (form **III**) showed remarkably higher binding affinity to their DNA complement than form **I** (Figure 7 A; T_m values are at most 12 °C higher). With ssRNA, the binding affinity of form **III** was much higher than form **I** in the case of **15a**, whereas they were almost identical in case of **15b–d**. ON **15b** (form **II**) showed much lower T_m values than form **III** (Figure 7 B).

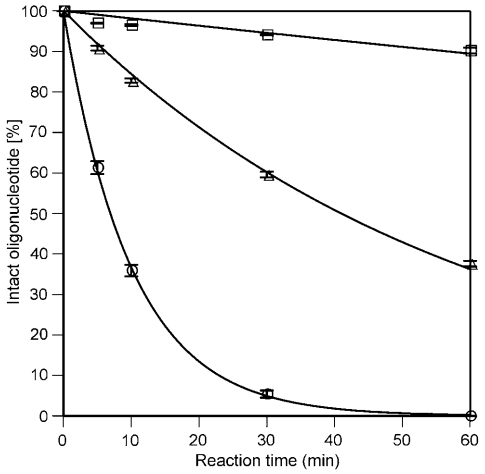


Figure 5. Time course of the reaction of ON **14c** in a solution at pH 3.0 (○; $t_{1/2}$ = 7 min), 4.0 (△; $t_{1/2}$ = 41 min), and 5.0 (□; $t_{1/2}$ > 300 min). Error bars indicate standard deviation (n = 3). Conditions: 25 mM citrate phosphate buffer and 10 μM ON **14c**; reaction temperature = 37 °C.

We also evaluated the triplex-forming ability of ONs containing BA-BNA with target dsDNA (Table 2). BA-BNAs (form **I**) showed low binding affinity, especially if they had three modifications (T_m value < 20 °C). In addition, no triplex formation was observed with 6-NV^B and 2-NB^B after photoirradiation (form **II**), whereas a stable triplex was formed (T_m of 40 °C) after conversion into 4′-*C*-hydroxymethyl RNA (form **III**).

Nuclease resistance of BA-BNA-modified ONs: We examined the resistance of ONs modified with a single BA-BNA unit (ON **18a–d**) toward 3′-exonuclease (*Crotalus adamanteus* venom phosphodiesterase (CAVP)) degradation and compared it with natural ONs. ONs were incubated with CAVP and the percentage of intact ONs was analyzed at several time points by RP-HPLC (Figure 8). The natural oligothymidylates were essentially digested within 20 min, whereas BA-BNA-modified ONs (ON **18a–d**; form **I**) were remarkably stable under these conditions, with about 95 % of each

Table 1. T_m Values [°C] of duplexes formed by BA-BNA-modified ONs with complementary ssDNA/RNA.[a]

Duplexes **X**=	T_m [°C]						
	6-NV^B (**I**)	6-NV^B (**II**)	2-NB^B (**I**)	2-NB^B (**II**)	B^B (**I**)	4-NB^B (**I**)	4′-*C*-hydroxymethyl RNA (**III**)
14a–d/ssDNA	41	39	43	42	45	44	44
15a–d/ssDNA	–[b]	–[b]	23	–[b]	–[b]	24	32
14a–d/ssRNA	39	37	43	39	45	45	43
15a–d/ssRNA	–[b]	–[b]	38	–[b]	34	41	37

[a] Target strand: 5′-AGCAAAAAACGC-3′. Conditions: 10 mM sodium phosphate buffer solution (pH 7.2) containing 100 mM NaCl; each strand concentration = 4 μM; scan rate of 0.5 °C min^{-1} at 260 nm. **X** = BA-BNA. The number is average of three independent measurements. [b] T_m < 20 °C or not detectable.

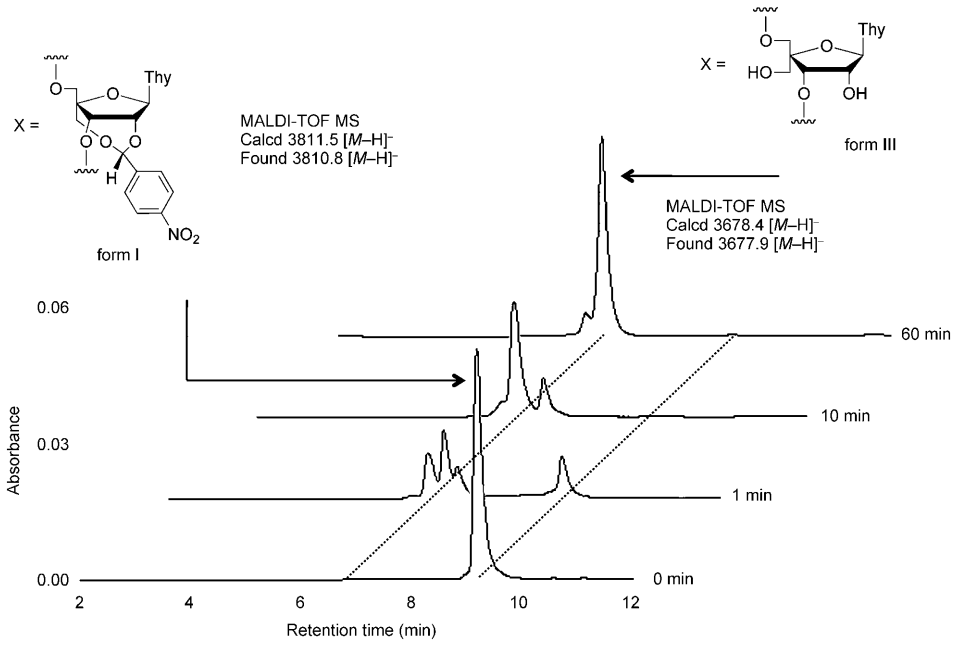


Figure 6. RP-HPLC analysis of ON **14c**. ON **14c** (10 μM) was treated with sodium dithionite (25 mM) for a given time at 37 °C. The reaction mixture was analyzed by RP-HPLC using an XTerra MS C18 column (4.6× 50 mm) with a linear gradient of CH_3CN (6% to 24% over 15 min) in 0.1 M triethylammonium acetate (pH 7.0).

Table 2. T_m Values [°C] of triplexes formed by BA-BNA-modified ONs with target dsDNA.[a]

Oligonucleotides **X**=	T_m [°C]						
	6-NV^B (**I**)	6-NV^B (**II**)	2-NB^B (**I**)	2-NB^B (**II**)	B^B (**I**)	4-NB^B (**I**)	4′-*C*-hydroxymethyl RNA (**III**)
16a–d	24	26	32	27	25	35	41
17a–d	_[b]	_[b]	_[b]	_[b]	_[b]	_[b]	40

[a] Target strand: 5′-d(GCT*AAAAAGAAAGAGAGA*TCG)-3′/3′-d(CGA*TTTTTCTTTCTCTCT*ATC)-5′; the italic portion indicates the target site for triplex formation. Conditions: 7 mM sodium phosphate buffer solution (pH 7.0) containing 140 mM KCl and 10 mM $MgCl_2$; each strand concentration = 1.5 μM; scan rate of 0.5 °C min^{-1} at 260 nm. **X** = BA-BNA; ^mC = 2′-deoxy-5-methylcytidine. The number is average of three independent measurements. [b] $T_m < 20$ °C or not detectable.

ON remaining intact after 20 min of exposure (form **I**: $t_{1/2} >$ 30 h). The nuclease resistance of ON **18a** and **18b** decreased drastically upon photoirradiation (form **II**; $t_{1/2} = 24$ min). Conversion to 4′-*C*-hydroxymethyl RNA (form **III**) also decreased nuclease resistance, but form **III** was still more stable than natural ON ($t_{1/2} = 11$ min *vs* 5 min), in agreement with the report by Wengel et al.[14]

Discussion

Orthogonal reactivity of BA-BNAs to each stimulus: The synthesized BA-BNAs respond to each stimulus (light, acid, or reductant) orthogonally by undergoing cleavage of the bridge according to the type of aryl moiety introduced into the acetal-bridged structure. 6-NV^B and 2-NB^B have a photoreactive 6-nitroveratryl[24] and 2-nitrobenzyl group,[25] respectively. Because an aryl moiety containing an *ortho*-nitro group is required for photoreactivity,[26] the bridge in B^B and 4-NB^B could not be cleaved by light (Figure S22 in Supporting Information). Moreover, a nitro group on the aryl moiety also affects the stability of the acetal bridge against acid. Benzylidene acetal is more sensitive to acid than methylene acetal because of the presence of the electron-donating phenyl group;[27] therefore, B^B loses its bridge under mild acidic conditions. In addition, it was impossible, in our hands, to synthesize ONs with a BA-BNA that had a 2,5-dimethoxybenzyl group because the acetal bridge was degraded by the acid treatment required for detritylation by the automated DNA synthesizer (data not shown). In contrast, all of the other BNAs were stable under these acidic conditions because the introduction of a nitro group into the aryl moiety reduced the electron density of the acetal bridge (Figure S23 in the Supporting Information). Furthermore, the position of the nitro group on the aryl moiety affected the reactivity with the reducing agent. 4-NB^B could effectively react with sodium dithionite and the resulting 4-aminobenzylidene acetal-bridged structure was easily hydrolyzed under neutral conditions. However, reduction of the nitro group in 2-NB^B under the same conditions was less effective (Figure S24 in the Supporting Information). It has been reported that the less-hindered *para*-nitro group in 2,4-dinitroaryl derivatives was preferentially reduced by some reducing agents;[28] therefore, reduction of the *para*-nitro group by sodium dithionite proceeded faster than that of the *ortho*-nitro group.

Change in hybridization properties of each BA-BNA-modified ONs: The hybridizing abilities of ONs **15a–d**, which have three BA-BNAs, to ssDNA were dramatically increased by converting form **I** into form **III**. In general, ONs containing a BNA with a seven-membered bridged ring had low hybridization affinities for their DNA complement.[29] In addition to the seven-membered bridge, BA-BNAs have a bulky aryl moiety in the minor groove, which may substantially destabilize the duplex structure. Transformation from form **I** to form **III** did not affect the hybridizing abilities of these ONs with ssRNA, except for 6-NV^B. Since the sugar

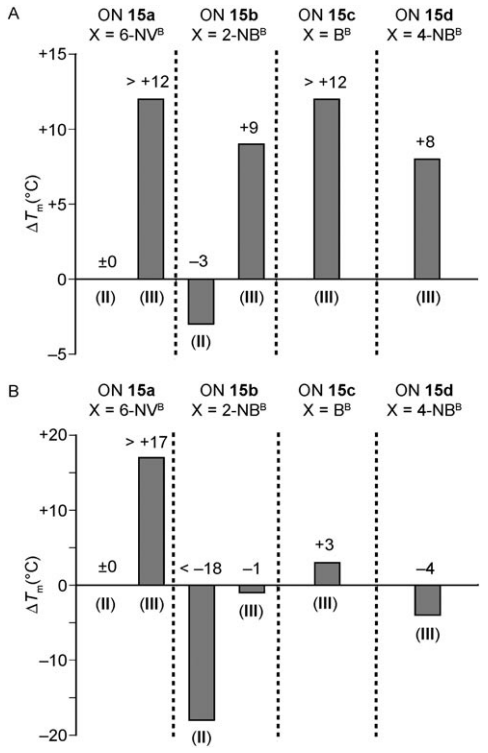


Figure 7. ΔT_m values of ONs **15a–d** (form **II** and **III**) relative to that of form **I** for A) ssDNA and B) ssRNA. ON **15** sequence: 5′-d(GCG**X**T**X**T**X**TGCT)-3′.

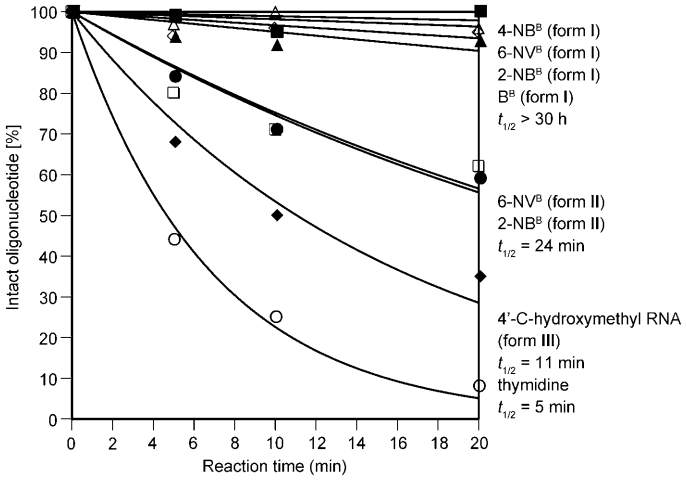


Figure 8. Nuclease resistance of 5′-d(T_8**X**T)-3′ against CAVP (Amersham Pharmacia Biotech); **X** = thymidine (○), 6-NV^B (form **I**) (△), 6-NV^B (form **I**) (□), 2-NB^B (form **I**) (◇), 2-NB^B (form **II**) (■), B^B (▲), 4-NB^B (■), 4′-*C*-hydroxymethal RNA (◆). Hydrolysis of the oligonucleotides (7.5 μM) was carried out at 37 °C in buffer (100 μL) containing 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, and CAVP (0.02 μg).

conformation of BA-BNA (form **I**) is restricted to N-type, which is the major conformation in the A-form of the RNA duplex structure,[30] these compounds can bind to RNA despite the presence of a large hydrophobic group in the minor groove.[31] 6-NV^B (form **I**) has a methoxy group at the 3-position of the aryl moiety that may hinder formation of a stable duplex due to steric repulsion (based on a modeling study; Figure S26 in the Supporting Information). Form **III** retained the ability to bind to ssRNA despite a flexible sugar conformation due to a reduction in steric hindrance. 6-NV^B and 2-NB^B (form **II**) lack sugar conformational restriction, yet have bulky substituents; hence, ONs containing form **II** are unable to bind to ssRNA. These results demonstrate that ONs with 6-NV^B and 2-NB^B will have different behavior following photoirradiation and subsequent thiol treatment in two stages.

The hybridizing abilities of all BA-BNAs to target dsDNA were changed upon exposure to the appropriate stimulus. Molecular modeling of triplex ON units helped clarify this change in affinity (Figure S27 in the Supporting Information) and showed that the aryl moiety of BA-BNAs (form **I** and **II**) in a triplex is positioned near the phosphodiester linkage. The resulting steric hindrance would decrease their ability to hybridize with dsDNA. Form **III** has a high triplex-forming ability due to its reduced steric hindrance.

Nuclease resistance of BA-BNA-modified ONs: Previous studies have shown that the substituent on the bridge structure, and the bridge size, contribute to the nuclease resistance of 2′,4′-BNA/LNA analogues and sterically hindered structures increase the nuclease resistance of modified ONs.[29a,b,d,32] In this study, ONs modified with BA-BNA (form **I**) showed high nuclease resistance against CAVP, probably due to steric hindrance of the aryl-substituted seven-membered bridged moiety. On the other hand, ONs with forms **II** and **III**, which were generated upon cleavage of the bridge, showed decreased nuclease resistance.

General overview: Several properties of ONs containing BA-BNAs can be regulated by various external stimuli, suggesting that BA-BNAs may be applicable to a variety of biotechnological applications. For example, the binding affinities of ONs containing 6-NV^B or 2-NB^B for ssRNA could be changed in two stages: first, by light and then with a thiol. Interestingly, the behavior of an ON could be changed according to which BNA is introduced (Figure 9A). These BA-BNAs could be useful for trapping or releasing functional ssRNA (mRNA, miRNA, or ribozyme) in a low thiol concentration or high thiol concentration environment at the desired time.[33] ONs modified with B^B or 4-NB^B would be able to bind ssRNA regardless of sugar conformation restriction by the bridge, although nuclease resistance is destroyed upon cleavage of the bridge (Figure 9B). These characteristics have a potential application as a molecular system in degradable devices, the properties of which could change in a controlled fashion at a specific location in the molecule.[34]

Many nucleic acid materials have been developed by focusing on base-pair hydrogen-bonding interactions,[35] stacking within DNA helix,[36] the inversion of helicity,[37] the activity of the 2′-hydroxyl group,[38] and recognition of the

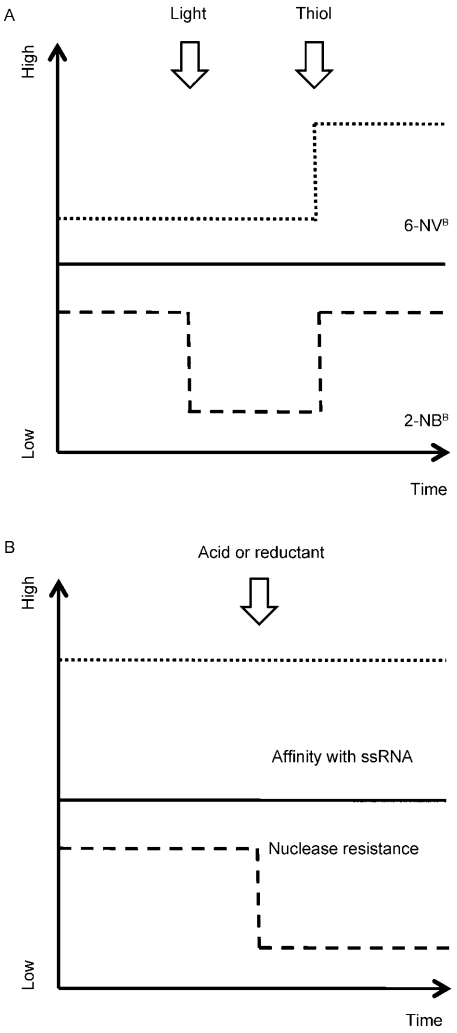


Figure 9. Basic concept behind changing the properties of BA-BNA-modified ONs by external stimuli. A) Change in the affinity of 6-NV^B- or 2-NB^B-modified ONs for ssRNA. B) Change in the affinity of B^B- or 4-NB^B-modified ONs for ssRNA and the nuclease resistance of B^B- or 4-NB^B-modified ONs.

phosphodiester linkage.[39] In this study, we have demonstrated that regulation of sugar conformational restriction is also an effective strategy for controlling the properties of nucleic acids. Molecular devices that exhibit similar responses to different stimuli are unique and attractive candidates for new types of molecular tools. Furthermore, the development of various types of BA-BNAs, in which each responds to a different stimulus, could be easily designed and simply achieved by altering the type of aryl moiety in the bridged structure.

Conclusion

We have synthesized BA-BNAs with a cleavable 2′,4′-bridged structure containing an aryl moiety. Each BA-BNA responds orthogonally to a specific external stimulus. The bridged structures of 6-NV^B and 2-NB^B were cleaved by photoirradiation and subsequent treatment with thiol triggered a change in the secondary structure to generate 4′-*C*-hydroxymethyl RNA. In contrast, the bridged structure of B^B was hydrolyzed under acidic conditions and that of 4-NB^B was hydrolyzed under reducing conditions. The sugar conformational restriction of these BA-BNAs was lost upon cleavage of the bridge. In conclusion, the duplex- and triplex-forming abilities and nuclease resistance of these BA-BNA-modified ONs could be changed at will in response to an appropriate external stimulus.

Experimental Section

Synthesis and characterization of compounds: General aspects and instrumentation: All moisture-sensitive reactions were carried out in well-dried glassware under an N_2 atmosphere. Acetonitrile, pyridine, and toluene were distilled from CaH_2. 6-NV^B and 2-NB^B were stable under ambient light conditions and did not need to be specially protected from light. ^1H, ^{13}C, ^{31}P, NOESY, and H–H COSY spectra were recorded on JEOL JNM-EX270, JEOL JNM-ECS400, and JEOL JNM-LA500 spectrometers. Chemical shifts are reported in parts per million referenced to $CHCl_3$ ($\delta = 7.26$ ppm) and CH_3OH ($\delta = 3.30$ ppm) for ^1H NMR spectra, and $CDCl_3$ ($\delta = 77.0$ ppm) and CD_3OD ($\delta = 49.0$ ppm) for ^{13}C NMR spectra. IR spectra were recorded on JASCO FT/IR-200 and JASCO FT/IR-4200 spectrometers. Optical rotations were recorded on a JASCO DIP-370 instrument. FAB mass spectra were measured on JEOL JMS-600 or JEOL JMS-700 mass spectrometers. MALDI-TOF mass spectra were recorded on a Bruker Daltonics Autoflex II TOF/TOF mass spectrometer. Fuji Silysia silica gel PSQ-100B (0.100 mm) and FL-100D (0.100 mm) were used for column chromatography, and silica gel PSQ-60B (0.060 mm) and FL-60D (0.060 mm) were used for flash column chromatography. For HPLC, SHIMADZU LC-10AT_{VP}, SHIMADZU SPD-10A_{VP} and SHIMADZU CTO-10$_{VP}$ instruments were used.

Compound 2: Under an N_2 atmosphere, a solution of 2-nitrobenzaldehyde (3.8 g, 25 mmol) in anhydrous toluene (5 mL) and zinc chloride (150 mg, 1.1 mmol) were added to **1**[13] (500 mg, 0.94 mmol) and the resultant mixture was stirred at room temperature for 37 h. After the addition of a saturated aqueous solution of $NaHCO_3$ at 0 °C, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (*n*-hexane/AcOEt 2/1) to give **2** as a white foam (400 mg, 65 %). M.p. 140–143 °C; $[\alpha]_D^{23} = -47.0$ ($c = 1.00$ in $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$): $\delta = 8.12$ (br s, 1H; N3-H), 8.02 (t, $J = 8$ Hz, 1H; Ar-H), 7.89 (t, $J = 8$ Hz, 1H; Ar-H), 7.66 (t, $J = 8$ Hz, 1H; Ar-H), 7.58 (s, 1H; H6), 7.49 (t, $J = 8$ Hz, 1H; Ar-H), 6.91 (s, 1H; acetal-H), 6.15 (s, 1H; H1′), 4.65 (d, $J = 6$ Hz, 1H; H3′), 4.41 (d, $J = 6$ Hz, 1H; H2′), 4.03 (d, $J = 13$ Hz, 1H; H5′), 4.00 (d, $J = 13$ Hz, 1H; H1“), 3.71 (d, $J = 13$ Hz, 1H; H1”), 3.67 (d, $J = 13$ Hz, 1H; H5′), 1.92 (s, 3H; CH_3), 1.13–1.08 ppm (m, 28H; TIPDS-H); ^{13}C NMR (67.8 MHz, $CDCl_3$): $\delta = 164.2$, 150.1, 148.0, 134.7, 133.6, 133.3, 129.4, 128.1, 124.2, 110.2, 98.1, 91.9, 89.3, 79.3, 70.6, 68.2, 59.6, 17.3, 17.3, 17.2, 17.1, 17.1, 17.0, 16.9, 12.7, 12.6, 12.4 ppm; IR (KBr): $\tilde{\nu}_{max} = 1274$, 1465, 1528, 1681, 2947 cm^{-1}; MS (FAB): *m/z*: 664 $[M+H]^+$; HRMS (FAB): *m/z* calcd for $C_{30}H_{46}N_3O_{10}Si_2$ $[M+H]^+$: 664.2722; found: 664.2728.

Compound 4: Under an N_2 atmosphere, a solution of 4-nitrobenzaldehyde (4.3 g, 28 mmol) in anhydrous HFIP (12 mL) and zinc chloride (130 mg, 0.94 mmol) were added to **1** (500 mg, 0.94 mmol) and the resultant mixture was stirred at room temperature for 18 h. After the addition of a saturated aqueous solution of $NaHCO_3$ at 0 °C, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (*n*-hexane/AcOEt 9/1→7/3) to give **4** as a yellow foam (370 mg, 60 %). M.p. 118–120 °C; $[\alpha]_D^{23} = -5.4$ ($c = 1.00$ in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 9.27$ (br s, 1H; N3-H), 8.21 (t, $J = 8$ Hz, 2H; Ar-H), 7.69 (t, $J = 8$ Hz, 2H; Ar-H), 7.54 (s, 1H; H6), 6.27 (s, 1H;

acetal-H), 6.14 (s, 1H; H1′), 4.67 (d, $J=6$ Hz, 1H; H3′), 4.42 (d, $J=6$ Hz, 1H; H2′), 4.06 (d, $J=13$ Hz, 1H; H5′), 3.93 (d, $J=13$ Hz, 1H; H1“), 3.80 (d, $J=13$ Hz, 1H; H1”), 3.69 (d, $J=13$ Hz, 1H; H5′), 1.91 (s, 3H; CH_3), 1.12–1.07 ppm (m, 28H; TIPDS-H); ^{13}C NMR (100.5 MHz, $CDCl_3$): $\delta=$ 163.6, 149.7, 148.1, 145.5, 135.1, 127.3, 123.5, 110.3, 102.2, 92.4, 88.9, 79.3, 77.2, 70.8, 68.7, 60.1, 17.3, 17.3, 17.2, 17.2, 17.1, 17.1, 16.9, 13.4, 12.7, 12.6, 12.4 ppm; IR (KBr): $\tilde{\nu}_{max}=1347$, 1525, 1695, 2946 cm^{-1}; MS (FAB): m/z: 664 $[M+H]^+$; HRMS (FAB): m/z calcd for $C_{30}H_{46}N_3O_{10}Si_2$ $[M+H]^+$: 664.2722; found: 664.2712.

Compound 5: TBAF (1.0 M in THF, 1.2 mL, 1.2 mmol) was added to a solution of **2** (370 mg, 0.60 mmol) in THF (17 mL) at 0 °C and the mixture was stirred at 0 °C for 1 h. The reaction mixture was concentrated and the crude product was purified by column chromatography (AcOEt/MeOH 30/1) to give **5** as a white powder (220 mg, 87 %). M.p. 153–156 °C; $[\alpha]_D^{27}=-59.9$ ($c=1.00$ in MeOH); ^{1}H NMR (270 MHz, CD_3OD): $\delta=8.04$ (d, $J=8$ Hz, 1H; Ar-H), 8.02 (d, $J=1$ Hz, 1H; H6), 7.86 (dd, $J=$ 1, 8 Hz, 1H; Ar-H), 7.70 (dt, $J=1$, 8 Hz, 1H; Ar-H), 7.56 (dt, $J=1$, 8 Hz, 1H; Ar-H), 6.91 (s, 1H; acetal-H), 6.14 (s, 1H; H1′), 4.60 (d, $J=6$ Hz, 1H; H3′), 4.37 (d, $J=6$ Hz, 1H; H2′), 3.95 (d, $J=12$ Hz, 1H; H1“), 3.76 (d, $J=12$ Hz, 1H; H1”), 3.72 (d, $J=12$ Hz, 1H; H5′), 3.70 (d, $J=12$ Hz, 1H; H5′), 1.87 ppm (d, $J=1$ Hz, 3H; CH_3); ^{13}C NMR (75.5 MHz, CD_3OD): $\delta=166.5$, 152.2, 150.0, 137.7, 135.0, 133.8, 130.6, 128.9, 125.0, 110.8, 99.2, 93.1, 90.6, 81.4, 72.0, 69.1, 60.8, 12.5 ppm; IR (KBr): $\tilde{\nu}_{max}=$ 1272, 1356, 1471, 1528, 1692, 3378 cm^{-1}; MS (FAB): m/z: 422 $[M+H]^+$; HRMS (FAB): m/z calcd for $C_{18}H_{20}N_3O_9$ $[M+H]^+$: 422.1220; found: 422.1171.

Compound 7: TBAF (1.0 M in THF, 0.96 mL, 0.96 mmol) was added to a solution of **4** (320 mg, 0.48 mmol) in THF (12 mL) at 0 °C and the resultant mixture was stirred at 0 °C for 1 h. The reaction mixture was concentrated and the crude product was purified by column chromatography (AcOEt/MeOH 30/1) to give **7** as a yellow powder (160 mg, 81 %). M.p. 164–167 °C; $[\alpha]_D^{24}=+59.9$ ($c=1.00$ in MeOH); ^{1}H NMR (400 MHz, CD_3OD): $\delta=8.24$ (d, $J=8$ Hz, 2H; Ar-H), 7.97 (s, 1H; H6), 7.76 (d, $J=$ 8 Hz, 2H; Ar-H), 6.46 (s, 1H; acetal-H), 6.21 (s, 1H; H1′), 4.62 (d, $J=$ 6 Hz, 1H; H3′), 4.35 (d, $J=6$ Hz, 1H; H2′), 4.02 (d, $J=12$ Hz, 1H; H1“), 3.85 (d, $J=12$ Hz, 1H; H1”), 3.78 (d, $J=12$ Hz, 1H; H5′), 3.71 (d, $J=$ 12 Hz, 1H; H5′), 1.88 ppm (s, 3H; CH_3); ^{13}C NMR (100.5 MHz, CD_3OD): $\delta=166.5$, 152.2, 149.4, 148.0, 137.8, 128.6, 124.3, 110.8, 103.0, 93.1, 90.6, 81.4, 71.9, 69.2, 60.9, 12.6 ppm; IR (KBr): $\tilde{\nu}_{max}=1349$, 1522, 1693, 3396 cm^{-1}; MS (FAB): m/z: 422 $[M+H]^+$; HRMS (FAB): m/z calcd for $C_{18}H_{20}N_3O_9$ $[M+H]^+$: 422.1220; found: 422.1171.

Compound 8: Under an N_2 atmosphere, DMTrCl (200 mg, 0.58 mmol) was added to a solution of **5** (190 mg, 0.44 mmol) in anhydrous pyridine (11 mL) and the resultant mixture was stirred at room temperature for 15 h. After the addition of a saturated aqueous solution of $NaHCO_3$, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4, and concentrated. The crude was purified by column chromatography (0.5 % triethylamine in *n*-hexane/AcOEt 1/2) to give **8** as a white foam (250 mg, 79 %). M.p. 140–142 °C; $[\alpha]_D^{26}=-24.0$ ($c=1.00$ in MeOH); ^{1}H NMR (270 MHz, $CDCl_3$): $\delta=9.27$ (s, 1H; N3-H), 7.97 (d, $J=8$ Hz, 1H; Ar-H), 7.76 (d, $J=8$ Hz, 1H; Ar-H), 7.54 (t, $J=8$ Hz, 1H; Ar-H), 7.47–6.76 (m, 15H), 6.10 (s, 1H; H1′), 4.74 (t, $J=5$ Hz, 1H; H3′), 4.46 (d, $J=6$ Hz, 1H; H2′), 3.93 (d, $J=13$ Hz, 1H), 3.79–3.72 (m, 2H), 3.72 (s, 6H; $OCH_3\times2$), 3.36–3.26 (m, 3H), 1.47 ppm (s, 3H; CH_3); ^{13}C NMR (67.8 MHz, $CDCl_3$): $\delta=163.7$, 158.2, 149.6, 147.4, 143.7, 135.1, 134.7, 134.6, 133.2, 132.8, 129.5, 128.9, 127.6, 127.5, 126.7, 123.6, 112.9, 109.9, 98.0, 92.2, 88.1, 86.4, 79.1, 70.9, 69.1, 61.4, 54.7, 11.7 ppm; IR (KBr): $\tilde{\nu}_{max}=1252$, 1693, 3008 cm^{-1}; MS (FAB): m/z: 746 $[M+Na]^+$; HRMS (FAB): m/z calcd for $C_{39}H_{37}N_3NaO_{11}$ $[M+Na]^+$: 746.2326; found: 746.2325.

Compound 9: Under an N_2 atmosphere, DMTrCl (150 mg, 0.45 mmol) was added to a solution of **6**[15] (130 mg, 0.35 mmol) in anhydrous pyridine (9 mL) and the resultant mixture was stirred at room temperature for 11 h. After the addition of a saturated aqueous solution of $NaHCO_3$, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (0.5 % triethylamine in *n*-hexane/AcOEt 1/1→1/2) to give **9** as a white foam (200 mg, 84 %). M.p. 165–168 °C; $[\alpha]_D^{26}=+7.80$ ($c=1.00$ in MeOH); ^{1}H NMR (270 MHz, $CDCl_3$): $\delta=8.52$ (br s, 1H; N3-H), 7.52–6.83 (m, 19H), 6.27 (s, 1H; acetal-H), 6.23 (s, 1H; H1′), 4.79 (dd, $J=4$, 6 Hz, 1H; H3′), 4.50 (d, $J=6$ Hz, 1H; H2′), 3.97 (d, $J=13$ Hz, 1H; H1“), 3.87 (d, $J=13$ Hz, 1H; H1”), 3.79 (s, 6H; $OCH_3\times$ 2), 3.43 (d, $J=11$ Hz, 1H; H5′), 3.34 (d, $J=11$ Hz, 1H; H5′), 2.77 (d, $J=$ 4 Hz, 1H; OH), 1.64 ppm (s, 3H; CH_3); ^{13}C NMR (67.8 MHz, $CDCl_3$): $\delta=164.3$, 158.6, 150.0, 144.2, 139.3, 136.3, 135.3, 135.2, 130.0, 128.8, 128.3, 128.0, 127.1, 126.1, 113.3, 110.3, 103.8, 92.6, 88.3, 86.7, 79.1, 71.1, 69.9, 62.3, 60.4, 55.2, 21.0, 14.1, 12.1 ppm; IR (KBr): $\tilde{\nu}_{max}=1252$, 1691, 2930, 3062, 3382 cm^{-1}; MS (FAB): m/z: 701 $[M+Na]^+$; HRMS (FAB): m/z calcd for $C_{39}H_{39}N_2O_9$ $[M+H]^+$: 679.2656; found: 679.2692.

Compound 10: Under an N_2 atmosphere, DMTrCl (150 mg, 0.45 mmol) was added to a solution of **7** (140 mg, 0.34 mmol) in anhydrous pyridine (9 mL) and the resultant mixture was stirred at room temperature for 21 h. After the addition of a saturated aqueous solution of $NaHCO_3$, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (0.5 % triethylamine in *n*-hexane/AcOEt = 1/1) to give **10** as a yellow foam (180 mg, 74 %). M.p. 160–162 °C; $[\alpha]_D^{24}=+5.2$ ($c=1.00$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=9.70$ (s, 1H; N3-H), 8.06 (d, $J=8$ Hz, 2H; Ar-H), 7.55 (d, $J=8$ Hz, 2H; Ar-H), 7.37–7.12 (m, 9H), 6.74 (d, $J=8$ Hz, 4H; Ar-H), 6.24 (s, 1H; acetal-H), 6.03 (s, 1H; H1′), 4.76 (s, 1H; H3′), 4.45 (d, $J=6$ Hz, 1H; H2′), 3.88 (s, 2H; H1“), 3.67 (s, 6H; $OCH_3\times2$), 3.32 (t, $J=12$ Hz, 2H; H5′), 2.15 (s, 1H; OH), 1.45 ppm (s, 3H; CH_3); ^{13}C NMR (100.5 MHz, $CDCl_3$): $\delta=164.5$, 158.6, 150.1, 147.9, 145.8, 144.2, 136.3, 135.2, 135.0, 130.0, 127.98, 128.01, 127.3, 127.1, 123.5, 113.3, 110.4, 102.0, 93.5, 88.4, 86.8, 79.3, 77.2, 71.3, 69.9, 62.2, 55.2, 12.1 ppm; IR (KBr): $\tilde{\nu}_{max}=1348$, 1511, 1691, 3211 cm^{-1}; MS (FAB): m/z: 746 $[M+Na]^+$; HRMS (FAB): m/z calcd for $C_{39}H_{37}N_3NaO_{11}$ $[M+Na]^+$: 746.2326; found: 746.2312.

Compound 11: Under an N_2 atmosphere, 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphane (0.29 mL, 0.90 mmol) and 4,5-dicyanoimidazole (0.25 M in CH_3CN, 1.3 mL, 0.33 mmol) were added to a solution of **8** (220 mg, 0.30 mmol) in anhydrous CH_3CN (4 mL) and the resultant mixture was stirred at room temperature for 17 h. After the addition of a saturated aqueous solution of $NaHCO_3$, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4, and concentrated. The crude was purified by column chromatography (0.5 % triethylamine in *n*-hexane/AcOEt 1/1) followed by the precipitation from *n*-hexane/AcOEt to give **11** as a white foam (102 mg, 37 %). M.p. 113–117 °C; ^{31}P NMR (202 MHz, $CDCl_3$): $\delta=150.7$, 150.2 ppm; MS (FAB): m/z: 924 $[M+H]^+$; HRMS (FAB): m/z calcd for $C_{48}H_{55}N_5O_{12}P$ $[M+H]^+$: 924.3585; found: 924.3575.

Compound 12: Under an N_2 atmosphere, 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphane (0.35 mL, 1.1 mmol) and 4,5-dicyanoimidazole (0.25 M in CH_3CN, 1.6 mL, 0.40 mmol) were added to a solution of **9** (250 mg, 0.36 mmol) in anhydrous CH_3CN (5 mL) and the resultant mixture was stirred at room temperature for 40 h. After the addition of a saturated aqueous solution of $NaHCO_3$, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4, and concentrated. The crude was purified by column chromatography (0.5 % triethylamine in *n*-hexane/AcOEt 1/1) followed by precipitation from *n*-hexane/AcOEt to give **12** as a white foam (220 mg, 68 %). M.p. 116–118 °C; ^{31}P NMR (202 MHz, $CDCl_3$): $\delta=151.2$, 150.5 ppm; MS (FAB): m/z: 879 $[M+H]^+$; HRMS (FAB): m/z calcd for $C_{48}H_{56}N_4O_{10}P$ $[M+H]^+$: 879.3734; found: 879.3712.

Compound 13: Under an N_2 atmosphere, 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphane (0.16 mL, 0.51 mmol) and 4,5-dicyanoimidazole (0.25 M in CH_3CN, 0.80 mL, 0.20 mmol) were added to a solution of **8** (130 mg, 0.17 mmol) in anhydrous CH_3CN (3 mL) and the resultant mixture was stirred at room temperature for 15 h. After the addition of a saturated aqueous solution of $NaHCO_3$, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (0.5 % triethylamine in *n*-hexane/AcOEt 1/1) followed by precipitation from *n*-hexane/AcOEt to give **13** as a white foam (120 mg, 77 %). M.p. 109–111 °C; ^{31}P NMR (161.8 MHz, $CDCl_3$): $\delta=151.2$, 150.6 ppm; MS

(FAB): *m/z*: 924 $[M+H]^+$; HRMS (FAB): *m/z* calcd for $C_{48}H_{55}N_5O_{12}P$ $[M+H]^+$: 924.3585; found: 924.3585.

Acknowledgements

This work was supported in part by a Grant-in Aid for Science Research from the Japan Society for the Promotion of Science (JSPS) and the Ministry of Education, Culture Sports, Science and Technology (MEXT), Japan, the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO), and a Grant for Industrial Technology Research from the New Energy and Industrial Technology Development Organization (NEDO) of Japan. K.M. is grateful for a Research Fellowship from the Japan Society for the Promotion of Science (JSPS) for Young Scientists.

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Received: February 18, 2011
Published online: June 3, 2011

