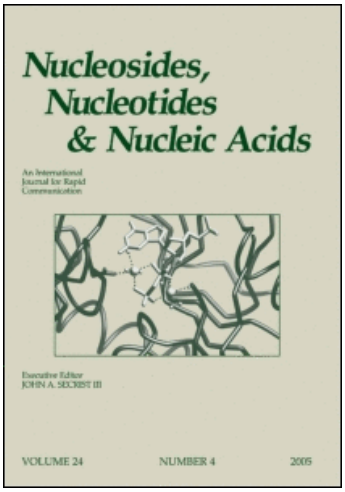


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Fluorine Substituted Analogs of Nucleosides and Nucleotides

Don Bergstrom[a]; Eric Romo[a]; Patrick Shum[a]
[a] Department of Chemistry, University of North Dakota Grand Forks, North Dakota

To cite this Article Bergstrom, Don , Romo, Eric and Shum, Patrick(1987) 'Fluorine Substituted Analogs of Nucleosides and Nucleotides', Nucleosides, Nucleotides and Nucleic Acids, 6: 1, 53 — 63
To link to this Article: DOI: 10.1080/07328318708056179
URL: http://dx.doi.org/10.1080/07328318708056179

NUCLEOSIDES & NUCLEOTIDES, 6(1&2), 53-63 (1987)

FLUORINE SUBSTITUTED ANALOGS OF NUCLEOSIDES AND NUCLEOTIDES

Don Bergstrom*, Eric Romo, and Patrick Shum
Department of Chemistry, University of North Dakota
Grand Forks, North Dakota 58202

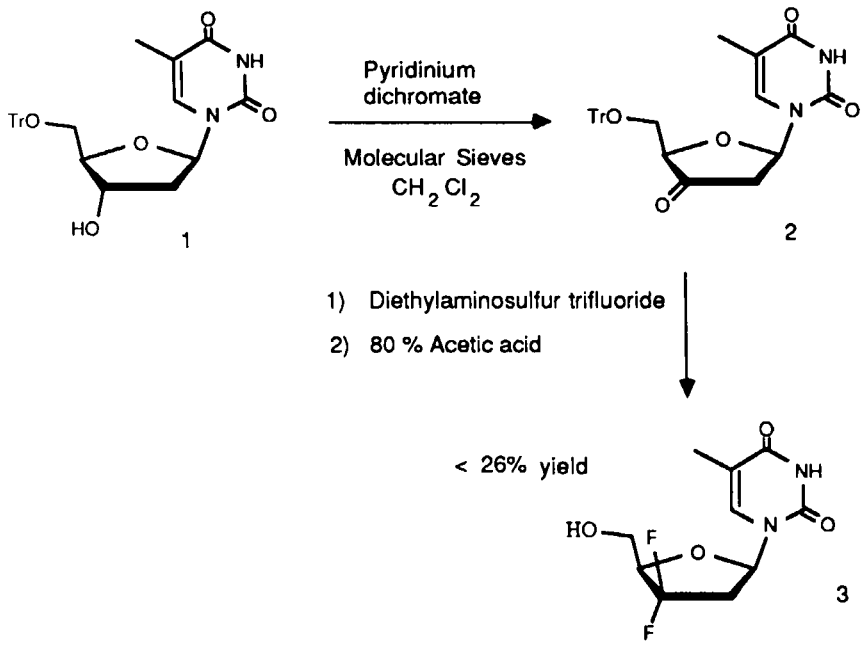
Abstract: The synthesis and ^{19}F NMR spectra of dTMP and TpT analogues in which the phosphate group is replaced by a difluoromethylphosphonate group are described.

Our laboratory is interested in creating new fluorine substituted analogs of nucleosides, nucleotides and oligonucleotides. With the introduction of high field NMR instrumentation capable of observing ^{19}F resonances at very low concentration with virtually no interfering signals, there is increasing justification to prepare new analogues of naturally occurring nucleosides and nucleotides with a variety of different substitution patterns for use as NMR-detectable probes both *in vitro* and *in vivo*.

As a substituent, fluorine is unique because it is relatively small and highly polar. An individual fluorine can serve as an isosteric replacement for hydrogen, while a CF_2 group can function as an isopolar and isosteric substituent for oxygen. Many examples of nucleoside analogs in which fluorine is substituted for hydrogen or hydroxyl on a carbohydrate carbon are known and are of considerable interest because of their biological activities. Much less effort has been devoted to the incorporation of CF_2 groups in place of oxygen or carbon.

Our early efforts addressed the problem of transforming the 2' and 3' carbons to *gem*-difluoro groups. Success was rather limited. 2',3'-Dideoxy-3',3'-difluorothymidine (**3**) can be obtained from thymidine (**1**) in four steps via the 3'-keto derivative, **3** (Scheme 1), however attempts to substitute 2'-ketonucleosides via reaction with diethylaminosulfur trifluoride proved unsuccessful.[1] An alternative strategy is to construct a *gem* -difluoro-substituted sugar followed by linkage to a nucleic acid base utilizing conventional methodology. Progress in this area has recently been reported.[2]

Substantial effort has been devoted to the synthesis of nucleotide analogs in which a phosphate linked oxygen is replaced by an isosteric group such as CH_2 [3] or an isopolar group such as NH or S.[4] However, relatively little effort has been devoted to analogues wherein the oxygen is replaced by a group that is both an effective isosteric and isopolar replacement for oxygen. The most sustained effort in this area has come from G. Michael Blackburn's laboratory. Analogues of nucleoside di- and tri-phosphates have been



Scheme 1

prepared in which a fluoromethylene or difluoromethylene group replaces one of the bridging oxygens.[5,6]

Nonionic sequence specific analogues of oligonucleotides are currently of interest as inhibitors of mRNA expression.[7,8] Oligo(deoxyribonucleoside methylphosphonates) have been studied most extensively. However, it appears that substantially larger side chains on phosphorus can be tolerated and even enhance binding to complementary oligonucleotides.[9]

One of the problems introduced by linking a substituent to the bridging phosphorus is the introduction of chirality at the phosphorus atom. Standard synthetic methodology for the preparation of dimers leads to approximately equal amounts of two diastereomers. Miller et al. have shown that when a mixture of the diastereomeric dimers is incorporated into oligonucleotides, the oligonucleotides differ substantially in the strength of their binding to complementary sequences.[10] Oligonucleotides containing phosphonate linkages with random chirality show a broad melting curve. Oligonucleotides possessing the same properties but with high specificity and narrow melting ranges would definitely be more useful. Possible solutions to this problem include devising stereospecific syntheses of chiral phosphonates or phosphotriesters. Alternatively, it may be possible to find substituents which do not differ substantially in backbone conformation for the R_P and S_P stereoisomers. More research in this area is needed to more precisely define the structural requirements at phosphorus for optimum binding to complementary sequences.

Phosphonates constructed with an HCF_2 group in place of methyl could be particularly interesting because of the HCF_2 's isopolar-isosteric equivalence with an OH group.

In a study with diethyl difluoromethylphosphonate (**5**) we determined that the proton on the HCF_2 group interacts strongly with the Lewis base pyridine (Figure 1). The dependence of the proton chemical shift on pyridine concentration resembles the data obtained in hydrogen bonding studies with chloroform.[11]

Initially, we sought to synthesize the CF_2 phosphonate analogues of thymidine 3'- and 5'-monophosphates (**8** and **9**). Structurally related nucleoside 5'-phosphonate analogues, in which one of the phosphate oxygens was replaced by methyl, hydroxymethyl and chloromethyl have been prepared in other laboratories and their activities as substrates for various nucleases determined.[12,13] None were substrates for alkaline phosphatase or snake venom phosphodiesterase, although uridine 5'-(hydroxymethyl)phosphonate was a substrate for 5'-nucleotidase.

Phosphonylation of thymidine with $HCF_2P(O)Cl_2$ (synthesis outlined in Scheme 2) in triethylphosphate, following a procedure similar to that outlined by Yoshikawa,[14] gave a mixture of thymidine 3'-O- and 5'-O-monophosphonates, **8** and **9**, and thymidine 3', 5'-bis-O-(difluoromethylphosphonate) (**10**, Scheme 3). The monophosphonates, **8** and **9** could not be cleanly separated, even by analytical HPLC on either a C-18 reverse phase column (H_2O, MeOH elution, retention times 5.6 and 6.4 min) or on a Whatman SAX ion exchange column. The ratio of **8** to **9** was 70:30. The bis-phosphonate **10**, which accounted for half of the total product yield, was readily separated on a DEAE-Sephadex column eluting with a linear aqueous ammonium bicarbonate gradient. The NMR spectra of **8-10** are typical of other difluoromethylphosponates. In the ^1H NMR spectrum of **10**, the two protons adjacent to phosphorus occur as a *dt* centered at 5.8 ppm (J_{HF} = 48 Hz, J_{HP} = 24 Hz). There are two sets of fluorine *dd* 's in the ^{19}F NMR. One is centered 130.25 ppm upfield from $CFCl_3$ and the other is located at 130.75 ppm. J_{HF} is about 48 Hz for each set while the downfield set has J_{FP} at about 79 Hz and the upfield set J_{FP} around 81 Hz.

3'-O- and 5'-O-t-butyldimethysilylthymidine were synthesized and independently subjected to phosphonylation under the same reaction conditions. Selective phosphonylation was not observed. A similar product mixture was obtained, apparently because the t-butyldimethylsilyl group was cleaved under the reaction conditions. In contrast, a relatively clean product was obtained when 5'-O-t-butyldimethysilylthymidine (**11**) was treated with difluoromethyl-O,O-bis(1-benzotriazolyl)phosphonate (**7**) in THF (Scheme 4). The latter reagent was prepared in three steps from diethyl phosphite, by the steps outlined in Scheme 2, and used immediately. Work is currently underway to determine whether analogues **8-10** are either substrates or inhibitors of various nonspecific nucleases.

In order to determine the feasibility of constructing oligonucleotides linked as difluoromethylphosphonate esters, the simplest possible dimer, $Tp_{CF_2}HT$ (**23**, Scheme 6) was chosen as the initial synthetic target. Model studies with diethyl difluoromethylphosphonate revealed that hydrolysis occurred too rapidly in concentrated ammonia or aqueous pyridine to allow use of the usual ammonia labile protecting groups in

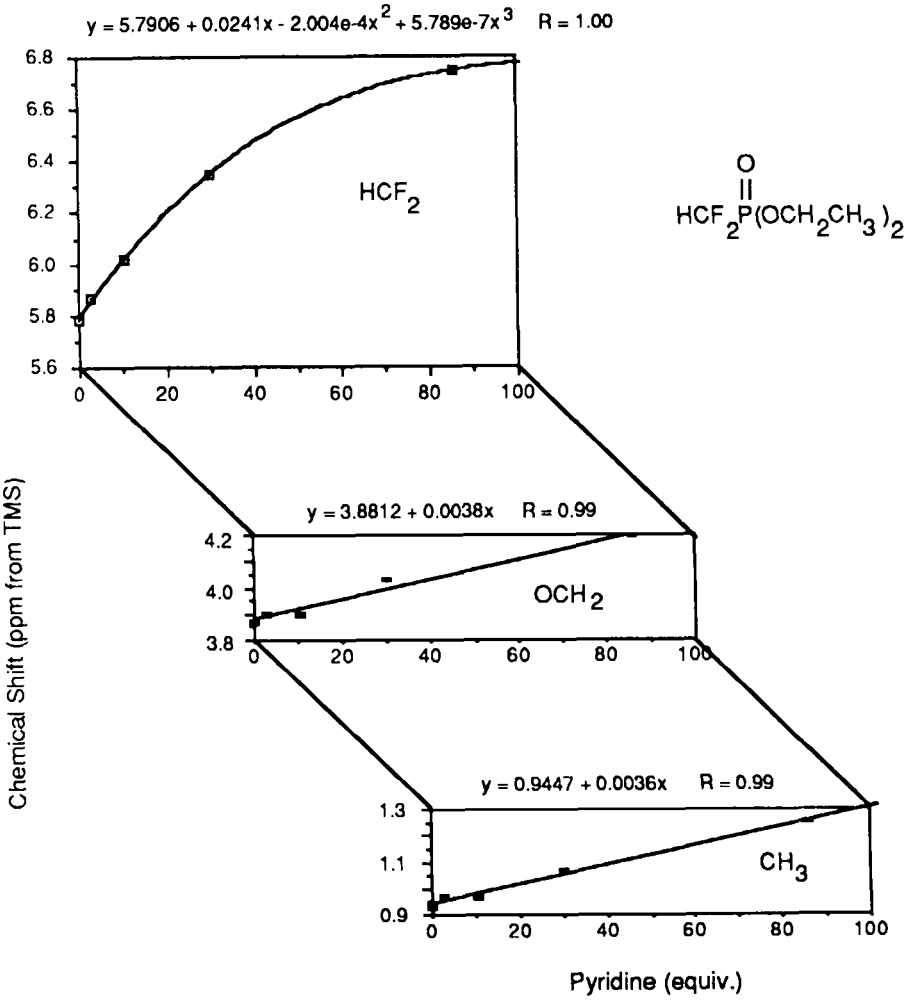
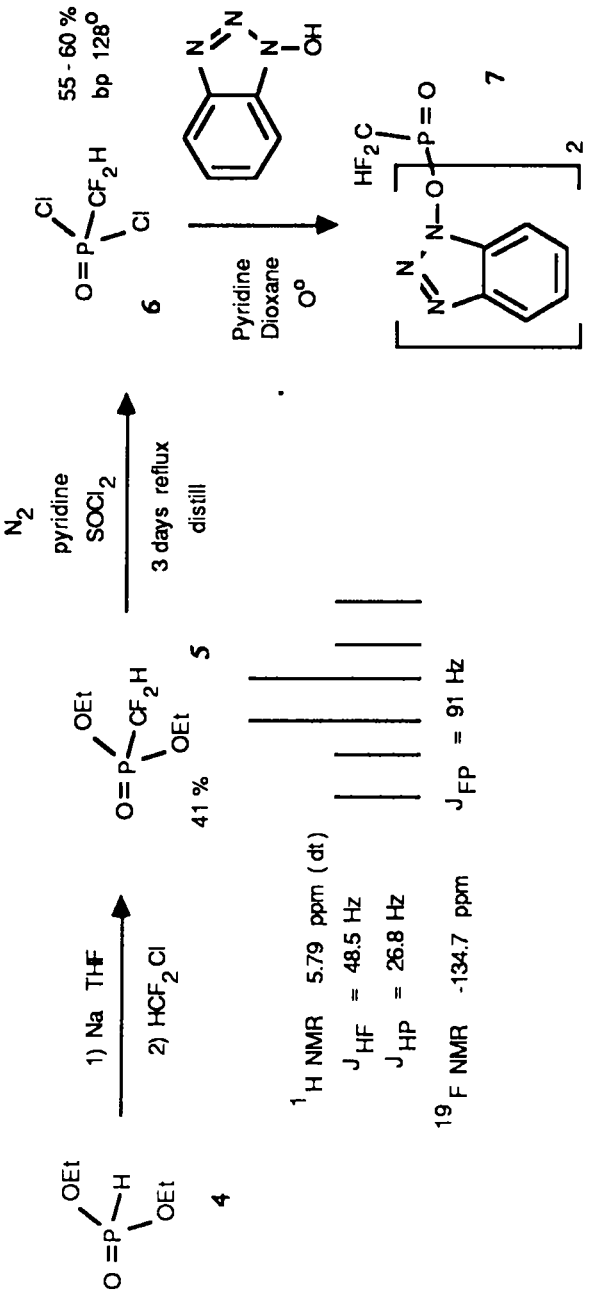


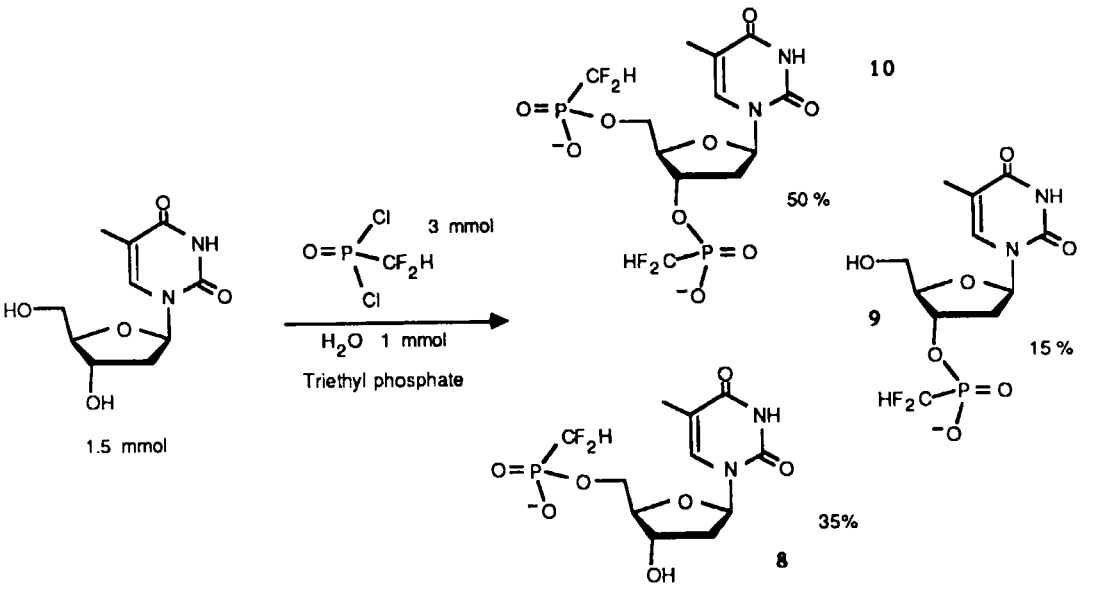
Figure 1 Diethyl difluoromethylphosphonate proton chemical shifts as a function of Pyridine Concentration in C_6D_6

the synthesis. However, the phosphonate was stable towards 80 % acetic acid-water, and to 1M hydrazine in 3/2 pyridine/acetic acid, reagents which, respectively, can be used to remove dimethoxytrityl and levulinyl protecting groups.

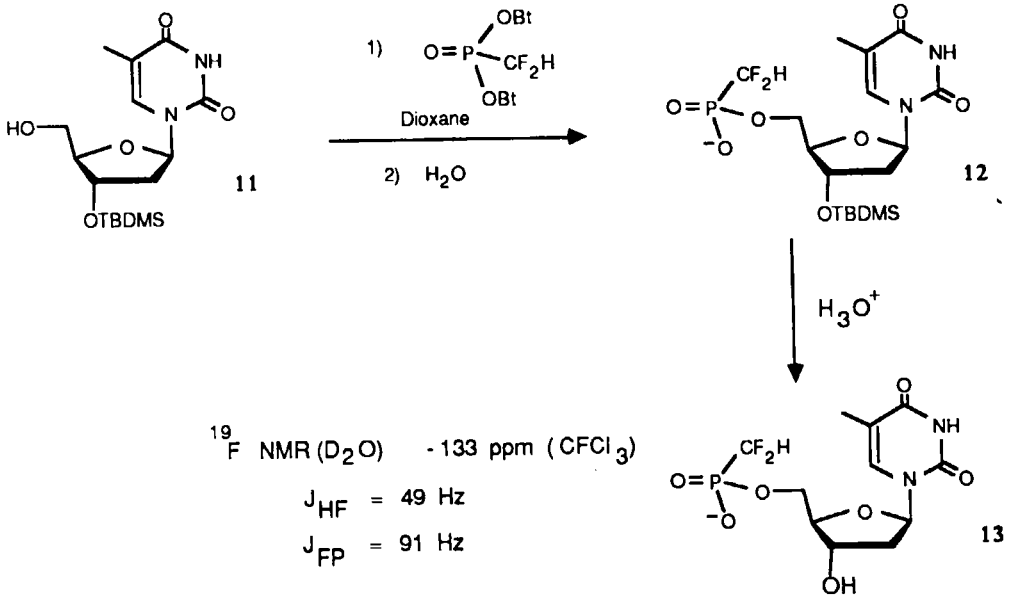
Following a procedure established by van Boom and coworkers for the preparation of oligonucleotides using 1-hydroxybenzoletriazole activated phosphotriester intermediates,[15]



Scheme 2



Scheme 3

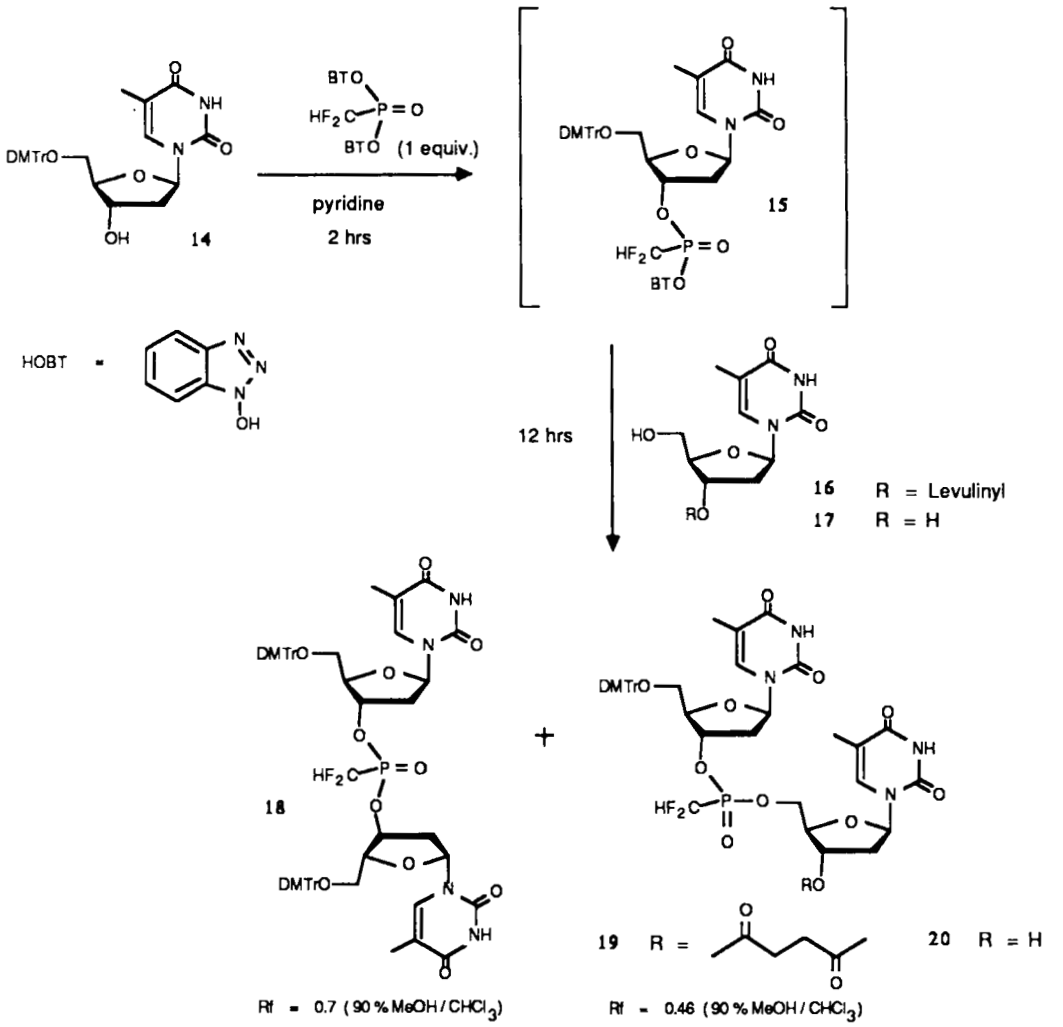


^{19}F NMR (D_2O) -133 ppm ($CFCl_3$)

J_{HF} = 49 Hz

J_{FP} = 91 Hz

Scheme 4



Scheme 5

we determined that the difluoromethylphosphonate linkage could be introduced as a 3'-5' linkage. Sequential reaction of difluoromethyl-O,O-*bis* (1-benzotriazolyl) phosphonate (**7**) with a 5'-O-dimethoxytrityl-2'-deoxythymidine (**14**) and 3'-O-levulinyl-2'-deoxythymidine (**16**) gave two dimeric products (Scheme 5).

The principal product was the symmetrical 3'-3' dimer **18** which could be separated from the unsymmetrical 3'-5' dimer **19** (10 % yield) by chromatography on silica gel (gradient elution with $MeOH/CHCl_3$). In the ^{19}F NMR, **18** showed two sets of four peaks (dd) separated by 6 Hz (Figure 2). Each set represents one of the fluorines, which are diastereotopic and hence show different chemical shifts in the spectra. Two additional

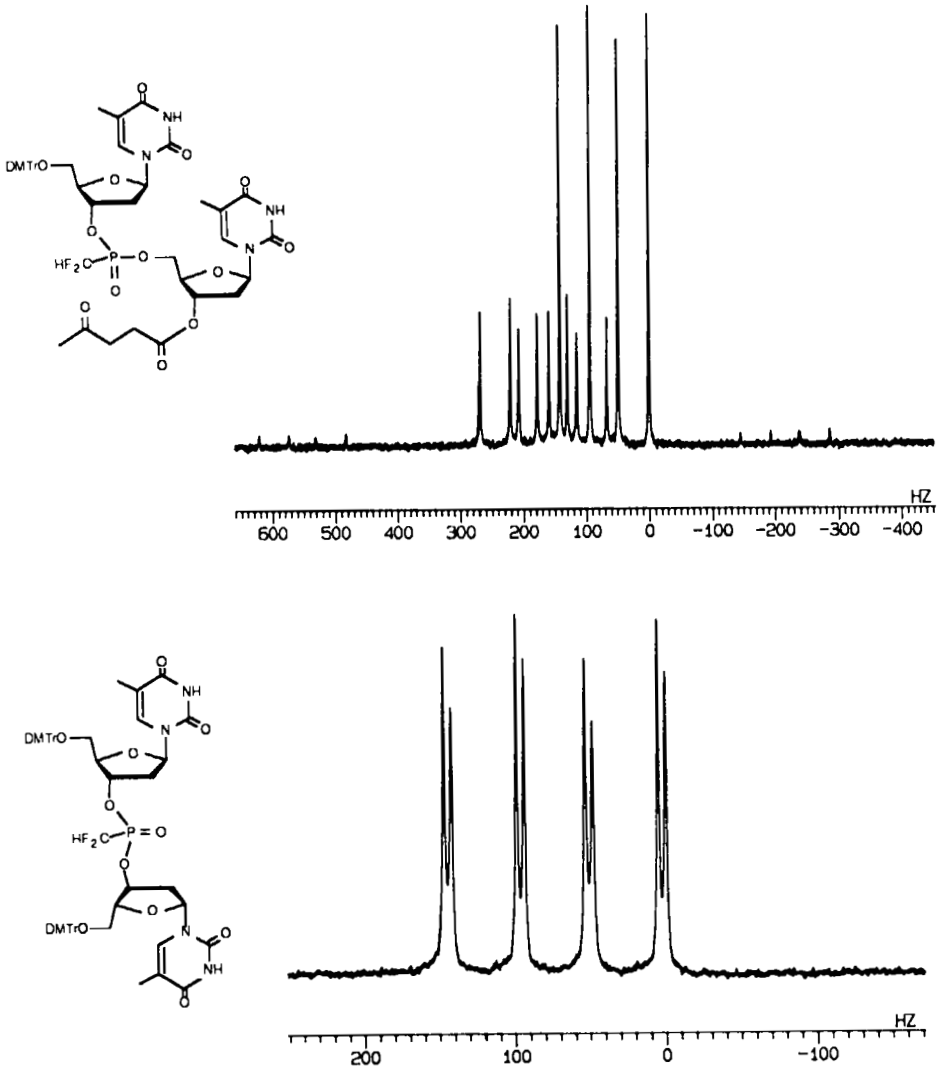


Figure 2 ^{19}F NMR Spectra (376.05 MHz) of the 3'-3' dimer **18** and the 3'-5' dimer **19**.

sets of four peaks of very low intensity should theoretically be found about 460 Hz upfield and downfield from these resonances due to fluorine-fluorine coupling. However these were not detected above the threshhold noise. The ^{19}F NMR spectrum of the 3'-5' dimer, **19** was more complex. At a field of 376.05 MHz, 20 peaks were observed. The precise pattern and peak size ratios remained constant even when the synthesis was repeated under a range of conditions.

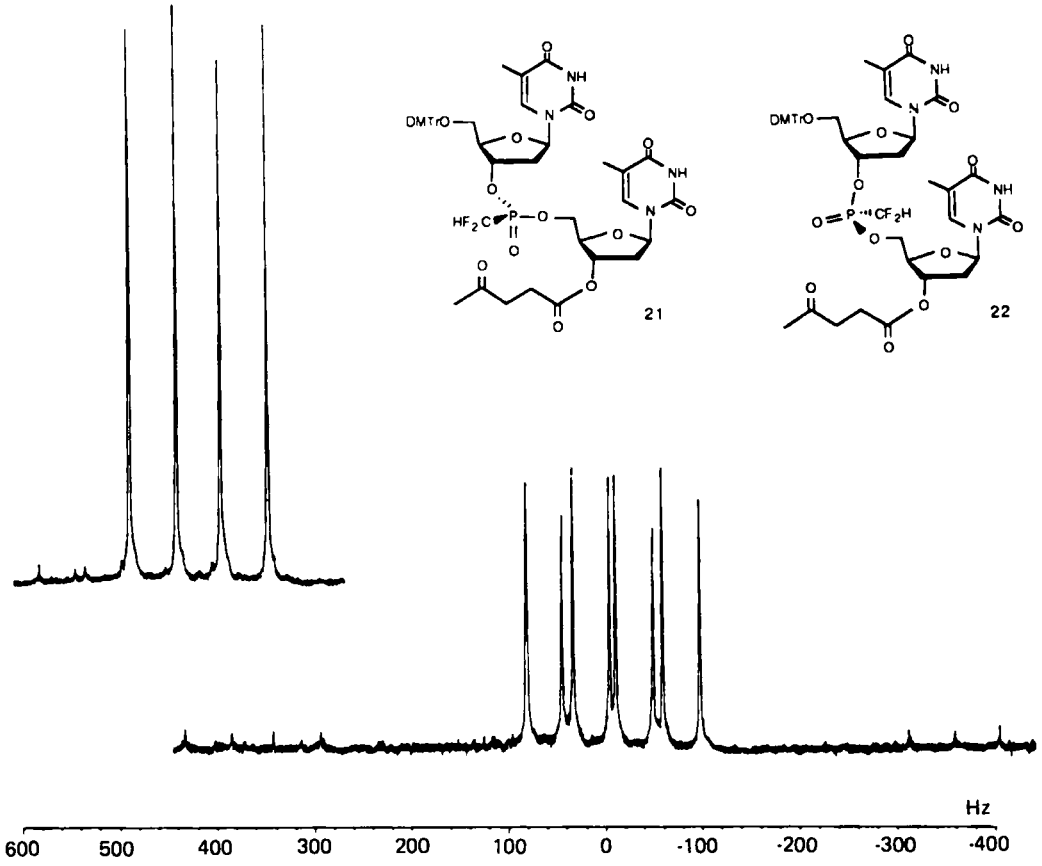
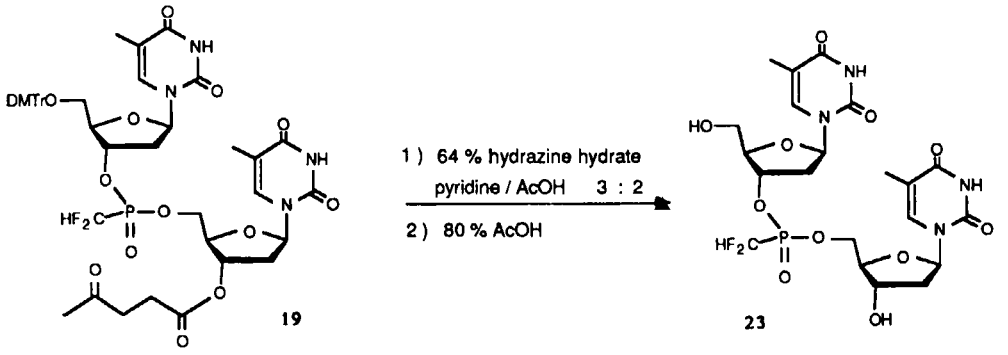


Figure 3 ^{19}F NMR Spectra (282 MHz) of Diastereomers **21** and **22**.

Again, following precedent established in van Boom's laboratory,[16] the 3'-5' linked dimer could be separated into two diastereomers (Rf = 0.13 and 0.18, ethyl acetate, silica gel) by chromatagraphy on silica gel eluting with an ethyl acetate (0.5 % Et_3N) / methanol gradient. The separated diastereomers (**21** and **22**) were characterized by ^{1}H, ^{31}P, ^{13}C, and ^{19}F NMR spectroscopy. Currently the information provided by the NMR spectra is not sufficient to allow absolute assignment of stereochemistry. But the ^{19}F NMR spectra (Figure 3) are very different and must reflect the distinct environments of the CF_2H groups. One of the isomers shows only four narrow peaks (dd, J_{HF} = 48 Hz and J_{FP} = 94 Hz) in the proton undecoupled spectrum. The two fluorine atoms have exactly the same chemical shift despite their stereochemical uniqueness. The second isomer shows sixteen peaks as a result of the chemical shift difference between the two diastereotopic fluorines (two ddd, J_{HF} = 48 Hz, J_{FP} = 93 Hz, and J_{FF} = 462 Hz).



Scheme 6

With the purified protected 3'-5' dimer in hand, deprotection by conventional methods was found to proceed cleanly (Scheme 6). No cleavage of the difluoromethylphosphonate linkage of $Tp^{CF_2H}T$ (**23**) was observed. Finally, $Tp^{CF_2H}T$ was found to be stable to methanolic ammonia under reaction conditions necessary for the removal of the protecting groups most commonly used to protect the bases in the phosphotriester and phosphoramidite methods of oligonucleotide synthesis.

Work is currently in progress to further characterize the diastereomers of $Tp^{CF_2H}T$ and to synthesize dimers composed of other bases. Physicochemical and biochemical studies to address the issue of phosphorus stereochemistry and complementary sequence binding are planned as well.

Acknowledgement:

This work was supported by PHS Grant AI20480 awarded by the National Institutes of Health.

REFERENCES

1 D. E. Bergstrom, E. H. Romo, and A. W. Mott, Abstr. CARB 38, 185th ACS National Meeting, Seattle, Washington, March 20-25, 1983.

2 J. S. Kroin, L. W. Hertel, and J. W. Misner, Abstr. ORGN 44, 191st ACS National Meeting, New York, New York, April 13-18, 1986.

3 Robert Engel, Chem. Reviews, 1977, 77, 349-367.

4 F. Eckstein, Ann. Rev. Biochem., 1985, 54, 367-402.

5 G. Michael Blackburn, Fritz Eckstein, David E. Kent, and Timothy D. Perree, Nucleosides and Nucleotides , 1985, 4 , 165-7.

6 G. M. Blackburn and M. J. Parratt, J. Chem .Soc. Chem. Commun., 1982, 1270; 1983, 886.

7 Paul S. Miller, Cheryl H. Agris, Laure Aurelian, Kathleen R. Blake, Akira Murakami, M. Parameswara Reddy, Sharon A. Spitz, and Paul O. P. Ts'o, Biochimie, 1985, 67, 769-776.

8 Cynthia C. Smith, Laure Aurelian, M. Parameswara Reddy, Paul S. Miller, and Paul O. P. Ts'o, Proc. Natl. Acad. Sci., USA, 1986, 83, 2787-2791.

9 R. L. Letsinger, S. A. Bach, and J. S. Eadie, Nucleic Acids Research, 1986, 14, 3487-3499.

10 P. S. Miller, Cheryl H. Agris, Kathleen R. Blake, Akira Murakami, Sharon A. Spitz, Parameswara M. Reddy, and Paul O. P. Ts'o, in "Nucleic Acids:Vectors of Life," 521-534, B. Pullman and J. Jortner, eds., D. Reidel Publ., 1983.

11 Gary R. Wiley and Sidney I. Miller, J. Amer. Chem. Soc., 1972, 94, 3287-3293.

12 Alan W. Nichol, Akihiko Nomura, and Alexander Hampton, Biochemistry, 1967, 6, 1008-1015.

13 A . Holy and Ng D. Hong, Coll. Czechoslov. Chem. Commun., 1972, 37, 2066-2076.

14 M. Yoshikawa, T. Kato, and T. Takenishi, Bull. Chem. Soc. Japan, 1969, 42, 3505-3508.

15 J. E. Marugg, L. W. McLaughlin, N. Piel, M. Tromp, G. A. van der Marel and J. H. van Boom, Tetrahedron Lett., 1983, 37, 3989-3992.

16 J. E. Marugg, E. de Vroom, C. E. Dreef, M. Tromp, G. A. van der Marel and J. H. van Boom, Nucleic Acids Research, 1986, 14, 2171-2185.