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A Nonafluoro Nucleoside as a Sensitive ^{19}F NMR Probe of Nucleic Acid Conformation

Nivrutti B. Barhate,[†] Rekha N. Barhate,[†] Pavol Cekan,[‡] Gary Drobny,[†] and Snorri Th. Sigurdsson*[,‡]

Department of Chemistry, University of Washington, Seattle, Washington 98195, and University of Iceland, Science Institute, Dunhaga 3, 107 Reykjavik, Iceland

snorrisi@hi.is

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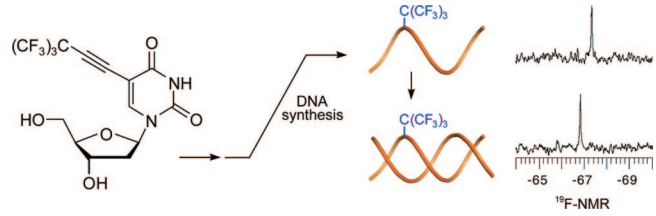
ABSTRACT



A nucleoside carrying a perfluorinated *tert*-butyl group (4) was prepared by a Sonogashira coupling of 5-iodo-2′-deoxyuridine with 4,4,4-trifluoro-3,3-bis(trifluoromethyl)-1-butyne in nearly quantitative yield and subsequently incorporated into DNA oligomers. Thermal denaturation studies showed that 4 had a negligible effect on duplex stabilty when compared to thymidine. Transition from single strand to duplex was monitored by ^{19}F NMR spectroscopy at micromolar concentrations of oligomers, demonstrating the sensitivity of 4 as an NMR reporter nucleoside.

Fluorine has many advantages as a probe for NMR spectroscopy of nucleic acids and other biopolymers.[1−3] The fluorine atom has a single naturally occurring isotope (^{19}F), which has a spin of one-half. High gyromagnetic ratio contributes to high sensitivity of ^{19}F (approximately 83% of the sensitivity of ^{1}H) and facilitates long-range distance measurements through dipolar−dipolar coupling. For example, ^{19}F−^{31}P rotational-echo double-resonance (REDOR) SSNMR has been used to monitor conformational changes in DNA and RNA upon ligand binding.[4,5] Moreover, the near-nonexistence of fluorine atoms in biological systems enables ^{19}F NMR studies without background signal interference. Furthermore, the chemical shift of ^{19}F has been shown to be very sensitive to its environment. For example, conformational equilibria of bistable RNAs,[6] Mg^{2+}-induced folding of the hammerhead ribozyme,[7] and metal ion binding to the TAR RNA[8] have been studied by ^{19}F NMR by incorporation of fluorinated nucleosides (**1** and/or **2**, Scheme 1) and monitoring changes in their ^{19}F chemical shift. Heteronuclear ^{1}H−^{19}F and homonuclear ^{19}F−^{19}F NOE experiments have also been performed on RNA[9] and long-range ^{1}H−^{19}F scalar couplings correlated with torsion angles of glycosidic bonds.[10]

[†] University of Washington.
[‡] University of Iceland.

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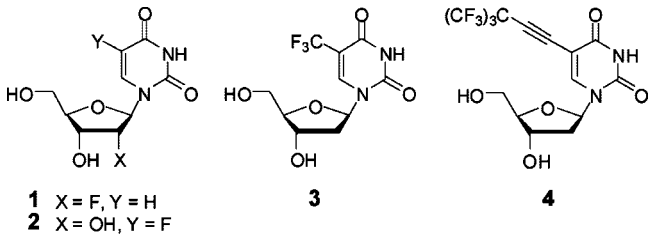
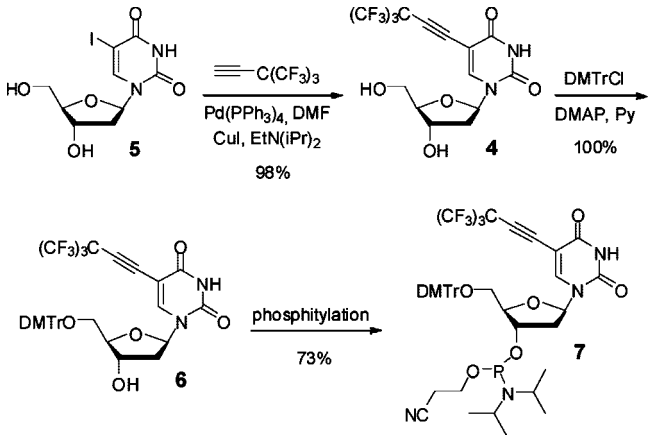
Scheme 1. Fluorinated Nucleosides **1**−**4**



Incorporation of multiple magnetically equivalent fluorine atoms into nucleic acids enables ^{19}F NMR experiments to be performed at lower concentrations, thereby reducing the material requirements and minimizing potential aggregation problems. More equivalent fluorine atoms would also enable longer distance measurements by ^{19}F−^{31}P REDOR SS-NMR.[11,12] We have previously incorporated nucleoside **3** into DNA but found that the CF_3 group was not stable.[13] In this paper, we describe the synthesis of nonafluoro nucleoside **4**, which contains nine magnetically equivalent fluorine atoms, incorporation into DNA, and its use for studying nucleic acid conformation at micromolar concentrations by ^{19}F NMR spectroscopy.

Nucleoside **4** was conveniently synthesized in one step by palladium(0)-catalyzed Sonogashira coupling of 5-iodo-2′-deoxyuridine with 4,4,4-trifluoro-3,3-bis(trifluoromethyl)-butyne (Scheme 2). No N3-alkylation was observed, unlike

Scheme 2. Synthesis of Nonafluorophosphoramidite **7**



the reaction of 3,3,3-trifluoropropyne with 5-iodo-2′-deoxyuridine, which yielded exclusively the N-alkylated product under similar conditions.[14] Tritylation and phosphitylation furnished phosphoramidite **7** in 72% overall yield (three steps).

The DNA sequence 5′-d(G**4**GCGCAC) was prepared by automated solid-phase synthesis using phosphoramidite **7**. After deprotection and purification of the DNA by denaturing polyacrylamide gel electrophoresis, mass spectrometric- and HPLC-analysis of the enzymatic digest verified incorporation of **4** into the oligomer (Supporting Information). We have previously shown that the CF_3-group in **3** is converted to 5-cyano-2′-deoxyuridine in aqueous ammonia at 55 °C, commonly used for deprotection of DNA prepared by chemical synthesis.[13] On the other hand, **4** is stable under those conditions for extended periods of time (>15 h, data not shown). Furthermore, we have not noticed any decomposition of **4**, or oligonucleotides containing **4**, that have been stored at 25 °C in buffered aqueous solutions at neutral pH 7 for up to a week. The effects of nucleoside **4** on duplex stability were determined by thermal denaturation experiments on DNA duplexes containing **4** (Table 1). Incorporation of **4** had a very minor effect on duplex stability when compared to oligomers containing T in the same positon(s). For one or two modifications per duplex, the differences in T_M were ≤1.2 °C. All the modified duplexes had a slightly higher T_M, as observed for 5-alkyno-modified pyrimidines in DNA.[15]

Table 1. Melting Temperatures of DNA Duplexes **I**−**VI**[a]

duplex	sequence	T_M(°C)	ΔT_M[b] (°C)
I	5′-d(GTGCGCAC) 3′-d(CACGCGTG)	49.5 ±0.5	
II	5′-d(G**4**GCGCAC) 3′-d(CACGCG**4**G)	50.0 ±0.5	+ 0.5
III	5′-d(GACCTCGCATCGTG) 3′-d(CTGGAGCGTAGCAC)	60.6 ±0.3	
IV	5′-d(GACC**4**CGCATCGTG) 3′-d(CTGGAGCGTAGCAC)	61.2 ±0.5	+ 0.6
V	5′-d(GACCTCGCATCGTG) 3′-d(CTGGAGCG**4**AGCAC)	61.5 ±0.4	+ 0.9
VI	5′-d(GACC**4**CGCATCGTG) 3′-d(CTGGAGCG**4**AGCAC)	61.8 ±0.4	+ 1.2

[a] 2 μM duplex in 10 mM sodium phosphate, 100 mM NaCl, 0.1 mM Na_2EDTA, pH 7.0. [b] ΔT_M is the difference in the T_M value between the duplex having a modified base and that having a natural base.

To evaluate the usefulness of nucleoside **4** for the study of nucleic acid conformation, a solution of 5′-d(CACGA**4**GCGAGGTC) was treated with aliquots of the complementary oligonucleotide 5′-d(GACCTCGCATCGTG) and the ^{19}F NMR spectra recorded. The single strand (Figure 1, top spectrum) has a chemical shift of −67.36 ppm, relative to CCl_3F. Addition of 0.4 equiv of the complementary strand yielded a new peak at −66.91 ppm, which integrates for ca. 40% of the fluorine. Upon further addition, the signal observed for the single strand decreased, along with the

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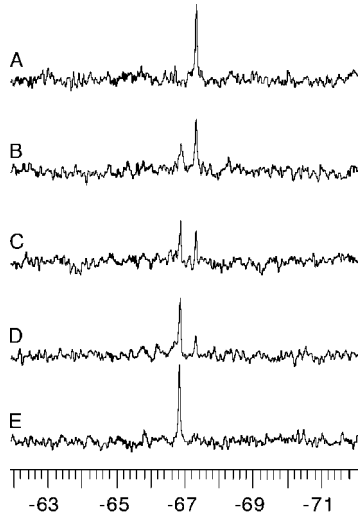

Figure 1. DNA duplex formation monitored by ^{19}F NMR spectroscopy. ^{19}F NMR spectra of single stranded 5′-d(CACGA**4**GCGAGGTC) (A) and after addition of 0.4 equiv (B), 0.6 equiv (C), 0.8 equiv (D), and 1.0 equiv (E) of the complementary strand 5′-d(GACCTCGCATCGTG). Spectra were recorded in deuterated 10 mM phosphate buffer, pH 7.0, 100 mM NaCl, 0.1 mM EDTA. The concentration of the fluorinated oligomer was 3.3 μM. Recorded on a Bruker NMR DRX 500 at 298 K.

concomitant increase in the signal at −66.91 ppm, and disappeared after addition of 1 equiv of the complementary strand. These data are consistent with formation of a duplex (Table 1) that has a different ^{19}F chemical shift than the single strand.

The NMR titration experiment illustrates two aspects of the sensitivity of nucleoside **4** as a probe of nucleic acid conformation. First, the NMR experiments can be carried out at micromolar concentrations of the fluorinated oligomer using a 500 MHz spectrometer. Second, the chemical shift of the perfluorinated *tert*-butyl group is sensitive to its environment. The ^{19}F chemical shift of the duplex differed from that of the single strand by ca. 0.5 ppm. The reason for this difference might be placement of the fluorines close to a phosphodiester in the duplex (Figure 2). The fact that the fluorine atoms yield a single signal in the duplex indicates that there is free rotation around both single bonds flanking the triple bond. Restriction of this rotation due to nucleic acid conformational change or intermolecular complex formation would likely give multiple fluorine signals, which is another potentially useful feature of this probe.

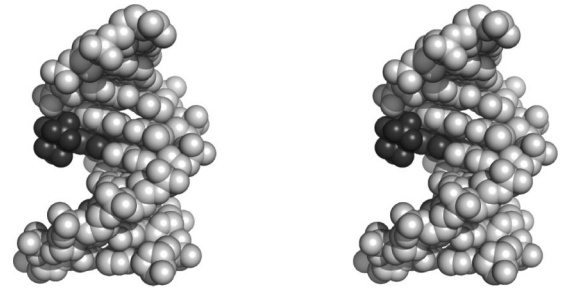

Figure 2. Molecular model of a duplex DNA containing nucleoside **4** (stereoview), showing the position of the perfluorinated *tert*-butyl group (dark gray) in the major grove of the DNA duplex.

In summary, we have described a short and efficient synthesis of a stable nonafluorinated nucleoside that does not perturb the stability of DNA duplexes. To our knowledge, this is the first example of conjugation of a perfluorinated *tert*-butyl group to biopolymers. We have demonstrated that nucleoside **4** is a sensitive NMR probe of nucleic acid conformation, both in terms of changes in the ^{19}F chemical shift and signal intensity arising from nine magnetically equivalent fluorine atoms. Modeling studies indicate that the perfluorinated *tert*-butyl group is accommodated in the major groove of RNA (data not shown) and if verified experimentally, this probe should be applicable for RNA studies, for example to investigate conformational equilibria of bistable RNAs.[6] This reporter group might also be useful for studying conformational changes in nucleic acids due to tertiary folding or ligand binding.[16] Fluorine has been shown to alter physiochemical and pharmacokinetic properties of organic compounds.[17] Incorporation of nucleoside **4** into nucleic acid oligomers, perhaps at multiple sites, could enhance the efficacy of cellular delivery and activity of therapeutic nucleic acids.

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Supporting Information Available: Synthetic protocols and spectral data for compounds **4**, **6**, and **7**; preparation and characterization of fluorinated DNA. This material is available free of charge via the Internet at http://pubs.acs.org.

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