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CONFORMATIONALLY LOCKED NUCLEOSIDES. SYNTHESIS AND HYBRIDIZATION PROPERTIES OF OLIGODEOXYNUCLEOTIDES CONTAINING 2',4'-*C*-BRIDGED 2'-DEOXYNUCLEOSIDES[1]

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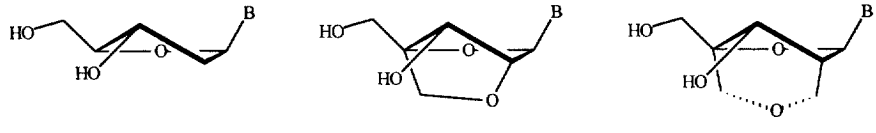
Abstract: A conformationally locked, 2',4'-*C*-bridged 2'-deoxyribofuranoside[2] was condensed with silylated pyrimidines to give 2',4'-*C*-bridged bicyclonucleosides, which were converted to the phosphoramidites and incorporated into oligodeoxynucleotides (ODNs). The hybridization data of the modified ODNs to DNA and RNA are presented. © 1999 Elsevier Science Ltd. All rights reserved.

Oligonucleotides (ONs) as antisense inhibitors of gene expression have been intensively explored in the past decade.[3–6] Recently, oligonucleotides containing conformationally locked nucleosides have drawn considerable attention.[7–17] It was well established that the sugar pucker of DNA in the natural DNA–RNA duplex tends to adopt 3'-endo conformation (**a**) while the 2'-endo sugar pucker predominates in the natural DNA–DNA duplex.[18] It is anticipated that conformationally restrained 3'-endo nucleosides would enhance hybridization of ODNs to the complementary RNA while conformationally restrained 2'-endo nucleosides would enhance hybridization to the complementary DNA. Recently, 2'-*O*,4'-*C*-methyleneribonucleosides (**b**), which have a locked 3'-endo conformation, were synthesized and incorporated into ONs.[13–16] Hybridization studies showed that these conformationally locked bicyclonucleosides dramatically enhanced hybridization of the modified ONs to the complementary RNA and DNA.[14,16] Other conformationally locked nucleosides that have been incorporated into ODNs include those having 4',6'- and 1',6'-methanocarbocyclic nucleosides,[7–9] 3',5'-ethanonucleosides,[10–12] and 2'-*O*,3'-*C*-linked arabinonucleosides.[17] Some of these modifications also have favorable hybridization to DNA and RNA, but to a less extent. It seems that conformationally restrained nucleosides are promising candidates as building blocks of ONs. Recently, we have independently explored conformationally locked nucleosides as building blocks for antisense ODNs. In this communication, we will present synthesis and hybridization properties of ODNs containing 2',4'-*C*-bridged 2'-deoxynucleosides (**c**). Compared to **b** in which the strain in the new ring affects the sugar pucker and orientations of C5'and the nucleoside base, **c** does not have such a strain in the new ring and, therefore, the orientations of C5' and the nucleoside base should not deviate much from those in the typical 3'-endo form (**a**). Hopefully, modified ODNs containing **c** would retain certain natural ODNs' favorable properties such as efficient binding affinity and capability to induce RNase H activity while they are anticipated to be more stable to cellular nucleases.



a **b** **c**

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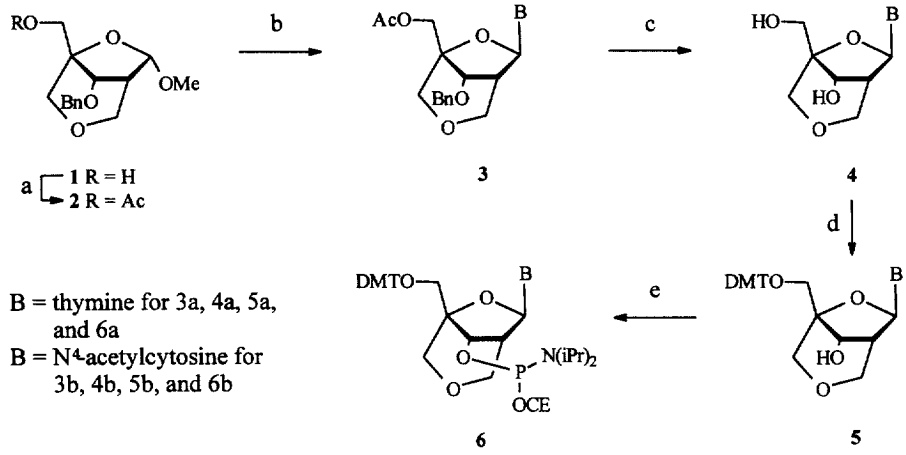
Synthesis of the 2',4'-*C*-bridged 2'-deoxynucleosides and their phosphoramidites is shown in Scheme 1. Preparation of 2',4'-*C*-bridged 2'-deoxyribofuranoside **1** was reported in a previous communication.[2] Acetylation of **1** afforded **2**, which was condensed with the silylated thymine and N^4-acetylcytosine with tin (IV) chloride as coupling reagent to give **3a** and **3b**, respectively. When B is thymine, a mixture of **3a** (β-anomer) and its α-anomer were obtained, with a β:α ratio of ~4:1. For cytidine derivative, **3b** was contaminated only by a minor amount of its α-anomer, with a β:α ratio of ~9:1. The α-anomer could be readily separated from **3b** by chromatography. When trimethylsilyl triflate was used as the coupling reagent, the reactions of the silylated pyrimidines with 1-*O*-acetyl derivative of **2** gave the α-nucleosides as the major or exclusive products (not shown). Treatment of **3a** (containing ~20% α-anomer) and **3b** with boron trichloride afforded **4a** (containing ~20% α-anomer) and **4b**, respectively. Compound **4a** could be separated from its α-anomer by chromatography. Compounds **4a** and **4b** were protected with DMT at O5' and then converted to the corresponding phosphoramidites **6a** and **6b**, respectively.

Scheme 1.



(a) Ac_2O, pyridine, rt, 99%; (b) di- or tri(trimethylsiyl)pyrimidines, $SnCl_4$, $(CH_2)_2Cl_2$, reflux, 1–2 h, 83% for **3a** and its α-anomer; 89% for **3b**; (c) BCl_3, CH_2Cl_2, rt, overnight, 93% for **4a**; 56% for **4b**; (d) DMT-Cl, pyridine, rt, 15 h, 82% for **5a**; 87% for **5b**; (e) Cl-P(OCH_2CH_2CN)N(i-Pr)$_2$, (i-Pr)$_2$NEt, CH_2Cl_2, rt, 2 h, 76% for **6a**; 4 h, 89% for **6b**.

The stereochemical assignments of the 2,4-*C*-bridged 2-deoxyribofuranoside **1** was described in the previous communication.[2] The conformations of the nucleosides resulting from the condensation reaction of **2** and the silylated pyrimidines can be assigned by the same token.[2] As can be seen from a ball-stick model, in **4a** and **4b** (β-nucleosides), the protons (H1' and H2') at C1' and C2' direct to the different directions and the torsion angle of H1'-C1'-C2'-H2' is around 90°. As expected, no coupling between the H1' and H2' in **4a** and **4b** was observed. However, a coupling constant of 4.2 Hz was observed in the α-anomer of **4a** in which the H1' and H2' are on the same side of the ribose ring and nearly parallel. X-ray crystallography of **4a** (Fig. 1) further confirmed the conformational assignments from NMR data. The X-ray crystal structure also demonstrates that the thymine base has the anti orientation.

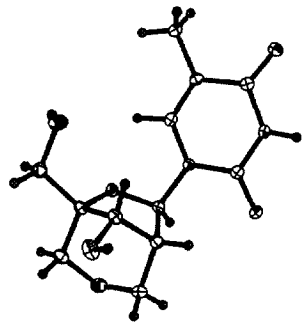


Figure 1. X-ray crystal structure of **4a**

Modified ODNs containing the bicyclonucleosides were synthesized by using the phosphoramidites **6a** and **6b** by a standard procedure[19] except that a more concentrated solution and a prolonged coupling time were used. The solution for the modified phosphoramidites were 0.11 M that is 10% more concentrated than those for the unmodified phosphoramidites (0.1 M). Ten minutes coupling time was used for the modified phosphoramidites and five minutes for the unmodified phosphoramidites next to the modified ones. The coupling yields for the modified phosphoramidites are slightly lower (95–99%) than, or comparable to, the unmodified (98–99%). The modified ODNs were purified by reverse-phase HPLC and characterized by mass spectrometry.

Table 1. Hybridization data of ODNs containing the 2',4'-*C*-bridged bicyclonucleosides

Sequence	Tm °C DNA	ΔTm °C/Mod.	Tm °C RNA	ΔTm °C/Mod.
1. 5'-d(ATCTCTCCGCTTCCTTTC)-3'	58.3		64.4	
2. 5'-d(ATCTCTCCGCTTCCTTTC)-3'	61.9	+0.7	78.1	+2.8
3. 5'-d(ATCTCTCCGCTTCCTTTC)-3'	64.7	+0.8	~82	~+2.2
4. 5'-d(ATCTCTCCGCTTCCTTTC)-3'	57.1	-0.4	71.7	+2.4
5. 5'-d(ATCTCTCCGCTTCCTTTC)-3'	57.3	-0.1	77.5	+1.9
6. 5'-d(CTTCCTGTCTGATGGCTTC)-3'	60.4		63.0	
7. 5'-d(CTTCCTGTCTGATGGCTTC)-3'	61.0	+0.3	69.5	+3.3
8. 5'-d(CTTCCTGTCTGATGGCTTC)-3'	64.5	+1.0	76.2	+3.3
9. 5'-d(CTTCCTGTCTGATGGCTTC)-3'	66.1	+0.7	81.4	+2.3

T = 2',4'-*C*-bridged thymidine (**4a**), **C** = 2',4'-*C*-bridged cytidine (**4b**). The samples for Tm measurements contain 2.0 μM of modified ODNs and 2.0 μM of either complementary DNA or RNA in a buffer (10 mM sodium phosphate, 0.1 mM EDTA, and 0.1 M sodium chloride, pH 7.0).

Hybridization of the modified ODNs to DNA and RNA was studied through the thermodynamic melting measurements.[20] As can be seen in Table 1, the modifications enhance hybridization to RNA significantly. For the sequences containing the bicyclic thymidine **T**, the increases in Tm values are in the range of 2.2–3.3 degrees per modification. It seems that the modified **T** in the middle region of the sequences has more effects

than in the 3'- and 5'-region. The Tm value of Sequence 8 that contains four **T** in the middle region was increased by 3.3° per modification while that of Sequence 2 that has five **T** in the region other than in the middle was increased by 2.8° per modification. The sequences containing the bicyclic cytidine **C** also have higher Tm values than the unmodified ODN, 2.4° higher per modification for Sequence 4 and 1.9° higher per modification for Sequence 5. For the sequences in which all the T and C are replaced by **T** and **C**, the Tm values (>90°) were increased further so that it was not possible to obtain the accurate Tm values in our measurement system. As expected, these bicyclonucleosides do not significantly affect hybridization of the modified ODNs to DNA. A moderate increase in Tm for the sequences containing **T** and a slight decrease in Tm for the sequences containing **C** were observed.

In summary, we have reported synthesis and conformational assignments of the pyrimidine nucleosides having 2',4'-*C*-bridged 2'-deoxyribofuranose as the sugar moiety. The 2',4'-*C*-bridged nucleosides were converted to the phosphoramidites and incorporated into ODNs. Hybridization studies have shown that these bicyclonucleosides significantly increase hybridization of the modified ODNs to the complementary RNA. Further studies are underway in this laboratory.

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References and Notes

1. Part of this work was presented as an oral presentation at *the International Round Table, Nucleosides, Nucleotides, And Their Biological Applications,* Montpellier, **1998**.
2. Wang, G.; Gunic, E. A communication letter was accepted and will appear in *Nucleosides Nucleotides* **1999**.
3. Crooke, S. T.; Lebleu, B. *Antisense Research and Applications*; CRC: Boca Raton, 1993.
4. Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543.
5. Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, 49, 6123.
6. Sanhvi, Y. S.; Cook, P. D. *Carbohydrate Modifications in Antisense Research; ACS Symposium Series, No. 580*; American Chemical Society: Washington, D.C., 1994.
7. Marquez, V. E.; Siddiqui, M. A.; Ezzitouni, A.; Rusa, P.; Wang, J. *J. Med. Chem.* **1996**, *39*, 3739.
8. Altmann, K-H.; Kesserring, R.; Francotte, E.; Rihs, G. *Tetrahedron Lett.* **1994**, *35*, 2331.
9. Altmann, K-H.; Imwinkelried, R.; Kesselring, R.; Rihs, G. *Tetrahedron Lett.* **1994**, *35*, 7625.
10. Tarkoy, M.; Bolli, M.; Leumann, C. *Helv. Chim. Acta.* **1994**, *77*, 717.
11. Litten, J. C., Epple, C., Leumann, C. J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1231.
12. Litten, J. C.; Leumann, C. *Helv. Chim. Acta.* **1996**, *79*, 1129.
13. Obika, S.; Nanbu, D.; Hari, Y.; Morio, K-i.; In, Y.; Ishida, T.; Imanishi, T. *Tetrahedron Lett.* **1997**, *38*, 8735.
14. Obika, S.; Nanbu, D.; Hari, Y.; Andoh, J-i., Morio, K-i.; Doi, T.; Imanishi, T. *Tetrahedron Lett.* **1998**, *39*, 5401.
15. Singh, S.; Nielsen, P.; Koshkin, A. A.; Wengel, J. *J. Chem. Commun.* **1998**, 455.
16. Koshkin, A. A.; Singh, S.; Nielsen, P.; Rajwanshi, V. K.; Kumar, R.; Meldgaard, M.; Olsen, C. E.; Wengel, J. *Tetrahedron* **1998**, *54*, 3607.
17. Christensen, N. K., Petersen, M., Nielsen, P., Jacobsen, J. P., Olsen, C. E., Wengel, J. *J. Am. Chem. Soc.* **1998**, *120*, 5458;.
18. Saenger W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.
19. A prorocol for ABI 394 Synthesizer from Perkin-Elmer (1994).
20. Wang, G.; Middleton, P. J.; He, L.; Stoisavljevic, V.; Seifert, W. E. *Nucleosides Nucleotides* **1997**, *16*, 445.