



The Preparation of New Phosphorus-Centered Functional Groups for Modified Oligonucleotides and Other Natural Phosphates

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Abstract: Efforts to develop synthetic methodologies allowing the preparation of α,α -difluorophosphonothioates, α,α -difluorophosphonodithioates, α,α -difluorophosphonotrithioates, and α,α -difluorophosphinates are reviewed in the light of applications in the field of modified oligonucleotides and cyclitol phosphates. Two successful approaches have been developed, based either on the addition of phosphorus-centered radicals onto *gem*-difluoroalkenes or on a process involving the addition of lithiodifluorophosphonothioates **91** onto a ketone and the subsequent deoxygenation reaction of the adduct. The radical route successfully developed a practical route to α,α -difluoro-*H*-phosphinates which proved to be useful intermediates to a variety of phosphate isosters. The ionic route led to the first preparation of phosphonodifluoromethyl analogues of nucleoside-3'-phosphates.

Keywords: Phosphate isoster; difluorophosphonates; difluoro-*H*-phosphinates; nucleotide analogue; cyclitol analogue.

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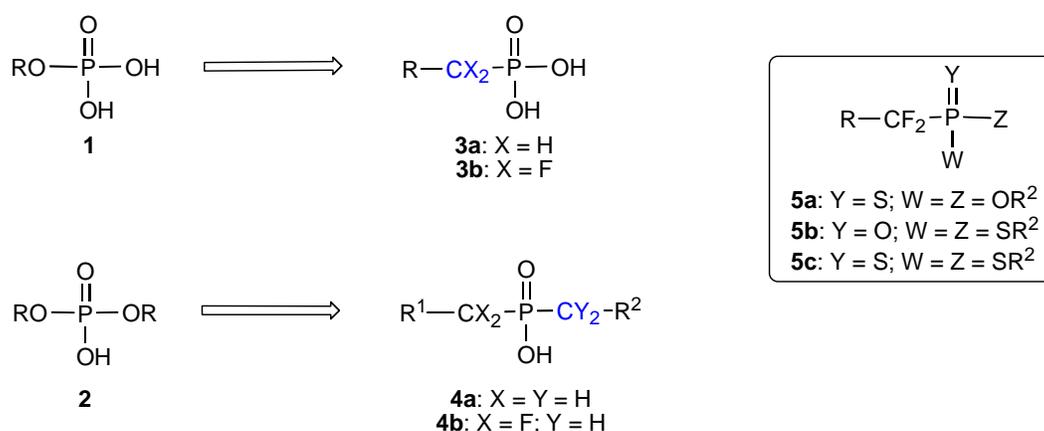
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Introduction

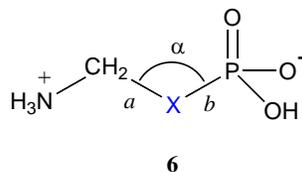
The ubiquitous presence of the phosphate group in molecules involved in life processes has focused the attention of many organic and medicinal chemists in their efforts to design and generate non natural molecules to interfere with biochemical transformations. The phosphate group is mainly found in biomolecules in the form of its monoesters **1** or diesters **2** (Scheme 1), which are produced and broken down by the action of enzymes such as kinases, phosphorylases, phosphatases or nucleases, for instance. Hence the early design of efficient enzyme inhibitors has often relied on the use of phosphonates **3a**, in which the esterified oxygen atom of the phosphate is replaced with a CH₂ unit [1]. The pioneering work of Blackburn and McKenna in the early eighties resulted in the design and use of the α,α -difluoromethylphosphonates **3b** as closer isosters of the phosphate [2]. Structural and electronic studies by Chambers and O'Hagan on the zwitterionic species **6** have since then confirmed this fact and firmly established the monofluorophosphonates and difluorophosphonates as closely related, hydrolytically and enzymatically stable mimics of the phosphate (Table 1) [3].

Scheme 1



Thus, the pK_{a2} as well as the bond lengths and angles obtained from X-ray crystallography for the various functional groups clearly indicated the closer analogy between both **6c** and **6d**, on the one hand, and the phosphate **6a**, on the other hand. Indeed, the literature reports numerous examples of enzyme inhibitors encompassing the above fluorinated phosphonates. Among these, analogues targeting phospholipase C (PLC), purine nucleoside phosphorylase (PNP) and protein phosphotyrosine, phosphoserine or phosphothreonine phosphatases have been published [4]. This functional group has also been successfully used to mimic the phosphate in nucleotide monophosphates and triphosphates: analogues of adenosine monophosphate, cyclic adenosine monophosphate, adenosine triphosphate and adenosyl adenosine triphosphate, as well as structurally related potent inhibitors of the reverse transcriptase of Human Immunodeficiency Virus (HIV), have been described in literature [5].

Table 1.



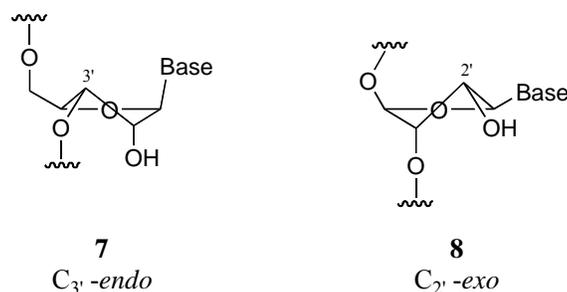
Entry	Compound	X	α ($^\circ$)	<i>a</i>	<i>b</i>	<i>a+b</i>	pKa ₂
1	6a	O	118.7	1.43	1.59	3.02	6.4
2	6b	CH ₂	112.1	1.807	1.51	3.32	7.6
3	6c	CHF	113.3	1.82	1.50	3.32	6.5
4	6d	CF ₂	116.5	1.85	1.496	3.35	5.4

In the case of phosphate diesters **2**, the replacement of both esterified oxygen atoms with two methylene units generates a phosphinate **4a**, a functional group which has been used in the past to generate analogues of the natural parent phosphates. (Scheme 1) [6].

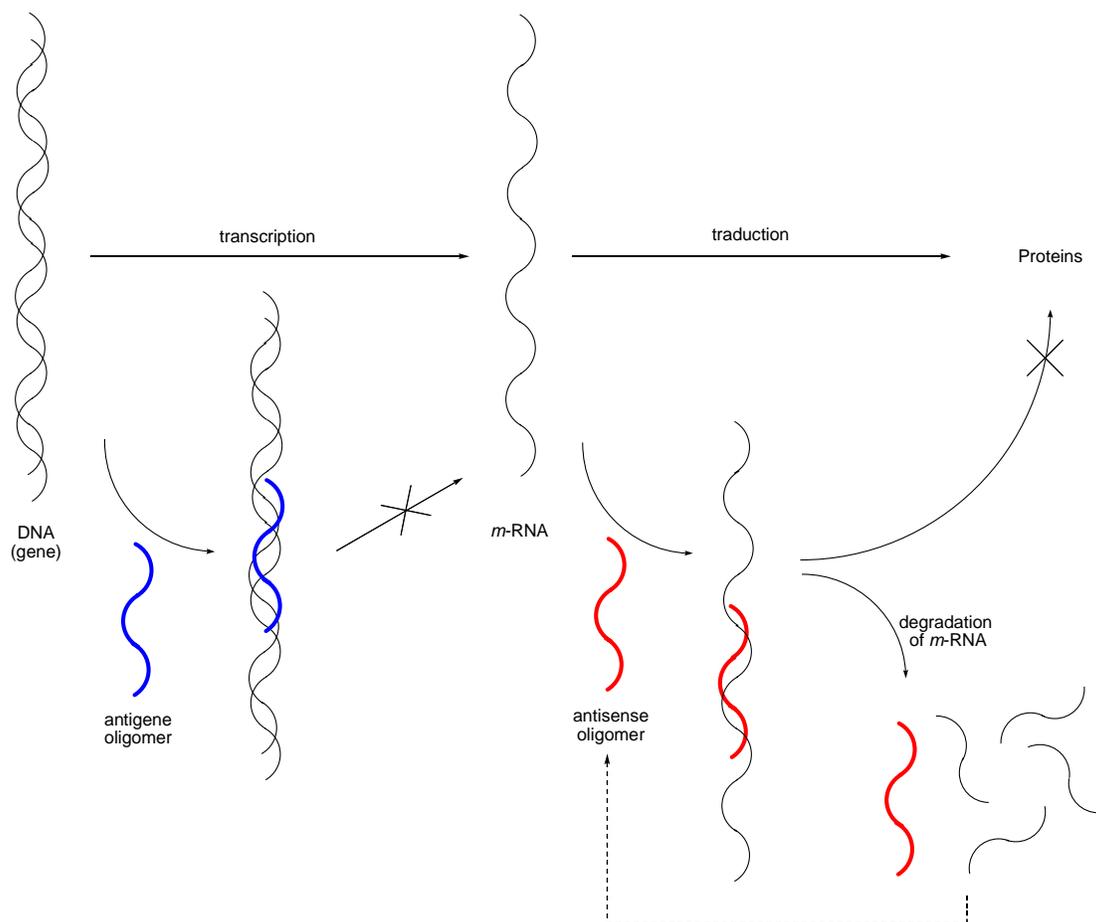
Our interest in new variants of the phosphonates led us to develop efficient preparations of the α,α -difluorophosphonothioate **5a**, α,α -difluorophosphonodithioate **5b** and α,α -difluorophosphonotrithioate **5c**, in which the various oxygen atoms of the parent phosphonate become replaced with sulfur. More recently, we have focused on the phosphinate functional group, and the hitherto poorly developed fluorinated analogues **4b**.

Among the many possibilities for application are the antisense and antigene strategies, and the inositol cycle. Briefly, the antisense and antigene strategies rely on the formation of a triplex between the DNA and a modified oligonucleotide (MON), and of a duplex between the messenger RNA (*m*-RNA) and a MON, respectively, to keep the transcription (antigene) or the translation (antisense) from occurring (Scheme 2) [7]. This should result in the regulation of gene expression and open the door to a conceptually new therapeutic treatment of diseases. Analysis of the requirements for an optimal analogue of the monomeric unit in MONs points the finger at the necessity to keep the 3'-endo conformation **7** of the ribose cycle that favors pairing of the strands (Figure 1), an optimal geometry and electronic features of the phosphate mimic, and a stability towards the action of nucleases; this allows the MON to target another molecule of *m*-RNA molecule, once the first one has been lysed by enzymes (antisense strategy; 4'-endo conformation).

Figure 1

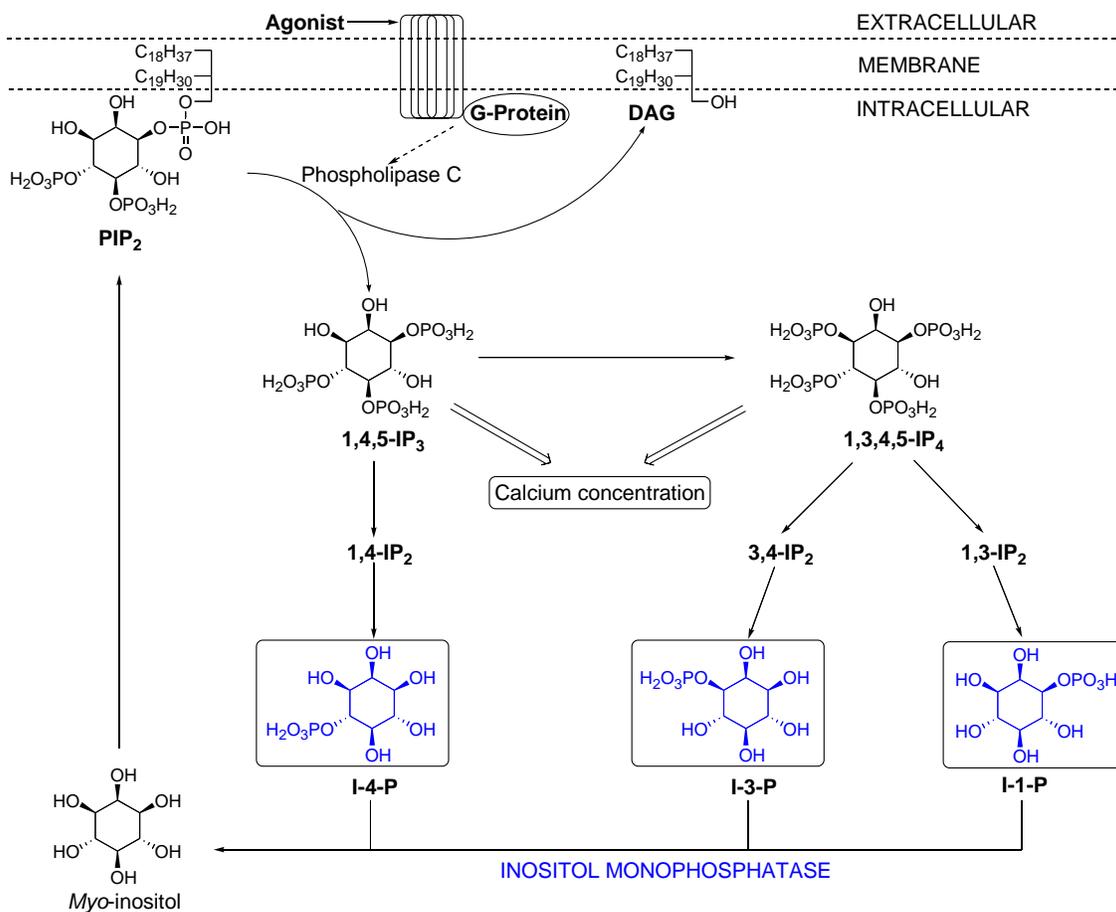


Scheme 2



Numerous studies aiming at understanding the inositol cycle have underlined its importance [8]. The cycle is part of an important cell signalling system which helps regulate the intracellular calcium level. A very simplified view of the cycle (Scheme 3) shows how the second messenger inositol-1,4,5-trisphosphate (1,4,5-IP₃) released from phosphatidylinositol-4,5-diphosphate (PIP₂) by the action of a phospholipase C, undergoes a sequential series of phosphorylations of the hydroxyl groups and hydrolysis of the phosphates to produce, among others, three different inositol monophosphates (I-4-P, I-3-P and I-1-P). These are hydrolysed by a single enzyme, inositol monophosphatase (IMPase) to yield *myo*-inositol which is then converted into PIP₂, thereby closing the cycle. Inositol monophosphatase is a homodimeric enzyme and has been postulated as a possible target to treat manic depressive illness [9].

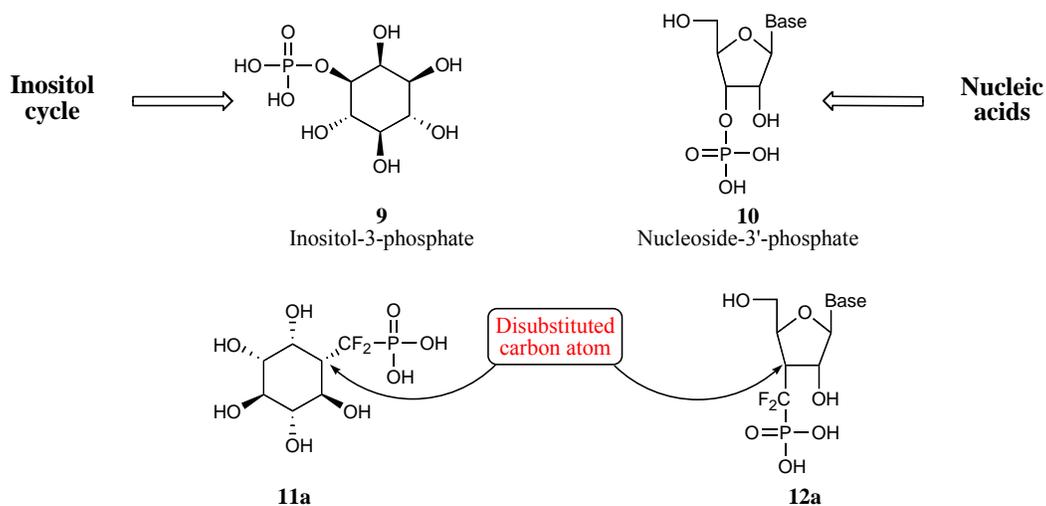
Scheme 3



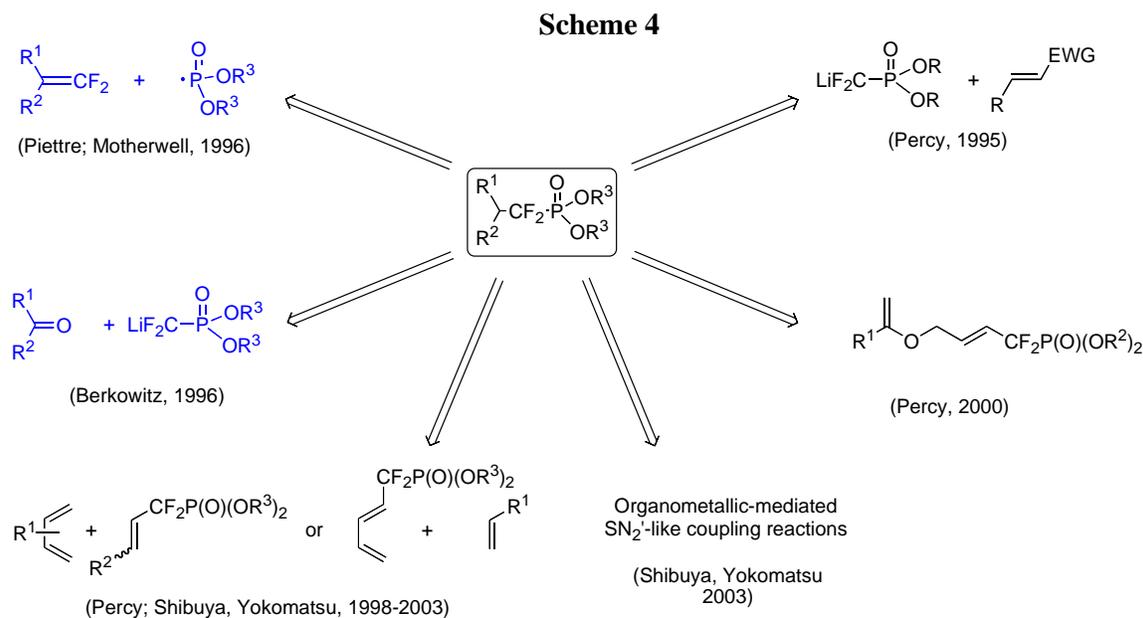
Results and Discussion

The target molecules we elected to work on are depicted on Figure 2. Thus both inositol-3-phosphate 9 and nucleoside-3' phosphate 10 can be related to analogues 11 and 12, respectively, encompassing phosphono(thio)difluoromethyl units.

Figure 2



A particular feature of these molecules is the disubstitution of the carbon atoms bearing the CF₂ moiety. A number of methodologies allowing the preparation of compounds characterized by this particular substitution pattern have been published and are outlined in Scheme 4 [10]. Early on, we chose to develop our own radical approach, and to use Berkowitz methodology relying on the sequential addition of phosphonodifluoromethyl anion on ketone and radical mediated deoxygenation process of the adduct.

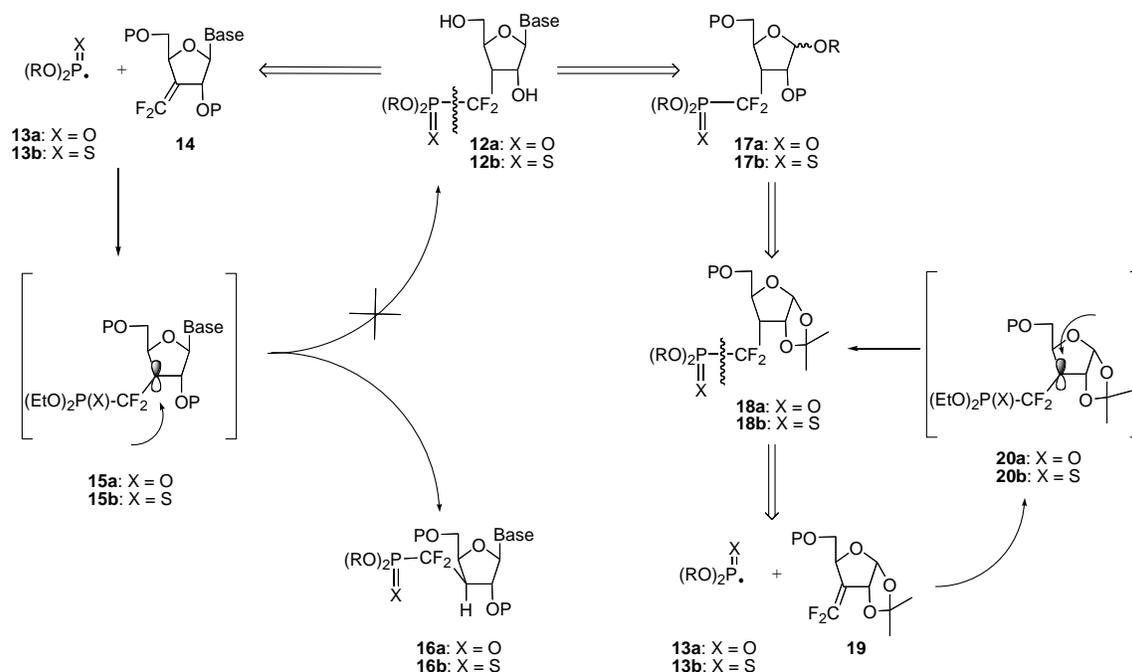


A) MONs

a) Radical approach

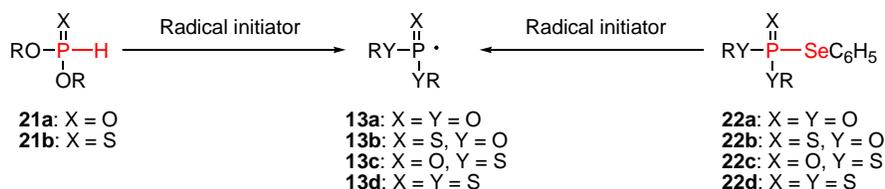
Retrosynthetic analysis of the target nucleoside-3'-phosphate analogues **12** pointed the finger at the probable wrong stereodirecting effect of the purine or pyrimidine base in the hydrogen quench of the radical adducts **15** generated from the addition of phosphonyl or phosphonothioyl radicals **13** onto difluoroalkene **14** (Scheme 5); this would produce the undesired phosphono(thio)difluoromethyl derivative **16**. Hence it was decided to start from a carefully protected furanose derivative **19**. A 1,2-isopropylidene protecting group was chosen to take advantage of the steric hindrance generated by the angular methyl group and thus favor the formation of the desired diastereomer **18** [11].

Scheme 5



For synthetic purposes, phosphonyl **13a** and phosphonothioyl **13b** radicals are usually generated either from the corresponding phosphite **21a** and thiophosphite **21b**, respectively. These radicals, along with phosphonodithioyl **13c** and phosphonotrithioyl **13d** radicals, can also be produced from the selenophosphate derivatives **22** (Scheme 6) [11,12].

Scheme 6



Radicals **13a** and **13b** have been shown to add onto β,β -disubstituted- α,α -difluoroalkenes under radical-chain conditions to yield the expected adducts in fair to good yields [**10b-c**, **10h**, **10j**]. The use of thiophosphite usually results in the formation of adducts in substantially higher yields (when compared to phosphites). Radicals **13c** and **13d**, however, were found to be unreactive towards these substrates.

In our case, the readily available glucufuranose derivative **23** was selected as starting material and oxidized into **24** [13], which was then conveniently transformed into the corresponding difluoroalkene **25** using literature methods (Scheme 7) [14]. However, attempts to add phosphorus-centered radicals **13** onto this substrate by either method completely failed to furnish the desired adducts **26**, the substrate being recovered [15]. Other cyclic difluoroalkenes (e.g. **19**, **27** and **28**) displayed a similar behavior (Figure 3). The successful addition of phosphonyl radicals onto *gem*-difluoro enol ether

derivatives **29-31** by the groups of Motherwell and Sinaÿ may be indicative of the electrophilic nature of the radicals [**10c**, **10h**, **10j**].

Scheme 7

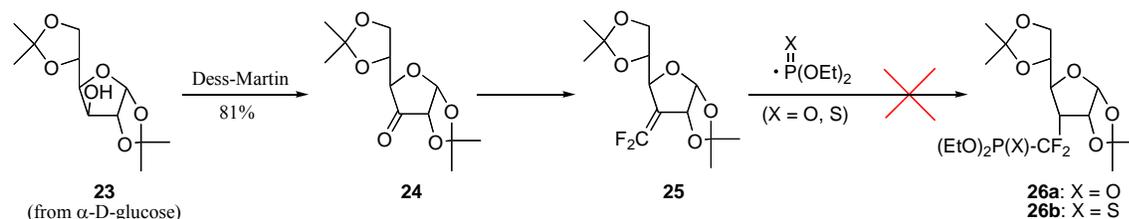
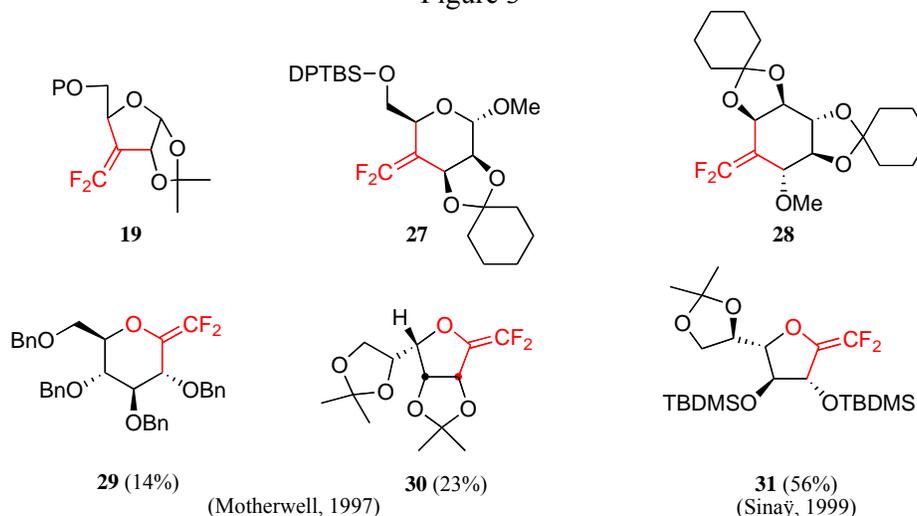
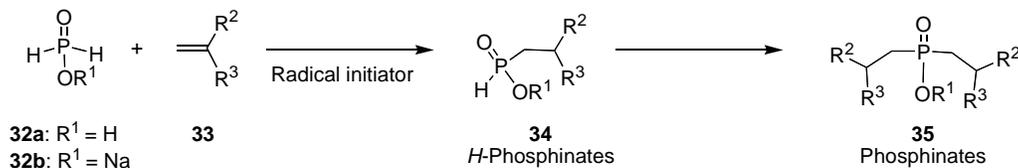


Figure 3



A literature search for alternate precursors of phosphorus-centered radicals quickly led to the identification of hypophosphorous acid **32a**, featuring two P-H bond and only one phosphorus–oxygen single bond. Indeed, the work of Nifant'ev has demonstrated the possibility of adding hypophosphorous acid onto alkenes **33** under radical-chain reaction conditions, thereby producing *H*-phosphinates **34** (single addition) or phosphinates **35** (double addition) (Scheme 8) [16].

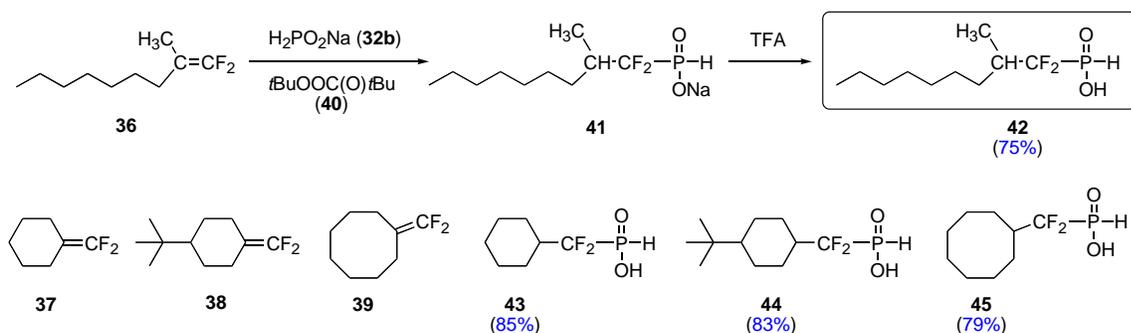
Scheme 8



Nifant'ev also showed that the sodium salt **32b** of hypophosphorous acid behaves similarly, a feature of importance in the context of acid-sensitive protected substrates. In addition, we were especially interested in testing hypophosphorous acid (or its sodium salt) addition onto *gem*-difluoroalkenes, as this would open the door to both hitherto unreported α,α -difluoro-*H*-phosphinates, and α,α -difluorophosphinates, a functional group for which no general method of synthesis has been reported to date.

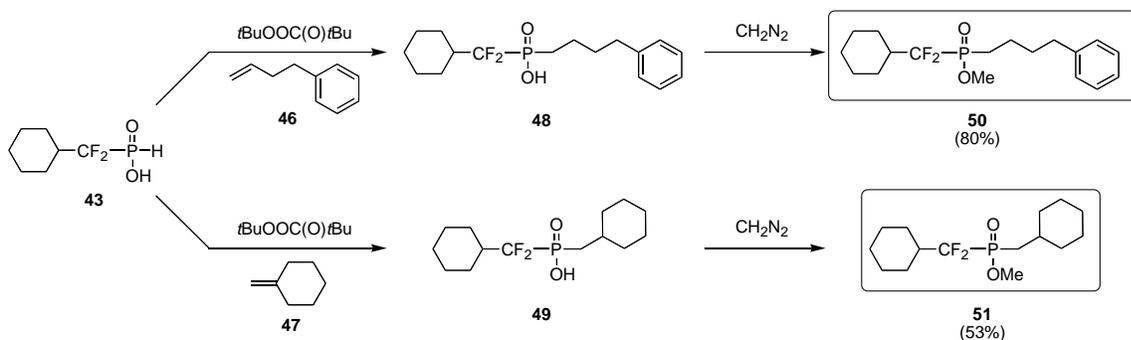
Thus, refluxing a solution of either difluoroalkene **36-39** and **32b** in methanol in the presence of a catalytic amount of *tert*-butyl peroxyphosphate (**40**) resulted in a complete consumption of the substrates. Hydrolysis and work-up led to the isolation of the first α,α -difluoro-*H*-phosphinates **42-45** in good yields (Scheme 9). The compounds were found to be both air and room temperature stable for extended periods of time and displayed a one-bond P-H coupling constant of about 560 Hz (^{31}P -NMR spectrometry). Infrared spectra featured both P=O and P-H bonds signals, indicative of a P(V) species. Other successful radical initiators included commercially available *tert*-butyl 2-ethylhexyl peroxyphosphate and the triethylborane/air system [17].

Scheme 9



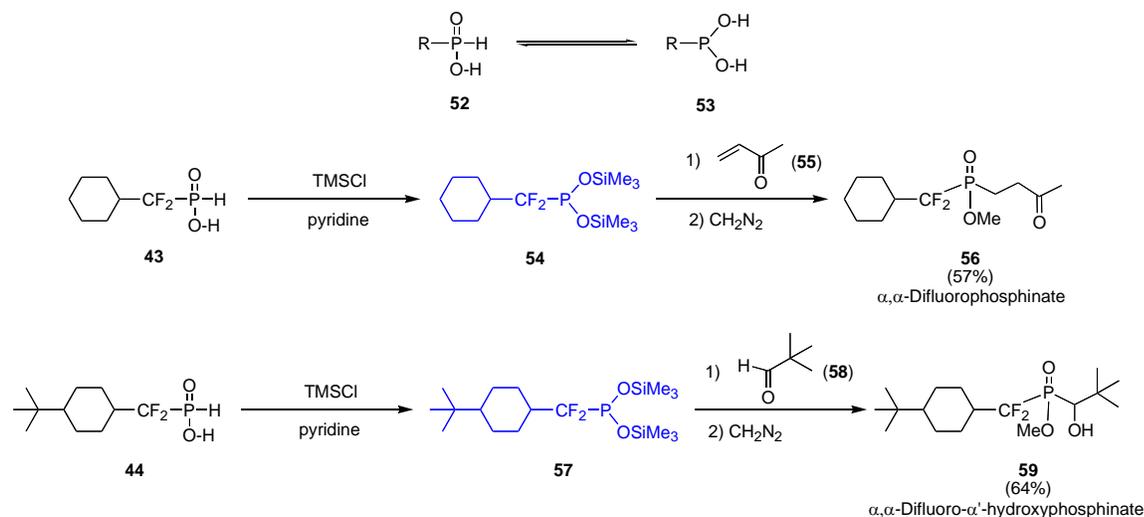
Homolytic cleavage of the phosphorus hydrogen single bond present in difluoro-*H*-phosphinates was achieved by heating the compounds in the presence of both an alkene (1 equiv) and a radical initiator. Thus, for instance, *H*-phosphinate **43** sequentially reacted with either alkene **46** or **47**, and diazomethane (CAUTION) to deliver methyl α,α -difluorophosphinates **50** and **51** in good isolated yield (Scheme 10).

Scheme 10



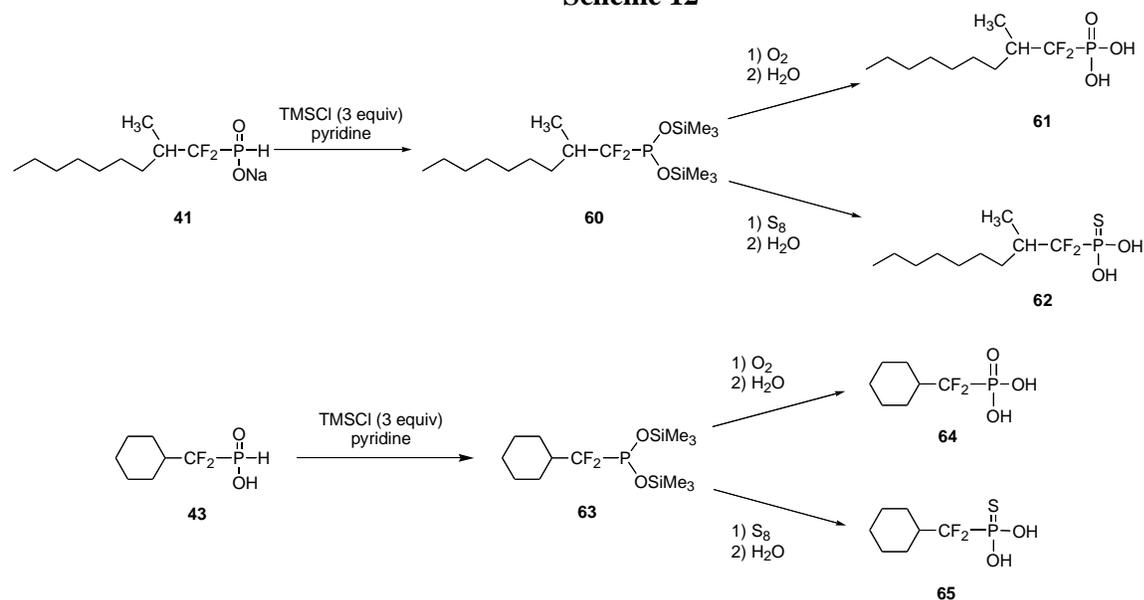
The documented tautomeric equilibrium between *H*-phosphonates **52** and phosphites **53** led us to develop alternate ionic routes to α,α -difluorophosphinates [18]. Treatment of α,α -difluoro-*H*-phosphinates **43** and **44** with trimethylsilyl chloride in the presence of pyridine afforded the air-sensitive *O,O*-bissilylated phosphites **54** and **57**, which were then subjected into interaction with methyl vinyl ketone **55** and pivalaldehyde **58**, respectively (Scheme 11). Hydrolysis and esterification with diazomethane (CAUTION) cleanly delivered difluorophosphinates **56** and **59** [19,20]. Of particular note is the fact that the two fluorine atoms present in the *O,O*-bissilylated phosphites did not prevent the phosphorus atom from reacting with electrophilic centers.

Scheme 11



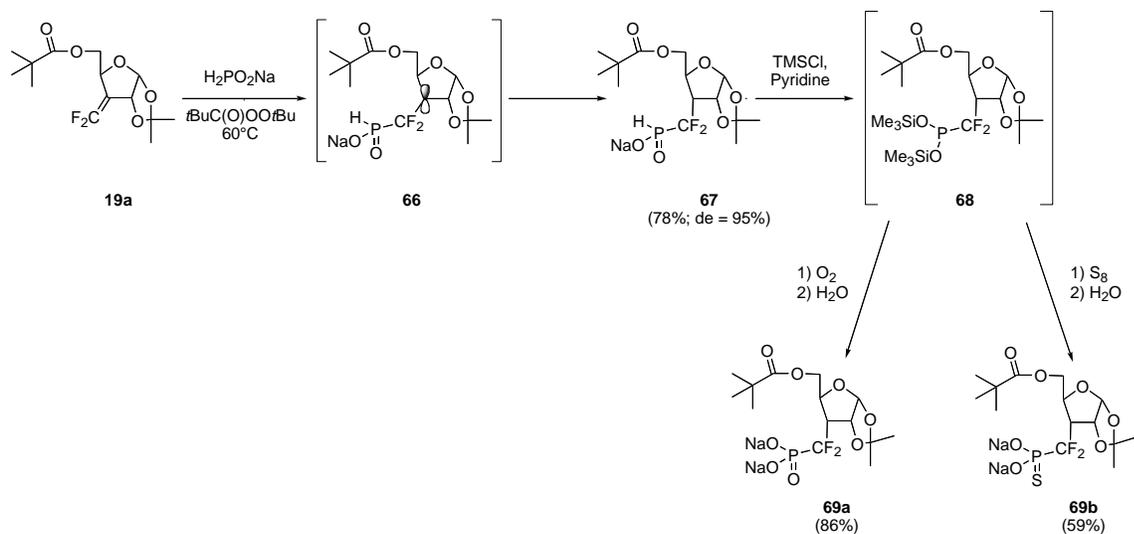
Alternatively, treating these bisilylated phosphites with either oxygen or sulfur led to the isolation of difluorinated phosphonic and phosphonothioic acids, respectively, as exemplified in Scheme 12.

Scheme 12



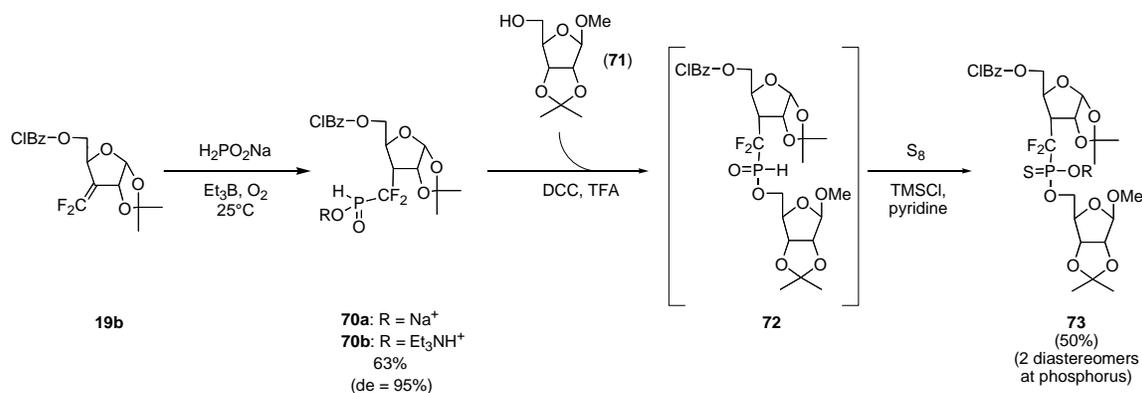
More importantly, when hypophosphorous sodium salt **32b** was reacted with difluoroalkene **19a** (a substrate inert to the action of both phosphite and thiophosphite) under the above conditions, a clean reaction occurred and adduct **67** was isolated in 78% yield as a single diastereomer (Scheme 13). ¹H-NMR spectrometry indicated the structure to be as depicted, indicative of the efficiency of the angular methyl group of the 1,2-isopropylidene unit in keeping hydrogen quenching of radical-adduct **66** from occurring on the concave face [12]. Transformation into *O,O*-bisilylated phosphite **68** and sequential treatment with oxygen and water delivered the expected phosphonic acid, isolated in the form of its disodium salt **69a** in excellent yield. An analogous sequence involving sulfur in place of oxygen afforded the corresponding phosphonothioate **69b**.

Scheme 13



Similarly, difluoroalkene **19b** and hypophosphorous acid sodium salt reacted together at room temperature in presence of Et_3B and oxygen (air) as the radical initiator to furnish the α,α -difluoro-*H*-phosphinate, sodium salt **70a**, conveniently purified as its triethylammonium salt **70b** by ion exchange and simple extraction with ethyl acetate (Scheme 14) [111]. An additional step forward was accomplished when this compound could be esterified with alcohol **71** in the presence of trifluoroacetic acid and dicyclohexyl carbodiimide (DCC). Treatment of the resultant *H*-phosphinate **72** with a mixture of sulfur, TMSCl and pyridine gave difluorophosphonothioate **73**, isolated in 50% yield as a 1:1 diastereomeric mixture at the phosphorus center [17].

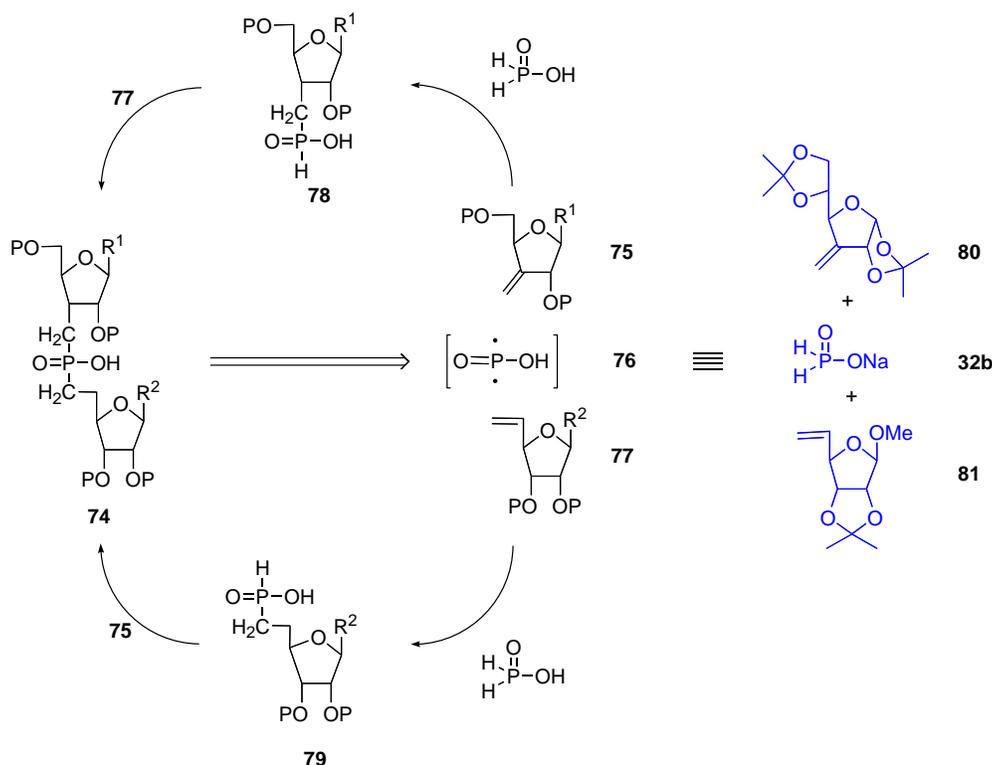
Scheme 14



The possibility of using the Nifant'ev protocol to link two furanoses on position 3 and 5 with a dimethylphosphinoyl (i.e. generate compound **75**, Scheme 15) was also verified. A double disconnection of the two phosphorus-carbon bonds points the finger at alkenes **75** and **77** as protected starting substrates and at a synthetic equivalent of diradical **76**. As demonstrated earlier, a process using hypophosphorous acid sodium salt in two sequential radical addition reactions would achieve this. Two possible syntheses exist, depending on which alkene **75** or **77** is used first. Here again, a stereocontrol is needed in the hydrogen quench of any radical-adduct generated from alkene **75**. The

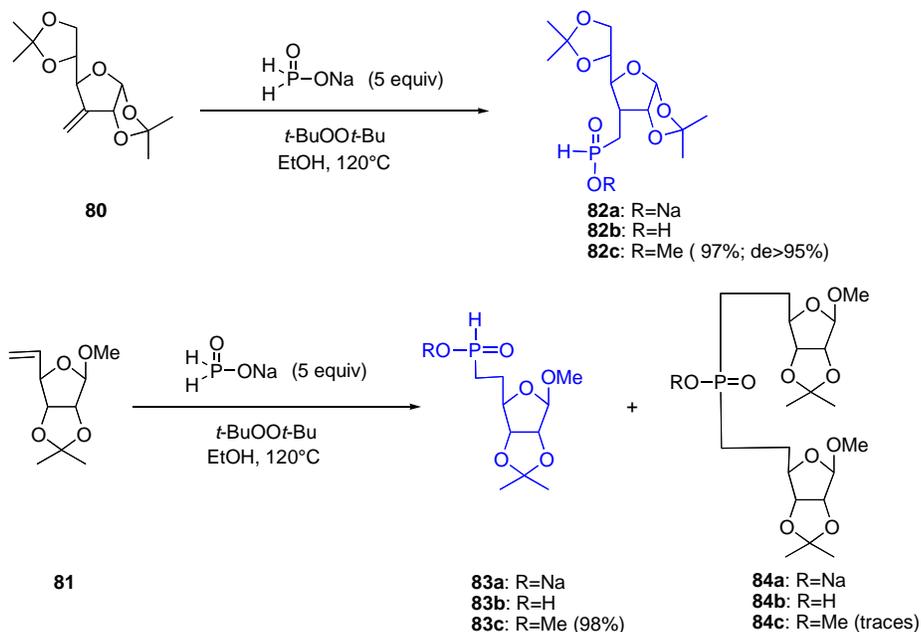
demonstrated efficiency of a 1,2-isopropylidene unit (see above) led us to consider furanose **80**, along with **32b** and **81**.

Scheme 15



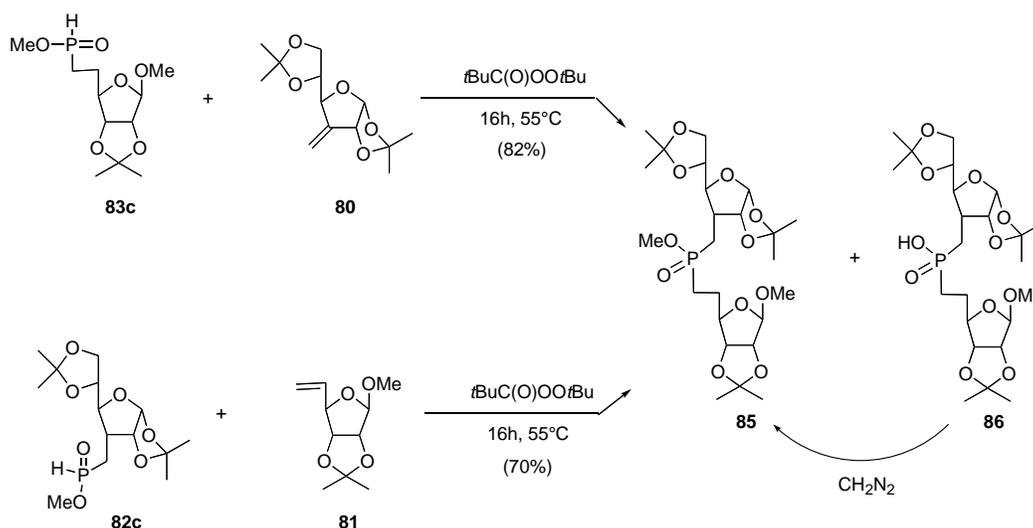
Reaction of a solution of alkene **80** in EtOH with hypophosphorous acid sodium salt **32b** (5 equiv) in the presence of di-*tert*-butyl peroxide at 120°C resulted in the quantitative formation of *H*-phosphinate **82a** isolated in the form of its methyl ester **82c** (diazomethane) as a single diastereomer (Scheme 16). Similarly, alkene **81** generated the expected adduct **83c** in virtually quantitative yield [21].

Scheme 16



Both *H*-phosphinate **82c** and **83c** were then subjected to the action of *tert*-butyl peroxyphosphate in the presence of alkene **81** and **80**, respectively. A 1:3 mixture of the expected methyl phosphinate **85** and phosphinic acid **86** were obtained in each case, which was treated with diazomethane (CAUTION) to deliver methyl ester **85** in 82 and 70% isolated yield (Scheme 17). Phosphinic acid **86** was probably formed through internal hydrogen quenching of the radicals–adducts by the methyl group of phosphinic ester, loss of formaldehyde, hydrogen quenching of the thereby-formed phosphorus radical and oxidation of the resultant *H*-phosphine oxide (through its P(III) tautomer) during work-up. These results thus demonstrated that the Nifant'ev protocol can be efficiently exploited to prepare 3-furanosyl–5'-furanosylphosphinate through a tandem sequential radical process.

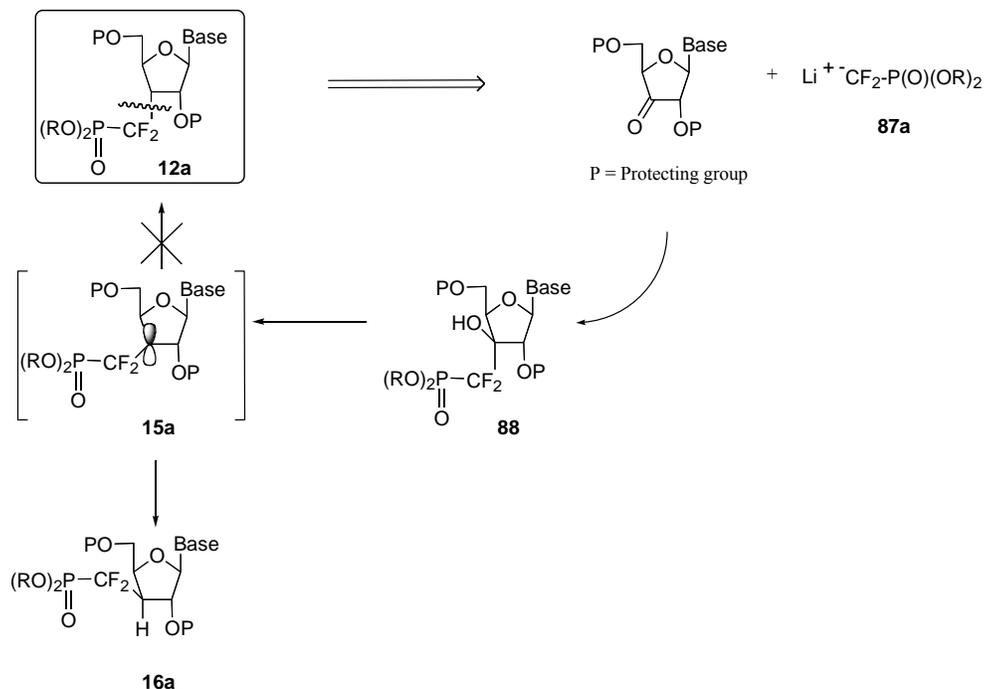
Scheme 17



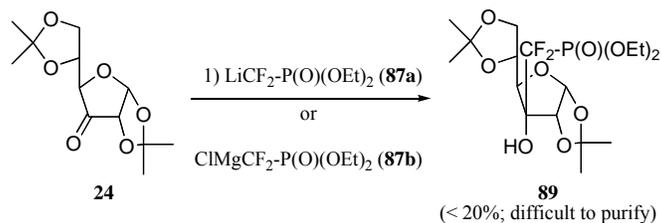
b) Ionic approach

An alternate approach, relying on the ionic introduction of the phosphonodifluoromethyl unit was also considered. The retrosynthetic analysis of our target molecules **12a** is outlined in Scheme 18. Based on Berkowitz' work, a two-step process calling for (i) the addition of reagent **87a** onto a keto group and (ii) a radical-based deoxygenation of the resultant adduct was developed [10d]. Here again, the stereodirecting effect of the base was expected to result in a predominant attack of nucleophilic species **87a** on the convex face of the molecule to generate **88**. However, the same stereodirecting effect acting on the hydrogen quenching of radical intermediate **15a** during the deoxygenation step would probably provide the undesired diastereomer **16a**. As this last step would probably play a crucial role in installing the requisite configuration on carbon $\text{C}_{3'}$, it was decided to start from a furanose derivative featuring a hindered concave face to force the hydrogen quenching to occur on the convex face (thereby positioning the phosphonodifluoromethyl unit on the desired face of the ring), and to introduce the base in a subsequent step, thus paralleling the radical approach (see above). However, interacting either **87a** or the analogous organomagnesium reagent **87b** [22], with ketone **24** never resulted in a clean transformation, and product **89** was isolated in yields lower than 20%, along with substantial amounts of bis(*O,O*-diethylphosphono)difluoromethane and the hydrate of the starting ketone (Scheme 19) [15].

Scheme 18

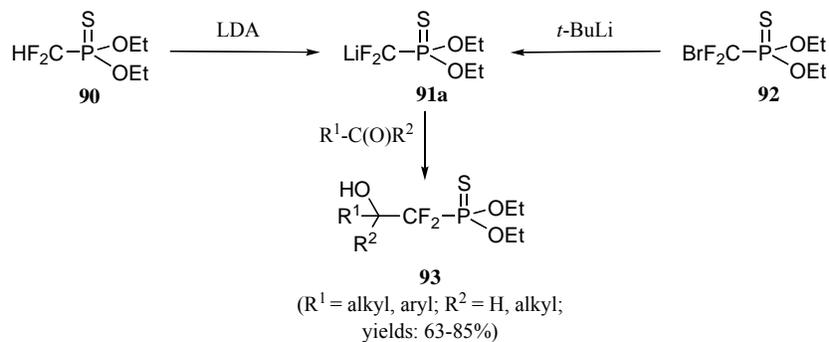


Scheme 19



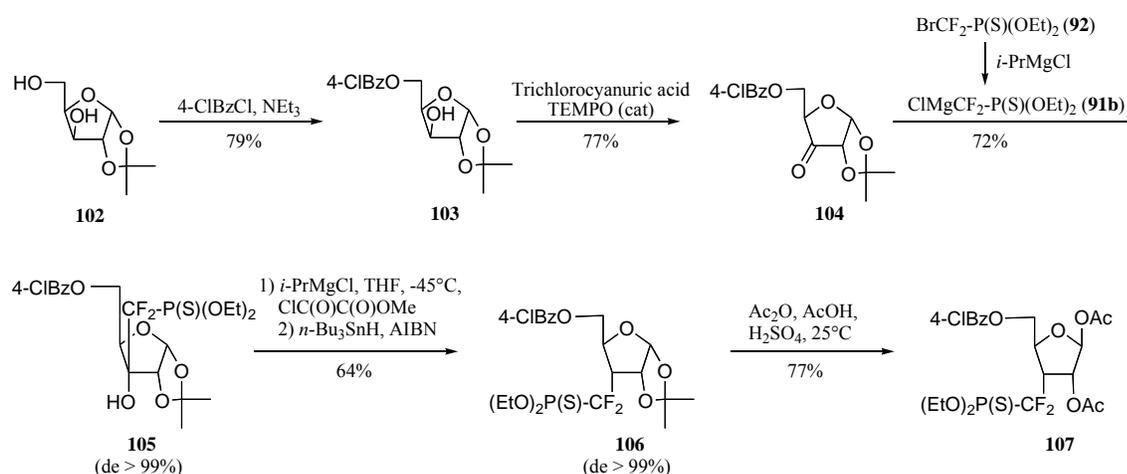
We next considered the thioanalogues **91a-91c** of reagent **87a** (Scheme 20, Figure 4). The thermally more stable lithiated reagent **91a** is conveniently prepared either by treatment of difluoromethylphosphonothioate **90** with lithium diisopropylamide, or by interacting bromodifluorophosphonothioate **92** and *tert*-butyllithium [23]. Reagent **91a** has been shown to be stable at temperatures up to -20°C , thus favorably comparing to phosphonate **87a**, stable only at -78°C or below [24].

Scheme 20



Attempts to improve both the synthesis and the overall yield started with a critical analysis of this route. This led to the identification of several drawbacks impeding its use in larger scale preparation of **101**. Thus, LDA or *tert*-butyllithium were used as base, and chromatography had to be used to obtain several intermediates in the pure form. The involvement of the somewhat hazardous Dess–Martin periodane was also considered as a limitation to the development of a larger scale preparation. Moreover, the 6-carbon starting substrate implied additional steps to obtain the five-carbon skeleton of ribofuranose **101**. An improved synthesis was thus worked out, starting from α -D-xylose derivative **102** (Scheme 22). The required 1,2-isopropylidene protection was completed by the installation of a *para*-chlorobenzoyl unit on the 5'-hydroxyl group. An additional feature of *para*-chlorobenzoate derivatives lies in their propensity to crystallize (see below) [26].

Scheme 22



Alcohol **103** was oxidized by a mixture of trichloroacetic acid and 2,2,6,6-tetramethylpiperidine *N*-oxide (TEMPO) in catalytic amount to deliver ketone **104** in 77% isolated yield [27]. Lithium reagent **91a** was replaced with the organomagnesium reagent **92b**, readily prepared by treating bromide **92** with 1.1 equivalents of freshly prepared *iso*-propyl magnesium chloride in diethyl ether [22]. For the reason cited above, adduct **105** was obtained as a single diastereomer. Again, radical deoxygenation of this adduct resulted in a clean stereochemical inversion of the phosphonothio-difluoromethyl moiety, and phosphonothioate **106** was obtained in 64% isolated yield (two steps) as a single diastereomer. Treatment of this product with acetic anhydride in a mixture of acetic acid and sulfuric acid removed the 1,2-acetonide and furnished triester **107** as a single stereoisomer. This scheme resulted in the formation of a slightly different precursor *with an improved overall yield of 21%* from xylose derivative **102**. Noteworthy is the fact that no chromatography separation is needed, and that the preparation of **107** has been carried out on a 10-gram scale [28].

Stereoselective installment of the pyrimidine bases was achieved by using Vorbruggen's modification of the Hilbert–Johnson protocol while the purine bases were introduced by using the procedures of Saneyoshi and Robins (Table 2) [29,30]. Isolated yields were good to excellent and a complete diastereoselectivity was observed, as a result of the steric hindrance generated by both the 2-acetoxy group and the 3-phosphonothiodifluoromethyl unit. It might be suggested that the longer P=S bond (1.90 Å versus 1.53 Å for the P=O bond) allows the latter group to participate in the stabilisation

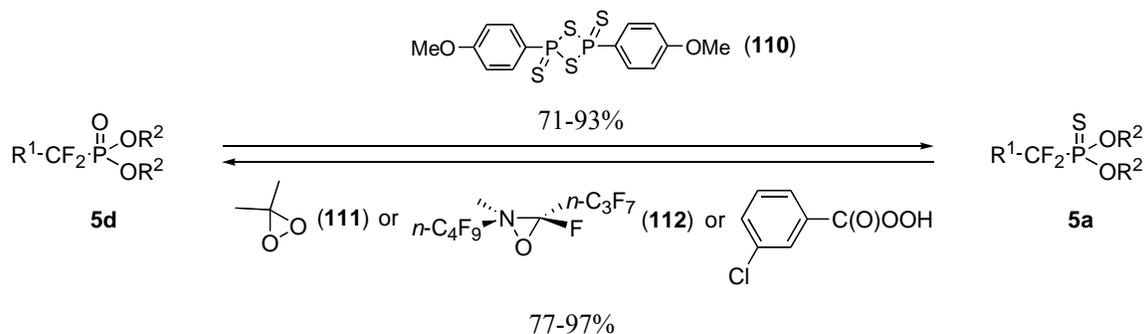
of the intermediate cation. Such an analogous participation has recently been described with 2,3-dideoxy-3-phosphonothiofuranose derivatives [31]. Of particular note is the excellent regiochemical control observed in the induction of the purine bases: a single regioisomer was found to have formed.

Table 2

Entry	Base	Reagent	Conditions	Products	Yields (%)	Products	Yields (%)
1	(T)		TMSOTf, ClCH ₂ CH ₂ C I, 25°C	108a	92	109a	77
2	(U)		TMSOTf, ClCH ₂ CH ₂ C I, 25°C	108b	81	109b	54
3	(C)		TMSOTf, ClCH ₂ CH ₂ C I, 80°C	108c	95	109c	91
4	(A)		SnCl ₄ , CH ₃ CN, 25°C	108d	87	109d	73
5	(G)		TMSOTf, ClCH ₂ CH ₂ C I, 80°C	108e	66	109e	66

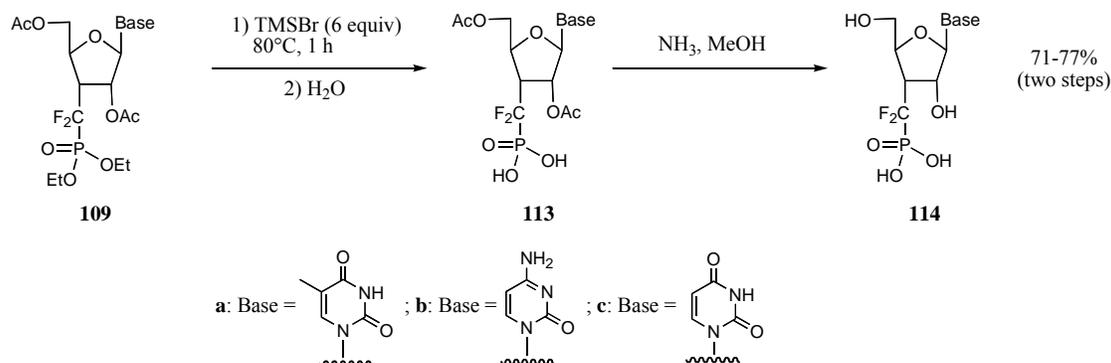
Interconversion of difluorophosphonates **5d** and difluorophosphonothioates **5a** can easily be achieved in high yields and require either the action of Lawesson's reagent **110** (P=O→P=S) or a mild oxidizing agent (P=S→P=O) such as dimethyl dioxirane **111**, perfluorooxaziridine **112** or *m*-CPBA (Scheme 23) [32].

Scheme 23



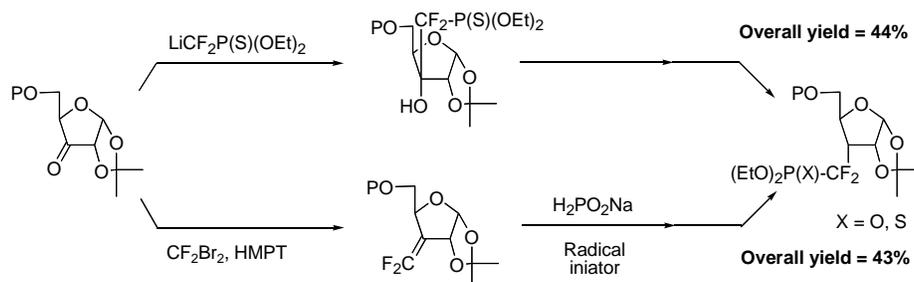
In this program, nucleotide analogues **108**, encompassing the phosphonothiodifluoromethyl unit, were easily transformed into the corresponding phosphonates **109** in good to excellent yields by simple treatment with *m*-CPBA (Table 2). Pyrimidine derivatives **109a-109c** were then fully deprotected by reaction with trimethylsilyl bromide, hydrolysis and treatment with methanolic ammonia; the final products **114a-114c** were isolated as their disodium salts in yields ranging from 70-77% (Scheme 24).

Scheme 24



Comparison of both radical and ionic approaches shows them to be equally effective in stereoselectively producing a protected furanose derivative bearing a phosphono(thio)difluoromethyl unit on carbon 3 (Scheme 25).

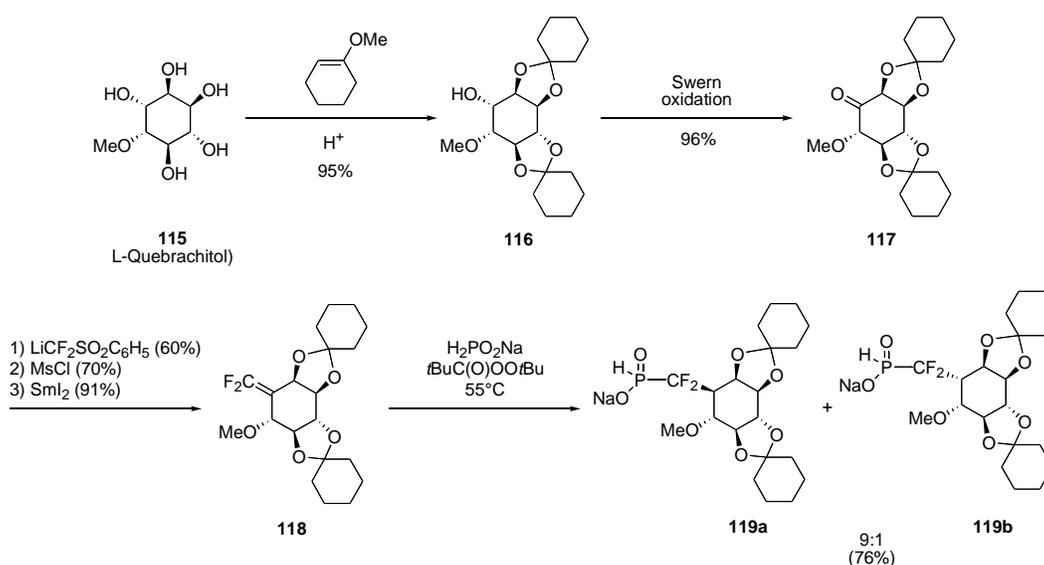
Scheme 25



CyclitolsRadical approach

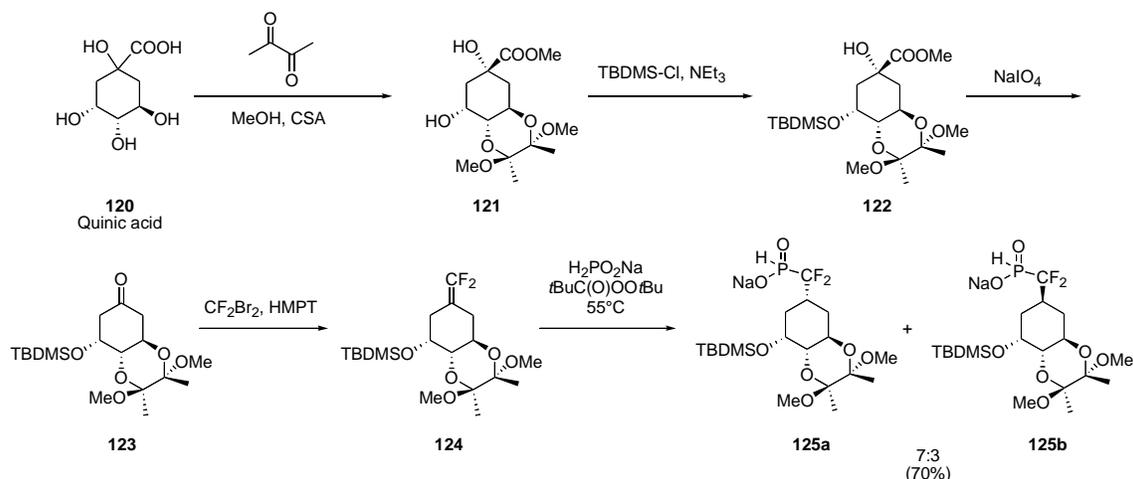
L-Quebrachitol (**115**) is a waste-product of the natural rubber industry and can be obtained from the serum (aqueous phase) of the latex [33]. Protection of the natural product as bicyclohexylidene acetal derivative **116** and oxidation of the remaining hydroxyl group under the conditions of Swern delivered ketone **117** with five of the six stereocenters present in inositol-3-phosphate. Ketone **117** was next transformed into *gem*-difluoroalkene **118** by using the three-step procedure published by McCarthy [34]. When a mixture of hypophosphorous acid sodium salt **32b** and substrate **118** were heated at 55°C in the presence of *tert*-butyl peroxopivalate as radical initiator, a complete consumption of the substrate was observed and products **119a** and **119b** were formed (9:1 diastereomeric ratio) (Scheme 26). The neighboring axial ether group was thus efficient in directing the hydrogen quenching to occur on the face opposite to that carbon-oxygen bond. The major product **119a** constitutes a key intermediate *en route* to the phosphono(thio)difluoromethyl analogues of inositol-3-phosphate.

Scheme 26



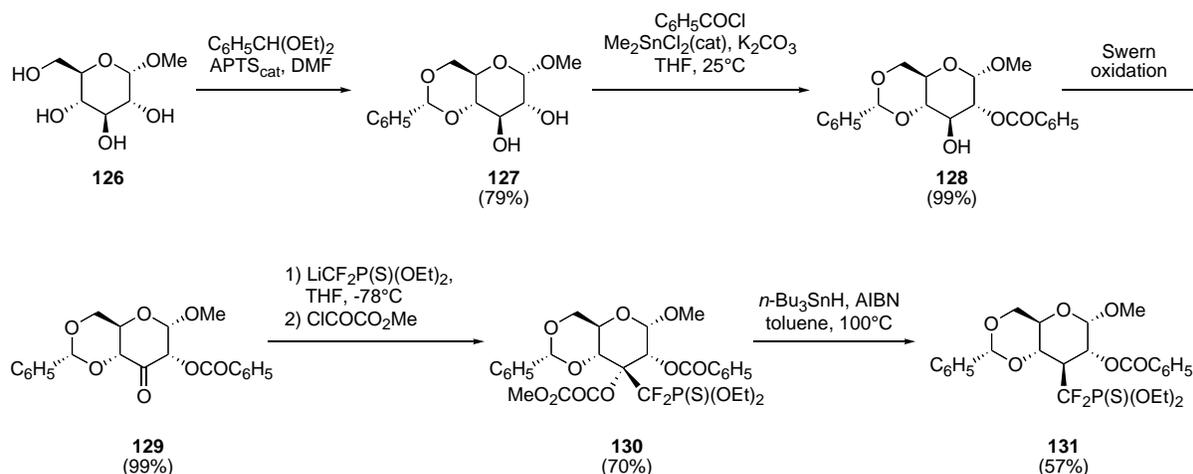
Quinic acid **120** also represents a useful chiral source for the preparation of modified cyclitols (Scheme 27) [35]. Protection of the three secondary hydroxyl groups was accomplished using literature procedure and the resultant methyl ester **122** was subjected to a decarboxylating oxidation to deliver ketone **123**. Transformation into *gem*-difluoroalkene **124** yielded the substrate on which the radical methodology was applied. The phosphorous-centered radical generated from **32b** by the standard procedure once again delivered the expected difluoro-*H*-phosphinates **125**, this time as a 7:3 diastereomeric mixture due to the relative remoteness of the stereodirecting, axial silyl ether group.

Scheme 27

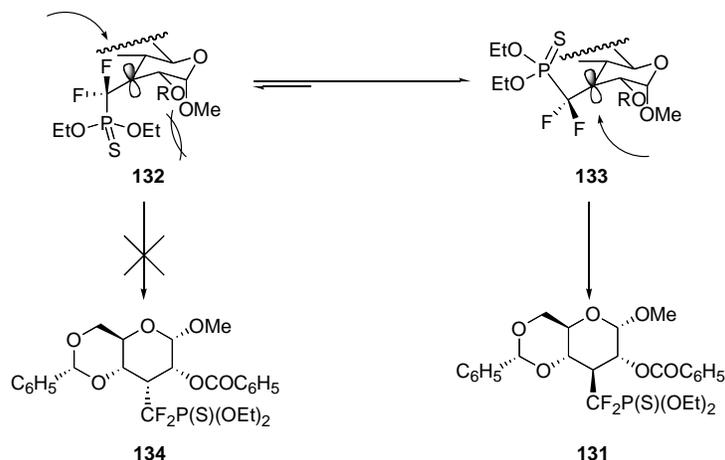
Ionic approach

The ionic approach was also tested on a pyranose derivative. Indeed, the possible use of the Ferrier reaction makes of pyranose derivatives a potentially useful entry into the preparation of cyclitol analogues [36]. Thus, α -D-glucose derivative **126** was protected and oxidized using a three-step sequence of transformations depicted in Scheme 28. The resultant ketone **129** was subjected to the action of lithiated reagent **91a** at -78°C , and the corresponding alcoholate was quenched with *O*-methyl oxalyl chloride. Difluorophosphonothioate **130** was isolated in 70% yield as a single diastereomer. Treatment of **130** with tri-*n*-butyltin hydride and AIBN in toluene at 100°C gave exclusively product **131** in 57% isolated yield. No stereochemical inversion of the phosphonothio-difluoromethyl group occurred this time, however. Apparently, the steric hindrance generated by the methoxy group forces the radical-adduct to adopt conformation **133** rather than **132**, thereby leading to **131** and not **134** (Scheme 29).

Scheme 28

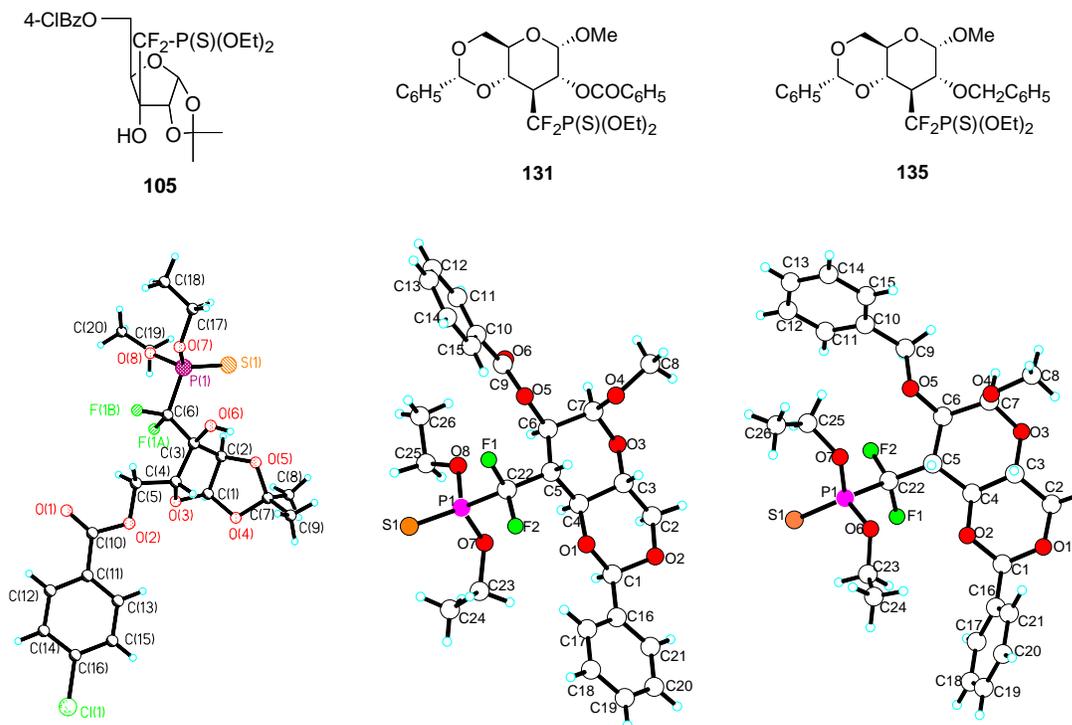


Scheme 29

*X-ray analyses*

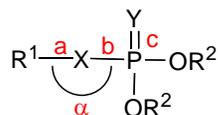
Three of the above α,α -difluorophosphonothioates (**105**, **131**, and the benzyl analogue **135** of **131**) were crystalline solids. Careful crystallization led to the growth of monocrystals suitable for X-ray analysis. Figure 5 shows the structures and ORTEP graphics of these three compounds and Table 3 include selected data obtained from the X-ray analyses, along with data published by Chambers and O'Hagan on compounds [3a,3c].

Figure 5



Although the three phosphonothioates are structurally and electronically different than zwitterions **6a** and **6d**, the data clearly indicate a widening of the angle formed by C–CF₂ and CF₂–P bonds (Table 3). The bond lengths *a* and *b* themselves do not vary much from the values reported for difluorophosphonates (e.g. **6d**). The length *c* of the phosphorus-sulfur double bond is, expectedly much larger than the oxygenated counterpart in **6a** and **6d**.

Table 3



6a: R¹ = H₂NCH₂; X = O; R² = H
6b: R¹ = H₂NCH₂; X = CF₂; R² = H

Compound	X	Y	<i>a</i> (Å)	<i>b</i> (Å)	<i>c</i> (Å)	α (°)
105	CF ₂	S	1.88	1.51	1.90	120.3
131	CF ₂	S	1.86	1.52	1.91	122.5
135	CF ₂	S	1.86	1.55	1.91	117.5
6a	O	O	1.59	1.43	1.52	118.5
6d	CF ₂	O	1.85	1.50	1.53	116.5

Conclusions

Our journey into the field of new phosphate isosters related to the difluorophosphonates has allowed the preparation of new functional entities such as the difluorinated phosphonothioates, phosphonodithioates and phosphonotrithioates, in which the sulfur atom(s) may either play a positive role in the course of a reaction, or have a deleterious effect on the outcome of a desired transformation. Thus the presence of the double-bonded sulfur atom in reagents **91** induced a dramatic, positive effect in the condensation on ketone **19**, **24** and **129**, which ultimately translated into a stereocontrolled synthesis of the phosphonodifluoromethyl analogues of nucleoside-3'-phosphates. Despite the positive effect of sulfur on the addition of phosphonothioyl radical – when compared to the fully oxygenated analogues –, it was simply not enough to induce an addition reaction on functionalized, cyclic *gem*-difluoroalkenes (e.g. **19**, **27** or **28**). Hypophosphorous acid sodium salt **32b** brings a solution to this problem and displays a reactivity that allows the easy preparation of a variety of phosphate isosters through the intermediary of α,α -difluoro-*H*-phosphinates. It also allows to stereoselectively link two furanose units on carbon 3 and 5' with a phosphinyl or a difluorophosphonothioyl unit, and to produce new fully protected analogues of inositol phosphates. Work is in progress in this laboratory to fully explore the possibilities offered by this chemistry.

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