Tetrahedron 64 (2008) 6153–6190

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Tetrahedron report number 838

Fluorinated organophosphates for biomedical targets

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ARTICLE INFO

Article history:
Received 9 April 2008
Available online 22 April 2008

Keywords:
Organophosphates
Fluorinated organophosphates
Fluorinated phosphate esters
Biophosphates
Dialkyl phosphorofluoridates
Fluorinated nucleotides
Synthesis
Biomedical application

Contents

1. Introduction

Phosphate esters are of great importance because of the ubiquity of phosphate-containing molecules in biological processes including signalling pathways, information storage and energy transfer. As a result, they have been a topic of interest for many

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0040-4020/$ – see front matter
doi:10.1016/j.tet.2008.04.064

years, with applications mainly directed towards bioorganic and medicinal chemistries.[1,2] The chemistry of fluorinated organophosphates is a relatively new area of research, which has been developed mostly in the last 20 years. In general, incorporating fluorine as either a bioisosteric replacement for hydrogen (small size) or an isoelectronic replacement for the hydroxyl group (similar polarity) has considerable impact on the behaviour of a phosphate in a biological environment. The high electronegativity of fluorine, small size and ability to form hydrogen bonding (as an acceptor) can have dramatic mechanistic consequences, which can lead to mechanistic deviations and enzyme inhibition.[3–6] In fact, many of the fluorinated species are good enzyme inhibitors and have been developed into clinically useful chemotherapeutic agents. Consequently, they have become important in the treatment of cancers (eniluracil, fludarabine), HIV infection (alamifovir) and life-threatening fungal infections (fosfluconazole).[7–9] Using fluorinated phosphate esters has also enabled researchers to obtain crucial information regarding the catalytic mechanism of enzymatic reactions.[10–13] Lastly, fluorine substitution has been successfully employed in phosphate chemistry to increase bioabsorption and metabolic stability of biologically important compounds. In particular, the CF_3 group has a pronounced lipophilicity, as reflected by its Hansch–Leo substituent parameter π of 0.88 ($-CH_3$: π=0.56).[14,15]

Although the field of classical phosphate esters is covered by a considerable number of monographs, the chemistry of fluorinated organophosphates has never been reviewed systematically. This report covers the synthesis and biomedical applications of fluorinated organophosphate esters from 1992 to the middle of 2007. The first part of the review surveys the general synthetic methods used for the preparation of fluorinated organophosphates. The second part consists of a presentation of the different types of fluorinated phosphates having significant biological importance. Here, primary emphasis will be on the synthesis with a brief description of the biological rationale for the design and the biological outcome. Discussion of the basic aspects of phosphate chemistry is reduced to minimum. In addition, no particular attention will be paid to the general features of fluorine substitution that have been discussed extensively in the literature.[15–17]

2. Methods for design and synthesis

Three major structural types of simple fluorinated organophosphates (Chart 1) have found application in the block-building syntheses of bioactive species: dialkyl phosphorofluoridates containing a direct P–F bond (**A**), fluoroalkyl phosphorochloridates and fluoroalkyl phosphoramidates (**B**) and symmetrical and unsymmetrical fluorine-containing trialkyl phosphates (**C**).

Readers are referred to the early literature[2,18–20] concerning the synthesis of simple fluorine-containing organophosphate esters.

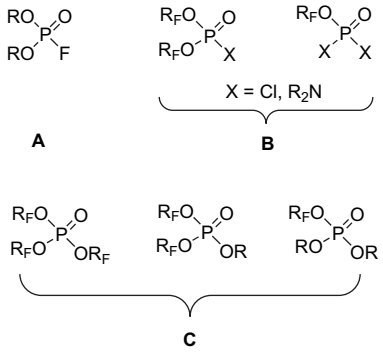


Chart 1. Structural types of simple fluorinated organophosphates.

Recent modifications of the classical methods as well as the development of new methodologies are outlined below.

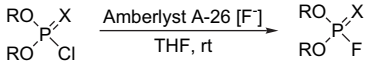
2.1. Dialkyl phosphorofluoridates

These compounds inhibit acetylcholinesterase (AChE), an enzyme, which controls nerve impulse transmission.[4] A variety of reactions are available for their synthesis, but three groups of methods are most frequently used. The first approach includes the halogen metathesis reactions of the corresponding chloridates $(RO)_2P(O)Cl$ using metal fluorides (e.g., NaF or KF); the second includes the reactions of phosphorus(V) species (mainly *H*-phosphonates) with an activated fluorine source (SO_2ClF, SOF_2, PhCOF, etc.) and the third approach is based on the alcoholysis of phosphoryl dichlorofluoride, $Cl_2P(O)F$.

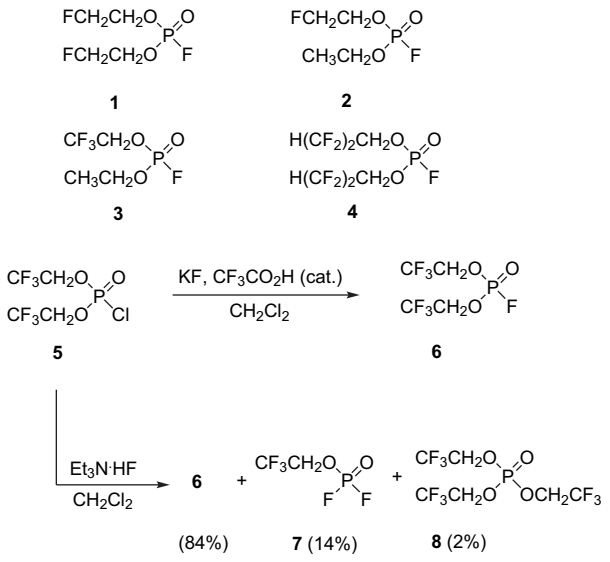
Chlorine-exchange fluorination reactions appear to be well adapted for the synthesis of phosphorofluoridates because they trade the weaker P–Cl for the stronger P–F bond. The original process developed during 1940s by Saunders in UK and by Schrader in Germany involves heating the corresponding phosphorochloridates with an alkali metal fluoride in an aprotic polar solvent such as benzene or acetonitrile.[4] Recently, Farooq has reported the nucleophilic fluorination of organochlorophosphorus(V) compounds with sodium hexafluorosilicate and other alkali metal salts of perfluorinated complex anions ($NaBF_4$, $NaPF_6$).[21,22] All the reactions occurred in a short time, but the yields of the phosphorofluoridates were highly variable (12–77%). In related work, treatment of thio- or selenophosphorus acids $(RO)_2P(S/Se)SH$ with aqueous silver fluoride in chloroform resulted in the formation of compounds $(RO)_2P(O)F$. The fluorination is accompanied by an efficient oxidation and so the $P(S/Se)\rightarrow FP(O)$ conversion can be achieved in a one-pot reaction. Analogous results were obtained with other phosphorothio(seleno)ates such as esters, amidates or halides.[23] Effective conversion of phosphorus(V) chlorides into the corresponding phosphorofluoridates was also achieved utilising a solid-supported source of fluoride ion. Thus, in THF at room temperature, the equimolar reaction of phosphorus(V) chlorides with the ion exchange resin Amberlyst® A-26 with a fluoride counterion afforded the corresponding phosphorus(V) fluorides in very good yields (Table 1). This methodology represents a simplified procedure over those methods previously reported, avoiding the need to use commercially unavailable starting materials.[24]

Dialkyl phosphorofluoridates with fluoroester groups **1**–**4** are accessible from either the reaction of alcohols with phosphoryl dichlorofluoride $Cl_2P(O)F$ or the direct interaction of fluorinated alcohols with phosphorus pentoxide.[4] Attempts to prepare bis-(trifluoroethyl) phosphorofluoridate **6** by heating phosphorochloridate **5** with NaF or KF in dichloromethane did not result in fluorination, but the chloridate **5** has been transformed into the fluoridate **6** using KF and a catalytic amount of trifluoroacetic acid. Reaction of **5** with a triethylamine hydrofluoride complex in dichloromethane has afforded a mixture of the fluorinated products **6**–**8** (Scheme 1).[25]

Table 1
Synthesis of dialkyl phosphonofluoridates using a solid-supported reagent[24]



R	X	Isolated yield (%)
Me	O	82
Et	O	81
Et	S	91
Pr^i	O	76
Ph	O	70



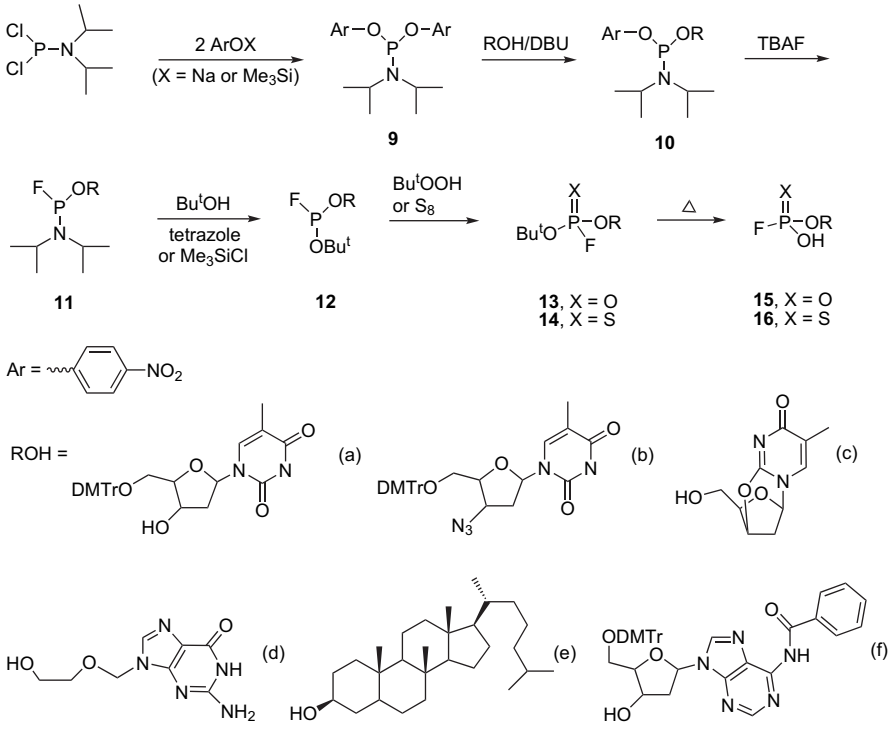
Scheme 1.

A new and general approach to phosphorofluoridates and phosphorofluoridothioates is depicted in Scheme 2. A 4-nitrophenolate group in phosphoramidite **9**, which is readily available from commercial *N*,*N*-diisopropyldichlorophosphoramidite, can be substituted by an alkoxy group through a reaction with an alcohol (ROH) in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The phosphoroamidites **10** are formed as a 1:1 mixture of diastereomers, but their conversion into the phosphorofluoroamidites **11** proceeds with some stereoselectivity. Coupling of **11** with *tert*-butanol in the presence of tetrazole or trimethylchlorosilane leads to the phosphorofluoridites **12**. Subsequent oxidation by *tert*-butyl hydroperoxide or addition of elemental sulfur gives the corresponding phosphorofluoridate **13** or **14**. Thermal elimination of 2-methyl-1-propene from **13** and **14** afforded the desired compound **15** or **16**, respectively (Scheme 2).[26]

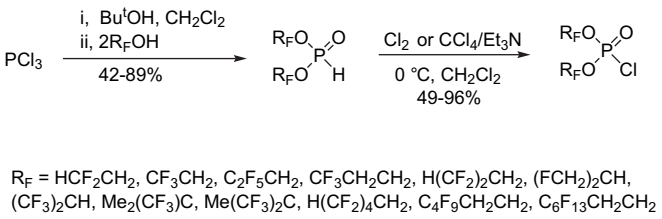
2.2. Fluoroalkyl phosphorochloridates and phosphoramidates

Alkyl phosphorochloridates $(RO)_{3-n}P(O)Cl_n$ (n=1, 2) have been a topic of interest for many years, with applications mainly directed towards the synthesis of bioactive phosphates. The interest in fluorinated alkyl phosphorochloridates, as building blocks, where fluorine is attached to the alkyl substituents has appeared much more recently.[25,27,28] The most simple route to fluoroalkyl phosphorodichloridates $R_FOP(O)Cl_2$ involves the alcoholysis of phosphorus oxychloride with removal of HCl by passage of nitrogen through the reactants. This method can be used only in cases where the fluoroalcohol contains few fluorine atoms. As an example, 2-fluoroethanol (pK_a 14.4) reacts with $POCl_3$ to give $FCH_2CH_2OP(O)Cl_2$ in 34% yield. Polyfluorinated alcohols such as trifluoroethanol (pK_a 12.4) do not react under these conditions and a base is required to initiate the phosphorylation. In fact, phosphorochloridates $CF_3CH_2OP(O)Cl_2$ and $(R_FCH_2O)_2P(O)Cl$ (R_F=CF_3 and C_2F_5) were prepared from phosphorus oxychloride, fluoroalcohols and triethylamine, but the selective substitution was difficult.[28] A much better synthesis of bis(fluoroalkyl) phosphorochloridates is based on the dialkyl phosphite methodology first investigated by Mahler in the late 1970s.[29] Chlorination of bis(fluoroalkyl) *H*-phosphonates $(R_FO)_2P(O)H$ gives the chloridates $(R_FO)_2P(O)Cl$ in good yield and in a state of high purity. Except when R_F=$(CF_3)_3C$, the starting bis(fluoroalkyl) *H*-phosphonates can be easily obtained by the reaction of fluoroalcohols with phosphorus trichloride and *tert*-butylamine. Examples of numerous syntheses disclosing detailed conditions were reported by Timperley and co-workers (Scheme 3).[25,30]

The presence of fluorine atoms in the ester groups imparts a high thermal stability to the forming phosphorochloridates. Thus, phosphorochloridate $[(ClCH_2)_2CHO]_2P(O)Cl$ decomposes readily during distillation and phosphorochloridate $(Me_3CO)_2P(O)Cl$ decomposes spontaneously at room temperature after several hours. The fluorocarbon analogues $[(FCH_2)_2CHO]_2P(O)Cl$ and $(Me_2CF_3CO)_2P(O)Cl$ could be distilled without appreciable decomposition.[25]



Scheme 2.



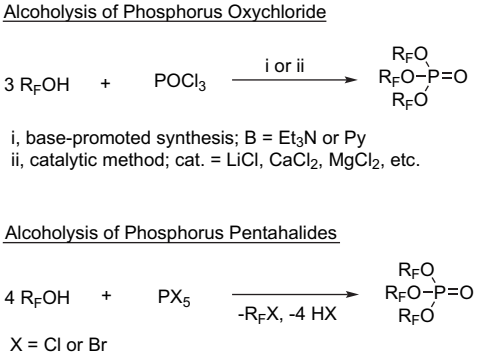
Scheme 3.

Interestingly, treatment of bis(trifluoromethyl) *H*-phosphonate with bromine or iodine gave the bromidate $(CF_3CH_2O)_2P(O)Br$ and iodidate $(CF_3CH_2O)_2P(O)I$ in 51 and 46% yield, respectively.[25,31]

Treatment of bis(fluoroalkyl) phosphorochloridates with nitrogen nucleophiles opens up a general route to bis(fluoroalkyl) phosphoroamidates, $(R_FO)_2P(O)NR^1R^2$. Recently, 40 phosphoramidates $(R_FO)_2P(O)X$ were prepared in 10–91% yield by treating phosphorochloridates $(R_FO)_2P(O)Cl$ (R_F=HCF_2CH_2, $HCF_2CF_2CH_2$, $H(CF_2)_4CH_2$, CF_3CH_2, $C_2F_5CH_2$, $C_3F_7CH_2$, $(CF_3)_2CH$, $(FCH_2)_2CH$ and Me_2CF_3C) with amines HX (X=NH_2, NHMe, NMe_2, NHEt and NEt_2). The bulky chloridate $[Me_2(CF_3)_2CO]_2P(O)Cl$ reacted with ammonia, methylamine, dimethylamine and ethylamine, but not with diethylamine—even on heating in the presence of 4-dimethylaminopyridine—due to steric hindrance at phosphorus.[32]

A simple method for producing dialkyl *N*-(fluoroalkyl)phosphoramidates $(RCH_2O)_2P(O)NHCH_2R_F$ (R=Me, CF_3, CCl_3; R_F=CF_3, C_2F_5), which involved the reaction between dialkyl phosphorochloridates and fluoroamines has been described.[33] Introduction of fluoroester groups on the phosphorus atom makes the P–Cl bond more susceptible to nucleophilic attack. In fact, bis(fluoroalkyl) phosphorochloridates $(R_FO)_2P(O)Cl$ [R_F=$C_2F_5CH_2$, $C_3F_7CH_2$, $(CF_3)_2CH$] easily reacted with 2,2,2-trifluoroethylamine to give the compounds $(R_FO)_2P(O)NHCH_2CF_3$ in yields greater than those with the corresponding dialkyl phosphorochloridates.[34] An alternative route to phosphoramidates $(RO)_2P(O)NHCH_2CF_3$ via dichloride $Cl_2P(O)NHCH_2CF_3$ was also explored. *N*-(2,2,2-Trifluoroethyl)phosphoramidic dichloride, however, failed to react cleanly with *n*-propanol or isopropanol in the presence of triethylamine, or with sodium *n*-propanolate or isopropanolate.[34]

The conversion of $(CF_3CH_2O)_2P(O)Cl$ into the fluorinated species $(CF_3CH_2O)_2P(O)NHOMe$ and $(CF_3CH_2O)_2P(O)N_3$ was affected by nucleophilic substitution with methoxyamine and azide ion. Bis(trifluoromethyl) phosphoroisocyanate $(CF_3CH_2O)_2P(O)NCO$ has been prepared from the corresponding phosphoramidate and oxalyl chloride in 10% yield.[32]



Scheme 4.

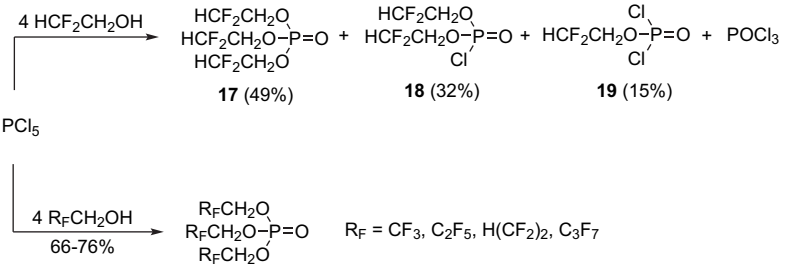
2.3. Symmetrical and unsymmetrical fluorinated alkyl phosphates

α-Replacement of protons with fluorine atoms alters the chemical and physical properties of organophosphates and this is well illustrated in the case of simple tris(fluoroalkyl) phosphate esters. While trimethyl phosphate $(MeO)_3P$=O is a highly thermally stable alkyl phosphoryl compound, the fluorinated trimethyl phosphates $(FCH_2O)_3P$=O and $(F_2CHO)_3P$=O, in sharp contrast, are hypothetical species. Tris(trifluoromethyl) phosphate $(F_3CO)_3P$=O is moderately stable and disproportionates over 3 days at 60 °C to give COF_2 and POF_3. β-Fluoroalkyl phosphates and trialkyl phosphates with more remote fluorine atoms exhibit high thermal stability and are easily available.

Most symmetrical fluoroalkyl phosphates have been prepared either by fluoroalcoholysis of phosphorus oxychloride or by interaction of phosphorus pentachloride or pentabromide with a fluoroalcohol (Scheme 4).

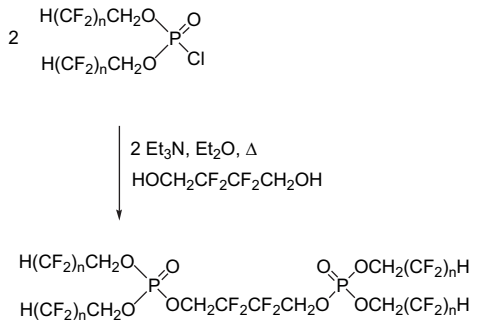
The first approach was successful for a wide range of R_F groups. Phosphorus oxychloride is, however, normally unreactive towards fluoroalcohols under neutral or acidic conditions and, therefore, the reactions require the use of a stoichiometric amount of a base.[28] In the catalytic method, the use of Groups I–III metal salt or a dipolar compound of the type R_2S=O or R_3P=O, which increases the positive charge on phosphorus by coordination to the oxygen atom of the P=O group, is essential for the combination of a phosphoryl chloride with a fluoroalcohol.[35–37] The second approach utilises the alcoholysis of phosphorus pentachloride or phosphorus pentabromide with 4 mol of a fluoroalcohol. In this case, a requirement for a high yield of the fluoroalkyl phosphate is the presence of many fluorine atoms in the fluoroalcohols. In one example, the reaction of 2,2-difluoroethanol with PCl_5 yielded a mixture of tris(difluoroethyl) phosphate **17**, phosphorochloridates **18** and **19** and phosphorus oxychloride. Primary fluoroalcohols containing three or more fluorine atoms, however, reacted rapidly with PCl_5 to give the phosphates $(R_FCH_2O)_3P$=O in good yield (Scheme 5). Tertiary fluoroalcohols such as CF_3Me_2COH, $Me(CF_3)_2COH$ and $(CF_3)_3COH$ do not react with PCl_5 under these conditions. Hexafluoroisopropanol $(CF_3)_2CHOH$ produced a 3:7 mixture of the symmetrical phosphate $[(CF_3)_2CHO]_3P$=O and the chlorophosphorane $[(CF_3)_2CHO]_4PCl$.[38]

Unsymmetrical fluoroalkyl phosphates are normally synthesised by triethylamine-promoted alcoholysis of the phosphorohalidates. Thus, the synthesis of the phosphates



Scheme 5.

$[H(CF_2)_nCH_2O]_2P(O)OR$ has been achieved by the interaction of bis(α,α,ω-trihydroperfluoroalkyl) phosphorochloridates with alcohols (R=Me, Et, Pr, ^{i}Pr) and triethylamine in ether. The reactions with isopropanol reached completion more slowly than those with the other alcohols and, when $n>1$, they required heating in the presence of a 4-dimethylaminopyridine (DMAP) catalyst.[38,39] When bis(α,α,ω-trihydroperfluoroalkyl) phosphorochloridates were allowed to react with 2,2,3,3-tetrafluorobutane-1,4-diol, the phosphates **20a–c** were isolated as colourless viscous liquids by vacuum distillation (Scheme 6).[39]



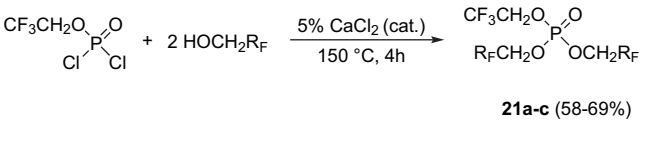
20a-c (22-65%)

n = 1 (**a**), 2 (**b**), 4 (**c**)

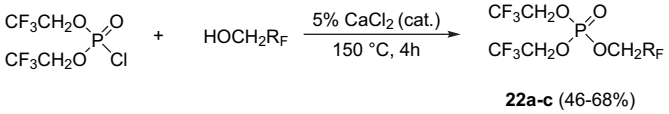
Scheme 6.

Interestingly, the experimental results indicate that bis(hexafluoroisopropyl) phosphorochloridate $[(CF_3)_2CHO]_2P(O)Cl$ ranks among the most reactive phosphorylating agents. Although it has a secondary fluoroalkyl group, it is more reactive than chloridates with primary fluoroalkyl groups and, in particular, unlike the other chloridates, bis(hexafluoroisopropyl) phosphorochloridate reacts readily with isopropanol in the absence of DMAP. It is assumed that the powerful electron withdrawal from the phosphorus atom by the hexafluoroisopropyl groups favours the attack of an oxygen nucleophile (kinetic effect) and the formation of a pentacoordinate phosphorane intermediate $[(CF_3)_2CHO]_2P(OH)(OR)Cl$ (thermodynamic effect).[39] The outstanding ability of the $(CF_3)_2CHO$ group to stabilise phosphoranes was exemplified by the isolation of $[(CF_3)_2CHO]_4PCl$, a stable compound that survived vacuum distillation.[38] Further examples on the synthesis of unsymmetrical fluoroalkyl phosphates by the route employing the catalytic alcoholysis of phosphoryl chlorides have been reported. Thus, heating trifluoromethyl phosphorodichloridate $CF_3CH_2OP(O)Cl_2$ and primary fluoroalcohols in the presence of 5% calcium chloride as catalyst affords the phosphates **21a–c** in good yield. Similarly, bis(fluoroethyl) phosphorochloridate $(CF_3CH_2O)_2P(O)Cl$ reacted with primary fluoroalcohols to give the unsymmetrical phosphates **22a–c** in isolated yields of between 46 and 68% (Scheme 7).[38] The major limitation of this method is that only primary fluoroalcohols react with phosphoryl chlorides under these conditions.

The reactions of fluoroalkyl phosphorochloridates with sulfur nucleophiles have also been investigated.[40] The synthesis of $CF_3CH_2OP(O)(SEt)_2$ in 30% yield was accomplished by treating $CF_3CH_2OP(O)Cl_2$ with 2 molar equiv of EtSH and Et_3N in ether. Bis(trifluoroethyl) phosphorochloridate and bis(pentafluoropropyl) phosphorochloridate, $(R_FCH_2O)_2P(O)Cl$ (R_F=CF_3 or C_2F_5), did not react with MeSH in ether in the presence of triethylamine. Ethanethiol and propanethiol reacted with fluoroalkyl phosphorochloridates in the presence of triethylamine to give the thiolates $(R_FO)_2P(O)SR$ (R_F=CF_3CH_2, $C_2F_5CH_2$, $C_3F_7CH_2$ or $(CF_3)_2CH$) in 13–41% yield. Perfluoroalkylated alcohols containing at least a C_2H_4 spacer unit between the perfluoroalkyl and hydroxyl groups have



21a-c (58-69%)

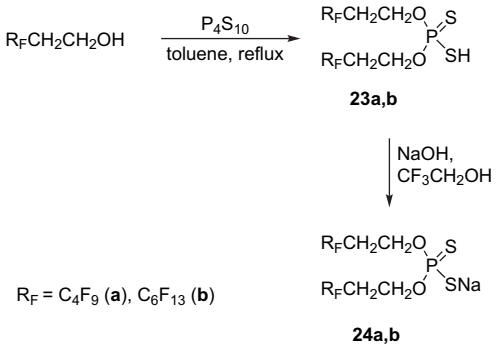


22a-c (46-68%)

R_F = C_2F_5 (**a**), HCF_2CF_2 (**b**), C_3F_7 (**c**)

Scheme 7.

been shown to react with P_4S_{10} to give the perfluoroalkylated dithiophosphoric acids (Scheme 8). 2,2,2-Trifluoroethanol or $C_7F_{15}CH_2OH$, containing only a single CH_2 spacer unit, or perfluoroalkylated phenols (e.g., 4-$C_6F_{13}C_6H_4OH$) did not undergo any reaction with P_4S_{10}. Both dithiophosphoric acids **23a**,**b** reacted with metal hydroxides in 2,2,2-trifluoroethanol to give the corresponding alkali metal salts **24a**,**b**.[41]



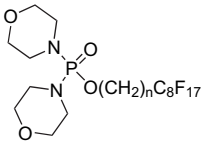
R_F = C_4F_9 (**a**), C_6F_{13} (**b**)

23a,b

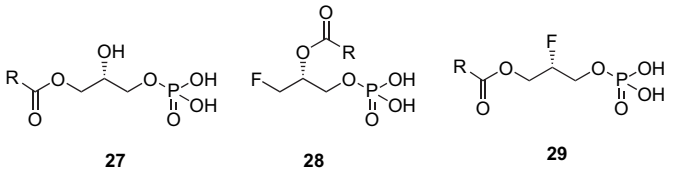
24a,b

Scheme 8.

The effect of fluorination of the ester groups in dialkyl phosphates on anticholinesterase activity has been little studied. Timperley and co-workers attempted to determine the bimolecular rate constants (K_i values) for the inhibition of bovine erythrocyte acetylcholinesterase (AChE) employing four fluoroalkyl phosphates: $(CF_3CH_2O)_3P{=}O$, $(C_2F_5CH_2O)_3P{=}O$, $(C_3F_7CH_2O)_3P{=}O$ and $(CF_3CH_2O)_2P(O)OCH_2C_2F_5$.[42] Under the experimental conditions used (37 °C, pH 7.4), none of these compounds inhibited AChE and it was only possible to define their K_i values as being <7–16 M^{-1} min^{-1}. They are, therefore, at least 10^5-fold less potent inhibitors than the nerve agents, sarin or soman. The low affinity of the fluoroalkyl phosphates for acetylcholinesterase can be explained by their hydrophobic character and the poor leaving ability of the $-OCH_2R_F$ group. Interestingly, compounds **25** and **26**, in which the perfluoroalkyl group on phosphorus is similarly attached through an $-OCH_2$ spacer, also had very low acute toxicity to mice when administered by intraperitoneal or intravenous injection. These compounds have been proposed as components of fluorocarbon emulsions for pulmonary applications in medicine.[43]



25, n = 2, LD_{50} ip mice > 2 g/kg
26, n = 11, LD_{50} iv mice > 2 g/kg



27 **28** **29**

Chart 2. Lysophosphatidic acid and its fluorinated analogues.

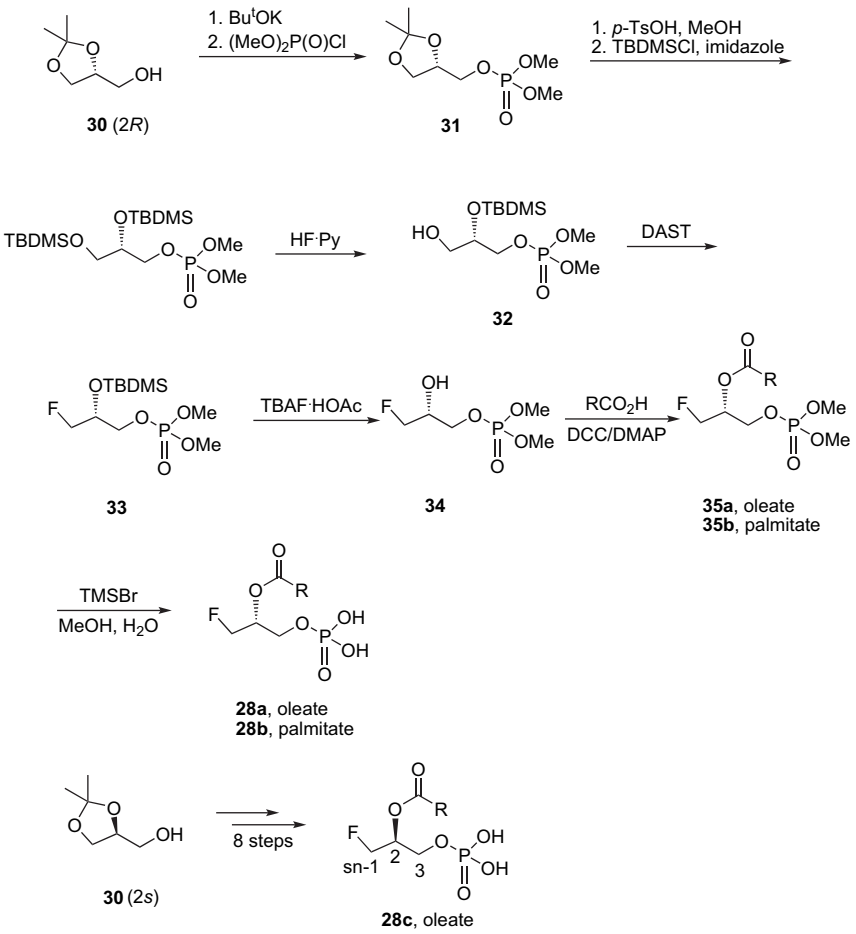
3. Types of biologically active fluorinated organophosphates

3.1. Analogues of *sn*-glycerol phosphate and phospholipids

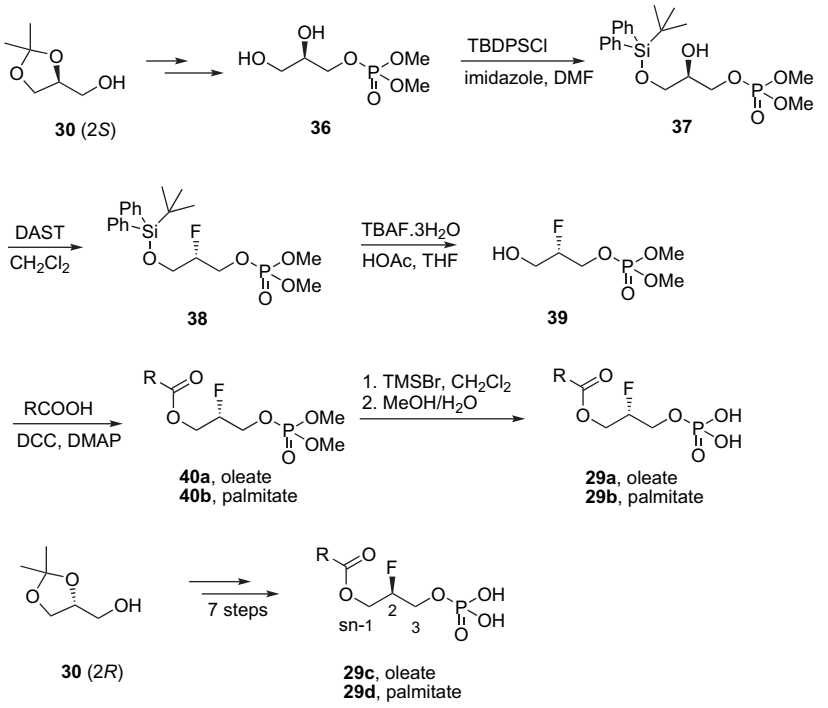
Prestwich and co-workers have developed a program to test the hypothesis that acyl migration-blocked fluorinated analogues of lysophosphatidic acid **27** (LPA; 1- or 2-acyl-*sn*-glycerol 3-phosphate), particularly with fluorine in the *sn*-1 or *sn*-2 position, might mimic LPA as a biological ligand (Chart 2).[44–46] LPA is generated by activated platelets and tumour cells, and elicits a wide range of biological effects. With a growing understanding of the involvement of LPA in both normal physiology and pathology, it is evident that LPA receptor agonists and antagonists may have therapeutic potential in treating various diseases.[47]

The key step for the synthesis of 1-fluorodeoxy-2-acyl-*sn*-glycerol 3-phosphate **28** and 1-acyl-2-fluorodeoxy-*sn*-glycerol 3-phosphate **29** was the stereoselective introduction of fluorine at the C-1 or C-2 position of the glycerol backbone. Phosphates **28a** and **28b** were synthesised from commercially available (*R*)-isopropylideneglycerol **30**. The latter compound was phosphorylated with dimethyl phosphochloridate in the presence of ^{t}BuOK to give the phosphate **31** in 92% yield. By using an optimised selective deprotection, the phosphate **31** was converted into the *tert*-butyldimethylsilyl (TBDMS) ether **32**, which, in turn, was then fluorinated with (diethylamino)sulfur trifluoride (DAST) in dichloromethane to give the corresponding 1-fluorodeoxy-(2*R*)-glycerol 3-phosphate **33**. The 2-hydroxyl group in **33** was unmasked with the use of tetra(*n*-butyl)ammonium fluoride (TBAF) in THF. Dicyclohexylcarbodiimide (DCC)-promoted esterification of compound **34** with either oleic or palmitic acid afforded good yields of the esters **35a** and **35b**. Finally, treatment of each ester, **35a** and **35b**, with bromotrimethylsilane (TMSBr) and the subsequent addition of 5% aq methanol provided the fluorinated LPA analogues **28a** and **28b**. The (2*S*)-LPA analogue **28c** was prepared from *S*-isopropylideneglycerol (Scheme 9).[48]

The 1-acyl-(2*S*)-fluorodeoxy-*sn*-glycerol 3-phosphates **29a** and **29b** were prepared from (*S*)-isopropylideneglycerol **30**. The primary hydroxyl group in the alcohol **36** was selectively protected as the *tert*-butyldiphenylsilyl (TBDPS) ether **37**. Nucleophilic deoxyfluorination of **37** afforded good yields of the 2-fluorinated glycerol 3-phosphate **38**. Deprotection of **38** with TBAF in THF gave the alcohol **39**, which was esterified with either oleic or palmitic acid to give the desired protected LPA derivatives **40a** and **40b**. Treatment of the phosphorotriesters **40a**,**b** with TMSBr provided the LPA analogues **29a** and **29b**. Similarly, the enantiomeric (2*R*)-2-fluorodeoxy LPA analogues **29c** and **29d** were synthesised from (*R*)-isopropylideneglycerol **30** (Scheme 10).[48] The fluorinated LPA analogues were tested in insect cells expressing LPA_1, LPA_2 or LPA_3 receptors. While compounds **28a**, **28b** and **29a**–**d** failed to show



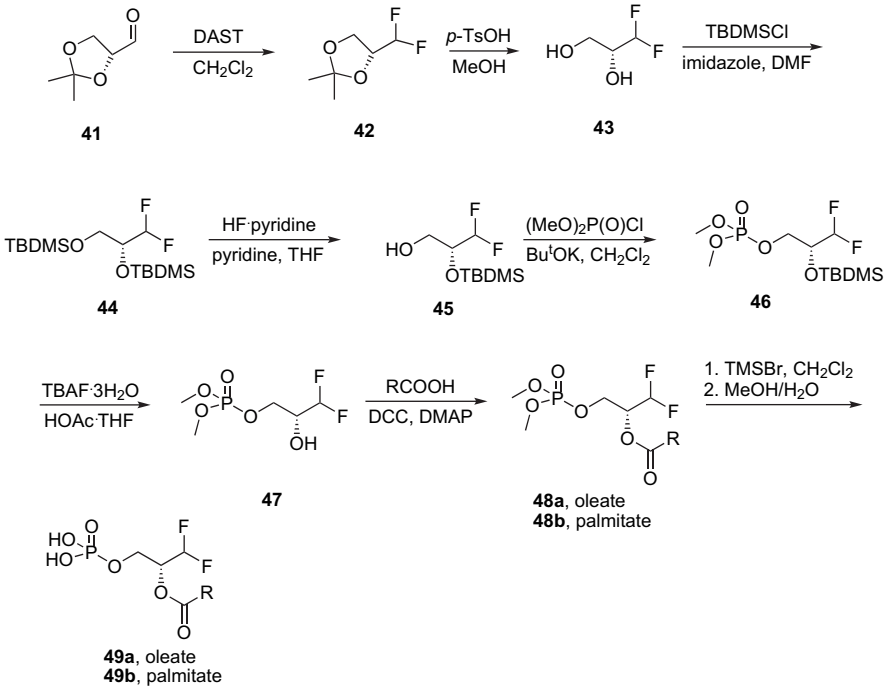
Scheme 9.



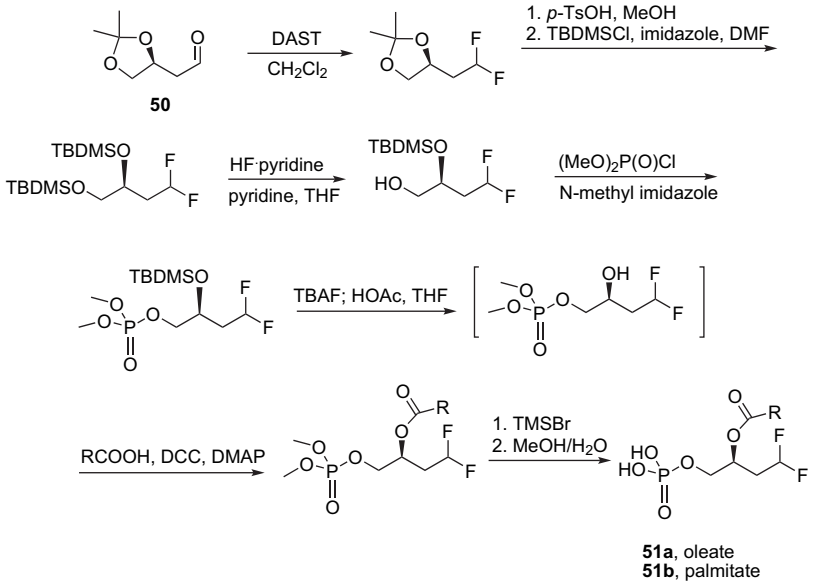
Scheme 10.

either significant agonist or antagonist activity for any of the three isoforms, **28c** was found to be more potent than natural LPA for the LPA_3 receptor.[45,48]

Other examples of acyl migration-blocked analogues of LPA are the 1,1-difluorodeoxy derivatives of (2*R*)-acyl-*sn*-glycerol 3-phosphates **49a**,**b**. (2*R*)-3,3-Difluoro-1,2-propanediol 1,2-acetonide **42** has been obtained in 83% yield by the addition of DAST to a solution of the aldehyde **41** in dichloromethane. The diol **43** obtained after removal of the acetonide was converted into the bis-*tert*-butyldimethylsilyl (TBDMS) ether **44**, and the more labile TBDMS-ether of the primary alcohol was cleaved selectively with a solution of pyridinium hydrofluoride in a mixture of pyridine and THF. The primary alcohol **45** was phosphorylated with dimethyl phosphorochloridate in the presence of ^{t}BuOK to give a good yield of the phosphate **46**. After deprotection of the 2-TBDMS-ether and DCC-promoted esterification of the alcohol **47** with oleic or palmitic acid, the esters **48a**,**b** have been obtained in good yields. Finally, treatment of **48** with bromotrimethylsilane and subsequent addition of 5% aq MeOH provided the difluorinated LPA analogues **49a** and **49b** (Scheme 11). These compounds failed to show either agonist or



Scheme 11.



Scheme 12.

antagonist activity when tested in cells expressing LPA_1, LPA_2 or LPA_3 receptors. Both compounds are, however, essentially equipotent with *sn*-1-oleoyl-LPA for the activation of the PPARγ nuclear receptor.[49]

By analogy with **41**, (*S*)-3,4-dihydroxybutanal acetonide **50** was converted into 1-difluoromethyl-deoxy-(2*S*)-acyl-*sn*-glycerol 3-phosphates **51a**,**b**, which retain the three-carbon glycerol backbone plus the difluoromethyl group mimicking the C-1 hydroxyl group (Scheme 12). It was found that these fluorodeoxy analogues **51a**,**b** induced luciferase expression from the acyl-CoA oxidase PPARG response element reporter. The effective concentrations for reporter activation by **51a**, **51b** and LPA were equivalent, so substitution of difluoromethyl for the hydroxyl of LPA was indeed an effective strategy to create a stabilised LPA mimetic for this receptor.[50]

1-*O*-Octadecyl-2-*O*-methyl-*rac*-*glycero*-3-phosphocholine **52** (edelfosine) is one of the most investigated of ether lipids, since it exhibits in vitro and in vivo cytotoxic activities against numerous human and murine tumour cell strains.[51] The racemic monofluorinated ether lipids **53** and **54** also exhibit cytotoxic activity.[52] The same holds for ilmofosine **55** and its oxygen analogue **56** (Chart 3).[53] The anticancer active ether lipid **62** bearing fluorine on its stereogenic centre has recently been synthesised by Haufe and coworkers (Scheme 13).[54] Treatment of compound **60**, obtained by a multistep procedure from **57** via **58** and **59**, with a threefold excess of 2-chloro-2-oxo-1,3-dioxa-2-phospholane and triethylamine in THF afforded the phosphoric acid triester intermediate **61**, which

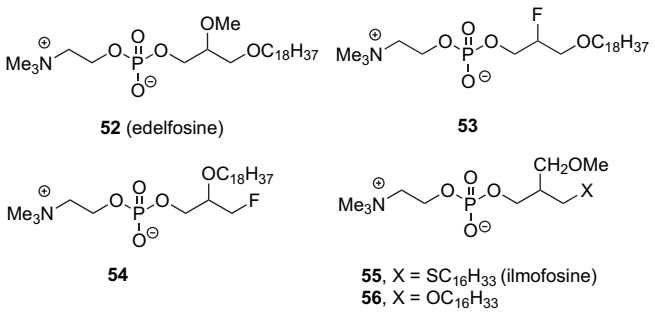


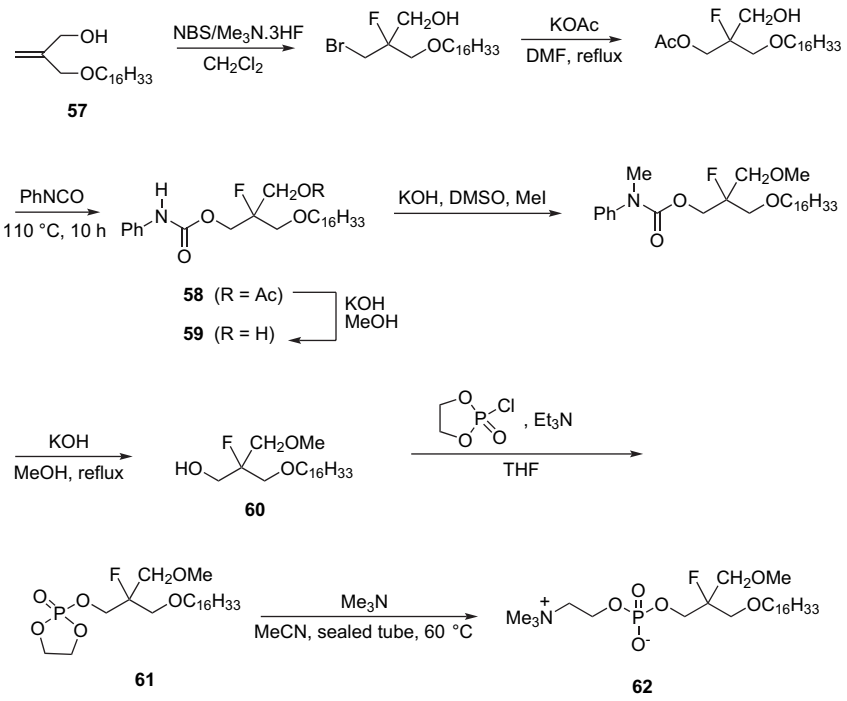
Chart 3. Edelfosine and its analogues.

was subsequently heated with trimethylamine in acetonitrile at 60 °C in a sealed tube. The resulting ester lipid **62** was isolated in 35% yield. The overall yield of **62** was 3% based on **57**. The anticancer activity of **62** has been found in an in vivo model of methylcholanthrene-induced fibrosarcoma in mice (IC_{50} 1.13 mg ml^{-1}, 48 h incubation time). This activity was, however, lower than that of cisplatin (IC_{50} 0.17 mg ml^{-1}) or ilmofosine (IC_{50} 0.23 mg ml^{-1}).[54]

In an effort to develop more potent antitumour agent than gemcitabine **63** (2′,2′-difluorodeoxyribofuranosylcytosine), currently marketed as Gemzar®, Alexander and co-workers prepared a lipid–nucleoside conjugate **64**, which comprised an alkyl phospholipid moiety covalently linked with gemcitabine (Chart 4).[55]

More recently, Ahmad's group has reported the development of a novel gemcitabine–lipid conjugate **66**.[56] To improve the half-life and reduce the toxicity of gemcitabine, the authors chose to conjugate gemcitabine with the ether analogue of cardiolipin **65**. The synthetic methodology, which was employed for the synthesis of cardiolipin, involves the application of the phosphoramidite approach (Scheme 14).[57,58] 1,2-Di-*O*-hexyl-*sn*-glycerol **67** reacted with the bifunctional phosphitylating reagent $^{i}Pr_2NP(OMe)Cl$ in the presence of diisopropylethylamine (DIPEA) to give the glycerol derivative **68**. The latter compound was not isolated, but was subsequently treated with 2-benzyloxy-1,3-propanediol in the presence of 1*H*-tetrazole to provide the phosphite triester **69**. In situ oxidation of **69** with *m*-chloroperbenzoic acid, followed by the hydrogenolysis of **70**, afforded the cardiolipin analogue **71**. Acylation of the central hydroxyl functionality of **71** with succinic anhydride yielded the phospholipids **72**. The gemcitabine–lipid conjugate **73** was obtained by the coupling of **72** with 4-*N*-3′-*O*-bis(*tert*-butoxycarbonyl)gemcitabine and the protecting groups were removed using trifluoroacetic acid in dichloromethane. The unprotected gemcitabine–lipid conjugate **66** was tested in a BxPC-3 human pancreatic tumour model in SCID mice and exhibited promising activity and lower toxicity, when compared with Gemzar®.[56]

Research on photoactivatable phospholipids containing fluorinated functionalities is an important aspect in phospholipid chemistry.[59,60] The presence of fluorine atoms allows the probing of specific lipid–lipid and lipid–protein interactions using ^{19}F NMR spectroscopy, which is highly suitable for investigating the structural and dynamic properties of biomembranes. One recent



Scheme 13.

example involves the synthesis of a phospholipidic probe **74** in which the photoactivatable tetrafluorophenylazido group is incorporated into the fatty-acid chain (Scheme 15). When exposed to light (≥300 nm), compound **74** generates highly reactive nitrene species, and this leads to a process of covalent crosslinkage with the protein or lipid present at the interaction site. Thus, this new phospholipid probe holds promise for further use to map the lipid-binding sites on proteins and biomembranes.[60]

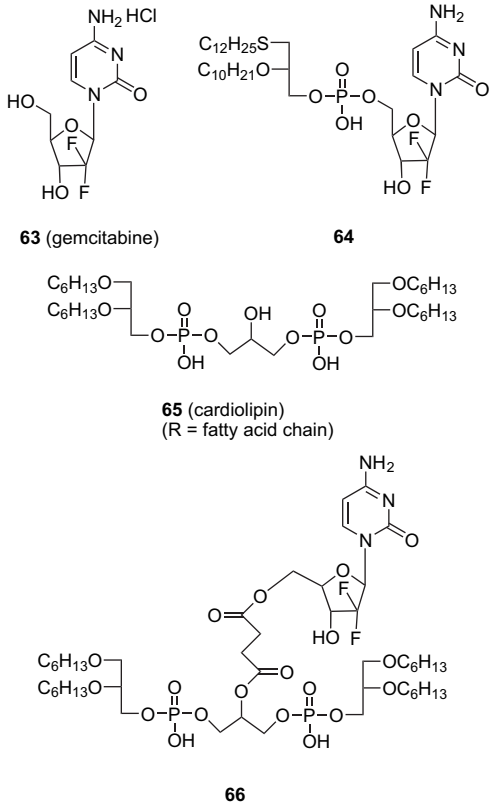


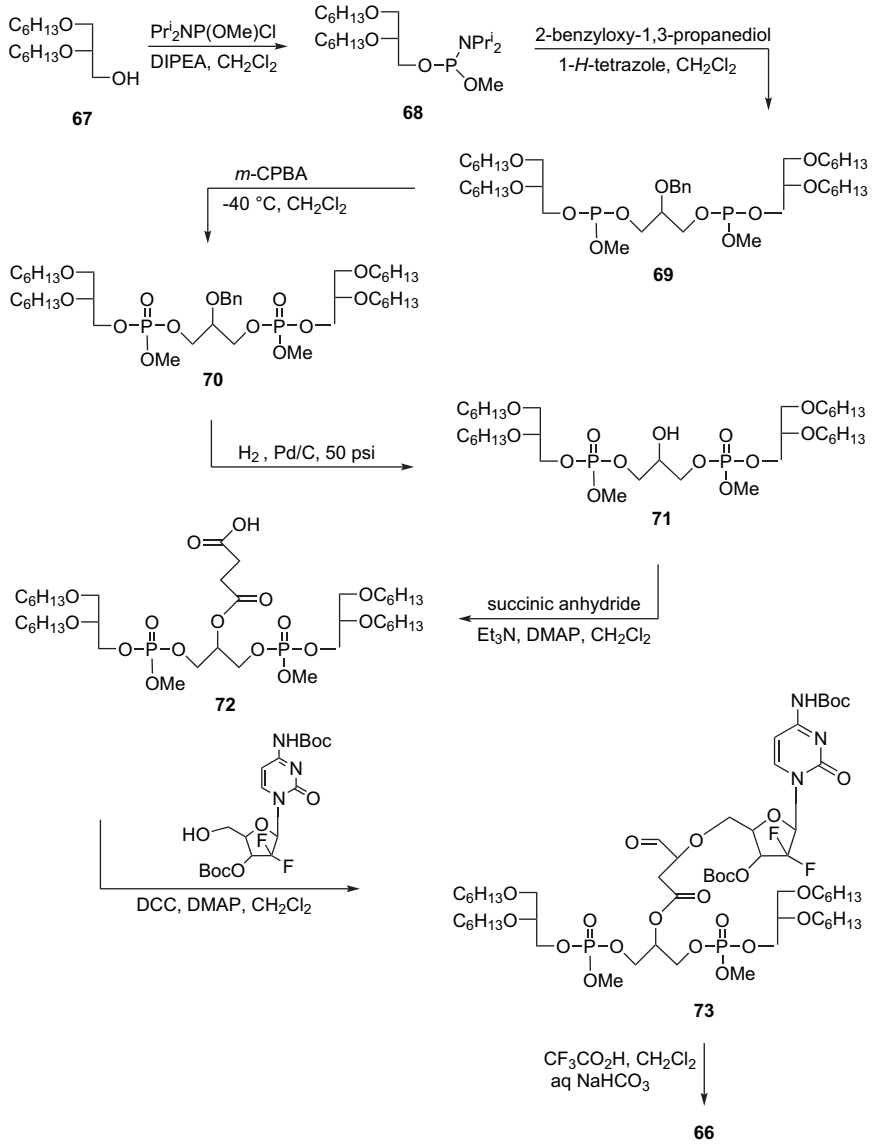
Chart 4. Gemcitabine and its lipid analogues.

Takagi and co-workers have described the synthesis of highly fluorinated single- and/or double-chain phospholipids **75**–**78** containing the perfluorooctyl group as the terminal segment of the hydrophobic chains and a phosphocholine moiety as the hydrophilic head group, in order to investigate the effect of fluorinated segments on the stability of phospholipids monolayers formed at the air–water interface (Chart 5). Judging from the equilibrium spreading pressures (π_es), all of the fluorinated phospholipids formed more stable monolayers than the corresponding non-fluorinated counterparts. The double-chain phospholipid **78** also formed fluid vesicle membranes in water. This observation suggests that the phospholipids **75**–**78** are promising materials for biotechnological and medical applications.[61]

3.2. Analogues of carbohydrate phosphates

Three aldose phosphofluoridates, D-glucose 6-phosphofluoridate (**79**), α-D-mannopyranosyl phosphofluoridate (**80**) and 2-deoxy-2-fluoro-α-D-glucopyranosyl phosphofluoridate (**81**), have been prepared from the parent phosphate and 2,4-dinitrofluorobenzene, and the mechanism of the fluorination has been studied. These compounds were found to be reversible inhibitors of phosphoglucomutase (Chart 6).[62]

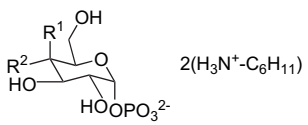
Considerable efforts have been expended in the design of fluorodeoxy sugar phosphates, primarily for use as analogues in probing enzymatic active sites. The history of the first successful synthesis of 2-deoxy-2-fluoro-D-glucose (2-FG) has been described.[63] This compound imitates the behaviour of naturally occurring glucose. It is transported into cells and converted into the corresponding 6-phosphate by the enzyme, hexokinase, in a manner similar to that of glucose. Due to the presence of the fluorine atom at C-2, however, this phosphate derivative does not undergo further glycolysis, but is metabolically trapped in the cell. Thanks to these properties, 8 years after the synthesis of 2-FG, its ^{18}F-labelled



Scheme 14.

derivative was successfully used in connection with positron emission tomography (PET).[64]

Chapeau and Frey have surveyed the overall synthesis of 4-deoxy-4-fluoro-α-D-galactopyranosyl 1-phosphate **82a** and 4-deoxy-4-fluoro-α-D-glucopyranosyl 1-phosphate **82b** and have described in detail the steps to compound **82a**. Both compounds were further used in the synthesis of the substrate analogues of UDP-galactose and UDP-glucose.[65]

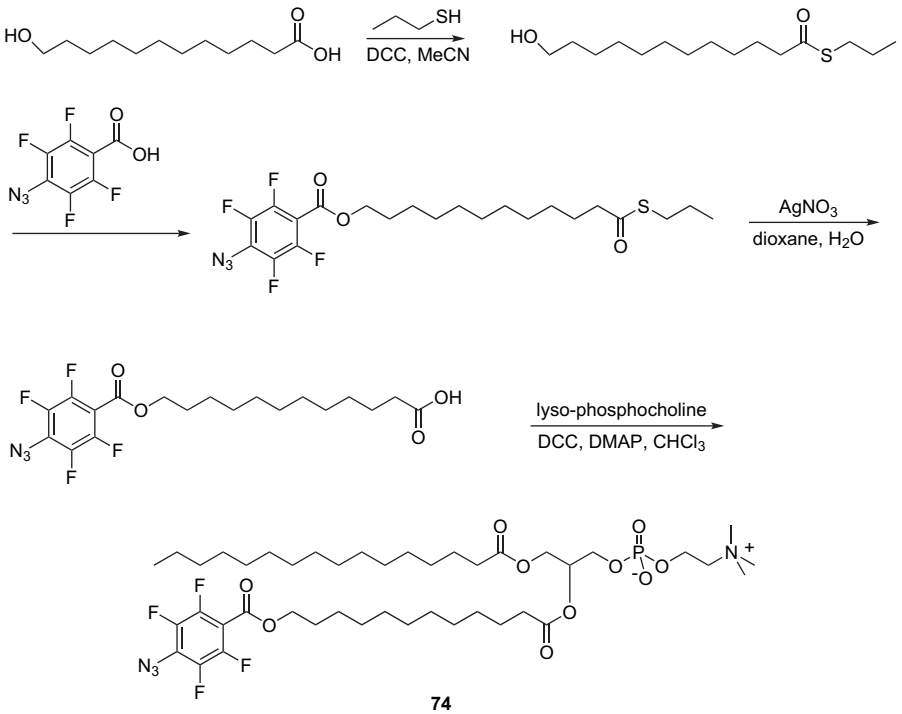


82a, R^1 = F, R^2 =H
82b, R^1 = H, R^2 = F

2-Deoxy-2-fluoro analogues of polyprenyl β-D-arabinofuranosyl phosphates **83**–**86** were synthesised via a route involving the synthesis of a protected β-D-arabinofuranosyl phosphate derivative, its coupling with a polyprenyl trichloroacetimidate and then deprotection of the resulting product (Scheme 16).[66]

Percy and co-workers have developed a total synthesis of conformationally locked difluorinated pentopyranose analogues and a pentopyranosyl phosphate mimetic.[67] The results concerning the introduction of the phosphate moiety into the diol **87** are summarised in Scheme 17. Deprotonation of **87** with *n*-BuLi, followed by the addition of tetrabenzyl pyrophosphate, afforded a mixture of **88** (14%), **89** (3%) and recovered **87** (35%). A higher yield of the monophosphate **88** (43%) could be achieved by changing the base to NaHMDS and adding the Na-selective 15-crown-5. Phosphorylation following secondary hydroxyl protection has also been studied. Exposure of NaHMDS to **90**, followed by the addition of tetrabenzyl pyrophosphate, allowed the isolation of **91**in 64% yield. Hydrogenolysis of the benzyl groups was followed by deacetylation, lyophilisation and column chromatography, allowing the isolation of the deprotected product **92**.

An interesting synthesis of 2-deoxy-2-fluoro phosphate saccharides has been proposed via a Selectfluor-mediated fluorination/nucleophilic addition sequence. This strategy involves the selective fluorination of glycals at the 2-position with concomitant nucleophilic addition of a dialkyl or diphenyl phosphate to the anomeric centre (Scheme 18). It was found that a judicious choice of protective-group strategy can improve the stereoselectivity of both

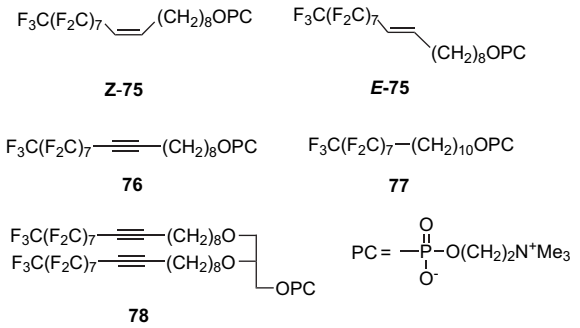


74

Scheme 15.

the fluorination and the nucleophilic addition. As an example, when diphenyl phosphate is used as a nucleophile in the fucose series, the reaction is α-specific, whereas a β-selectivity is observed when using dibenzyl phosphate.[68,69]

The synthesis of 5-fluoro *N*-acetylglucosamine pyrophosphates via epoxide fluoridolysis has been recently described by Hartman and Coward.[70] Oxidation of glycosyl phosphate **94**, derived from the glycoside **93**, with $NaIO_4$ followed by thermal selenoxide elimination in dihydropyran (DHP), gave the alkene **95** in good yield. Treatment of **95** with dimethyldioxirane (DMDO) provided a 3:2 mixture of the epoxides. Fluoridolysis with HF/pyridine, followed by acetylation of the resulting fluorohydrins, led to a mixture of separable 5-fluoro epimers **96**. 5-Fluoro glycosyl phosphate **96a** was further used in the synthesis 5-fluoro glycosyl pyrophosphate **97**. The latter compound was shown to be useful as a probe of transition-state charge development in several enzyme-catalysed reactions (Scheme 19).

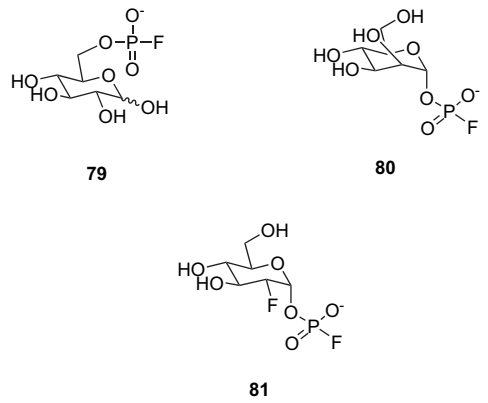


***Z*-75** ***E*-75**

76 **77**

78

Chart 5. Fluorinated phospholipids.



79 **80**

81

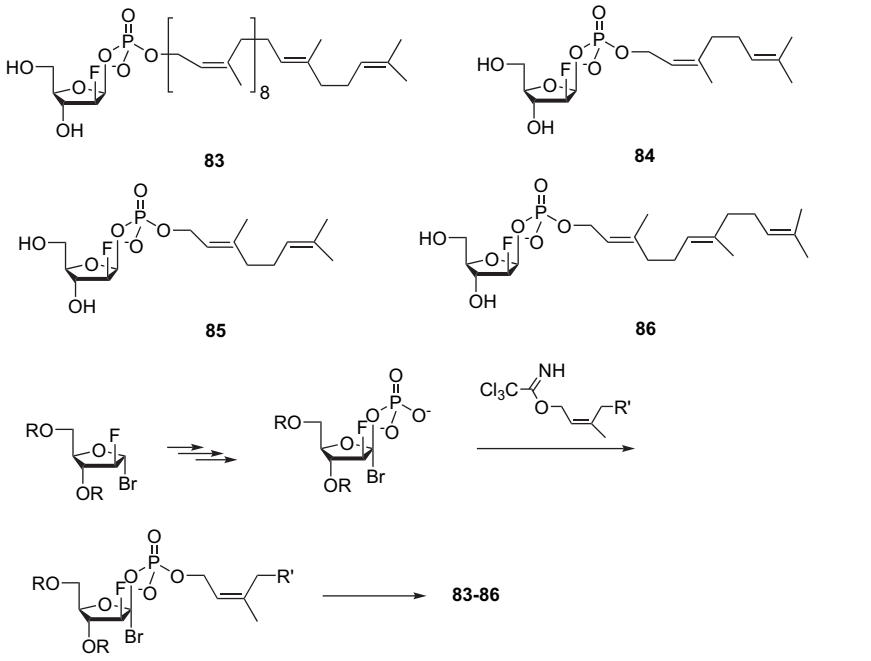
Chart 6. Reversible inhibitors of phosphoglucomutase.

The target of several groups was the use of fluorinated carbohydrate phosphites and phosphates in glycosylation reactions. Hashimoto and co-workers first reported that the coupling of 2,3-dideoxy-3-fluoro-D-*erythro*-pentofuranosyl diethyl phosphate **98** with 2,4-bis(trimethylsilyl)thymine in the presence of TMSOTf provides a facile and highly stereoselective entry to the 3′-deoxy-3′-fluorothymidine derivative **99** (Scheme 20).[71] Komatsu and co-workers reported a chemo-enzymatic synthesis of 2′-deoxy-β-D-nucleosides via the chemical synthesis of 2′-deoxy-α-D-ribose-1-phosphate.[72,73] This same strategy was also applied to the stereoselective synthesis of 2′,3′-dideoxy-3′-fluoro-β-D-guanosine.[74]

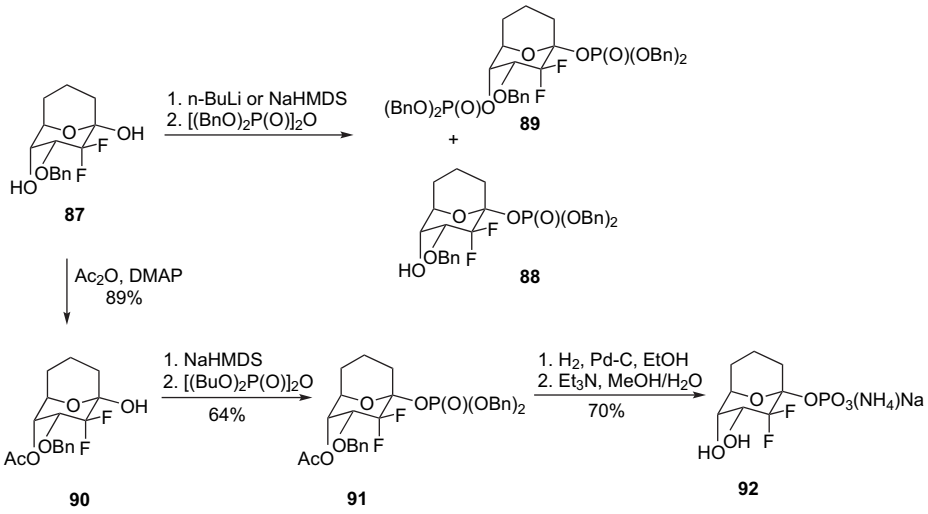
Finally, it should be mentioned that one of the most exciting recent studies in the area of fluorinated biophosphates is the identification of 5-fluoro-5-deoxy-D-ribose-1-phosphate as an intermediate in fluorometabolite biosynthesis in *Streptomyces cattleya*.[75,76]

3.3. Analogues of inositol 1,4,5-trisphosphates

The membrane phospholipid, phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2], regulates the activity of many ion channels and transporters.[77] Hydrolysis of PtdIns(4,5)P_2 by phospholipase C (PLC) releases two second messengers, D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] and diacylglycerol (Scheme 21). Ins(1,4,5)P_3 releases Ca^{2+} from non-mitochondrial stores to increase the cytoplasmic free Ca^{2+} concentration, whereas diacylglycerol is an activator of protein kinase C. Since some disease states



Scheme 16.



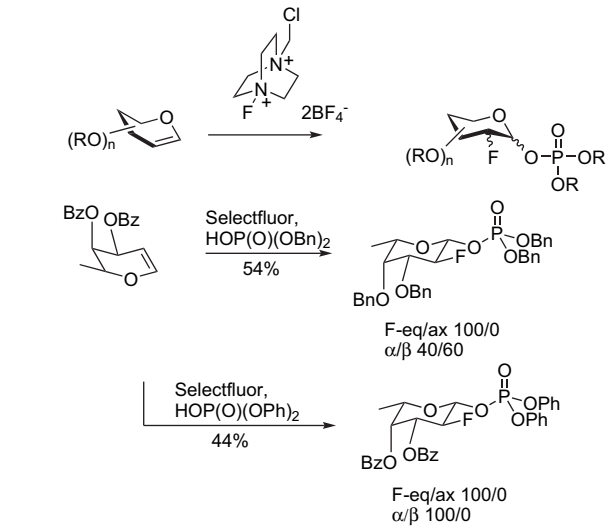
Scheme 17.

arise from the uncontrolled stimulation of PLC, inhibitors of enzymes of the phosphoinositide cascade, involved in the biosynthesis and degradation of Ins(1,4,5)P_3, are of medicinal interest. In order to study the biochemical and medicinal properties of these polyphosphates, fluorinated derivatives of Ins(1,4,5)P_3 **100**–**102** have been synthesised and evaluated (Chart 7).

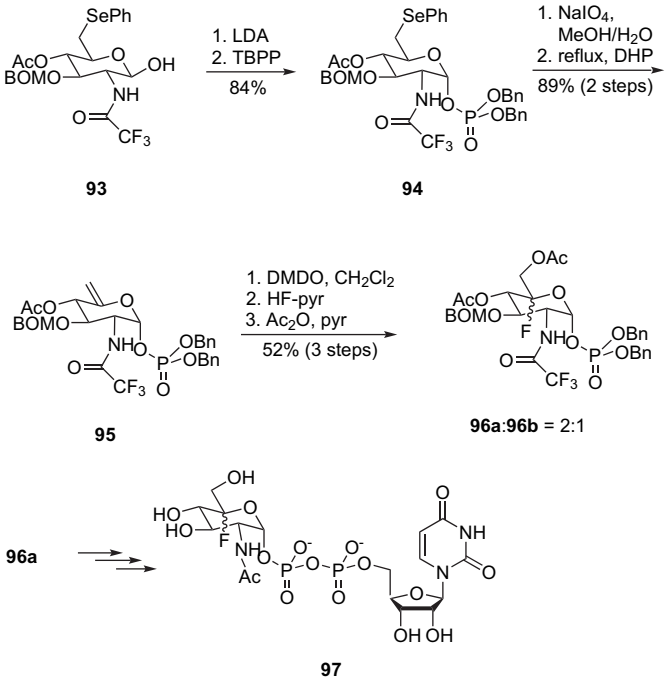
Potter and Sawyer have greatly contributed to the total synthesis of fluorinated analogues of inositol 1,4,5-trisphosphate.[78–81] The synthetic route to DL-2-deoxy-2-fluoro-*scyllo*-inositol 1,4,5-trisphosphate (**100**) and DL-2-deoxy-2,2-difluoro-*myo*-inositol 1,4,5-trisphosphate (**101**) is illustrated in Scheme 22. These compounds were prepared from the protected *myo*-inositol precursor **103** by the reaction sequence including DAST fluorination and removal of the non-benzylic protecting groups, followed by bis(2-cyanoethyl)-*N*,*N*-diisopropylaminophosphite phosphitylation, oxidation of the resulting trisphosphites with *tert*-butyl hydroperoxide and deprotection using sodium in liquid ammonia. The optical isomers of compound **101** [D-**101a** and L-**101b**] were prepared from the triols **104a**,**b** via chromatographic separation of the diastereomeric 1-camphanates, as shown in Scheme 23.

The interaction of compounds **100** and **101** with the Ins(1,4,5)P_3 receptor and the metabolic enzymes, 3-kinase and 5-phosphatatase, has been investigated.[82] 2*F*-Ins(1,4,5)P_3 **100** was a weak substrate for Ins(1,4,5)P_3 5-phosphatase, but (−)-D-2,2-F_2-Ins(1,4,5)P_3 (−)-D-**101a** was a potent Ca^{2+}-releasing agonist and a good substrate for Ins(1,4,5)P_3 5-phosphatase. (+)-L-2,2-F_2-Ins(1,4,5)P_3 (+)-L-**101b** was a potent competitive inhibitor of 3-kinase and 5-phosphatase.[83]

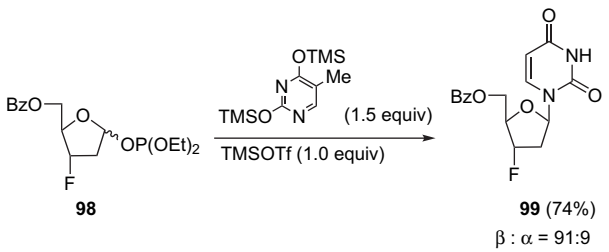
Kozikowski and Fauq employed quebrachitol as the starting material for the synthesis of D-3-deoxy-3-fluoro-*myo*-inositol 1,4,5-trisphosphate (**102**).[84] Quebrachitol was converted in two steps into 3-deoxy-3-fluoro-D-*myo*-inositol (**105**), and the latter



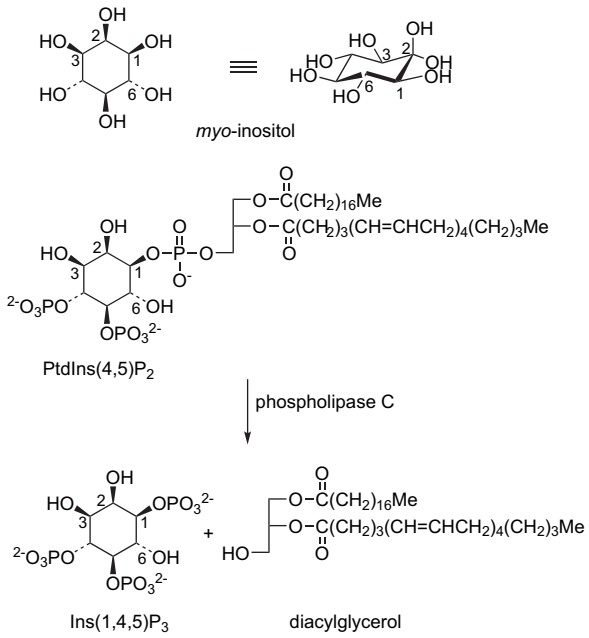
Scheme 18.



Scheme 19.



Scheme 20.



Scheme 21.

compound was reacted with 2-methoxypropene and camphorsulfonic acid to afford a 1:2.3 mixture of compounds **106** and **107**. These were separated by silica gel chromatography. Further treatment of **106** including protection/deprotection steps and phosphorylation afforded the stable hexasodium salt **108** (Scheme 24). 3*F*-Ins(1,4,5)P_3 **102** acted as a full agonist in releazing Ca^{2+} from 3T3 cells. The unnatural fluorinated $InsP_3$ analogue was found to be equipotent to natural $InsP_3$. Dextran sulfate, a potent blocker of the release of Ca^{2+} by Ins(1,4,5)P_3, also blocked the release of Ca^{2+} induced by 3*F*-Ins(1,4,5)P_3.

Chemical modification at C-2 of 3-deoxy-Ins(1,4,5)P_3 has been used to prepare the fluoro derivatives **109** and **110** (Chart 8). Both compounds were assayed for biological activity against activated pyruvate dehydrogenase phosphatase (PDH-Pase), inhibited pyruvate dehydrogenase kinase (PDH-K) and inhibited glucose 6-phosphatase (G6Pase), but none proved to be positive.[85]

A fluorinated phosphatidylinositol analogue **114** has been synthesised from D-3-deoxy-3-fluoro-*myo*-inositol **105** in order to examine its inhibitory effects on cell growth.[86] The intermediate **111** was deprotected at C-1 and phosphitylated with bis(diisopropylamino)benzyl phosphite to afford **112**. Next, **112** was coupled with 1,2-dipalmitoyl-*sn*-glycerol and the phosphite oxidised to phosphate **113**. Finally, all benzyl groups were removed by hydrogenolysis over $Pd(OH)_2$/C in *tert*-butanol (Scheme 25). The cell-growth-inhibitory properties of **114** towards wild-type NIH 3T3 and v-*sis* NIH 3T3 cells were studied. The IC_{50} value was found to be approximately 100 mM for each cell type. For comparison, D-3-deoxy-3-fluoro-*myo*-inositol was 10- to 70-fold less active as an inhibitor of cell growth.[87,88]

In order to dissect the effects of PtdIns(4,5)P_2 from those resulting from PLC-generated signals, the fluorinated PtdIns(4,5)P_3 analogues **122**–**126** were designed, in which the scissile O–P bond was replaced with a C–P bond that could not be hydrolysed by PLC. The key transformation was a Pd(0)-catalysed coupling of the *H*-phosphonate **115** with the 1-bromo-1-fluoroolefin **116** to form the desired C–P linkage. The acetal **117** was selectively deprotected to give the diol **118**. Acylation of the latter compound with octanoic acid, palmitic acid or oleic acid afforded the phosphonates **119**–**121**. Deprotection of **119** and **120** by hydrogenolysis, followed by treatment with ethanethiol, formed the α-fluoromethylenephosphonate analogues **122** and **123**. α-Fluorovinylphosphonates **124**–**126** were

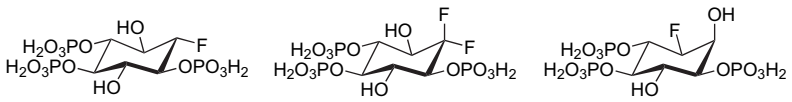
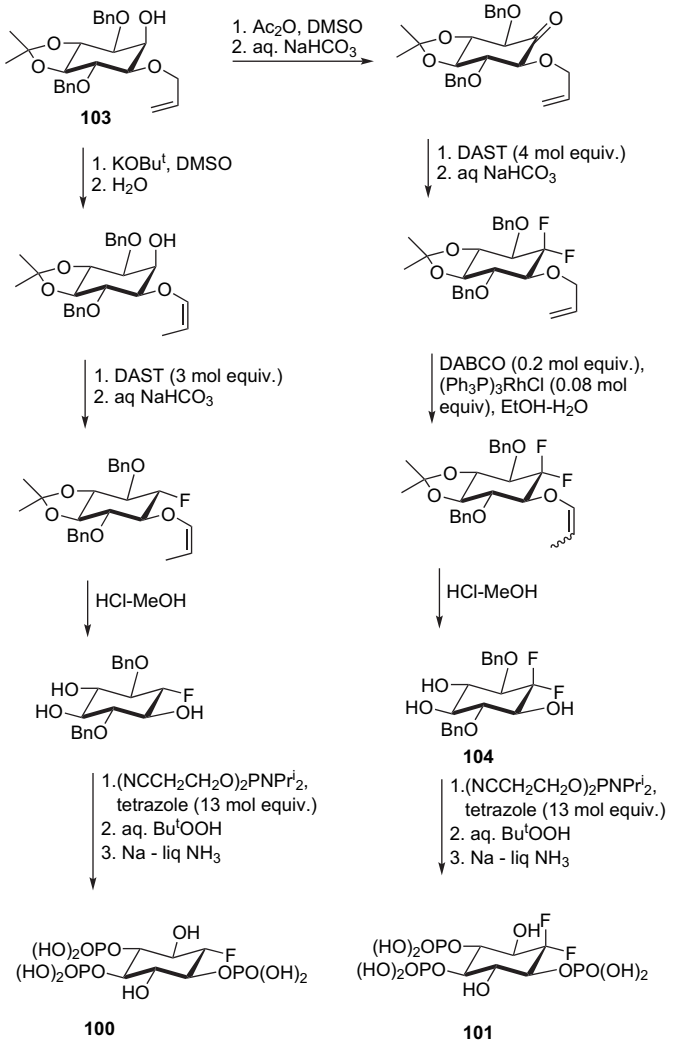
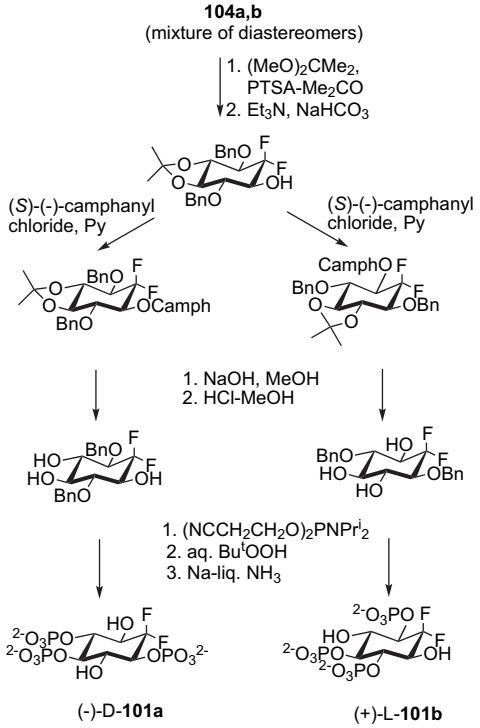


Chart 7. Fluorinated derivatives of D-*myo*-inositol 1,4,5-trisphosphate.



Scheme 22.



Scheme 23.

obtained by the reactions of compounds **119**–**121** with TMSBr/TMSI (Scheme 26). The two dioctanoyl-PtdIns(4,5)P_2 derivatives **123** and **125** were found to be effective in restoring the sensitivity of the TRPM4 channel to Ca^{2+} activation, but the α-fluorovinylphosphonate **126** was more potent.[89]

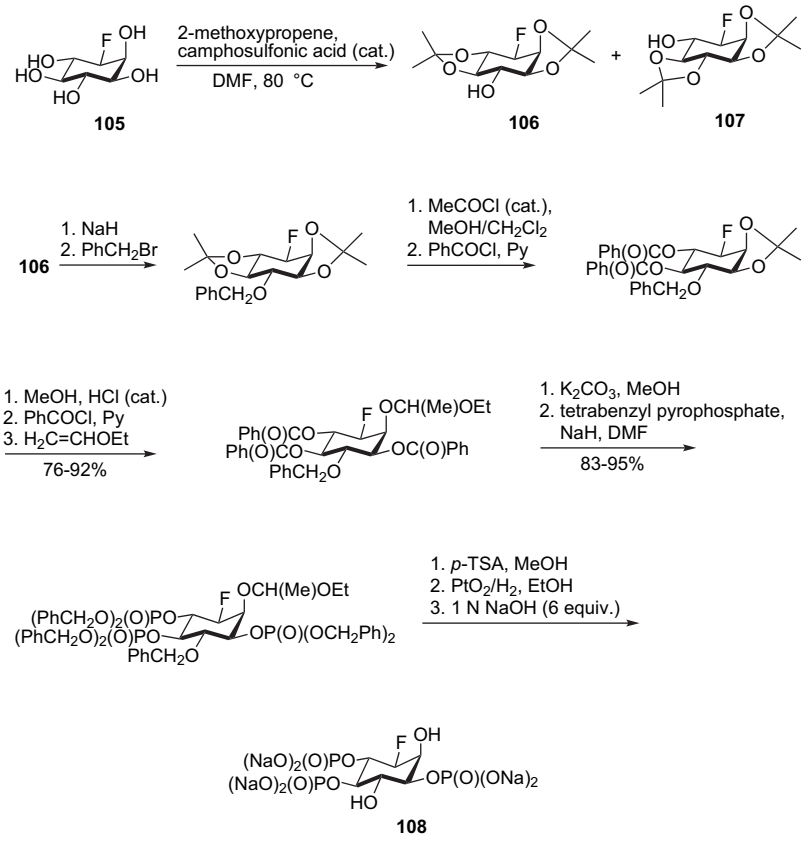
In addition to the strategy described above, the asymmetric total synthesis of phosphatase-resistant 3-(fluoromethyl)phosphonate analogues of PtdIns(3)P has been developed.[90] The secondary alcohol **127** was phosphorylated with methyl fluoromethylphosphonyl chloride in the presence of KO^{t}Bu to give compound **128** in good yield. The silyl group in the 1-position was removed with TBAF/HOAc, and the resulting alcohol **129** was treated with one of the three diacylglyceryl phosphoramidite reagents **130a**–**c**, followed by oxidation with Bu_4NIO_4. Deprotection of the methyl esters **131**–**133** was accomplished with TMSBr to give **134**–**136** (Scheme 27). The methoxymethyl (MOM) groups were removed using ethanethiol. It was found that the PtdIns(3)P analogues **134** and **135** having dioleoyl and dipalmitoyl chains were substrates for the 5-kinase enzyme PIK5.

3.4. Analogues of mevalonate 5-diphosphate and 1-deoxy-D-xylulose-5-phosphate

The terpenoid building block is a five-carbon unit, known as isoprene, which has been established to be derived from acetyl-CoA via the mevalonate pathway. In animal cells, the mevalonate pathway contains a series of three sequential ATP-dependent enzymes that convert mevalonic acid into isopentenyl pyrophosphate: mevalonate kinase (MVK), phosphomevalonate kinase (PMK), and mevalonate 5-diphosphate decarboxylase (MDD) (Scheme 28).[91]

Because of the importance of MVK and MDD in the regulation of cholesterol biosynthesis, two fluorinated MDD substrate analogues, 2-fluoromevalonate 5-diphosphate **137** and *P*′-geranyl-2-fluoromevalonate 5-diphosphate **138**, have been synthesised, as shown in Scheme 29.[92] Both compounds were found to be irreversible inhibitors of rat MDD. In addition, compound **138** shows good competitive inhibition of MVK. Thus, these studies provide an example of a single inhibitor carrying out sequential blocking of two enzymes in cholesterol biosynthesis.

More recently, an alternative isoprene biosynthetic pathway was discovered in some organisms including most bacteria, plants and the malarial parasite *Plasmodium falciparum*.[93] In this pathway, 1-deoxy-D-xylulose 5-phosphate (DXP) is formed from pyruvate and D-glyceraldehyde 3-phosphate (GAP) in a thiamine diphosphate-dependent reaction catalysed by DXP synthase (DXS).



Scheme 24.

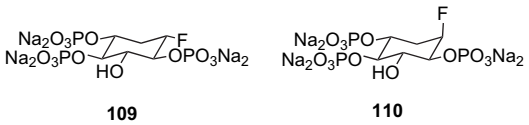


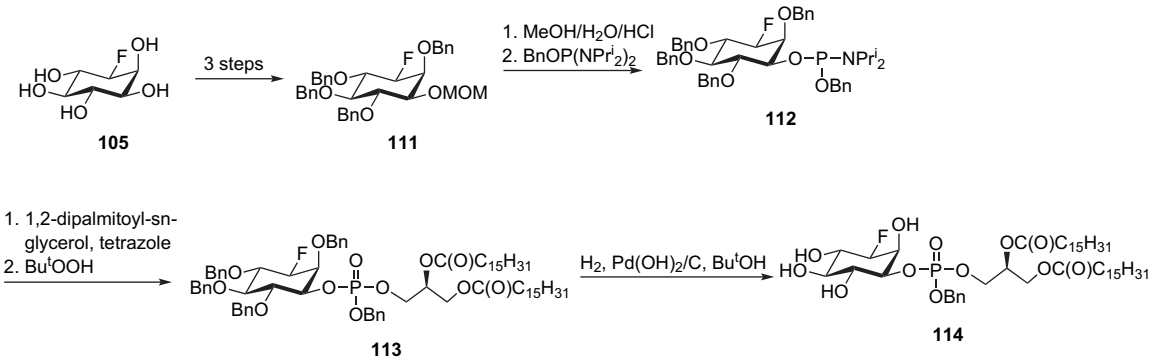
Chart 8. Fluorinated derivatives of 3-deoxy-D-*myo*-inositol 1,4,5-trisphosphate.

The rearrangement of DXP to 2*C*-methyl-D-erythritol 4-phosphate (MEP) is the first pathway-specific step en route to the basic isoprenoid unit. The enzyme catalysing the conversion of DXP into MEP is known as 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR) or MEP synthase (Scheme 30). Since this pathway appears to operate in most bacteria, but not in humans, each enzyme of the MEP pathway is a potential target for antibacterial or antiparasitic drugs. In fact, the natural product, fosmidomycin, $HC(O)N(OH)(CH_2)_2PO_3^{2-}$, which inhibits the conversion of DEX into MEP, has been shown to be effective for the treatment of malaria and other parasitic infections.[94,95]

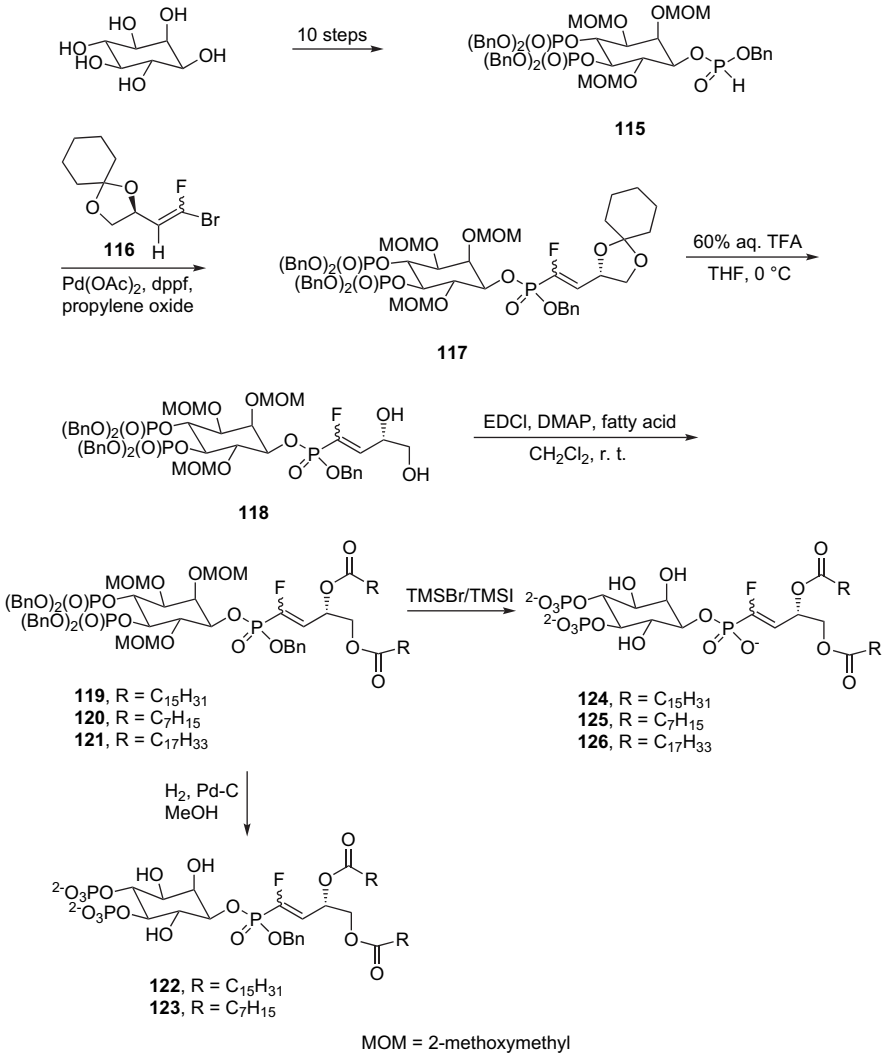
Two possible mechanisms for the conversion of DXP into MEP have been proposed: an α-ketol rearrangement (A) and retro-aldolisation/aldolisation (B) (Scheme 31). The first mechanism involves an α-ketol rearrangement that is mechanistically related to acetoin and pinacol rearrangements to give methylerythrose phosphate as an intermediate, followed by reduction of the aldehyde group by NADPH. In the second retro-aldolisation mechanism, initial deprotonation occurs at the *C*-4 hydroxyl group, followed by cleavage of the C3–C4 bond to form a bimolecular intermediate, which is identical to the aldehyde intermediate generated by the α-ketol rearrangement.

As part of an effort in the search of novel drugs acting on the MEP pathway, several fluorinated DXP analogues (**139**–**144**) have been synthesised and evaluated as alternative substrates and inhibitors of DXP reductoisomerase (Chart 9).

The reaction sequence used by Liu and co-workers to synthesise the 1-fluoro DXP analogue, 1-fluoro-1-deoxy-D-xylulose-5-phosphate (**139**), from 3-*O*-benzyl-1,2-*O*-isopropylidene-β-D-ara-



Scheme 25.

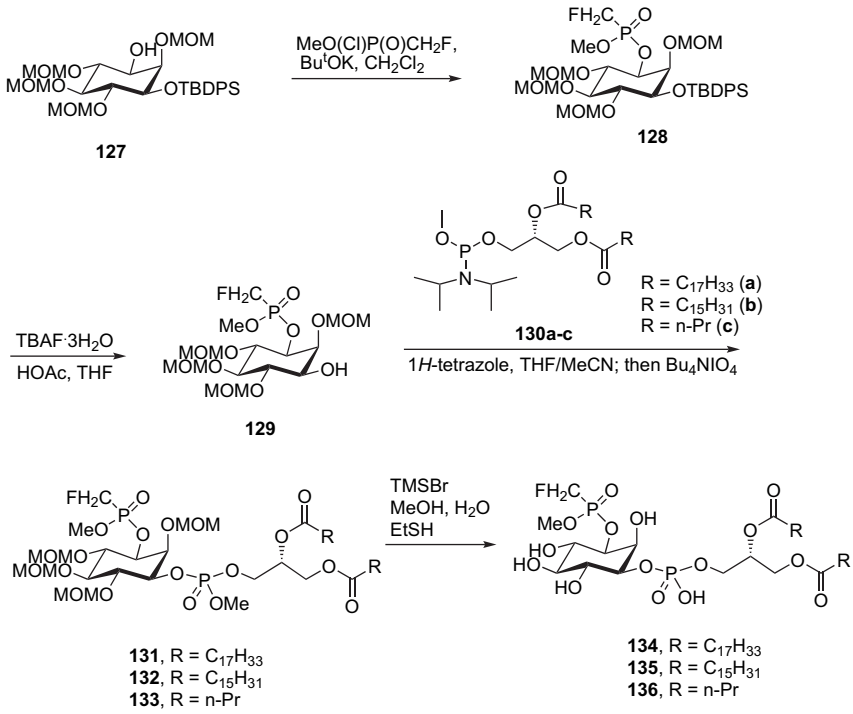


Scheme 26.

binofuranose **145** via compounds **146**, **147** and **149** is shown in Scheme 32.[96] The key intermediate **148** upon benzoylation and Swern oxidation gave the ketone **150**. Subsequent ketalisation and de-esterification of **150** led to the compound **152**. Phosphorylation of **152**, which was conveniently achieved by using trimethyl phosphite, 2,6-lutidine and tellurium tetrachloride, yielded the protected deoxyxylulose phosphate analogue **153**. Deprotection of **153** and purification over cellulose gave 1-fluoro-DXP **139**. An alternative synthesis of **139** from the commercially available (−)-2,3-*O*-isopropylidene-D-threitol **154** was reported by Fox and Poulter (Scheme 33).[97] At first, alcohol **155** was converted into the oxirane **156** by oxidation of the alcohol to the corresponding aldehyde, followed by treatment with dimethyloxosulfonium methylide. Under these conditions, compound **156** was formed as a 4:1 mixture of diastereomers in yields of 45–55% over two steps. The intermediate **157**, bearing a fluorine substituent at C-1, was prepared via a regioselective ring opening with diisopropylamine trihydrogen fluoride. The benzyl group was removed by hydrogenolysis and the resulting diol **158** was converted into phosphate **159**, followed by Dess–Martin oxidation, to give the fully protected 1-fluoro-DXP **160**. Deprotonation of **160** gave a 72:23 mixture of the ketone/hydrate **139**.

The 1-fluoro-DXP **139** was revealed as a good substrate for *Escherichia coli* DXR, with k_{cat}=38 s^{-1}, K_m=227 μM and k_{cat}/K_m=0.17 s^{-1} μM^{-1}. These values are similar to those for DXP, with k_{cat}=29 s^{-1}, K_m=50 μM and k_{cat}/K_m=0.58 s^{-1} μM^{-1}. The similar rates of reaction for DXP and 1-fluoro-DXP **139** are more consistent with the retro-aldolization mechanism for the rearrangement step catalysed by DXR. In the case of an α-ketol mechanism, the rearrangement would be slower for **139** because of the development of a positive charge at C-2 upon activation of the ketone by protonation.[96,97] Furthermore, a primary deuterium isotope effect was observed under single-turnover conditions when 1-fluoro-DXP **139** was incubated with 4*S*-[^2H]-NADPH ($^Hk/^Dk$=1.34±0.01), whereas no isotope effect was observed upon incubation with DXP and 4*S*-[^2H]-NADPH ($^Hk/^Dk$=1.02±0.02). The reaction did not exhibit burst kinetics for either substrate, indicating that the product release is not rate limiting. These studies suggest that the positive charge does not develop at C2 of DXP during catalysis. In addition, the isotope effect with 1-fluoro-DXP **139** and 4*S*-[^2H]-NADPH, but not with DXP, indicates that the rearrangement step, which precedes hydride transfer, is rate limiting for DXP, but becomes partially rate limiting for 1-fluoro-DXP. These observations are also consistent with a retro-aldol/aldol mechanism for the rearrangement during the conversion of DXP into MEP.[98]

The synthetic methods for preparing 3- and 4-fluoro-1-deoxy-D-xylulose 5-phosphate **140** and **141** were developed by Liu and coworkers (Scheme 34).[96] Transformation of compounds **161** and **166** into **140** and **141**, via intermediates **162**–**165** and **167**–**170**, respectively, followed the strategy used to make compound **139** from

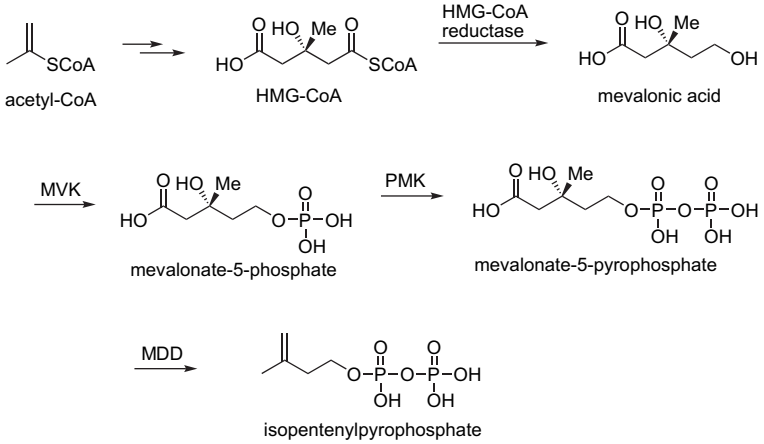


Scheme 27.

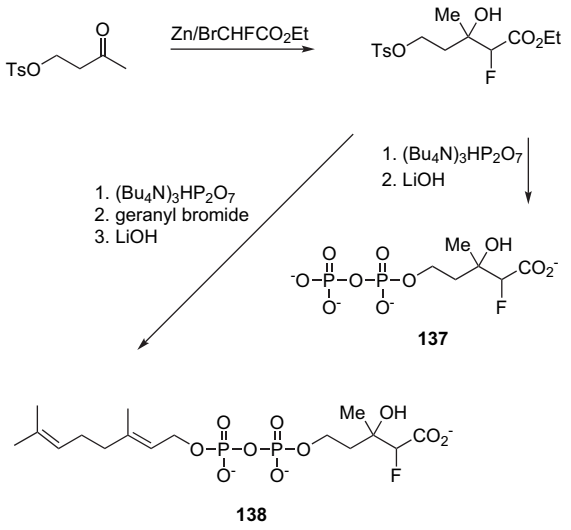
145. Incubation of compounds **140** and **141** in the presence of DXP, Mg^{+2} and NADPH with heterologously expressed DXR from *E. coli* showed that the fluorinated DXP analogues behave as non-competitive inhibitors, with K_i values of 444 and 733 μM, respectively. A possible explanation of the inhibition model observed is that **140** and **141**, both as DXP mimics, are capable of binding two distinct forms of the DXR enzyme. On the other hand, because the inability of DXR to process **140** and **141** is most likely a consequence of having chemically inert functional groups at the site of action preventing turnover to the product, the above results may be considered as a preliminary evidence implying that the retro-aldolisation/aldolisation mechanism for the isomerisation catalysed by DXR is operative.[96]

1,1-Difluoro-1-deoxy-D-xylulose 5-phosphate (**142**) was synthesised from (−)-2,3-*O*-isopropylidene-D-tartrate **171** following the reaction sequence outlined in Scheme 35. The first step of the synthesis is the desymmetrisation of **171** with $NaBH_4$. Phosphorylation of the primary alcohol **172** was accomplished using dibenzyl phosphoroiodidate (DBPI), generated in situ from tribenzyl phosphite and iodine. The resulting phosphorus triester **173** was then treated with $LiCF_2P(O)(OEt)_2$ to give difluoromethylphosphonate **174**, and this phosphonate was cleaved with NaOMe. The free acid **142** was obtained from **175** by catalytic hydrogenation, followed by hydrolysis of the isopropylidene group. The ratio of ketone to hydrate for **142** was 2:98, as judged by ^{19}F NMR. An enzyme study has established that 1,1-difluoro-DXP **142** was a poor substrate for *E. coli* DXR under catalytic conditions. Compound **142** was also a poor inhibitor of the enzyme, with an IC_{50} value of 3.4 mM, most likely because of the increase in steric bulk at C1 of DXP.[97,98]

In connection with 1,1,1-trifluoro-1-deoxy-D-xylulose 5-phosphate (**143**) and its reduced diastereomeric mixture analogues (**144**), Fox and Poulter described the synthesis of **143** in seven steps in 45% overall yield from the commercially available (−)-2,3-*O*-isopropylidene-D-threitol **154** via intermediates **176**–**180** (Scheme 36).[97] The product, >95% pure as judged by ^{19}F NMR spectroscopy, was identified in aqueous solution as the hydrate. More recently, the synthesis of **143** has been improved by a shorter reaction sequence, starting from the commercially available dimethyl 2,3-*O*,*O*-benzylidene-D-tartrate **181** in a global yield of 50% (Scheme 37).[99] The primary alcohol **182** was phosphorylated using the



Scheme 28.



Scheme 29.

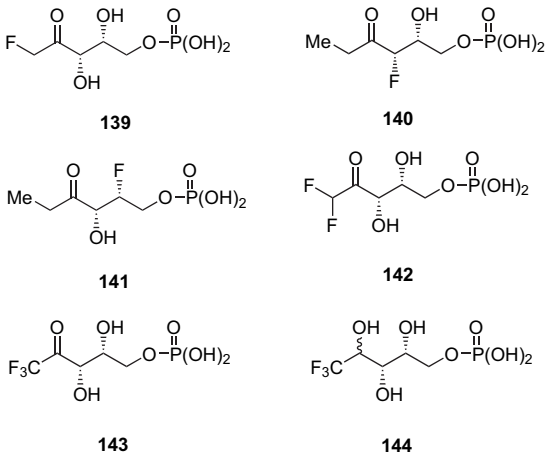
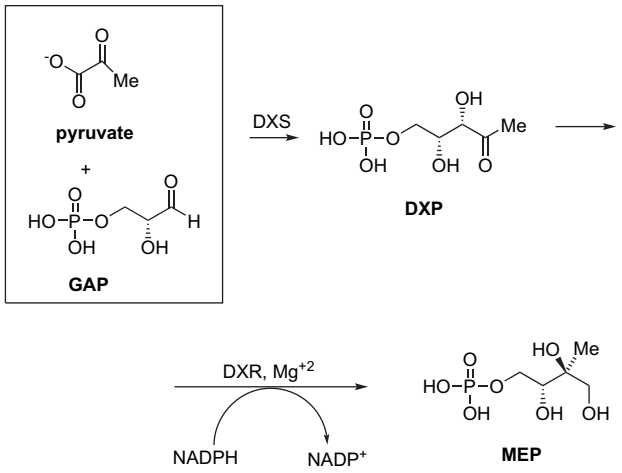


Chart 9. Fluorinated 1-deoxy-D-xylulose 5-phosphate analogues.

phosphoramidite method. Subsequent nucleophilic trifluoromethylation of the methyl ester **183** with Ruppert's reagent provided the trifluoromethylketone **184** in the hydrate form. Finally, the enantiomerically pure compound **143** was obtained quantitatively by catalytic hydrogenolysis of all benzyl-protecting groups.

In addition, the reduced diastereomeric mixture analogues, 1,1,1-trifluoro-1-deoxy-D-xylitol 5-phosphate and 1,1,1-trifluoro-1-deoxy-D-lyxitol 5-phosphate (**144**), were synthesised from the commercially available, (2*R*,3*R*)-2,3-*O*,*O*-dibenzyl-threitol **185** via compounds **186**–**190**, following the reaction sequence shown in Scheme 38. Attempts have been made to separate the diastereomeric mixture by silica gel chromatography, but all these failed.[99]

Evaluation of 1,1,1-trifluoro-DXP **143** has shown that this compound was not a substrate for *E. Coli* DXR under normal catalytic conditions and was, instead, a poor inhibitor.[97,99] Steric interactions at the active site are probably responsible for the poor inhibition observed for **143**. The hydration of the carbonyl group may also prevent the binding to the active site of the enzyme. The inhibitory activity of diastereomeric mixture **144** was also investigated and it was found to behave as a reversible non-competitive inhibitor with a K_i value of 360 μM.[99]

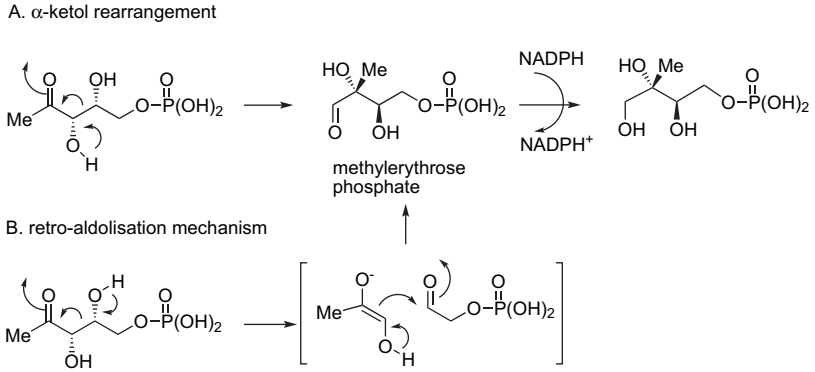


Scheme 30.

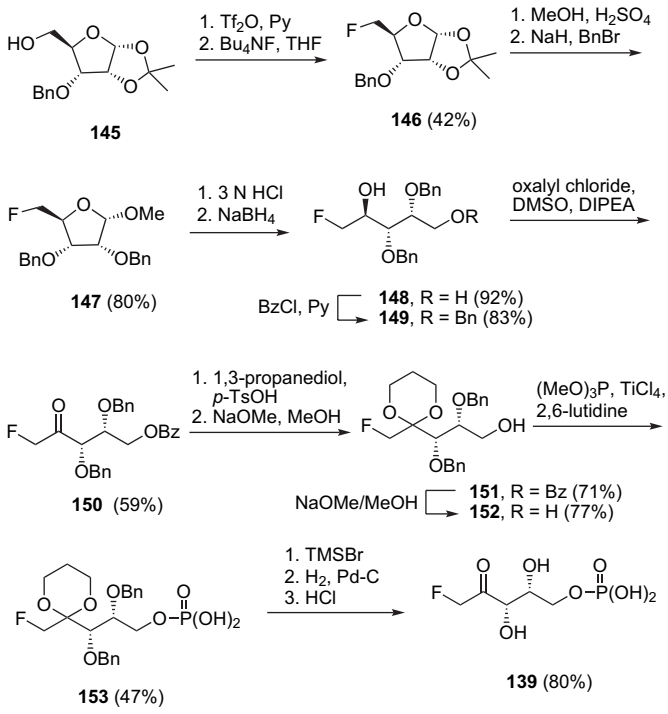
3.5. Analogues of phosphoenolpyruvate and 5-enolpyruvylshikimate-3-phosphate

Fluorinated phosphoenolpyruvate (PEP) analogues have been widely employed in studying the mechanisms of a variety of PEP-utilising enzymes.[100–103] Representative examples are the fluorinated derivatives **191**–**197** of a number of phosphates along the shikimic acid pathway that have been prepared synthetically and enzymatically (Chart 10).[104–111]

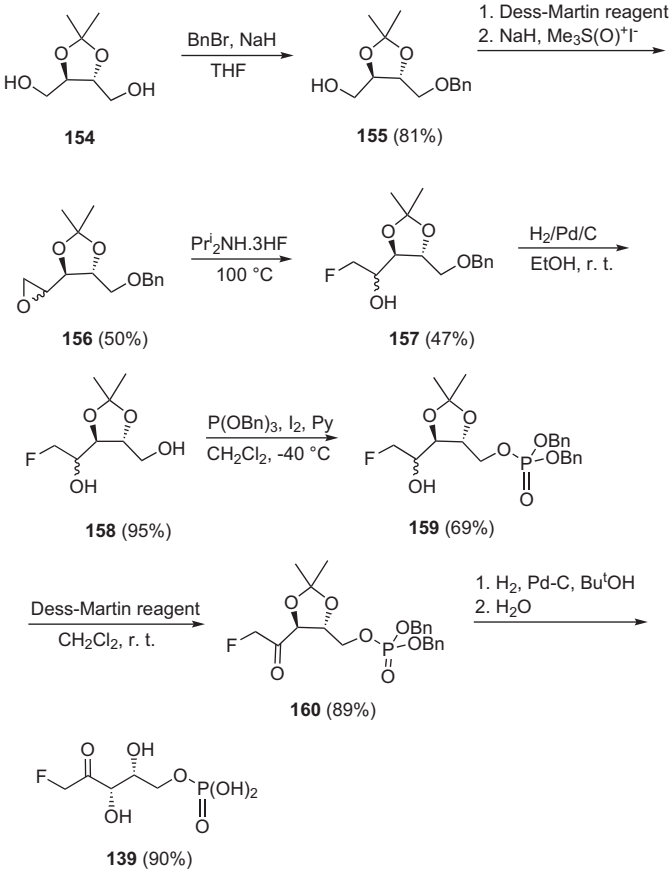
The catalytic mechanism of 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase has been studied using (*Z*)-3-fluorophosphoenolpyruvate [(*Z*)-F-PEP] as a pseudo-substrate. EPSP synthase normally catalyses the transfer of a carboxyvinyl moiety of PEP to the *C*-5 hydroxyl group of shikimate-3-phosphate (S3P). This enzymatic reaction proceeds through a single tetrahedral



Scheme 31. Conversion of DXP into MEP catalysed by 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR): α-ketol rearrangement versus retro-aldolisation mechanism.

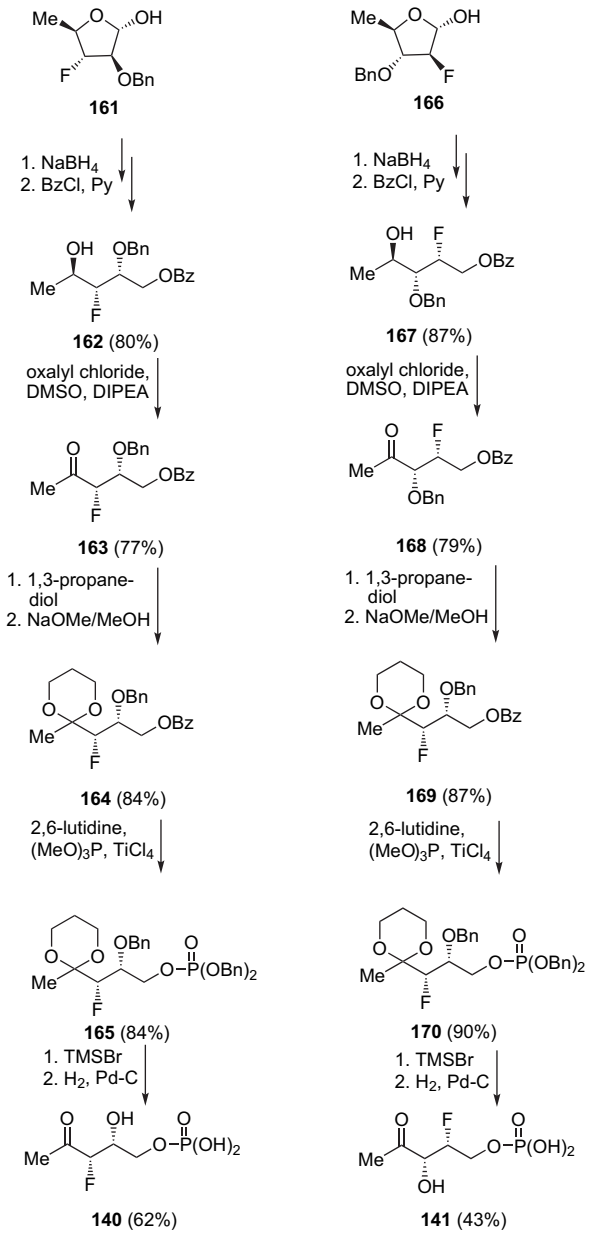


Scheme 32.



Scheme 33.

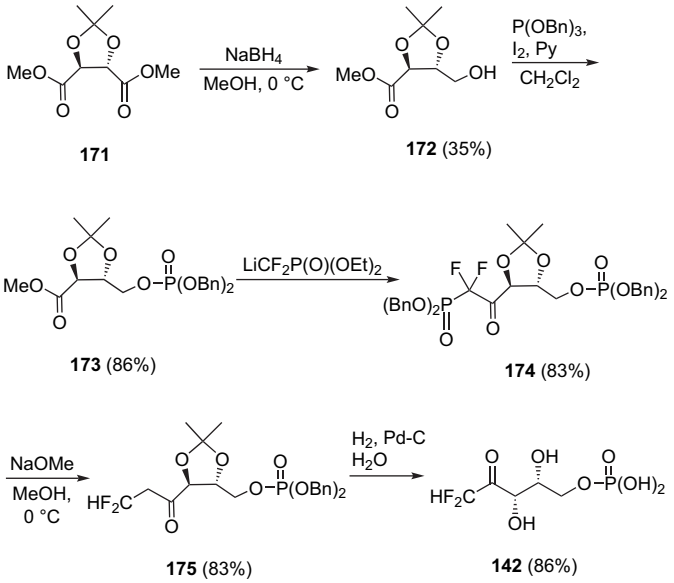
intermediate (TI), which presumably loses phosphate to give an oxonium ion, followed by deprotonation of the methyl group to form EPSP (Scheme 39).[112] Walker and co-workers reported that (*Z*)-3-fluoro-PEP, but not the (*E*)-isomer, is a pseudo-substrate for



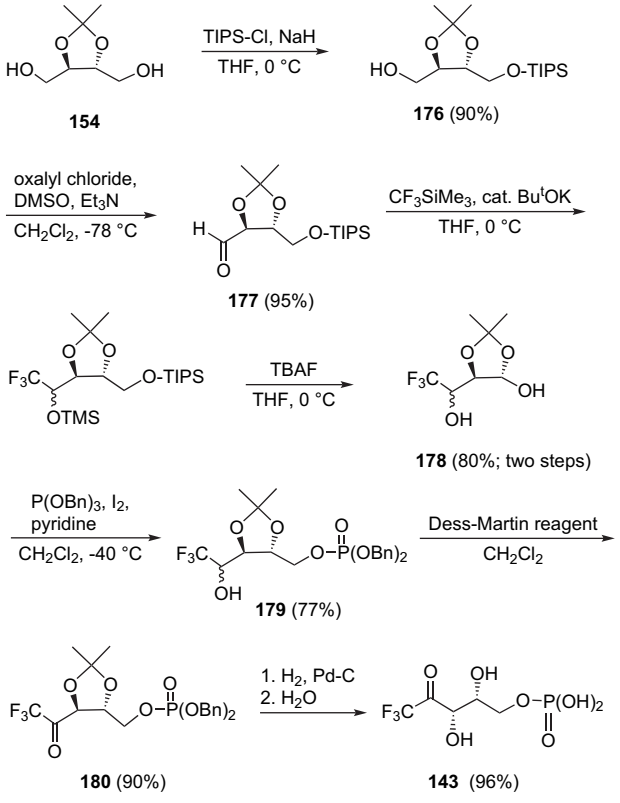
Scheme 34.

EPSP synthase from *E. coli*.[113] The enzyme catalyses the formation of a fluorinated tetrahedral intermediate (FTI) from (*Z*)-F-PEP and S3P, albeit in a reaction that is much slower than the conversion of the natural substrate. Additionally, subsequent conversion of the FTI into the corresponding (*Z*)-F-EPSP product was not observed (Scheme 40). The large reduction in the reaction rates with fluorinated species is consistent with destabilisation of the oxonium ion by the electron-withdrawing fluorine substituent. The use of (*Z*)-F-EPSP to probe the reverse reaction (formation of FTI by addition of PO_4^{3-} to F-EPSP) showed that (*Z*)-F-EPSP is not a substrate or pseudo-substrate for EPSP synthase. These results imply that the transition state for interconversion of the tetrahedral intermediate and EPSP has more cationic character than that for formation of the tetrahedral intermediate from PEP and S3P.[114]

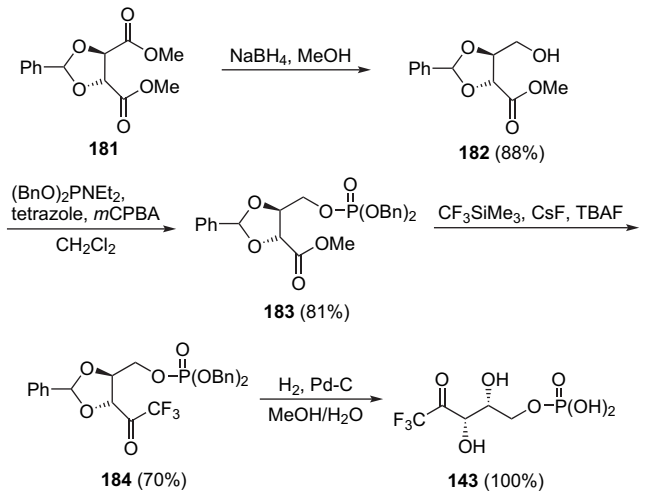
Using the fluorinated PEP analogues, (*Z*)-F-PEP and (*E*)-F-PEP, Walsh and co-workers were able to isolate and characterise a covalent phosphofluorolactyl-enzyme adduct **198** and a phosphofluorolactyl-UDP-GlcNAc tetrahedral adduct **199** upon incubation with UDP-GlcNAc enolpyruvyl transferase (MurZ) and UDP-GlcNAc



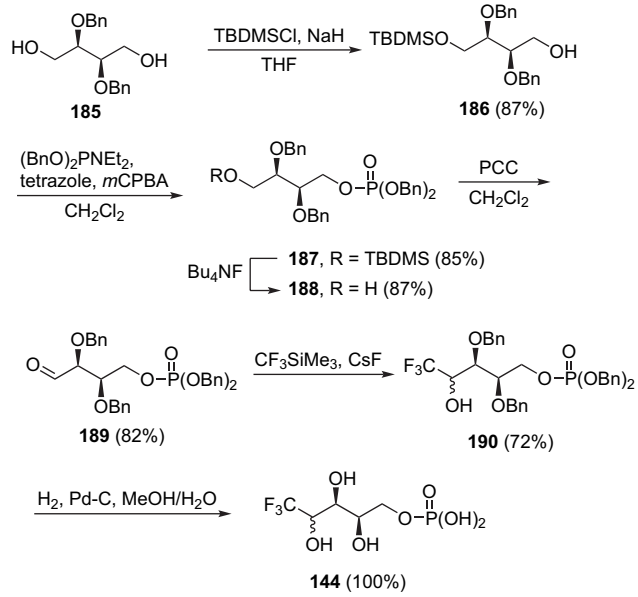
Scheme 35.



Scheme 36.



Scheme 37.



Scheme 38.

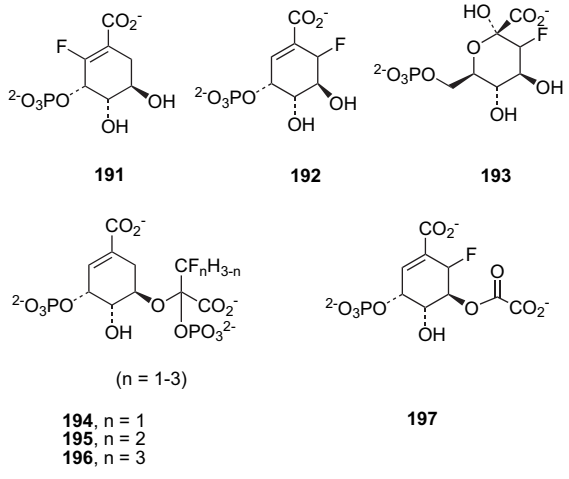
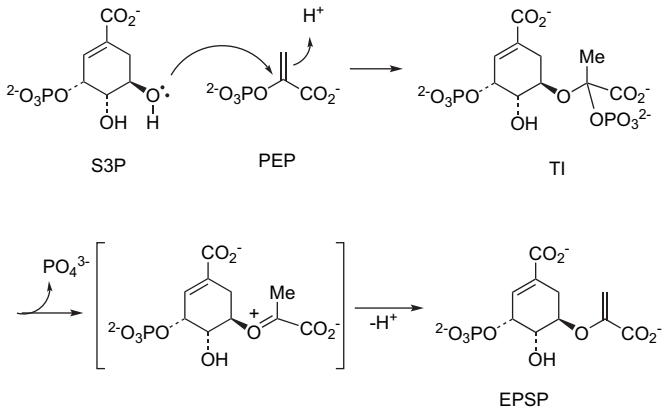
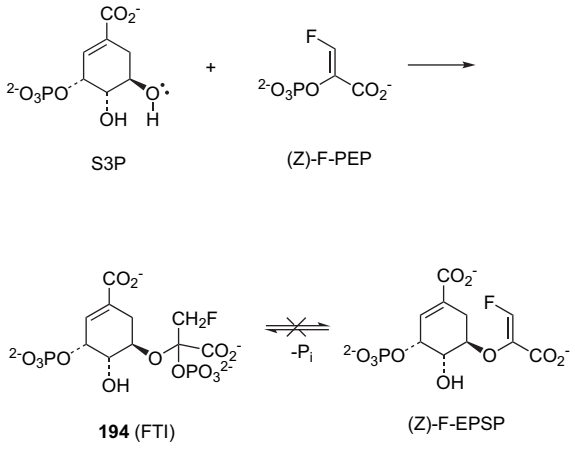


Chart 10. Fluorinated phosphoenolpyruvate analogues.

(Scheme 41).[115,116] Here, also, the rates of formation of **198** and **199** are roughly 10^4-fold slower than those of the analogous non-fluorinated substrates. Additionally, no subsequent conversion of **199** into the corresponding fluorinated enolpyruvyl-UDP-GlcNAc was observed.[116] Kinetic data indicated that the mechanism for normal MurZ catalysis involved the formation of two discrete oxonium ion intermediates. The substitution of fluorine for either of the vinylic hydrogens of PEP would destabilise the formation of an adjacent carbocation, which explains the suppressed rate of catalysis for UDP-GlcNAc enolpyruvyl transferase with fluorinated versions of phosphoenolpyruvate.[115] Analysis of the fluoromethyl group

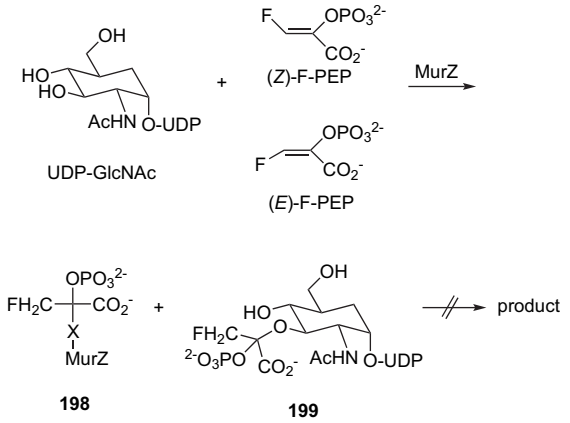


Scheme 39.



Scheme 40.

chirality established a common stereochemical course for the enolpyruvyl transfers catalysed by EPSP synthase and UDP-GlcNAc enolpyruvyl transferase.[117,118]



Scheme 41.

The design of analogues of the tetrahedral intermediate (TI) is an important approach to the invention of potent inhibitors of EPSP synthase.[106] The general strategy for the synthesis of trifluoromethyl ketal phosphates (*S*)-**196** and (*R*)-**196** is outlined in Scheme 42. In this reaction sequence, separation of the diastereomers (*S*)- and (*R*)-**200** is achieved prior to phosphorylation, which is accomplished with bis(*p*-nitrophenethyl)-*N*,*N*-diisopropylphosphoramidite and tetrazole. The configurations of (*R*)- and (*S*)-**196** were assigned by ^{1}H and ^{19}F heteronuclear Overhauser enhancement spectroscopy (HOESY).

The above strategy has also been employed for the synthesis of difluoromethyl ketal phosphates (*R*)-**195** and (*S*)-**195**. In contrast, attempts to prepare a monofluoromethyl ketal phosphate **194** by this route have not been successful. The less electrophilic methyl fluoropyruvate, $FCH_2C(O)–CO_2Me$, cannot be induced to undergo the one-pot condensation/phosphorylation with a shikimate alcohol derivative. Acid-catalysed condensation of fluoropyruvic acid with *trans*-1,2-cyclohexanediol as a model system, however, affords the adduct **201**. The latter compound can be phosphorylated with tetrabenzyl pyrophosphate to give the hemiketal phosphate **202**. Hydrogenolysis of the benzyl esters and alkaline hydrolysis of the lactone moiety affords the model structure **203** (Scheme 43).[106] Compounds (*R*)-, (*S*)-**195** and (*R*)-, (*S*)-**196** were evaluated as inhibitors of EPSP synthase from *Petunia hybrida*. These tetrahedral mimics are bound two- to threefold more tightly than the substrates EPSP or S3P. The most potent inhibitor is the (*R*)-stereoisomer of the difluoromethyl derivative (*R*)-**195** with a K_i value of 4 nM.

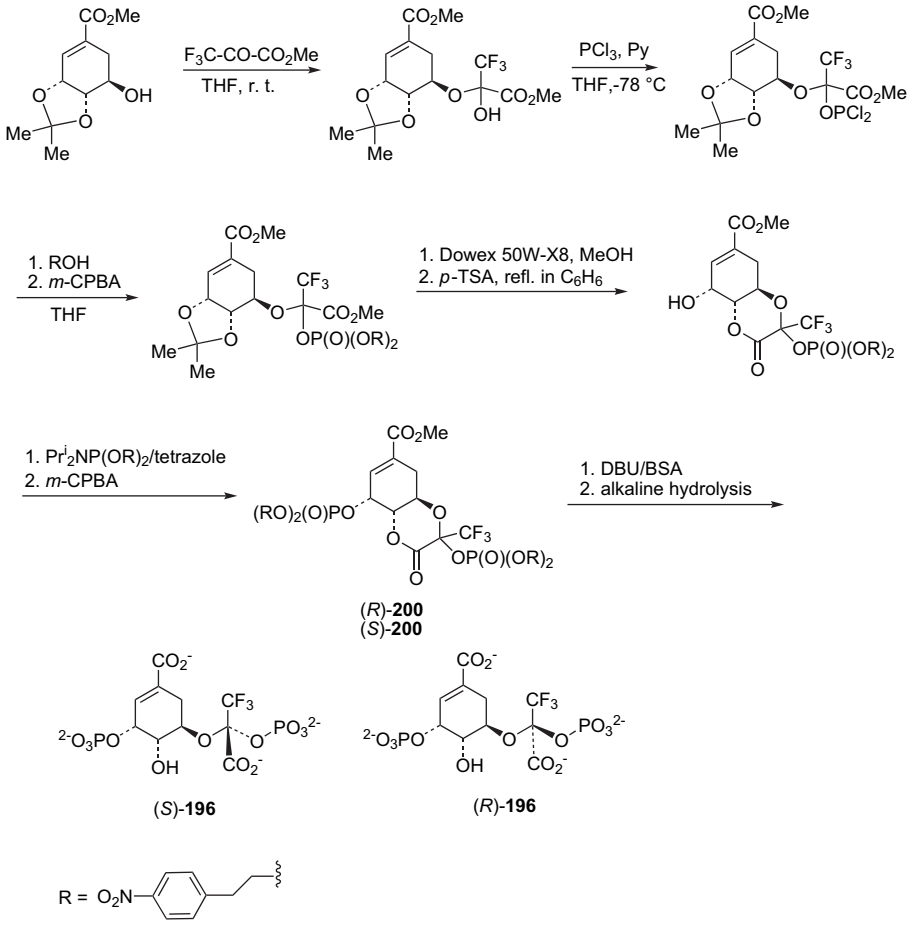
The synthesis of (*Z*)-9-fluoro-EPSP **208** was achieved in four steps from the malonyl ester **204**. The sodium salt of the malonate **204** reacted with difluorocarbene, generated from chlorodifluoromethane, to give the difluoromethyl derivative **205**. Subsequent conversion of **205** into the hydroxy lactone **206**, formation of the dibenzyl phosphate **207**, TMS–Br cleavage and alkaline hydrolysis afforded (*Z*)-9-fluoro-EPSP **208** in 60% overall yield from the carbene adduct **205** (Scheme 44). The EPSP analogue (*Z*)-**208** proved to be a modest inhibitor of EPSP synthase, with an affinity that was reduced sixfold in comparison with EPSP.[106]

Recently, the synthesis of new PEP analogues with modifications in the phosphate and the carboxylate function has been reported.[119] Included in this study is compound $H_2C{=}C(CF_3)OPO_3H_2$, which was prepared by a Perkow reaction. The commercially available α-trifluoromethyl ketone $F_3CC(O)CH_2Br$ was reacted with trimethyl phosphite, giving enolphosphate dimethyl ester. Subsequent replacement of the phosphate methyl ester by trimethylsilyl groups by treating with bromotrimethylsilane and final methanolysis furnished the desired product. Interestingly, this PEP derivative did not inhibit, but, instead, stimulated the activity of PEP carboxylase in the presence of Mg^{2+}. β-Trifluoromethylenol phosphates $F_3CCH{=}C(R^1)OP(O)(OR^2)_2$ have also been designed and prepared by several methods. Some of these phosphates showed good insecticidal activities.[120]

3.6. Fluorinated ketones bearing a phosphate group

Fluorinated ketones incorporating a phosphate moiety have been recognised as potent suicide enzyme inhibitors. In particular, 1-hydroxy-4-fluoro-2-butanone phosphate (**209**) was synthesised and studied as a potential inhibitor of ribulose-1,5-bisphosphate carboxylase (Scheme 45). Compound **209** is unstable at neutral pH and rapidly undergoes hydrolysis to 1-hydroxy-3-buten-2-one phosphate (**210**) by elimination of HF ($k_{obs}=2.51\times10^{-2}$ min^{-1}, at pH 8.2). Inactivation of spinach ribulose-1,5-bisphosphate carboxylase/oxygenase by **209** arises from the spontaneous generation of **210**, which reacts with sulfhydryl groups of the enzyme.[121]

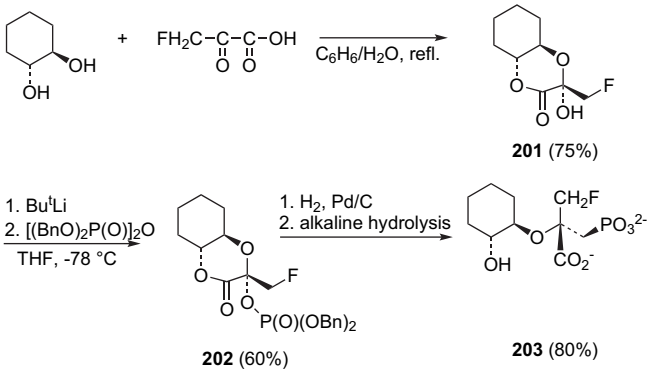
Advances have been made in understanding the nature and reactivity of acylphosphonates as acyl anion precursors in reactions with carbonyl compounds and in the development of a highly practical method for the synthesis of phosphorylated benzoins.[122] The treatment of a 4-fluorobenzoylphosphonate with benzaldehyde and 4-anisaldehyde catalysed by 10% KCN in DMF provided the disubstituted cross-benzoins **211a**,**b** in very good yields (Table 2, entries 1 and 2). Electron-rich 4-methoxybenzoylphosphonate



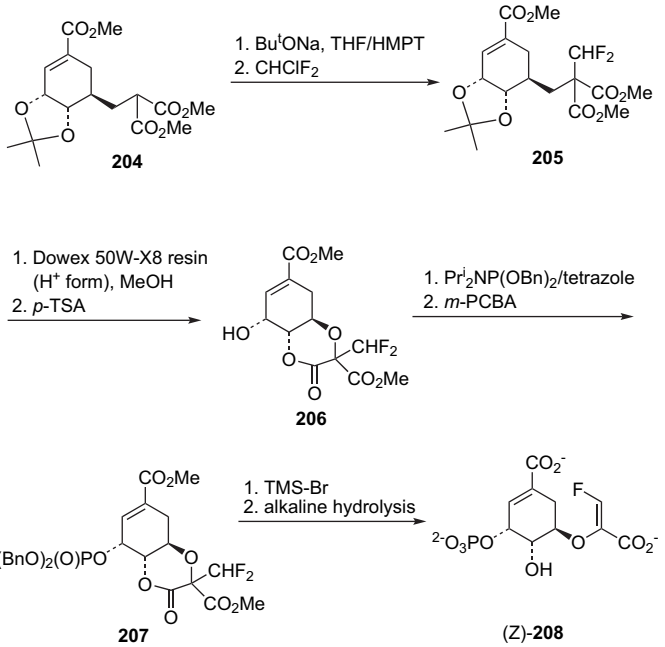
Scheme 42.

reacted very slowly with 4-fluorobenzaldehyde under these reaction conditions. Increasing the catalyst load (30% KCN), however, resulted in a smooth transformation, providing **211c** in 94% yield (Table 2, entry 3). Reactions of the same 4-fluorobenzoylphosphonate with aliphatic aldehydes (Table 2, entries 4 and 5) to give **211d**,**e** were carried out in a premixed DMF solution of 20% CsF and 30% Me_3SiCN. In a manner analogous to the synthesis of the benzoins **211a**–**e**, compound **212** has been prepared by the reaction of a benzoylphosphonate with 2,2,2-trifluoroacetophenone (Scheme 46). The same reaction with acetophenone provided poor yields, together with the recovered starting materials.

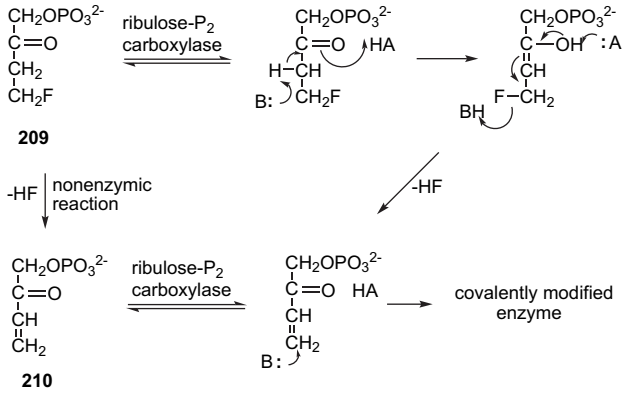
Mironov and co-workers[123–125] and Schmutzler and co-workers[126] have demonstrated the utility of cyclic phosphitylated derivatives of salicylic acid (salicylphosphites) in the preparation of 1,3,2-dioxaphosphepins incorporating a $C(O)C(CF_3)_2OP_i$ backbone. As an example, 6,7-benzo-1,3,2-dioxaphosphepan **214** was prepared from 2-L-menthyloxy-4-oxo-5,6-benzo-1,3,2-dioxaphosphorinane **213** and hexafluoroacetone (Scheme 47). The cyclic phosphate **214** was isolated as a mixture of diastereomers and was



Scheme 43.

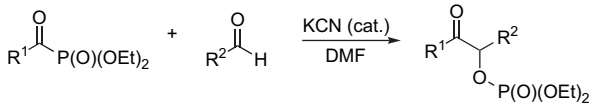


Scheme 44.



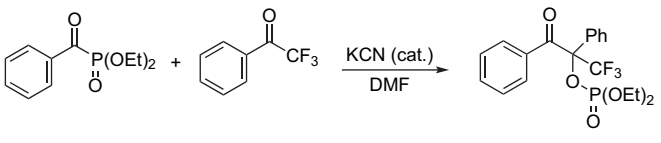
Scheme 45.

Table 2
Synthesis of phosphorylated benzoins via cyanide ion promoted generation of acyl anion intermediates[122]



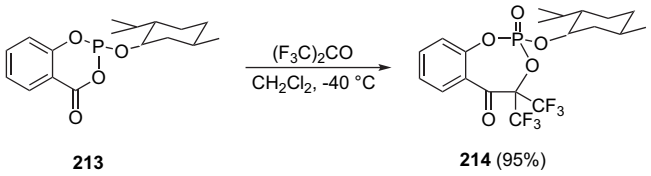
211a-e

Entry	R^1	R^2	Product	Yield (%)
1	4-FC_6H_4	Ph	**211a**	87
2	4-FC_6H_4	4-$MeOC_6H_4$	**211b**	93
3	4-$MeOC_6H_4$	4-FC_6H_4	**211c**	94
4	4-FC_6H_4	Cyclohexyl	**211d**	81
5	4-FC_6H_4	Benzyloxymethyl	**211e**	75



212 (87%)

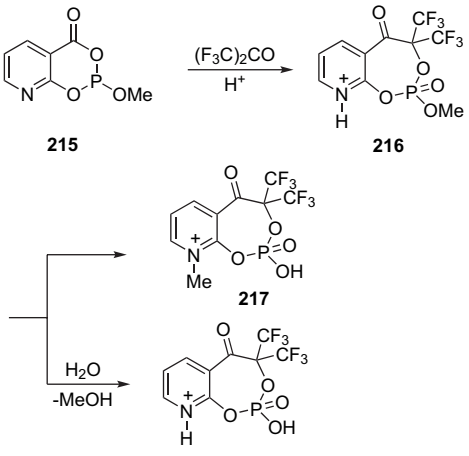
Scheme 46.



213 **214** (95%)

Scheme 47.

characterised by ^{13}C, ^{19}F and ^{31}P NMR spectroscopies.[127] The interaction of a phosphitylated derivative of 2-hydroxynicotinic acid **215** with hexafluoroacetone has been observed to take the course illustrated in Scheme 48. Under the usual conditions, i.e., in the presence of moisture, the reaction leads to the unstable cyclic phosphate **216**, which undergoes a further transformation into compounds **217** and **218**. The formation of the 1,3,2-dioxaphosphepin cycle presumably proceeds via an alkoxyphosphonium intermediate and its subsequent intramolecular rearrangement.[128]

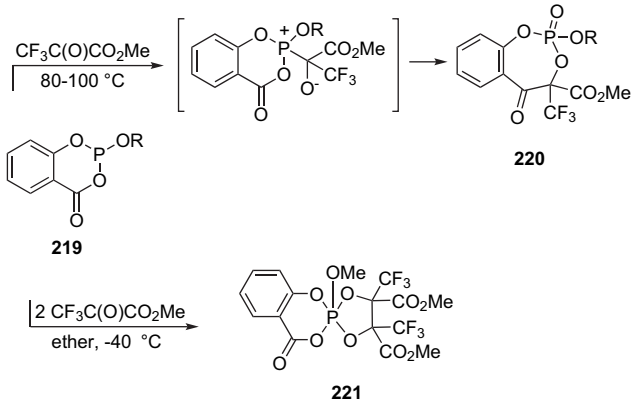


215 **216** **217** **218**

Scheme 48.

The easily accessible dioxaphosphorinanes **219** reacted with methyl trifluoromethylpyruvate at temperatures of 80–100 °C to give the phosphepins **220**. It is worth noting that, in ether at −40 °C, the major product was the kinetically controlled spirophosphorane **221** (Scheme 49).[123]

Fluorinated α,β-unsaturated ketones are excellent Michael acceptors and add a wide range of phosphorus nucleophiles, providing the functionalised organophosphorus compounds, which

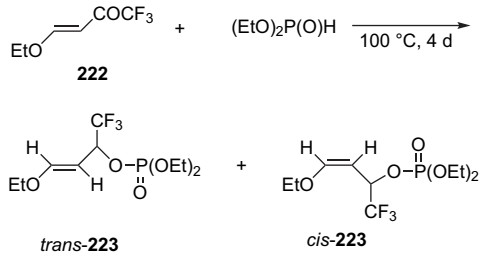


219 **220** **221**

R = OMe, OEt, OCH_2CF_3, $OCH_2CF_2CHF_2$, $OCH_2(CF_2)_4H$

Scheme 49.

are important intermediates in synthesis.[129,130] Recently, (*E*)-4-ethoxy-1,1,1-trifluoro-3-buten-2-one **222** has been utilised in the synthesis of a 1:1 cis- and trans-mixture of diethyl (1-trifluoromethyl-3-ethoxy)allylphosphate **223** (Scheme 50). The formation of **223** is consistent with the initial nucleophilic attack on the β-carbon atom of **222** by $(EtO)_2P(O)H$ and generation of a phosphonium intermediate, which subsequently undergoes isomerisation and 1,5-proton shift.[131]



Scheme 50.

3.7. Fluorinated nucleotides

The aim of this section is not to give a comprehensive account of all fluorine-containing nucleoside phosphates that have been synthesised, but to illustrate how fluorinated building blocks can be used either to tailor or to organise fluorinated oligonucleotides with a special emphasis upon the biomedical potential of new compounds. Various methods have been used to prepare fluorine-containing nucleoside phosphates (Fig. 1). Among the most common routes are: (a) introduction of fluorine into a carbohydrate moiety; (b) introduction of fluorine into a nucleobase moiety and (c) replacement of one of the oxygen atoms at phosphorus involved in the bridge by a fluorine atom or fluorine-containing group. Extensive studies on the introduction of fluorine atom(s) into sugars have been reviewed.[132,133] In addition, the field of fluorinated nucleosides has been reviewed and will not be discussed in this survey.[134]

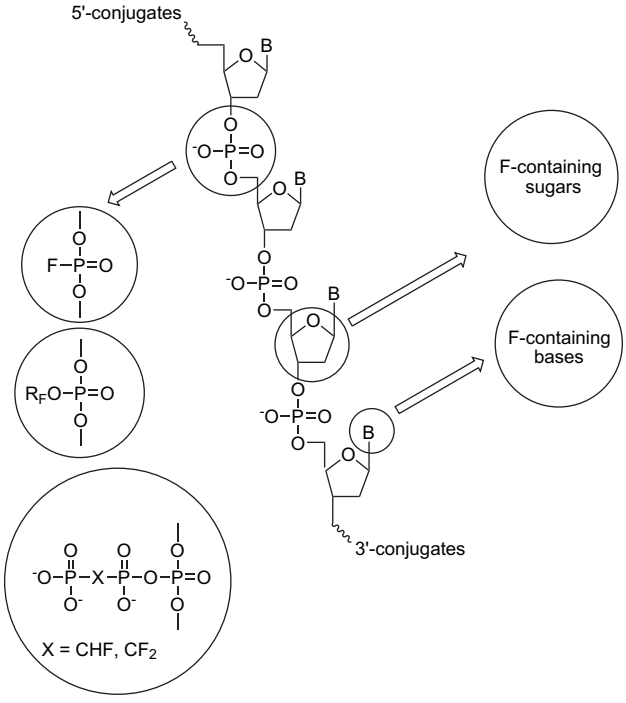


Figure 1. Possible synthetic approaches to fluorinated oligonucleotides.

3.7.1. Modification of a sugar moiety

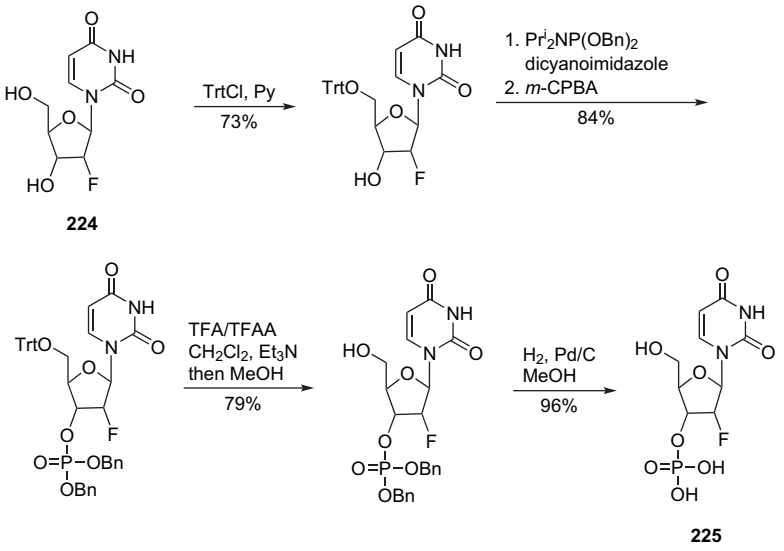
With the hope of finding highly effective antiviral and anticancer agents, a wide variety of carbohydrate-fluorinated nucleotides have been prepared. The synthesis of 2′-fluoro-2′-deoxyuridine 3′-phosphate (dU^FMP, **225**) was recently accomplished, starting from the unprotected nucleoside **224** by the route shown in Scheme 51.[135] The advantages of this synthesis include the use of commercially available reagents, the high yield of the phosphorylation step and the facile deprotection of the trityl group. dU^FMP was found to have higher affinity than uridine 3′-phosphate (3-UMP) or 2′-deoxyuridine 3′-phosphate (dUMP) for ribonuclease A (RNase A).

A recent study on the binding requirements of NAD kinase with modified substrate analogues was reported by Pankiewicz and co-workers.[136] NAD kinase catalyses a magnesium-dependent phosphorylation of the 2′-hydroxyl group of the adenosine ribose moiety of nicotinamide adenine dinucleotide (NAD) using ATP or inorganic phosphates as phosphoryl donors. It was found that NAD^+ analogues in which the *C*2′ hydroxyl group of the adenosine moiety was replaced by fluorine in the *ribo* or *arabino* configuration (**226** and **227**, respectively) could not be phosphorylated by NAD kinase. 2′-Fluoro *ribo* NAD **226** was synthesised by coupling the commercially available nicotinamide mononucleotide (NMN) with the 5′-monophosphate imidazolide of 2′-deoxy-2′-fluoro-adenosine. A similar coupling of 2′-deoxy-2′-fluoro-1-β-D-arabinofuranosyl adenine with NMN afforded 2′-fluoro NAD **227**, in which the 2′-fluoro atom is in the *arabino* configuration. The NAD analogue **226** inhibited 43% activity of human NAD kinase, but was inactive against *Mycobacterium tuberculosis* NAD kinase. In spite of its different conformation, the NAD analogue **227** showed a similar activity to that of **226**. Interestingly, the inversion of the configuration of the OH (such as in the analogue **228**) led to the inhibition of both the human and the bacterial enzyme (Chart 11).

In order to understand the influence of pre-organisation of *xylo*-configured monomers (XNA-monomers) on the hybridisation towards RNA and DNA complements, Poopeiko and co-workers have described the synthesis and binding properties of a novel conformationally restricted 2′-fluoro-2′-deoxy-β-D xylofuranosyl nucleotide **232**.[137] Condensation of 1-*O*-acetyl furanose **229** with persilylated thymine in the presence of Me_3SiOTf as catalyst afforded the protected anomeric nucleosides **230a** and **230b**, which were separated by column chromatography. Using standard transformations, the nucleosides **230a** and **230b** were deprotected using saturated methanolic ammonia to give the α-anomer **231a** and the β-anomer **231b**. Nucleoside **231b** was, via the 4,4′-dimethoxy trityl (DMT)-protected nucleoside, converted into the desired phosphoramidite derivative **232**, which was used for incorporation into XNAs (Scheme 52).

Recently, an efficient method for the synthesis of 5′-*O*-monomethoxytrityl-2′,3′-dideoxy-2′-fluoro-3′-thioarabinothymidine **237** and its 3-phosphoramidite derivative **238** suitable for automated incorporation into oligonucleotides has been described by Dahma and co-workers.[138] Sugar precursor **233** was converted into α-1-bromoarabinose **234** with retention of sugar C-1 configuration. Compound **234** was then used for glycosylation reaction with silylated thymine base, which resulted, after removal of 5′- and 3′-*O*-benzoyl groups, in the formation of nucleoside **235**. The latter compound was then 5-monomethoxytritylated and 3-mesylated before being converted into 2,3′-anhydronucleoside **236**. Direct ring opening of the 2,3′-anhydro linkage, in nucleoside **236**, with sodium thioacetate afforded the nucleoside **237**. Deprotection of **237**, followed by treatment with 2-cyanoethyl-*N*,*N*-diisopropyl-chlorophosphoramidite, resulted in the desired phosphoramidite derivative **238** (Scheme 53).

Gemcitabine (**63**) is a potent anticancer agent that exerts its cytotoxic activity, in part, through incorporation of its nucleoside triphosphate into DNA and perturbation of DNA-mediated

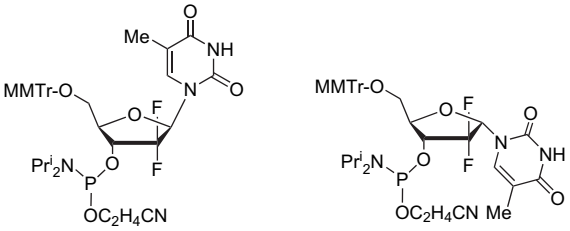


224 **225**

Scheme 51.

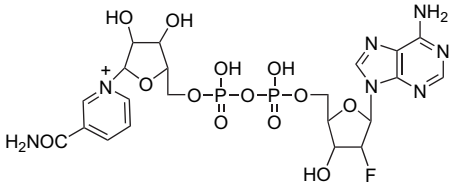
processes. A gemcitabine phosphoramidate prodrug **241**, designed for the intracellular delivery of gemcitabine 5′-monophosphate, was synthesised as shown in Scheme 54. The exocyclic amine moiety of Gemcitabine was protected with the allyloxycarbonyl group (AOC). AOC-2′,2′-difluorodeoxycytidine **239** was then converted into the prodrug **241** via allyloxycarbonyl derivative **240** according to a procedure that features phosphorylation of the nucleoside using a highly reactive phosphoramidic bis(1-benzotriazolyl) ester. The prodrug was about an order of magnitude less active than gemcitabine against wild-type cells. In deoxycytidine kinase(dCK)-deficient cell lines, however, the prodrug is about fourfold more active than Gemcitabine.[139]

A modification of gemcitabine was also reported by Viazovkina and co-workers.[140] These workers prepared the 3′,5′-benzoyl-protected difluorinated nucleoside as a mixture of α- and β-anomer upon coupling of the sugar precursor to the thymine base and then separated the anomers by selective crystallisation from ethanol. After appropriate deprotection, both anomeric nucleosides were converted into their 5′-monomethoxytrityl-protected 3′-phosphoramidite building blocks **242** for oligonucleotide assembly on solid support.

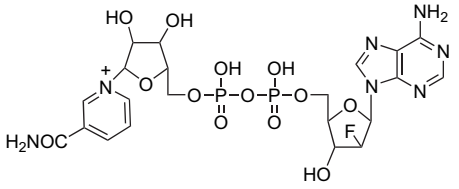


242

β-isomer α-isomer



226



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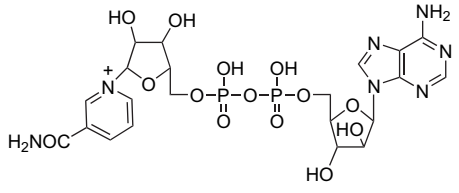
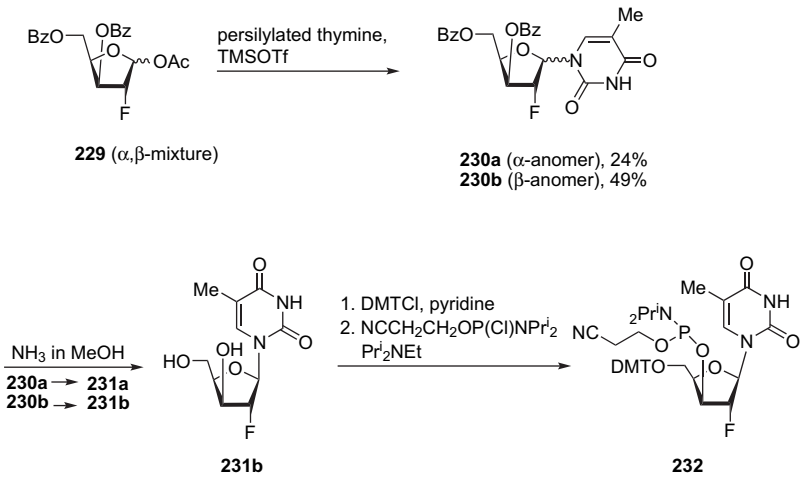


228

Chart 11. Modified substrate analogues of NAD kinase.

Vanheusden and co-workers, working with a series of 2′- and 3′-modified thymidine 5′-*O*-monophosphate analogues, found that a 2′-halogeno substituent (Hlg=Cl or F) and a 3′-azido group are the most favourable functions for the development of potent inhibitors of *M. tuberculosis* thymidylate kinase (TMPKmt).[141] The synthetic methods used to prepare the nucleotides **246**, **247** and **250** are outlined in Schemes 55 and 56. For the synthesis of 2′-fluorothymidine **245**, 2,2′-anhydrothymidine **243** was treated with 3,4-dihydropyran in DMF, followed by saponification, to yield its 3′,5′-bisprotected arabinosyl derivative. Fluorination with DAST, followed by deprotection with *p*-toluenesulfonic acid in MeOH, afforded **245**. The nucleosides **244** and **245** were converted into their monophosphates **246** and **247** using the procedure of Yoshikawa, involving treatment with $POCl_3$ (3 equiv) in $(MeO)_3PO$. 3′-Deoxy-3′-fluorothymidine 5′-*O*-monophosphate (**250**) was synthesised from compound **248** via treatment with DAST giving fluorinated nucleoside **249** and subsequent detritylation and phosphorylation. All nucleotides were tested on TMPKmt. Compound **246** exhibits appreciable affinity with a K_i value of 19 μM. Changing the chlorine of **246** for fluorine leads to a twofold drop in affinity. Replacement of the 3′-OH of dTMP by a 3′-F in **250** affords an analogue that behaves as a substrate with a K_m value of 30 μM.

To delineate the most appropriate length of the spacer between the 3′-carbon of the 2′,3′-deoxy thymidine monophosphate (dTMP) and the introduced functionality, 3′-deoxy-3′-fluoromethyl thymidine monophosphate **255** was also investigated for its affinity for

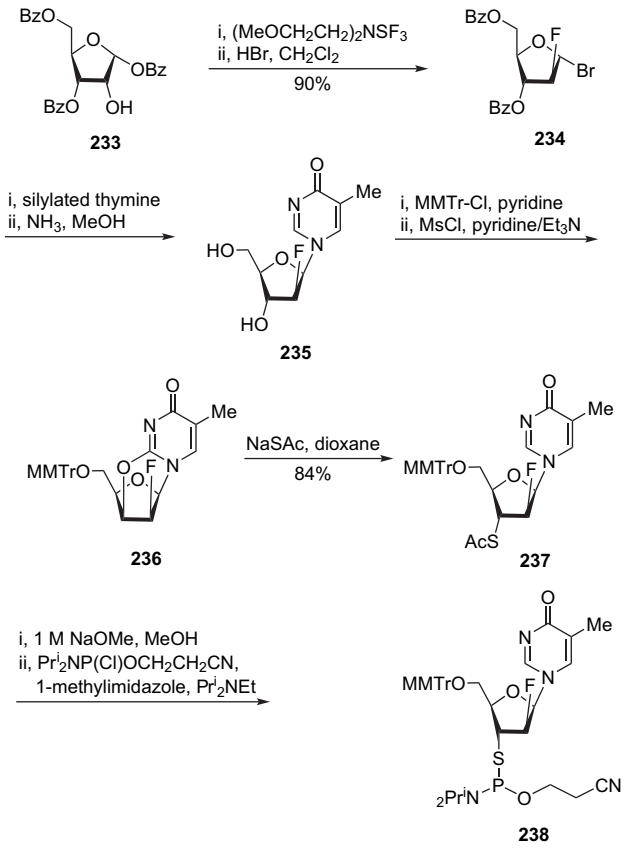


Scheme 52.

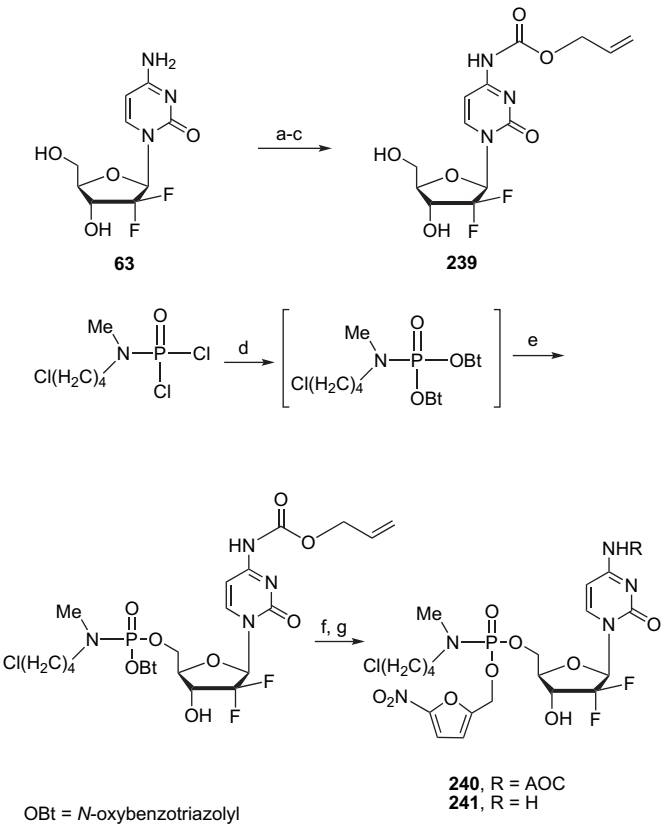
TMPKmt. The required compound was prepared starting from the nucleoside **251**. Removal of the 2′-hydroxyl function was performed by reduction of its phenoxythiocarbonate ester with AIBN and Bu_3SnH. Subsequent removal of the *p*-anisyl group with ammonium cerium(IV) nitrate gave the 2′,3′-dideoxy-3′-hydroxymethyl derivative **253**. Fluorination of **253** with DAST, followed by reductive debenzylation, afforded the desired 2′,3′-dideoxy-3′-fluoromethylnucleoside **254**. Phosphorylation of the 5′-hydroxyl group led to the corresponding nucleotide **255** (Scheme 57). This compound was proved to be potent inhibitor of TMPKmt with a K_i value of 15 μM.[142]

Since 2′,3′-dideoxy-3′-fluoroadenosine **256** and related analogues have shown promising activity against HIV and HBV, it was interesting to learn if their phosphoramidate protides would lead to better activity. Treatment of **256** with phosphorochloridates **257a**,**b** in pyridine in the presence of ^tBuMgCl afforded good yields of phosphoramidate protides **258** and **259** (Scheme 58). Compound **258** was found to be about 280-fold more potent than the parent nucleoside **256** against HIV and about 7800-fold more potent than **256** against HBV. Dimethylglycinylphosphoramidate protide **259** showed a 20-fold improvement in activity against HIV and 20,000-fold improvement for HBV.[143]

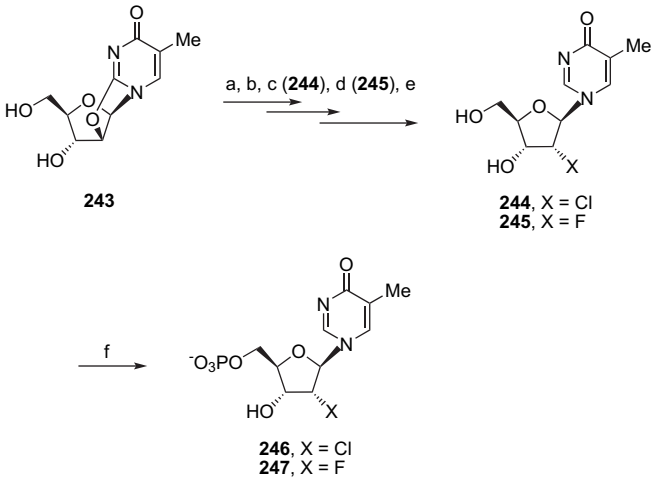
The synthetic entry into a new subclass of nucleosides bearing a branched ribose was described by Piccirilli and co-workers.[144] The glycosylating agent **263** was prepared in three steps from 1,3,5-tri-



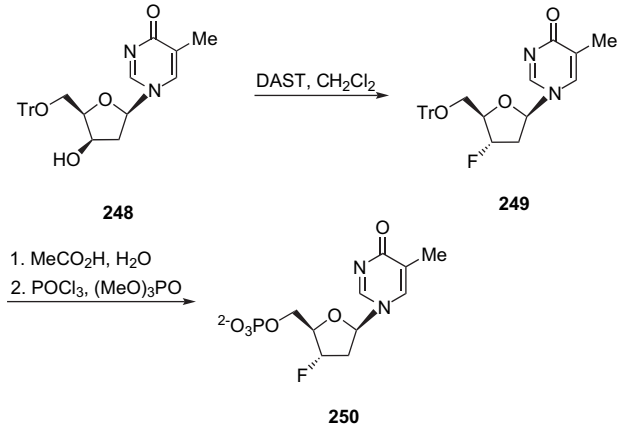
Scheme 53.



Scheme 54. Reagents and conditions: (a) $(Me_3Si)_2NH$, $(NH_4)_2SO_4$, dioxane, 2 h, reflux; (b) AOCCl, *N*-methylimidazole, CH_2Cl_2, 4 h, rt; (c) Et_3N, MeOH, overnight, rt; (d) HOBt, pyridine, THF, 4 h, rt; (e) **239**, *N*-methylimidazole, pyridine, 20 h, rt; (f) 5-nitrofurfuryl alcohol, DMAP, THF, overnight, rt; (g) $Pd(PPh_3)_4$, *p*-$MeC_6H_4SO_2Na$, THF/H_2O (2:1), 1 h, rt.



Scheme 55. Reagents: (a) 3,4-dihydropyran, *p*-TSA, DMF; (b) 1 N NaOH, MeOH; (c) HCl, dioxane; (d) DAST, CH_2Cl_2, pyridine; (e) *p*-TSA, MeOH; (f) $POCl_3$, $(MeO)_3PO$.



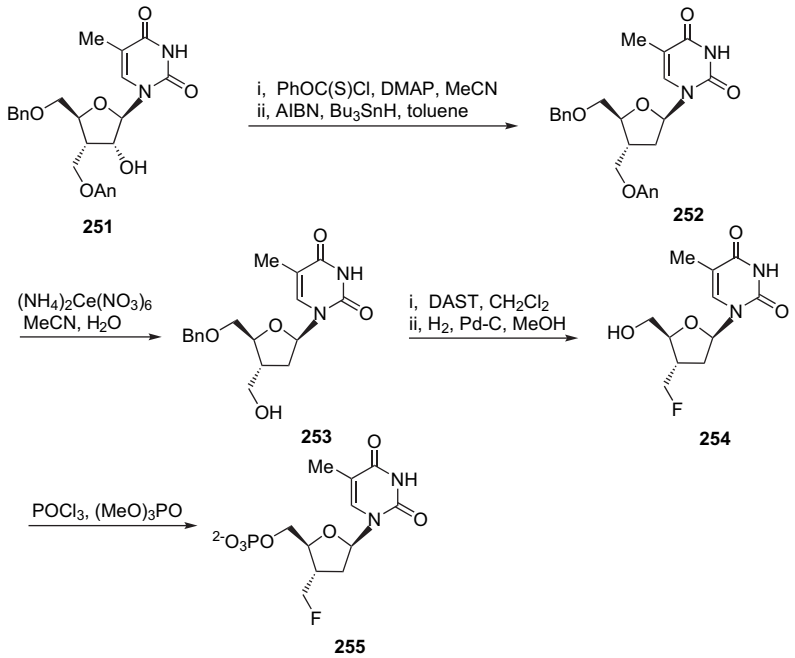
Scheme 56.

O-benzoyl-α-D-ribofuranose **260** via benzenesulfonyldifluoromethyl derivative **262**. The key step included nucleophilic addition of difluoromethyl phenyl sulfone to 2-ketoribose **261** followed by reductive desulfonation. Glycosylation of bis(trimethylsilyl)uracil with **263** in the presence of TMSOTf was unsuccessful and resulted in recovered starting material. Using a stronger Lewis acid ($SnCl_4$) at reflux in acetonitrile gave uridine **264** in 78% yield. Difluoromethyluridine 3′,5′-bisphosphonate **266** was synthesised from 2′-*C*-β-difluoromethyluridine **265** using diphosphoryl chloride as a phosphorylating agent (Scheme 59).

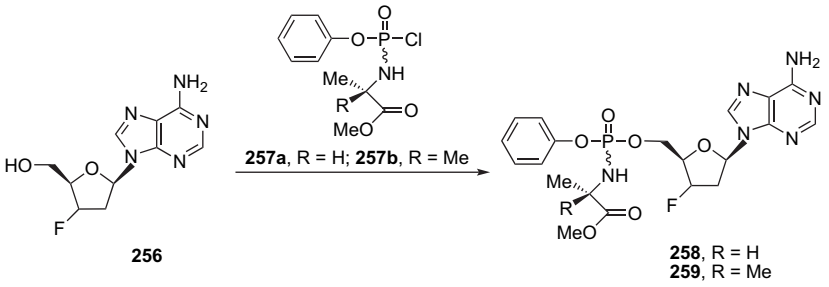
In order to create a novel inhibitor of bacterial growth, Nishimura and co-workers have concentrated on obtaining 4-fluorinated UDP-*N*-acetyl muramic acid (MurNAc) pentapeptide **270** (Scheme 60).[145] Coupling the pentapeptide **268** with the muramyl carboxyl group of **267** afforded the glycopeptide **269** in 66% yield for the two steps. The benzyl-protecting groups of the phosphate moiety in **269** were removed in the presence of trioctylamine. Subsequent reaction of the trioctylammonium phosphate salt with uridine 5′-monophosphormorpholidate and 1*H*-tetrazole in pyridine produced the protected UDP-4-F-MurNAc pentapeptide **270**. Removal of the protecting groups was accomplished by rapid treatment with aqueous sodium hydroxide. The UDP-4-F-MurNAc pentapeptide showed growth-inhibition activity against Gram-positive bacteria when it was added to growth media at 0.01 mg ml^{-1}.

Nucleoside phosphoramidites **275** bearing a fluorous dimethoxytrityl (FDMT) group were used to synthesise fluorous-tagged oligonucleotides, which were subjected to solid-phase extraction using a pH-stable fluorinated absorbent. Compounds **275** were prepared by a reaction sequence shown in Scheme 61. Grignard reaction of the commercially available **271** with *p*-methoxyphenylmagnesium bromide afforded a tertiary alcohol, which was converted into the fluorous dimethoxytrityl chloride **272**. Fluorous dimethoxytritylation of thymidine **273a** and suitably protected forms of dA, dC and dG (**273b–d**) proceeded normally to give **274a–d**. Phosphitylation afforded the desired phosphoramidites **275a–d**. From a synthetic standpoint, the FDMT group was found to behave identically to a DMT group throughout this sequence.[146]

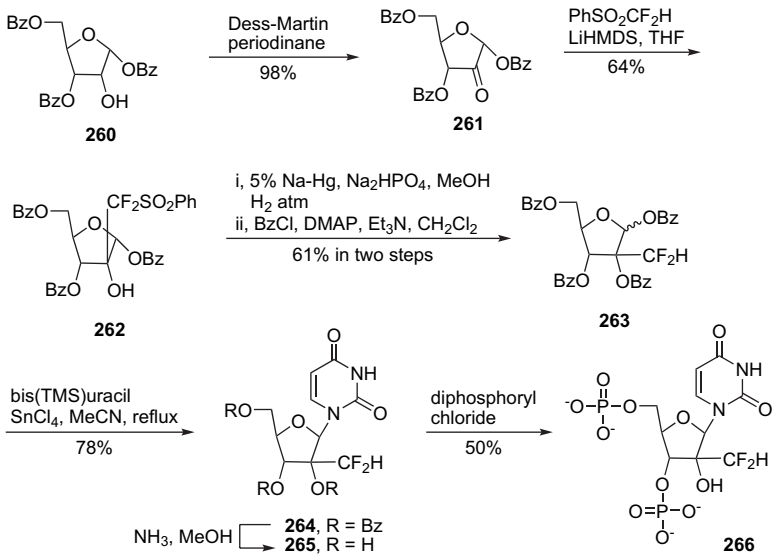
The Prakash group has performed a systematic study on the effect of 2′-sugar modifications [2′-*F*-2′-deoxy-, 2′-*O*-methyl- and



Scheme 57.



Scheme 58.



Scheme 59.

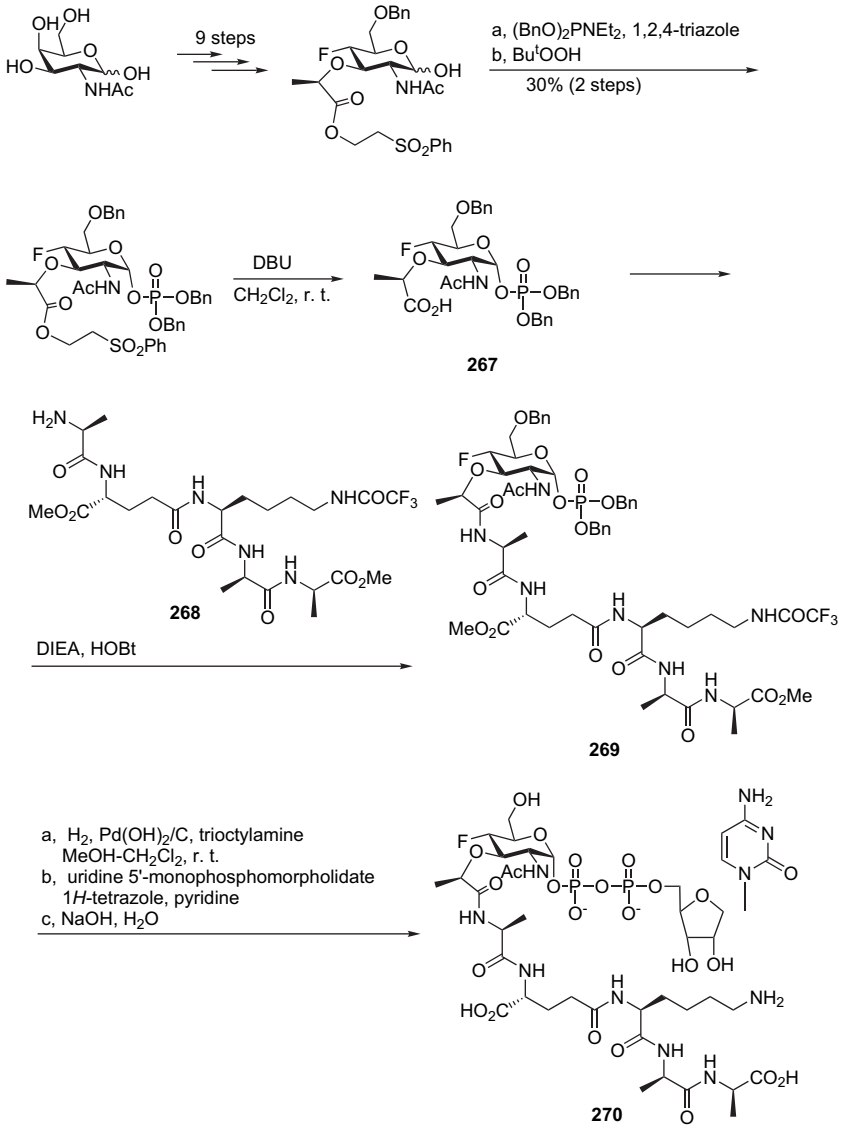
2′-*O*-(2-methoxyethyl)nucleoside residues] in the antisense and sense strands of short interference RNA (siRNA). The siRNAs with modified ribonucleotides at the 5′-end of the antisense strand were less active relative to the 3′-modified siRNAs. The 2′-*F* sugar was generally well tolerated on the antisense strand, whereas the 2′-OMe sugar showed a significant shift in activity, depending upon the position of modification. The 2′-*O*-methoxyethyl modification in the antisense strand resulted in less active siRNA constructs, regardless of the placement position in the construct.[147] The same authors identified an siRNA motif consisting entirely of 2′-OMe and 2′-*F* nucleotides that showed a remarkable improvement in the in vitro potency and stability, compared to the unmodified siRNA.[148]

Advances have been made in using ^{19}F NMR spectroscopy for the characterisation of RNA secondary structure equilibrium. The concept is based on the site-specific labelling of RNA with single 2′-fluoro-2-deoxy nucleosides.[149] A spectroscopic approach, namely ^{31}P–{^{19}F} FEDOR NMR, has been utilised to measure the distances between a CF_3 group and phosphodiester in nucleic acids.[150]

3.7.2. *Modification of a nucleobase*

5-Fluoropyrimidines such as 5-fluorouracil (5-FU), 5-fluoro-2′-deoxyuridine (Floxuridine) and their derivatives are known to be the most common and extensively investigated anticancer agents (Chart 12). The active form of these prodrugs is 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP), which is generated in vivo. 5-FU and its derivatives are potent mechanism-based inhibitors of thymidylate synthase (TS), an enzyme, which converts dUMP into 2′-deoxythimidine-5′-monophosphate (dTMP), utilising the coenzyme N^5,N^{10}-methylene-5,6,7,8-tetrahydrofolate as the source of the methyl group as well as a reductant. A number of detailed reviews on the mechanism of TS inhibition by 5-FU and its derivatives have been published.[7,10,151] The ethynyl analogue of FdUMP, 5-ethynyl-2′-deoxyuridine-5′-phosphate **276**, is a potent inhibitor of dihydropyridine dehydrogenase.[152] 5-Fluoropropynyl-dUMP **277**, an improved derivative of **276**, has been designed as a prototype of a new generation of dTMP synthase inhibitors.[153] Of the fluorinated adenosine antimetabolites, fludarabine (F-*ara*-AMP) **278** is a DNA polymerase inhibitor, which is a useful chemotherapeutic agent against a variety of cancers.[154]

The 2-thio derivatives of 5-fluoro-dUrd and their monophosphates were synthesised in 1993 by Kulikowski and coworkers.[155] The β- and α-anomer **280** and **281** were prepared by condensation of the di-TMS derivatives of 5-fluoro-2-thiouracil with 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-ribofuranosyl chloride **279** in the presence of a Lewis acid (Scheme 62). The ratio of the β- to the α-anomer increased with the strength of the Lewis acid catalyst, being 1:3 with TMS-triflate, 1:2 with $SnCl_4$ and 3:1 with $TiCl_4$. Each of the anomers was unblocked with MeOH–NH_3 to give the desired free anomeric nucleoside pair **282** and **283**. These compounds were selectively converted into the corresponding 5′-monophosphates **284** and **285** with the aid of the wheat shoot phosphotransferase system. β-5-Fluoro-2-thio-dUMP **284** proved to be a potent competitive, slow-binding inhibitor ($K_i \sim 10^{-8}$ M) of the purified enzymes from Ehrlich ascites carcinoma and L1210 cells, albeit 40- to 100-fold less effective than FdUrd. The α-anomer **285** is a much weaker inhibitor of tumour cell growth, with a K_i value in the millimolar range.



Scheme 60.

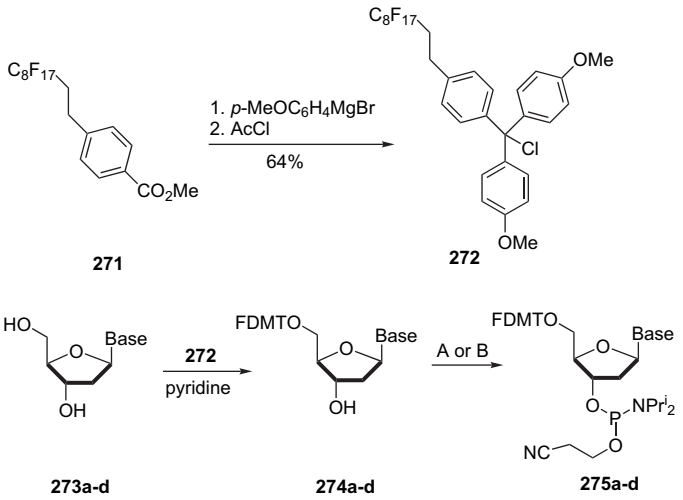
In order to overcome the poor cell membrane penetration of therapeutically useful nucleotides, Farquhar and co-workers have designed 5′-(1,3,2-dioxaphosphorinan-2-yl) and 5′-(1,3,2-oxazaphosphorinan-2-yl) derivatives of 2′-deoxy-5-fluorouridines **286a**,**b** (Scheme 63).[156] It was anticipated that these compounds would be oxidatively transformed by hepatic P-450-dependent mixed-function oxidases, in a manner similar to cyclophosphamide, to yield the labile hydroxylated intermediates **287a**,**b**. Unfortunately, compounds **286a**,**b** were only modestly effective at prolonging the life of mice bearing murine leukaemia P-388 and inactive against a P-388 variant that was resistant to 5-fluorouracil.[157] To circumvent the requirement for oxidative biotransformation of **286a**, the activating hydroxyl group has been introduced into the 4-position of the 1,3,2-dioxaphosphorinane ring in the form of a stable carboxylate ester derivative (**286c**). Compound **286c** was prepared by condensation of the cyclic phosphorinane with 5-FdUr, in the presence of the Mitsunobu reagent, as shown in Scheme 64. As anticipated, this derivative acted as a membrane-permeating precursor of FdUMP and inhibited the growth of Chinese hamster ovary (CHO) cells in culture at a concentration of 5×10^{-6} M. In the presence of 2-mercaptoethanesulfonic acid (an acrolein scavenger), **286c** was equally effective against a P-388 mutant cell line that was resistant to FU.[156]

Another interesting procedure for obtaining membrane-permeating prodrugs of 5-FdUMP comprises the incorporation of masking mononucleotides into amphiphilic dimers. In one example, the amphiphilic anticancer prodrugs **291** and **292** were synthesised according to the hydrogen phosphonate method by coupling the lipophilic cytosine derivatives **289** and **290** with 5-fluoro-2′-deoxyuridine **288** (Scheme 65).[158]

Aside from the amphiphilic dimers, some phospholipid–nucleoside conjugates containing a 5-fluorouracil moiety have been synthesised and studied.[159] As an example, glycerothiophospholipid–nucleoside conjugates **294** and **295** (Chart 13) were designed to take advantage of the good activity of 5-fluoro-1-(tetrahydro-2-furyl)uracil, an important antitumour drug **293** (tegafur). These compounds were reported to exhibit antitumour activity against bladder cancer cells.[160]

Analogues of UDP-glucose with fluorine modification in the uracil moiety have also been synthesised.[161] Thus, treatment of FdUMP with 1,1′-carbonyldiimidazole in DMF, followed by hydrolysis and condensation with a glucose 1-monophosphate tributylammonium salt, allowed the isolation of the corresponding nucleoside 5′-diphosphoglucose derivative **296** (Scheme 66).

The synthesis and biological evaluation of a triphosphate derivative **299** as a potential anti-HIV agent have been reported by



Series	Base	Yield of **274** (%)	Yield of **275** (%)	Phosphitidylation condition
a	thymine	73	79	A
b	N^6-benzoyladenine	91	71	B
c	N^4-benzoylcytosine	96	62	B
d	N^2-isobutyrylguanine	96	84	B

Phosphitidylation conditions: **A**: $Pr^i_2NP(Cl)O(CH_2)_2CN$, Pr^i_2NEt; **B**: $(Pr^i_2N)_2PO(CH_2)_2CN$, tetrazole

Scheme 61.

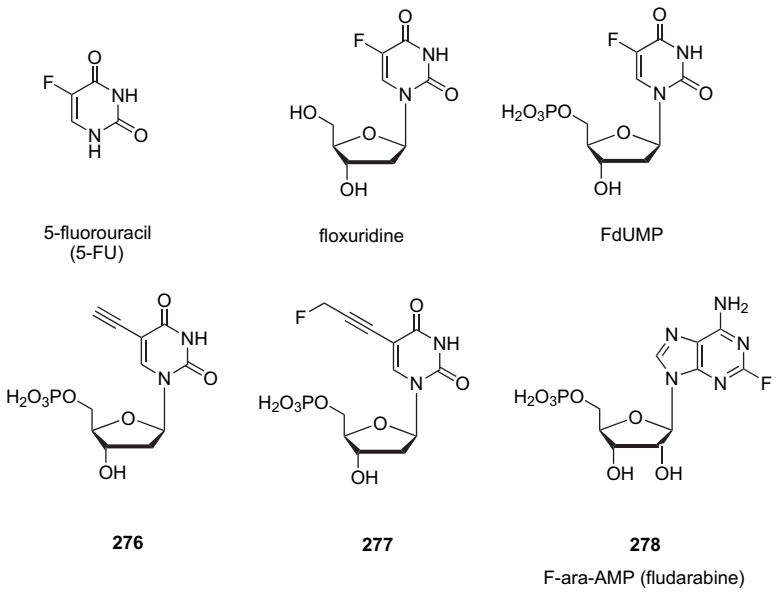
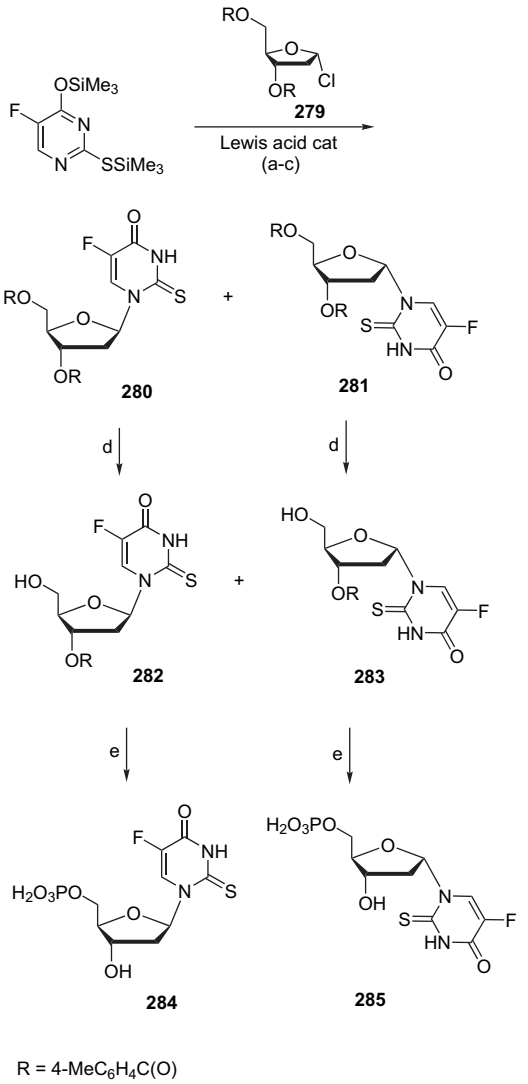


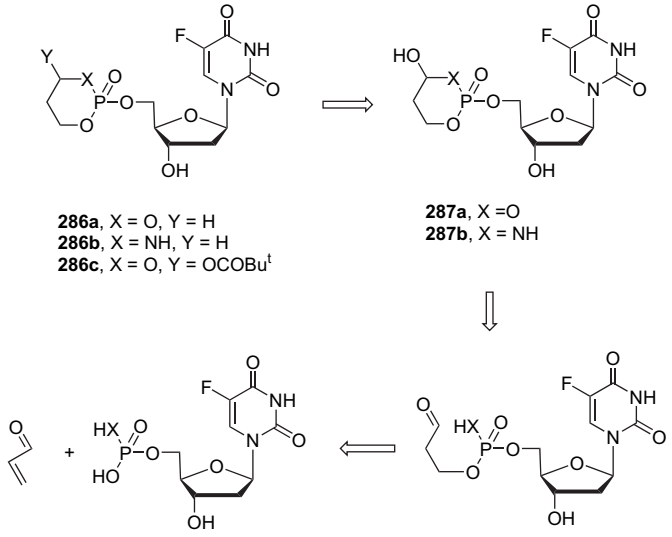
Chart 12. 5-Fluoropyrimidines and their analogues.

Liotta and co-workers (Scheme 67).[162] The monophosphate **298** was generated by the reaction of the nucleoside **297** with phosphorus oxychloride using trimethyl phosphate as the solvent. The monophosphate **298** was activated as its morphine phosphoramidate, which was subsequently combined with pyrophosphate to provide the triphosphate **299**. Although compound **297** was not active up to 100 mM, its triphosphate **299** exhibited comparable anti-HIV activity to 3TC-TP against recombinant HIV RT and wild-type HIV RT (IC_{50}=4.7, 6.9 μM). Furthermore, the triphosphate also showed very good activity against M184I and M184V mutant RT (IC_{50}=6.1, 6.9 μM), which were not inhibited by 3TC-TP.

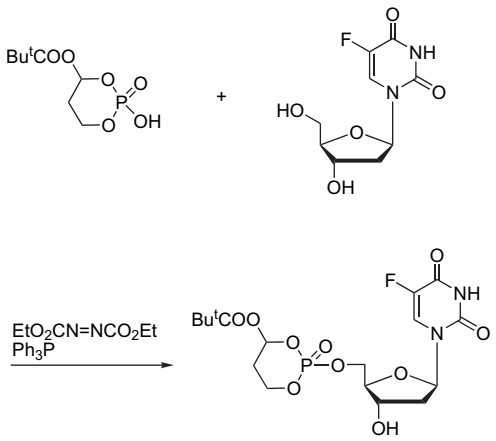
The interesting and very promising biological properties of 2-vinylinosine and its monophosphate have encouraged Nair's research group to synthesise 2-[2-(*Z*)-fluorovinyl]inosine 5′-monophosphate (2-FVIMP) **300** (Scheme 68).[163] This compound was found to be a potent inhibitor of inosine monophosphate



Scheme 62. Reagents: (a) TMS–triflate/dichloroethane, 10% yield, 16:18=1:3; (b) $SnCl_4$/dichloroethane, 30% yield, 16:18=1:2; (c) $TiCl_4$/dichloroethane, 70% yield, 16:18=3:1; (d) NH_3/MeOH, 20 °C; (e) *p*-nitrophenyl phosphate/wheat shoot phosphotransferase, 37 °C.



Scheme 63. Prodrug concept for 5'-(1,3,2-dioxaphosphorinan-2-yl) and 5'-(1,3,2-oxazaphosphorinan-2-yl)-2'-deoxy-5-fluoridines.

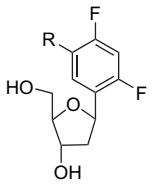


286c

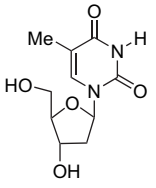
Scheme 64.

dehydrogenase (IMPDH), the enzyme, which catalyses the oxidation of inosine 5′-monophosphate to xanthosine 5′-monophosphate with the concomitant reduction of nicotinamide adenine dinucleotide (NAD^+) to NADH. Inhibition of IMPDH by 2-FVIMP appears to be irreversible with k_{inact} and K_i values of 0.0269 s^{-1} and 1.11 μM, respectively.

During the past decade, there has been a remarkable development in the biochemistry of unnatural *C*-nucleosides containing a fluorinated benzene moiety as a nucleobase analogue.[164] As an example, the non-polar hydrophobic 2,4-difluoro-5-methylphenyl isostere **301** (R=Me) was designed as an unnatural mimic with similar structural, steric and isoelectronic properties to the natural nucleoside thymidine **302**.[165,166]

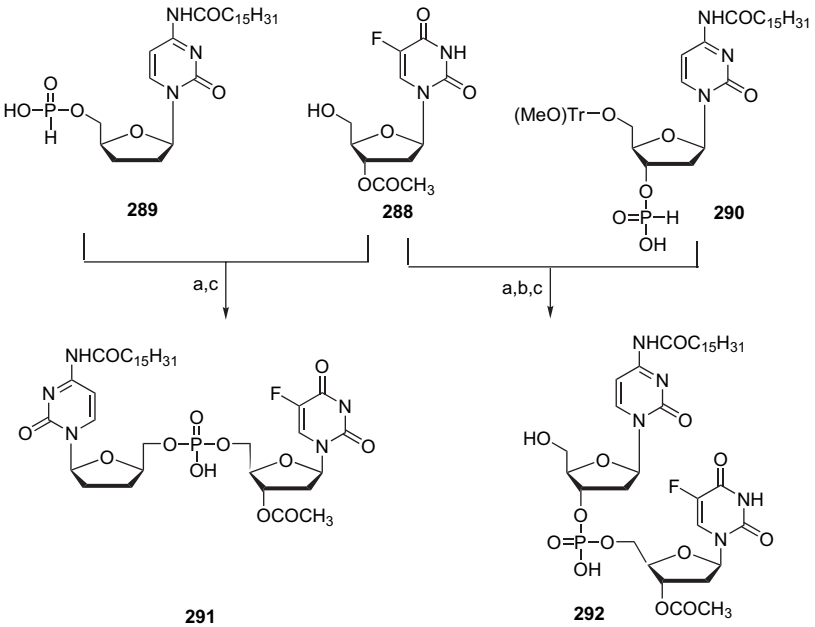


301, R = Me



302, Thymidine

More recent studies have shown that unnatural 5-substituted thymidine mimics **301** (R=H, Me, F, Cl, Br, I, CF_3, CN, NO_2, NH_2) exhibited weak or negligible anticancer and antiviral activity. One plausible explanation for the low cytotoxicity exhibited by these compounds could be their failure to undergo phosphorylation by thymidine kinase to the 5′-monophosphates. This motivated Knaus and co-workers to develop prodrugs that release nucleotides intracellularly thereby circumventing the requirements for intramolecular phosphorylation.[167] A synthesis of the (S_P)-, (R_P)-diastereomeric mixtures of 5′-*O*-*cyclo*-Sal pronucleotide phosphotriesters **305**–**310** starting from chlorophosphite **303a**–**c** and



Scheme 65. Reagents: (a) pivaloyl chloride in pyridine, then I_2 in THF/pyridine/water (16:1:1); (b) *p*-toluenesulfonic acid (2%) in $CHCl_3$/MeOH; (c) column chromatography on silica gel.

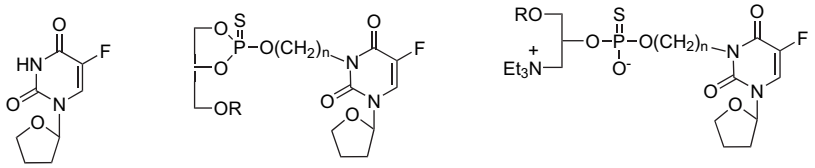
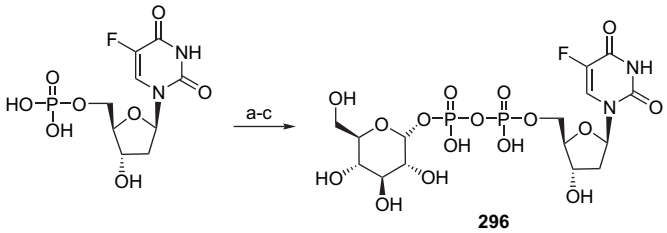
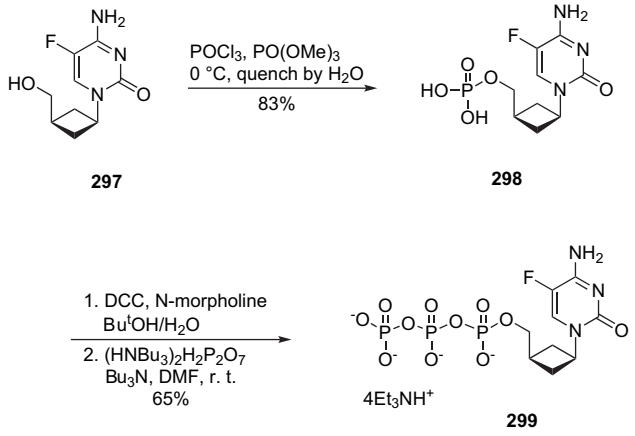


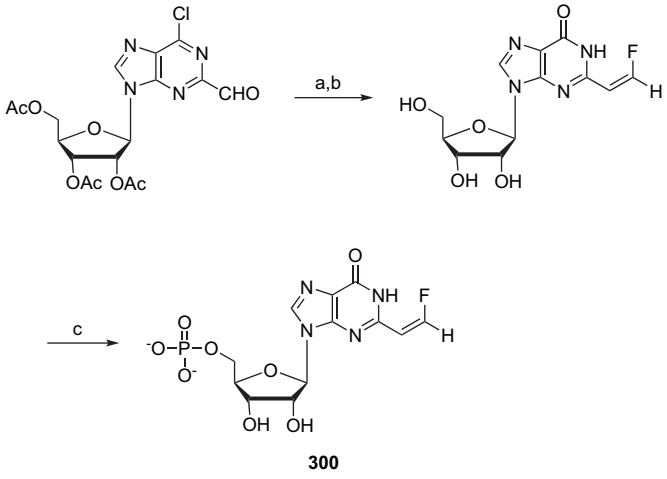
Chart 13. Tegafur and phospholipid–nucleoside conjugates containing 5-fluorouracil moiety.



Scheme 66. Reagents: (a) 1,1-carbonyldiimidazole, DMF, rt; (b) Et_3N 5% in H_2O/MeOH 1:1 rt; (c) glucose-1-monophosphate tributylammonium salt, DMF, rt.



Scheme 67.



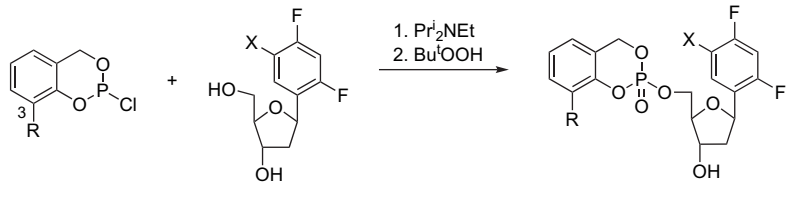
Scheme 68. Reagents and conditions: (a)Bu_3P, $CFCl_3$, DCM, 0 °C, rt; (b) 10% NaOH, rt, 24 h; (c) $POCl_3$, $(MeO)_3PO$.

nucleosides **304a**,**b** is illustrated in Scheme 69. Unfortunately none of these new *cyclo*-Sal pronucleotides showed the expected cytotoxic activities, except for **307** that was more potent than the reference drug, 5-iodo-2′-deoxyuridine.[167]

The easy access to the fluorine-substituted benzene nucleobase analogues resulted in an avalanche of studies on the stacking interactions in non-polar nucleotide isosteres. Examples include the synthesis of novel fluorine-substituted benzene nucleobase analogues and the structural characterisation of DNA-containing unnatural self-pairs,[168] the preparation of a series of non-polar thymidine analogues of increasing size and their incorporation into synthetic DNAs ,[169] the synthesis of 2′-aminoalkyl-substituted fluorinated nucleobases and their influence on the kinetic properties of Hammerhead ribozymes,[170] a study of pentafluorophenylphenyl interactions in biphenyl-DNA[171] and an investigation of fluorous base-pairing effects in the DNA polymerase active site.[172] In particular, Leumann and co-workers prepared the 4,4′-dimethoxytrityl (DMTr)-protected phosphoramidite building block **317** from 2,3,5-tri-*O*-benzyl-D-ribono-1,4-lactone and 4′-bromo-2,3,4,5,6-pentafluorobiphenyl via compounds **311**–**316** (Scheme 70). A thermodynamic analysis using isothermal titration calorimetry of a series of duplexes d(GATGAC(**X**)$_n$GCTAG)–d(CTAG-C(**Y**)$_n$GTCATC), in which **X** and **Y** designate biphenyl (bph) and pentafluorobiphenyl (^{5F}bph) *C*-nucleotides and *n* varies from 0–4, revealed lower duplex formation enthalpies (ΔH) in the ^{5F}bph/^{5F}bph case than in the bph/bph case, and confirmed the higher thermodynamic stability (ΔG) of the fluorinated duplex.[171]

3.7.3. Modification of a phosphate group

Nucleoside phosphorofluoridate monoesters were first prepared by Wittmann via the reaction of nucleoside phosphate monoesters with 2,4-dinitrofluorobenzene.[173] A more general approach to nucleoside 5′-*O*-phosphorofluoridates involves the reaction of the corresponding nucleoside with fluorophosphoric acid in the presence of *N*,*N*′-dicyclohexylcarbodiimide, 2,4,6-triisopropylbenzenesulfonyl chloride or mesitylene-2-sulfonyl-3-nitro-1,2,4-triazole.[62,174] Ribonucleotide analogues with a P–F linkage have been prepared using the reaction of tetra-*n*-butylammonium fluoride (TBAF) with nucleoside-*O*-aryl-3-alkylthiophosphates.[175] Another important procedure for the preparation of nucleoside phosphorofluoridate, phosphorofluoridothioate and phosphorofluoridodithioate monoesters is based on the oxidation of the corresponding *H*-phosphonate and *H*-phosphonothioate monoesters with iodine in pyridine in the presence of trimethylchlorosilane, followed by reaction with triethylamine trishydrofluoride.[176,177] Stec and co-workers have found that the 5′-*O*- or 3′-*O*-protected thymidine 3′-*O*- or 5′-*O*-(2-thiono-1,3,2-oxathiaphospholane) react with triethylammonium fluoride in the presence of DBU, leading to the appropriate phosphorofluoridothioates.[178] Perhaps the most versatile synthesis of nucleoside phosphorofluoridates and

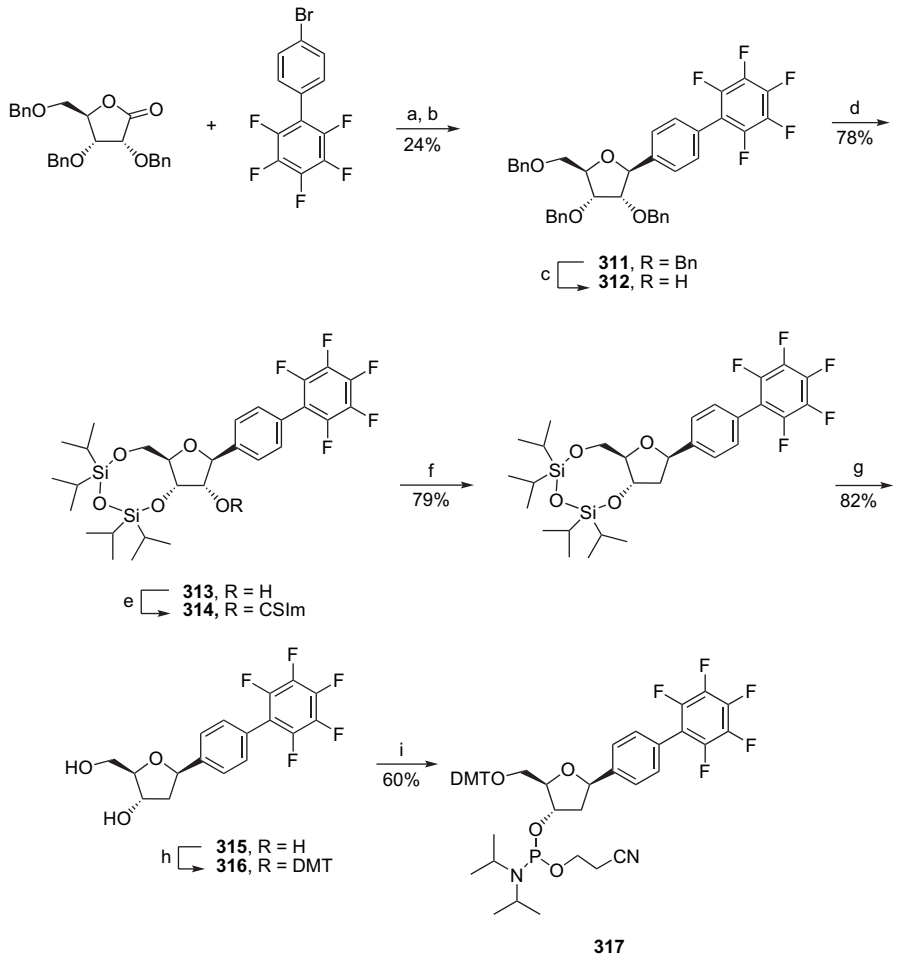


303a, R = Me
303b, R = OMe
303c, R = H

304a, X = I
304b, X = CF_3

305, X = I, R = Me
306, X = I, R = OMe
307, X = I, R = H
308, X = CF_3, R = Me
309, X = CF_3, R = OMe
310, X = CF_3, R = H

Scheme 69.

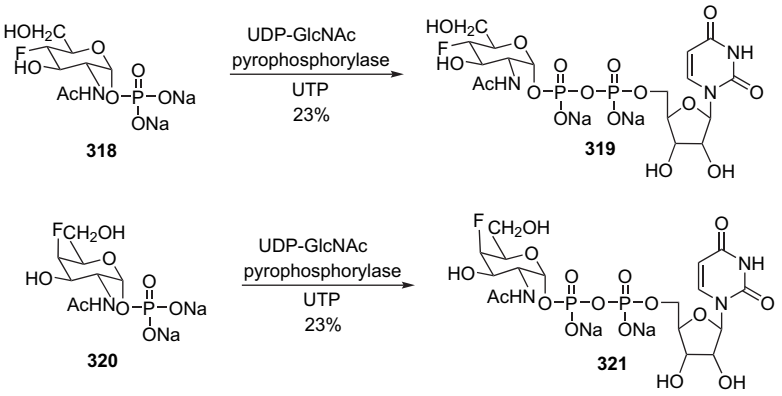


Scheme 70. Reagents: (a) **1** (1 equiv), BuLi (1 equiv), THF, then 2,3,5-tri-*O*-benzyl-D-ribono-1,4-lactone (1 equiv) in THF, −78 °C; (b) Et_3SiH (5 equiv), $BF_3{\cdot}OEt_2$ (5 equiv), CH_2Cl_2; (c) BBr_3 (3.5 equiv); (d) 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (1.2 equiv), pyridine; (e) 1,1′-thiocarbonyldiimidazole (1.2 equiv), MeCN; (f) AIBN (0.2 equiv), tris-(trimethylsilyl)silane, toluene; (g) $(HF)_3{\cdot}NEt_3$ (10 equiv), THF; (h) 4,4′-dimethoxytrityl (DMTr) chloride (1.2 equiv), pyridine; (i) $[(^iPr_2N)(NCCH_2CH_2O)P]Cl$ (1.5 equiv), $^iPr_2NEt$ (3 equiv), THF.

phosphorofluoridothioates is based on phosphoramidite methodology (see Scheme 2).[26]

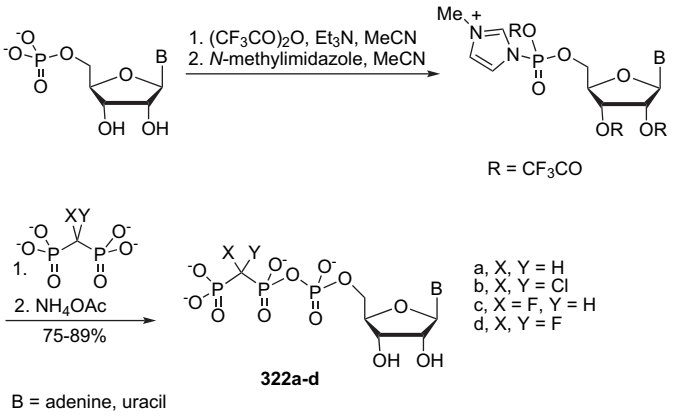
Nishimura and co-workers reported a practical method for the preparation of the fluorine-substituted 2-*N*-acetamidosugar nucleotides, uridine 5′-diphosphate (UDP) 2-acetamido-2,4-dideoxy-4-fluoro-α-D-glucopyranose (UDP-4-FGlcNAc) **321**, by employing a combined chemical synthesis and UDP *N*-acetylglucosamine (UDP-GlcNAc) pyrophosphorylase.[179] Treatment of 4-FGlcNAc-1-P (**318**) or 4-F-GalNAc-1-P (**320**) and uridine 5-triphosphate (UTP) with UDP-GlcNAc pyrophosphorylase affords the fluorinated 2-*N*-acetamidosugar nucleotides **319** and **321** (Scheme 71). These results imply that the fluorine-containing precursors **318** and **320** are accepted as substrates by UDP-GlcNAc pyrophosphorylase.

Further progress in the area of fluorinated nucleotide analogues results from structural modification of the triphosphate unit in oligonucleotides. Thus, 5′-β,γ-fluoromethylenetriphosphate nucleotide analogues have been developed to mimic nucleoside triphosphates. In this case, a fluoromethylene or difluoromethylene moiety replaces the β,γ-bridging oxygen in the terminal



Scheme 71.

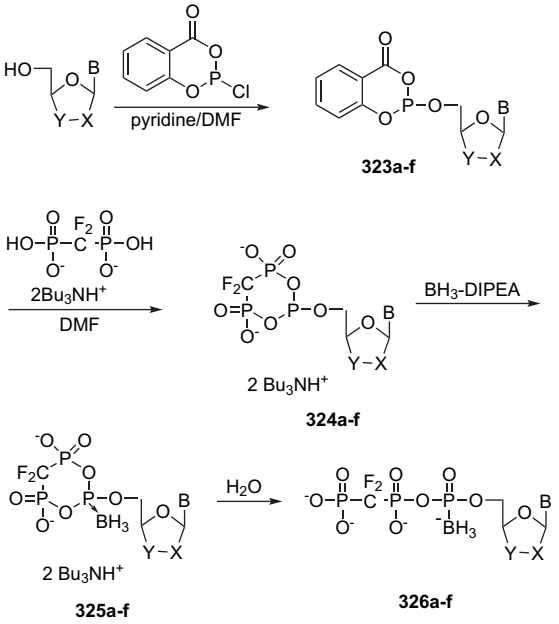
pyrophosphate function. Scheme 72 illustrates an improved method for the synthesis of nucleoside 5′-β,γ-methylene-triphosphates. Nucleoside monophosphates, when activated by trifluoroacetic anhydride and *N*-methylimidazole, efficiently couple with a variety of electron-deficient diphosphonates to give the desired nucleotide analogues **322a–d** in high yield.[180]



Scheme 72.

The synthesis of 2′,3′-dideoxynucleoside 5′-α-*P*-borano-β,γ-(difluoromethylene)triphosphates **326** was the subject of study by Wang's group.[181,182] Treatment of nucleosides with 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one yielded the activated phosphites **323a–f**, which were condensed with bis(tributylammonium) difluoromethylenediphosphonate to form the cyclic triphosphates **324a–f**. Reaction of **324a–f** with diisopropylethylamine–borane complex gave compounds **325a–f**, which after hydrolysis afforded the ddN 5′-αB-βγ-CF_2TPs **326a–f** in moderate yields (Scheme 73).

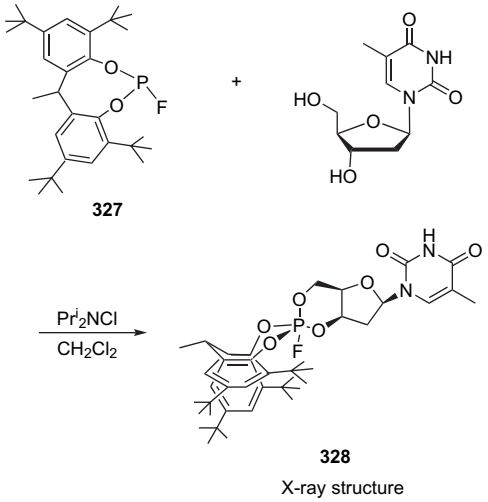
Recently, the first successful synthesis and structural characterisation of a nucleotidyl phosphorane have been reported



a, B = thymine, X = Y = CH_2; b, B = thymine, X, Y = CH=CH; c, B = 5-F-cytosine, X = CH_2, Y = S (L-ribose); d, B = uracil, X = Y = CH_2; e, B = adenine, X = Y = CH_2; f, = 7-deazaguanine, X = Y = CH_2

Scheme 73.

(Scheme 74). The nucleotidyl phosphorane **328** was synthesised in 90% yield by reacting 2,2'-ethylidenebis(4,6-di-*tert*-butylphenyl)-fluorophosphite **327** with thymidine in the presence of *N*-chloro-diisopropylamine. The phosphorane **328** may be considered as a model transition state in the hydrolysis of a structural part of DNA.[183]



Scheme 74.

4. Concluding remarks

Thanks to the important progress in synthetic methodologies that provide more ready access to fluorinated molecules, fluorine-containing phosphate esters are becoming more and more relevant in drug discovery. The various examples described in this report demonstrate that the introduction of fluorine has had a dramatic effect on the metabolism of a phosphate substrate. The complex structure–activity relationships within the active substrate make it difficult to predict sites where fluorine substitution will increase the favourable modes of interaction with enzymatic binding partners. Nevertheless, the research carried out by many chemists has already resulted in some impressive examples of the efficiency of the fluorine–phosphate strategy. Many new fluorine-containing phosphate esters exhibit in vitro and in vivo cytotoxic activities against numerous human and murine tumour cell strains. Another area of great promise is the synthesis of nucleoside phosphates featuring activity against HIV and HBV. Fluorinated phosphate substrates can also serve as mechanistic probes or inhibitors and play a key role in the elucidation of enzyme mechanisms. As an example, the fluorinated analogue of PEP, 3-fluorophosphoenolpuruvate (F-PEP), has been successfully employed in studying the mechanisms of a variety of PEP-utilising enzymes. Further studies to obtain additional details concerning the interactions of fluorinated phosphate substrates with enzymatic binding partners are likely to be the next challenge in organophosphate chemistry. There is an urgent need for coordinated efforts by different research groups to attain these goals. Thus, it can be safely predicted that many new biomedical applications of fluorinated organophosphates await discovery.

Acknowledgements

Financial support from the Science and Technology Center in Ukraine (Grant 3558) is gratefully acknowledged. V.D.R. thanks Centre National de la Recherche Scientifique (France) for a fellowship.

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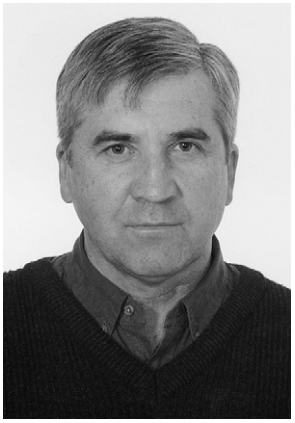
Biographical sketch



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