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The Synthesis and Hybridization Properties of an Oligonucleotide Containing Hexafluoroacetone Ketal Internucleotide Linkages

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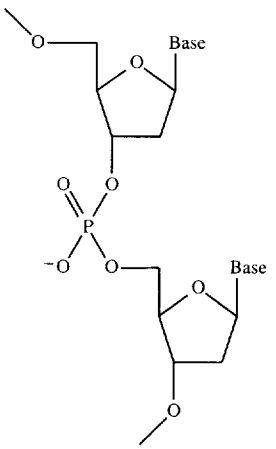
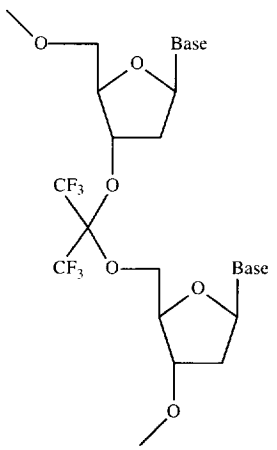
Abstract: The hexafluoroketal functionality is isosteric with the phosphate linkage in DNA and RNA. A thymidine:thymidine dimer bearing a hexafluoroacetone ketal internucleotide linkage has been synthesized from hexafluoroacetone using a Mitsunobu reaction. The resulting dimer has been incorporated into an oligonucleotide and has been shown to result in poorer binding to a complementary DNA and RNA relative to an unmodified control. Copyright © 1996 Elsevier Science Ltd

Neutral analogs of the phosphate linkage within oligonucleotides (ONs) have been investigated as potentially improved "antisense" reagents[1,2], as tools for the mechanistic understanding of enzymes such as polymerases[3] and as probes of protein-nucleic acid interactions.[4] Neutral analogs bearing the phosphorus atom, such as methyl phosphonate, are chiral and difficult to synthesize with stereo control.[5] Numerous achiral, non-phosphorus analogs have been synthesized most of which are not isosteric with the parent phosphate.[1,2] We report the synthesis and preliminary hybridization results of a carbon-based phosphate analog, the hexafluoroacetone ketal. This achiral derivative is sterically similar to a phosphate but without the negative charge. This analog can be synthesized directly via a Mitsunobu procedure capitalizing on the acidity of hexafluoroacetone hemiketals.[6]



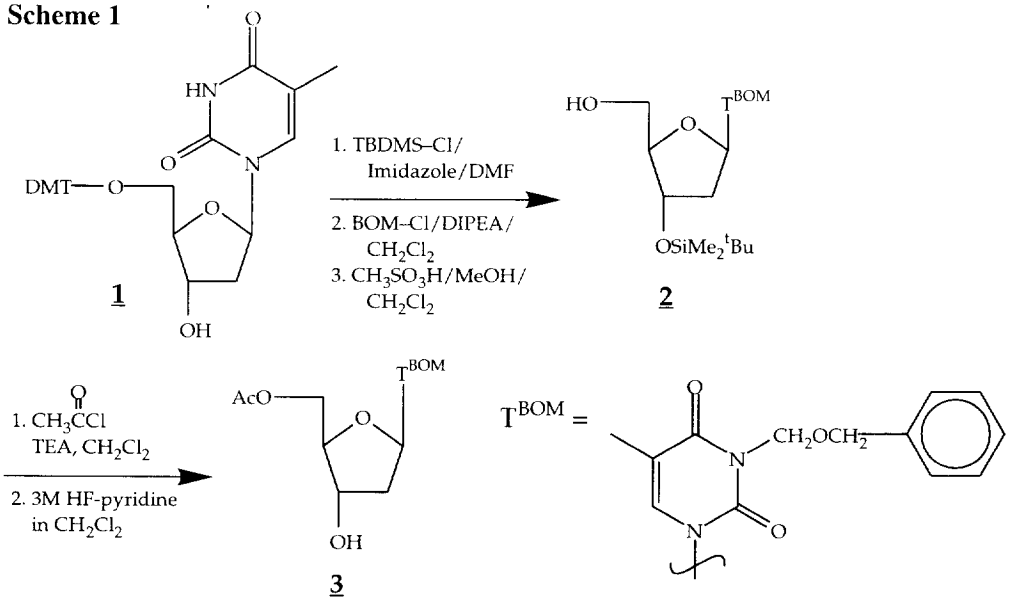
Phosphate



Hexafluoroacetone ketal

The synthesis of the hexafluoroacetone ketal dimer is outlined in Schemes 1 and 2.

Scheme 1



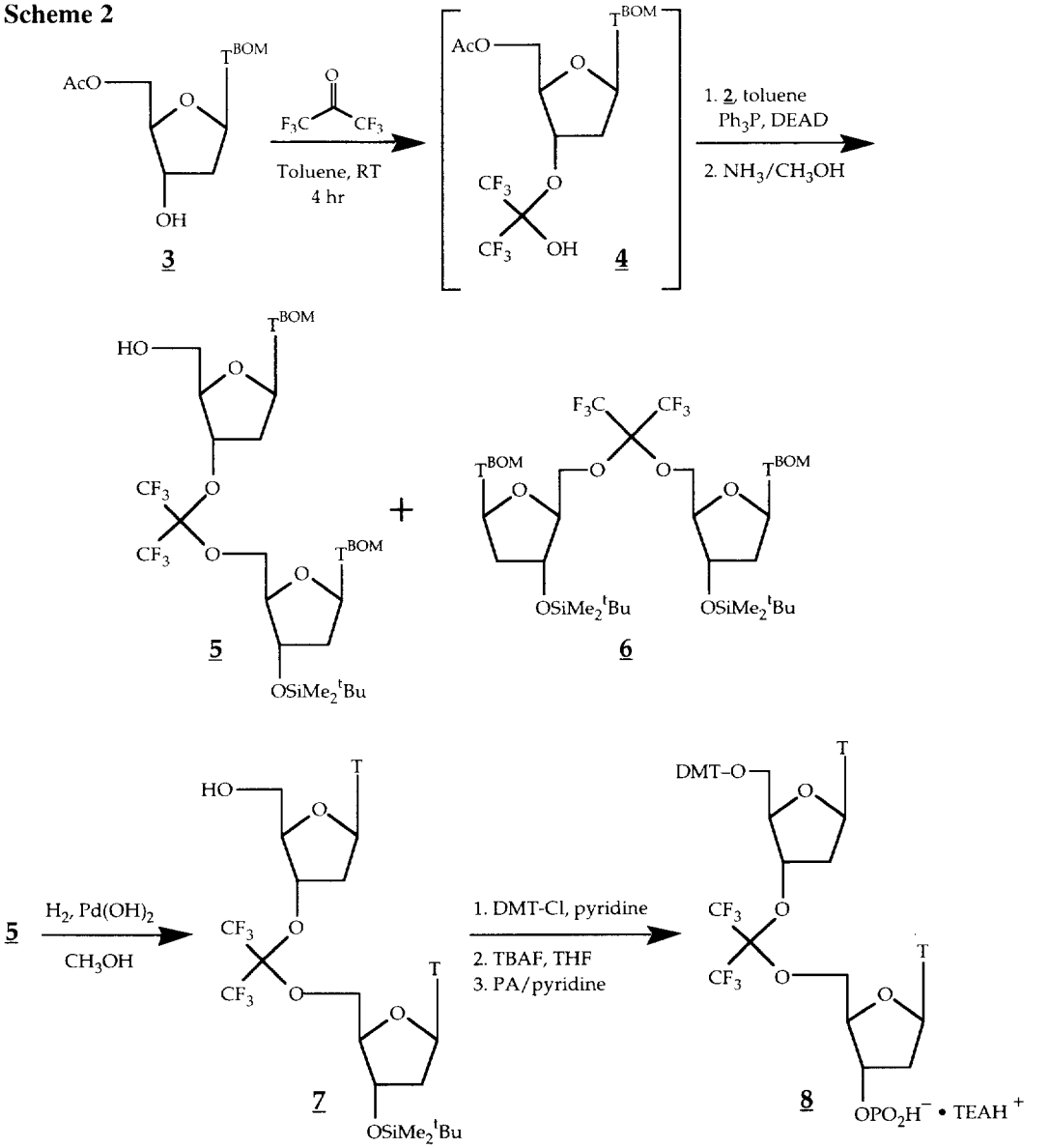
1 **2** **3**

The intermediate **3** was synthesized from commercially available 5'-*O*-DMT-thymidine in five steps. The 3'-hydroxy group was protected with the t-butyldimethylsilyl group (TBDMS). The thymine base was protected at the N^3 position with benzyloxymethyl chloride (BOM-Cl)[7] in the presence of diisopropyl-ethylamine (DIPEA) followed by the deprotection of the DMT group using 1.5 eq. of methanesulfonic acid in 10% methanol/methylene chloride to yield compound **2** (37% in 3 steps). The 5'-hydroxy group of **2** was masked with the acetyl group and the 3'-TBDMS group was removed with hydrogen fluoride-pyridine in methylene chloride (3M) to yield **3** (91%, 2 steps).

Hexafluoroacetone was bubbled into a toluene solution of compound **3** (Scheme 2). The resulting solution of the labile intermediate hexafluoroacetone hemiketal **4** was concentrated to dryness and azeotroped twice with toluene. **4** was redissolved in toluene, coupled with 0.9 eq. of compound **2** via a Mitsunobu reaction[8] by using 1.1 eq. of triphenylphosphine and diethylazodicarboxylate (16 hr). Treatment with saturated methanolic ammonia unmasked the acetyl group from the 5'-hydroxy of the dimer. Two major products were isolated, the desired 3'-5' dimer (**5**) and the 5'-5' dimer (**6**), 53% and 19%, respectively. The BOM group on N^3 was removed by hydrogenation in methanol in the presence of 20% palladium hydroxide on carbon.[9] Silica gel chromatography yielded 42% of 5'-hydroxy hexafluoroacetone 3'-5' ketal T-T dimer **7**.[10]

Compound **7** was converted to the H-phosphonate dimer by tritylation with DMT-Cl, desilylation of the 3'-TBDMS group with tetrabutylammonium fluoride, and phosphitylation[11] with 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one (PA) yielding the ON synthesis-ready synthon **8** (60% in 3 steps).

Scheme 2



T·T dimer **8** was incorporated into two positions of the ON sequence 5'-TCTCTCTCTCT·T T·TT via the standard H-phosphonate protocol.[12,13] The effect of the hexafluoroacetone ketal linkage on the hybridization affinity of ON to its complementary DNA and RNA was assessed by measuring the melting temperature (Tm), Table 1. The Tms resulting from the ON containing the hexafluoroacetone ketal T·T dimer **9** were lower than the Tms resulting from the duplexes derived from the control phosphodiester ON.

The helix destabilization observed with the hexafluoroacetone ketal linkage is not an inherent property of acetal and ketal linkages. The formacetal linkage has been previously investigated in precisely the same sequences and showed a ΔTm per linkage of less than -0.5°C for both RNA and DNA.[14] A likely reason for

the hexafluoroacetone ketal destabilization is the perturbation of water solvation. The non-bridging oxygens in phosphate linkages are highly hydrated and trifluoromethyl groups of this ketal likely are not. The current ON tested the hexafluoroacetone ketal in an alternating context with phosphodiesters. A superior context for the hexafluoroacetone linkage may be a continuous stretch of the fluorocarbon analog. Modeling studies suggest that an ON would have the phosphodiester spine of hydration replaced with a van der Waals contacted spine of fluorocarbon. Testing of this hypothesis awaits further synthesis.

Table 1. Tm Results.

	RNA Complement		DNA Complement	
ON	Tm (°C)	ΔTm (°C/Subst.)	Tm (°C)	ΔTm (°C/Subst.)
5'-TCTCTCTCTCTTTTT	62.0	—	54.5	—
5'-TCTCTCTCTCT•TT•TT	59.5	-1.2	52.5	-1.0

• = Hexafluoroacetone ketal, all other linkages are phosphodiester; T=thymine; C=5-methyl-2'-deoxycytidine. Tm values were assessed in 140 mM KCl/5 mM Na_2HPO_4/l mM $MgCl_2$, pH=7.2, at 260 nm, and the final concentrations of all ONs and RNA were approximately 2 μM. Tm values are ± 0.5°C.

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10. ^{1}H NMR ($CDCl_3$ + 10% CD_3OD) : δ 7.61 (s, 1H), 7.06 (s, 1H), 6.22–6.32 (m, 2H), 4.93 (s, 1H), 4.54 (q, 1H), 4.19 (s, 1H), 4.06 (d, 1H, J = 8.8 Hz), 3.85 –3.95 (m, 2H), 3.78 (d, 1H, J = 11.7 Hz), 3.55 (d, 1H, J = 11.7 Hz), 1.91 and 1.89 (2s, 6H), 0.9 (s, 9H), 0.10 (s, 6H). ^{19}F NMR ($CDCl_3$): 36.1 (q) and 35.8 (q) ppm. FABLR MS: 747 (calc. 747 calc. for M+H) $C_{29}H_{40}N_4O_{10}F_6Si$.
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