Phosphorus and Fluorine – The Union for Bioregulators

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The review demonstrates the very high efficiency and usefulness of the fluorine-phosphorus combination in order to synthesize organic molecules for purposes of modern life science. For biochemistry, the "P-F-union" in biomolecules enables investigation of the enzyme structure and mechanism of action more correctly, as well as creation of new anti-body enzymes. Enhancing or regulation of inhibitor properties of these compounds, their stability or selectivity allows creation of new drugs for treatment of numerous serious diseases, especially viral infections and cancer.

Key words: *Fluorine, phosphorus, difluoromethylene, phosphonates, phosphates, inhibitors, biomimics*

Introduction

Among various integration points of chemistry and biology the biomimic approach has already demonstrated its usefulness for biochemistry in a clarification of the structure and function of enzymes, "abzyme" synthesis, and, finally, construction of new highly active pharmaceuticals and bioregulators. In construction of numerous mimics, chemists and biochemists very often use organoelement compounds in order substitute some atoms or groups, and thus change electronically or sterically an interaction of a proper substrate with the target studied.

In the review* we would like to focuss attention of phosphorus chemists on some impressive results from fluorine and phosphorus chemistry areas directed at the synthesis of biologically important compounds. It's very easy to explain our choice – lately both these elements as constructive units of bioactive substances are very popular and, in many cases, very useful.

In contrast to natural phosphorus compounds, only few organofluorine compounds are found in nature (for example – Fig. 1). But fluorine is a small atom with a big "ago" and one of the smallest radii and the greatest electronegativity, which attracts high interest of scientists. The nearly 1.8 million C–F compounds are presented in Chemical Abstracts; in Derwent Drug File 1020 organofluorine compounds are included, almost 5 % of the total, and more than 150 fluorinated drugs currently in use. 10 % of newly registered pharmaceuticals and 40 % of new agrochemicals contain at least one fluorine atom. At the Winter Fluorine Conference of ACS in Florida, James R. McCarthy from Eli Lilly said: "Fluorine will continue to have a major impact in the design of biologically active molecules".

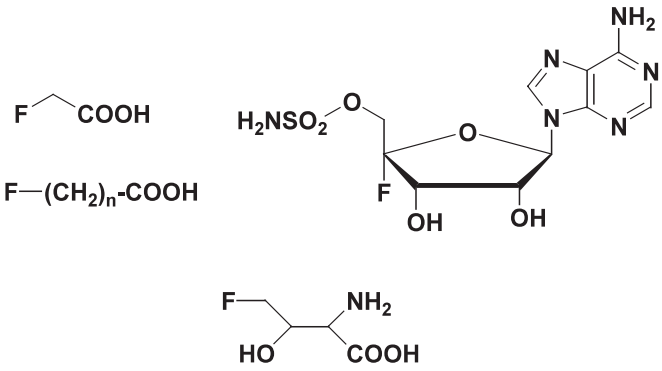

Fig. 1 – *Some natural fluorine-containing compounds*
Slika 1 – *Neki prirodni spojevi koji sadrže fluor*

Substitution of hydrogen by fluorine in organic compounds causes minimal steric effects due its small size (van der Waals radius of 1.35 A and 1.20 A for H) (Table 1), while important pharmacokinetic properties, such as a metabolic stability and absorption, can be modulated in a favorable way. In general, replacement of hydrogen by fluorine is considered as "a **bioisosteric replacement**". Today there are new data on the ligand binding affinity of organofluorine compounds, so called – **Fluorophilicity** and **Fluorophobicity**, that reflect an ability of C–F compounds to interact with H-bond donors, such as N–H or OH– groups of proteins. As well known, the CF_3 group has a pronounced lipophilicity, as reflected by its Hansch substituent parameter π_x of 0.88 (CH_3: $\pi_x = 0.56$, CH_2CH_3: $\pi_x = 1.02$). Thus, the introduction of F atoms into aromatic rings greatly affects aromatic-aromatic interactions by influencing the electronic nature of the rings. Fluorine NMR opens also the possibility to study protein stability, ligand-receptor interactions and other important structural and biochemical properties as well, as pharmacodynamics and accumulation of drugs in a body or tissues.

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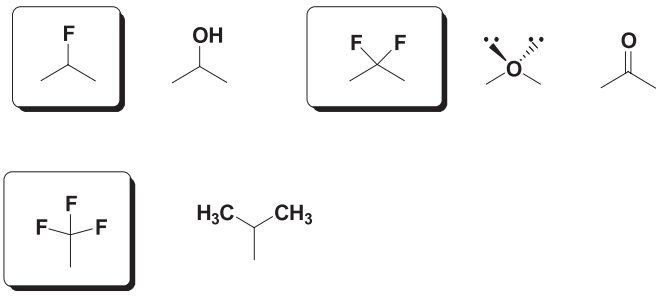
T a b l e 1 – *Comparison of some parameters of* C–H, C–F *and* C–OH *bonds*
T a b l i c a 1 – *Usporedba nekih parametara veza* C–H, C–F i C–OH

C–X	Ionization potential Ionizacijski potencijal kcal/mol	Electron affinity Afinitet za elektrone kcal/mol	Van der Waals radius, Å Van der Waalsov polumjer	Electron affinity Afinitet za elektrone	Bond energy Energija veze kcal/mol, CH_3–X	Bond length Duljina veze CH_3–X, Å
H	313.6	17.7	1.20	2.1	99	1.09
F	401.8	79.5	1.35	4.0	116	1.39
O (OH)	310.4	33.7	1.40	3.5	86	1.43

In addition to hydrogen bonding the lone electron pair of a fluorine atom can also play a significant role in coordination with metals in a similar manner. If we remember that many enzymes contain a metal ion in the active centre, we can imagine the possibility of a **C–F…Metal** interaction or additional binding of fluorine-containing substrates and some inhibitor properties of the molecules. Recently, investigations of a fluorine rigid inhibitor to map the fluorophilicity/fluorophobicity of the active site in thrombin by X-ray crystal-structure analysis of the protein-ligand complex revealed favorable C–F…H–C–C=O and C–F…C=O interactions of the 4-F substituent of the inhibitor with the backbone unit H–C–C=O of Asn-98.[1] The importance of these interactions was further corroborated by the analysis of small-molecule X-ray crystal-structure searches in the Protein Data Base (PDB) and the Cambridge Structural Database (CSD). The C–F…C=O interactions are observed for both aromatic and aliphatic C–F units and a variety of carbonyl and carboxyl derivatives. Similar orientation preferences are also seen in the dipolar interactions C–F…C=N, C–F…C–F, and C–F…NO_2, in which the F atoms interact at sub van der Waals distances with the electrophilic centers.

Due to the strong C–F bond energy, physiologically active fluorinated compounds can be resistant to metabolic degradation. Thus, fluorinated compounds are rendered resistant to oxidation because they have much greater bond energy than C–H or C–X (X = Br, Cl) bonds. The lipophilic properties of X–CF_3 group result in better passing of the fluorine-containing molecules through the cell membranes and reaching active sites without destruction. As a result, drug potency is increased and side effects are suppressed.

The fluorine atom has been used with great success as a replacement for a **hydroxy** group, and CF_2 group has been used as a mimic for the **oxygen atom**, especially, in phosphate mimics (Fig. 2). There are many publications on the impressive substitution of P–O–P bond by P–CF_2–P group in biologically important organic phosphates. David O'Hagan noted that "***Replacement of a methylene for a difluoromethylene group (CH_2 for CF_2) can be much more dramatic than the single substitution***".[2] Indeed, one of the most important peculiarities of fluorine and the fluorine-containing group is the electronic influence on adjacent surroundings that principally changes their chemical character, sometimes dramatically. As a consequence, fluorine introduction in a biomolecule may change the "normal" route of its interaction with a biological target or the direction of binding. That is why fluorine is more and more popular in construction of new pharmaceutical and biochemical tools.



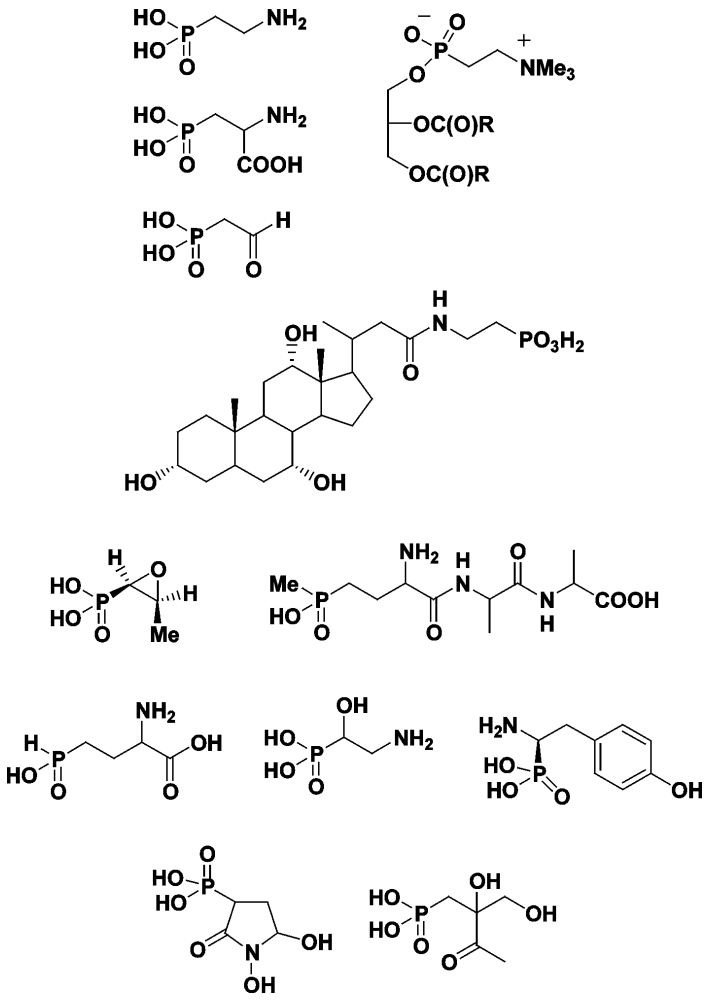
F i g. 2 – *Fluorine mimics of some functional groups*
S l i k a 2 – *Fluor "imitira" neke funkcionalne skupine*

The comparison of phosphorus with fluorine (by the manner early used for fluorine) is a very difficult task: in contrast to the fluorine atom as a single substituent near the carbon atom the phosphorus atom in organic substances is surrounded in most cases by additional groups influencing various characteristics of the residue. Phosphorus in organophosphorus compounds may be in various coordination numbers, as a rule – from three to five. The stereochemistry of phosphorus compounds is much more complicated in contrast to fluorine compounds. Bond energy and bond distance P–X in organophosphorus compounds strongly connected with a nature of X and surrounding at phosphorus and are changed in wide ranges. Phosphorus atom would play a role of the key reaction center and participates in direct interaction with a substrate in contrast to fluorine.

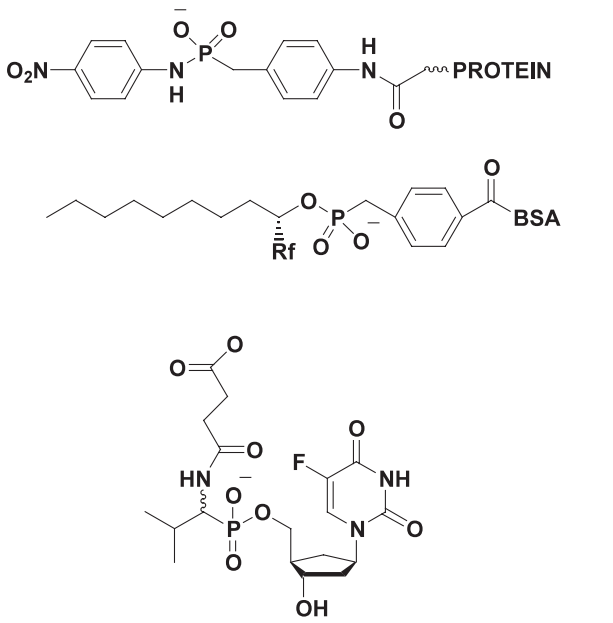
Organic phosphates are well-known to play the "key function" in various biochemical processes. Starting from 1959 after isolation of the first natural phosphonate AEP – aminoethylphosphonic acid by Horiguchi and Kandatsu the natural compounds with C–P bonds more than 20 classes were found in numerous organisms.[3] Some of them are shown in the Fig. 3.

These compounds were discovered in hundreds of animals and microorganisms, in free form or bound to structural components of lipids and proteins. Many of these compounds are attractive due to their antibacterial, antiviral, antibiotic, pesticide, anticancer and enzyme inhibitor properties. Mainly, this activity has been attributed to the structural similarity of phosphonic and phosphonic acids to biologically important phosphates and stability of P–C bond under hydrolysis.

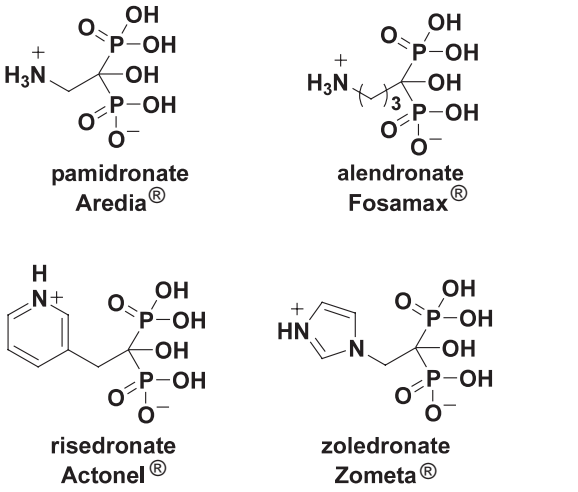
The well known so-called "old" area of application of organophosphorus compounds is agrochemistry and weapons.



F i g. 3 – *Some phosphorus-containing natural compounds*
S l i k a 3 – *Neki prirodni spojevi koji sadrže fosfor*



F i g. 4 – *Phosphorus mimics of tetrahedral transition state structure of some enzymes*
S l i k a 4 – *Fosfor "imitira" tetraedrijsku strukturu prijelaznog stanja nekih enzima*



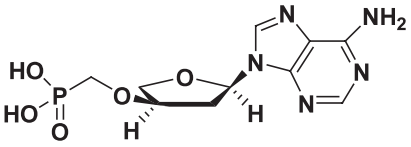
F i g. 5 – *Bisphosphonates of medical application*
S l i k a 5 – *Bisfosfonati koji se primjenjuju u medicini*

Only rare phosphonates have been applied as pharmaceuticals (*in comparison with fluorine*). But revently we can see a growing interest in organophosphonates as very powerful inhibitors or substrates of various enzyme and biochemical processes, and finally, new promising pharmaceuticals. Some phosphonic acid derivatives have been successfully applied as transition state mimics to construct "abzymes" or "catalytic antibodies" (Fig. 4). Phosphonic acid residue excellently mimics the tetrahedral transition state structure of some hydrolytic enzymes. Connection of these residues with proteins creates haptens, which induce antibody "library" production in cell with properties similar to natural enzymes.[4] These abzymes catalyze hydrolysis processes of various substrate, reduction and oxidation as well as prodrug activation to release active component.[5]

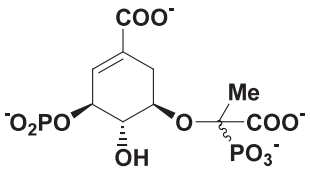
A new stimulus to search specific bioactivity in organophosphorus compounds was induced by bisphosphonates. Detailed biochemical studies in the bisphosphonate area resulted in discovering a new important class of drugs currently used to treat osteoporosis, Haget's disease, malignancy hypercalcemia (Fig. 5). These compounds are inhibitors of **farnesyl diphosphate synthase** (FPPS) in osteoclasts decreasing level of protein prenylation. Bisphosphonates demonstrate also anti-parasitic activity, stimulate human γ,δ-T cells and are interesting as vaccines for B cell malignancies.[6]

Indeed, in last years, numerous new data have been presented data on the various activities of organophosphorus compounds. For instance, hydroxyphosphonate derivative **1** (PMDTA) showed potent anti-HIV-1 and HIV-2 activity EC_{50} = 2.53 μM (PMDTA); the incorporated PMDTA fits very well in the active site pocket of **HIV-1 reverse transcriptase**.[7] Other compounds, also hydroxy phosphonic acid derivatives, which are mimics of tetrahedral transition state, display competitive inhibitor properties for enzyme ***5-enolpyruvylshikimate-3-phosphate synthase*** (EPSPS), which catalyzes the shikimate pathway and is the target of

the broad-spectrum herbicide glyphosate. The (*S*)-phosphonate **2** (K_{iS3P} = 750 nM), whose configuration corresponds to that of the genuine tetrahedral intermediate, is a much weaker inhibitor than the (*R*)-phosphonate analog (K_{iS3P} = 16 nM).[8] (Fig. 6).



1 (PMDTA)



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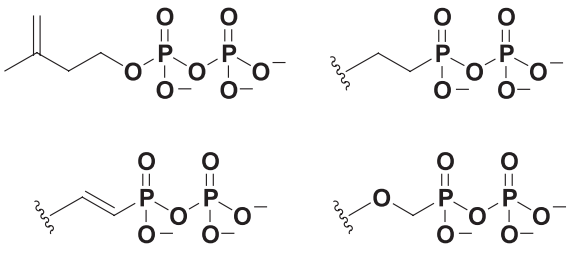
F i g. 6 – *Phosphonate inhibitors of HIV and EPSPS*
S l i k a 6 – Fosfonatni inhibitori HIV-a i EPSPS-a

Several prenyl and alkenyl pyrophosphonate with different chain lengths as new isosteric analogues of natural prenyl pyrophosphates (IPP) were tested as activators of γ,δ-T-cells of human blood lymphocytes (Fig. 7). Several of them appeared to be better activators of γ,δ-T-cell proliferation than IPP. These results open the perspective of a potential use of isoprenoid pyrophosphonates as specific immunoregulatory molecules. T cells of human blood lymphocytes are known produce and to promote strong cytotoxic activity against many pathogens that are implicated in several human infectious diseases.[9]

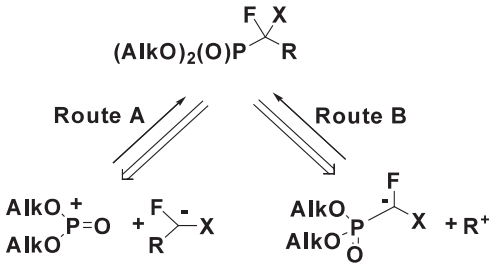


F i g. 7 – *Phosphonate analogues of natural prenyl pyrophosphates (IPP)*
S l i k a 7 – Fosfonatni analozi prirodnih prenil-pirofosfata (IPP)

Of course, the introduction of fluorine atoms or fluorinated groups into the "basic" structure of the organophosphorus molecule may be a powerful tool in biochemical research and development of new drugs, first of all using "mimic" properties of fluorine, its peculiar electronic effects. Phosphonate residue is used in many cases in order to substitute the labile phosphate ester oxygen by carbon to obtain stable phosphate analogs. About 25 years ago, G. Blackburn proposed the excellent idea to use fluoromethylene phosphonates as phosphate mimics, which is based on **isosteric** relationship of these compounds. Moreover, α-halophosphonates are argued to be also **isopolar** analogs of the parent phosphates.[10] *Ab initio* calculations been published by G. Thatcher and A. Campbell,[11] have shown some differences and similarities between fluoromethyl phosphonates and parent phosphates, but the conclusion was that the formers would be "***potentially potent transition-state analogs inhibitor for phosphoryl transfer enzymes***" and "***a powerful arsenal of biological probes for examination of the mechanism and active sites of enzyme***".

Syntheses and bioactivity of fluorine-containing phosphonates

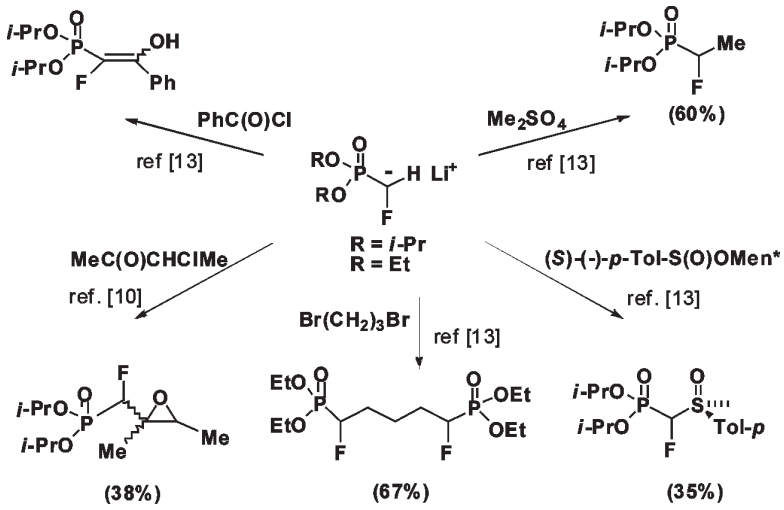
It is interesting also to note the next words from the conclusion: "**no general route for synthesis of the difluoromethylene phosphonates exists**". Today after 10 years, we can see numerous publications of synthesis, which open various possibilities for building desired fluorophosphonate analogs of natural and unnatural compounds as shown by the retrosynthetic Scheme 1. A comprehensive review on the synthesis of mono- and difluoromethylenephosphonates has been published recently.[12]



X = H or F; R = Alk, TMS, $(AlkO)_2P(O)$ et al.

S c h e m e 1/S h e m a 1

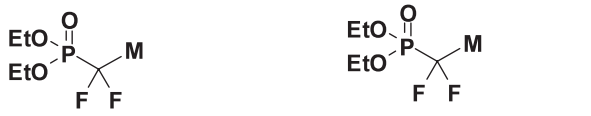
The **first route** (A) represents the carbon-phosphorus bond-forming strategy, and the **second route** (B) represents the carbon-carbon bond-forming reactions based on α-fluoroalkylphosphonate carbanion and functionalized carbon electrophile. In principle, these two strategies complement each other. In practice, the carbon-phosphorus bond-forming reactions are only useful for the preparation of simple fluorinated phosphonates. In the last decade, carbon-carbon bond-forming strategy based on the use of α-fluoro phosphonate carbanions has been widely recognized as a versatile approach to $(RO)_2P(O)CHF$– and $(RO)_2P(O)CF_2$-functionalized molecules. The anions $[(RO)_2P(O)CXF]^-$, where X = H, F or any suitable functional group, can be readily generated starting from the corresponding phosphonate esters by standard deprotonation or halogen/metal exchange reactions and attached to a range of organic electrophiles, Scheme 2).[13] Many of α-fluorophosphonate carbanions show enhanced stability compared to the corresponding non-phosphorylated carbanions and thus are easily handled and studied. Lastly, α-fluoro phosphonate carbanions allow shorter synthetic routes by avoiding, for example, the use of protection-deprotection steps as well as functional group interconversions.



Scheme 2/Shema 2

Synthetic possibilities allowed obtaining additional data on the similarity between $–CF_2$-group and –O– in phosphates. For instance, D. O'Hagan determined the crystal structures of the aminophosphonic acids **7**, **9** and **10**, and revealed that the P–C–C angle of the CF_2-phosphonate (116°) is closer than that of the $–CH_2–$ (112°) and –CHF– (113°) phosphonates to the C–O–P angle (118°) of the phosphate group of 8. Thus, the CF_2-phosphonate appears to approximate the geometry of the phosphate group most closely in the series, at least in the ground state. Therefore, on geometric and ionic grounds the CF_2-phosphonate emerges as a good phosphate mimic. On the other hand, the substitution of oxygen with CF_2 will have an increased steric impact, as the fluorine atoms are resident in the space previously occupied by the lone pairs of oxygen.

Chart 1/Dijagram 1



Nucleophilic substitution of alkyl halides with lithiodifluoromethylphosphonates has been used in the preparation of several analogs of naturally occurring phosphates such as the amino acid derivative **3** and the nucleoside phosphorylase inhibitor **4.**

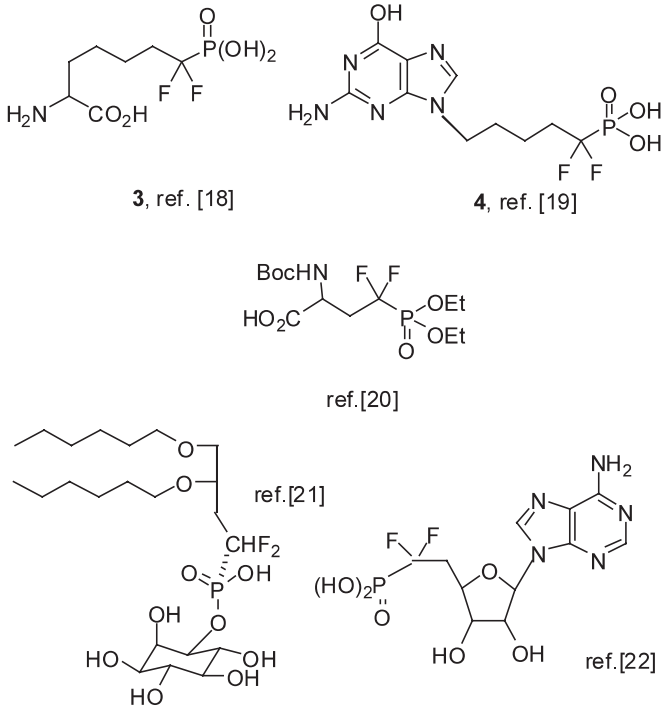


Fig. 8 – *Some difluoromethylene phosphonates prepared from lithiodifluoromethylphosphonate*

Slika 8 – *Neki difluormetilenski fosfonati pripravljeni iz litijeva difluormetil-fosfonata*

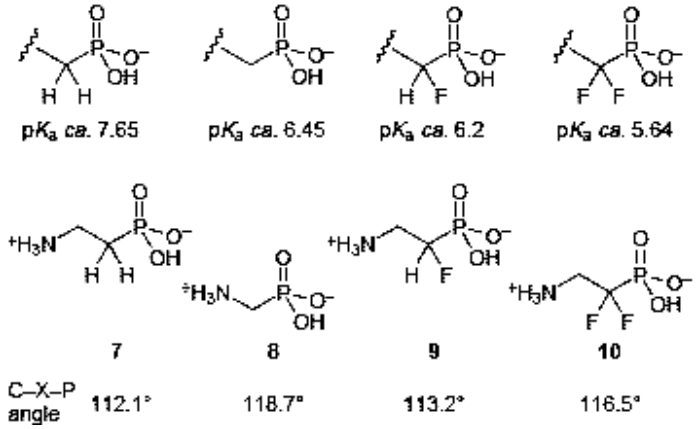


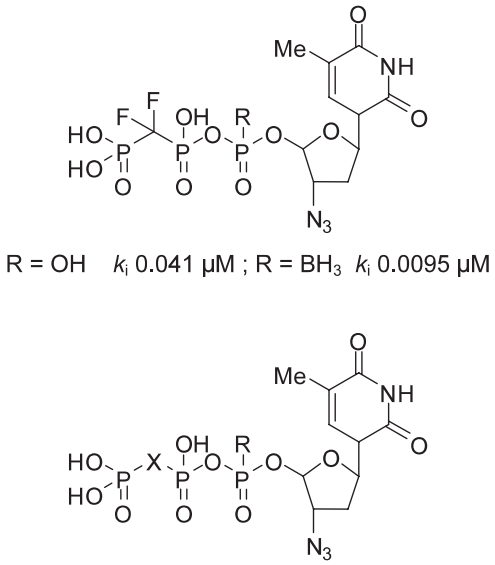
Fig. 9 – *Phosphate/phosphonate acidity (second deprotonation) and C–X–P angles as obtained from the X-ray structures of compounds **7–10***[2]

Slika 9 – Kiselost fosfat/fosfonata (druga deprotonacija) i kutovi C–X–P dobiveni iz rendgenskih struktura spojeva **7**-**10**[2]

$(RO)_2P(O)CF_2M$ species, where M = Li, MgCl, ZnBr, CdBr, and Cu, act as a masked $[(RO)_2P(O)\ CF_2]^-$ carbanionic equivalent and can be prepared by a variety of methods of which the main ones are: (i) deprotonation of $(RO)_2P(O)CF_2H$ with a suitable organometallic base, (ii) halogen-metal exchange between $(RO)_2P(O)CF_2Br$ and alkyllithium or Grignard reagent; (iii) direct insertion of metal (Zn or Cd) into the carbon-halide bond of $(RO)_2P(O)CF_2Br$ or $(RO)_2P(O)CF_2I$; and (iv) desilylation of $(RO)_2P(O)CF_2SiMe_3$ initiated by means of fluoride ion (Chart 1).[14–17]

Recently, a series of AZT triphosphate mimics (AZT P3Ms) with difluoromethylene and R-borano-*a*,*c*-difluoromethylene unit has been synthesized and their inhibitory effects evaluated on HIV-1 reverse transcriptase as well as their stability in fetal calf serum and in CEM cell extracts. A number of AZT P3Ms exhibited very potent inhibition of HIV-1 reverse transcriptase. Modifications at the *a*,*c*-bridge of triphosphate rendered the AZT P3Ms with varied activities

(K_i from 9.5 to 0.500 nM) while modification at the R,*a*-bridge of triphosphate led to weak AZT P3M inhibitors. The results imply that the AZT P3Ms were substrate inhibitors, as is AZT triphosphate. The most active compound, AZT 5.-R-*Rp*-borano-*a*,*c*-(difluoromethylene)triphosphate (AZT 5.-RB-*ac*CF2TP), is as potent as AZT triphosphate with a *Ki* value of 9.5 nM and at least 20-fold more stable than AZT triphosphate in the serum and cell extracts. Therefore, for the first time, a highly active and stable nucleoside triphosphate mimic has been identified, which is potentially useful as a new type of antiviral drug. The promising triphosphate mimic, 5.-R-borano-*a*,*c*-(difluoromethylene)-triphosphate, is expected to be valuable to the discovery of nucleotide mimic antiviral drugs.[23,24]

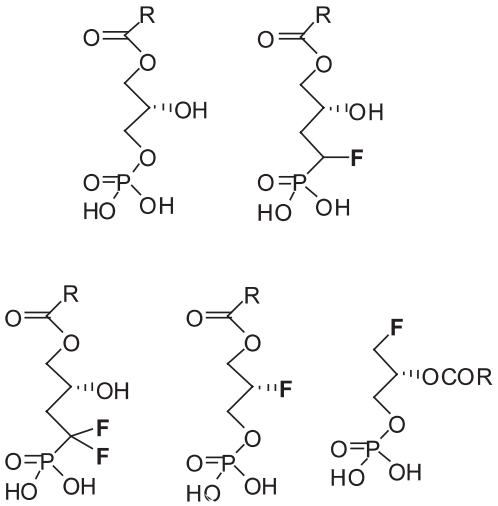


F i g. 10 – *Inhibition of HIV-1 Reverse Transcriptase by AZT triphosphate mimics*

S l i k a 10 – *Inhibicija reverzne transkriptaze HIV 1 trifosfatnim AZT-"imitatorima"*

Interest in phosphatase-resistant phosphonolipids as phospholipids analogs has grown substantially with the recognition that lysophosphatidic acid (LPA, 1-or 2-acyl-*sn*-glycerol 3-phosphate) is an important mitogenic signal in ovarian cancer and in normal cell proliferation and migration. Biology of LPA is mediated via interaction with seven-trans- membrane G-protein receptor and nuclear hormone receptor. In a series of papers, Prestwich and co-workers have prepared a variety of new phosphonate analogs of natural phospholipids, in which the bridging oxygen in the monophosphate was replaced with a CHF or CF_2 moiety (Chart 1).[25] PA is known to be an intracellular lipid second messenger that regulates a growing list of signaling proteins, including several protein kinases and phosphatases. PA has also been implicated as a mediator of the mitogenic action of various growth factors and hormones in mammalian cell.

CHF-phosphonate analog of LPA activated Ca-release in LPA-transfected insect cell at a concentration 1000-fold lower than 1-oleyl-LPA and has increased the half-life in cell culture. This activation was stereoselective – (2*S*)-enantiomer showed 100-fold more activity than (2*R*)-enantiomer.[26]

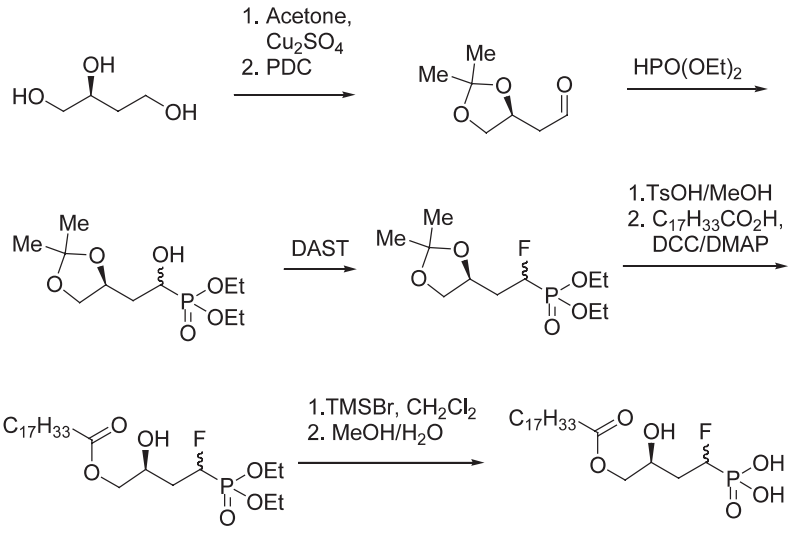


F i g. 11 – *sn-O-Acyl lysophosphatidic acid (left, LPA) and its fluorinated phosphonate analogs*

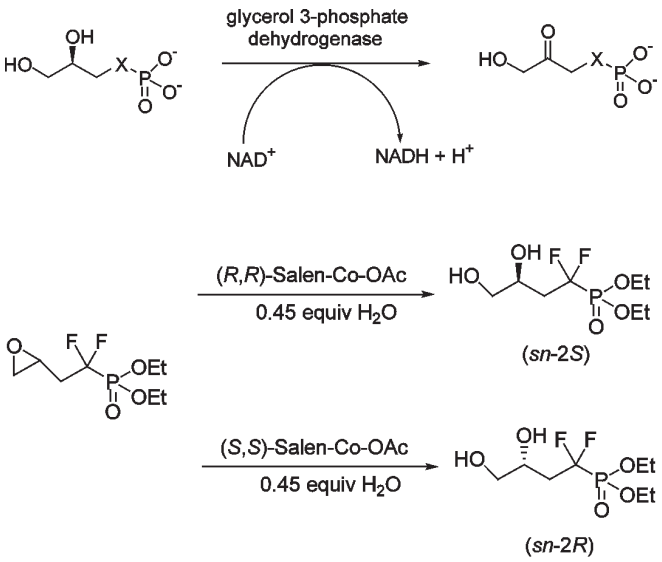
S l i k a 11 – *sn-O-Acil-lizofosfatidna kiselina (lijevo, LPA) i njezini fluorirani fosfonatni analozi*

The next Scheme 4 shows one of the routes to the chiral difluorophosphonates starting from fluorinated phosphonate carbanions. 3(*S*),4-Dihydroxybutylphosphonate **12** and the corresponding CHF- and CF_2-phosphonates **13** and **14**, the isoelectronic and isosteric analogs of biologically important *sn*-glycerol 3-phosphate **11**, are substrates for NADH linked *sn*-glycerol 3-phosphate dehydrogenase.[14]

G. Blackburn synthesized conformationally restrained bisphosphonate analogs of 1,3-*bis*-phosphoglyceric acid and evaluated as inhibitors of 3-PGK; binding showed good correlation with the state of ionization of the phosphonic acids. Some of the bis-α-fluorophosphonates **15-16** have submicromolar K_i values.[27]



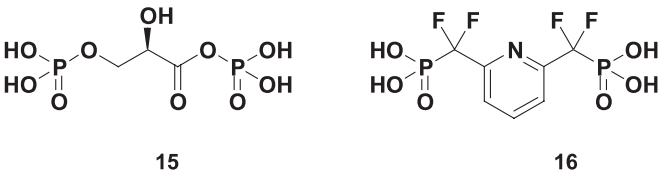
S c h e m e 3 / S h e m a 3



		K_m/mmol dm^{-3}	V_{rel}
11	X = O	0.2	1.0
12	X = CH_2	0.18	0.8
13	X = CHF	0.17	0.9
14	X = CF_2	0.73	0.8

Scheme 4/Shema 4

Chart 2/Dijagram 2



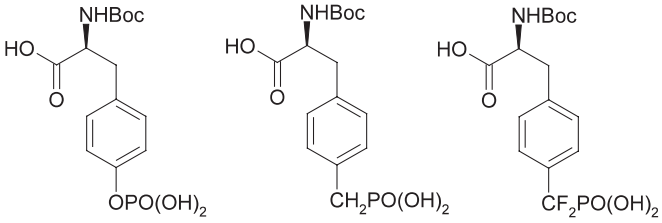
15 **16**

Amino acids with remote –CF_2–P(O)$(OH)_2$ residue are of interest as potential biologically active compounds and components of modified peptides, and are useful for elucidation of enzyme mechanisms and as enzyme inhibitors. This concept has been successfully applied for preparation of phosphatase resistant difluoromethylene analogs of phosphoserine β-aspartyl phosphate and phosphortyrosine.

Phosphotyrosyl (pTyr) residues play important roles in cellular signal transduction by facilitating recognition and binding necessary for critical protein-protein interactions, and for this reason pTyr motifs represent attractive starting points in the development of signaling antagonists. Although the phosphoryl moiety is central in these phenomena, its incorporation into signaling inhibitors is contradicted due to enzymatic lability and limited bioavailability associated with phosphate esters. To address these limitations, T. Burke realized an entire field of study devoted to the design and utilization of pTyr mimetics.[28]

Protein tyrosine phosphatases (PTPs) are signaling enzymes that control a diverse array of cellular processes. Malfunction of PTP activity is associated with a number of human disorders. Recent genetic and biochemical studies indicate that PTPs represent a novel platform for drug discovery.[29]

Chart 3/Dijagram 3



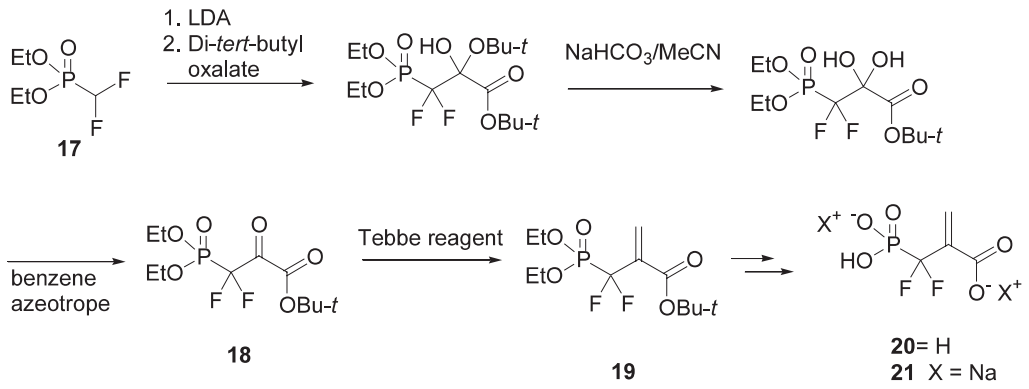
Phosphonate analogs of phosphotyrosine have been prepared and compared CH_2-, CHF- and CF_2-phosphonates directly in a biological system. The phosphonodifluoromethyl phenylalanine (F2Pmp) is superior to phosphonomethyl phenylalanine as a non-hydrolyzable phosphotyrosine mimetic.[30]

The difluoromethyl moiety increases the inhibitory potency of a F2Pmp-containing peptide over a Pmp-containing counterpart by 1000-fold toward the protein tyrosine phosphatase (PTPase), PTP1. Fluorine substitution at the methylene carbon have the double effect of lowering the phosphonate pK_a2, as well as introducing hydrogen bonding interactions similar to the phosphate ester oxygen in pTyr. Both the monoanion and the dianion forms of the phosphonate bind PTP1 with equal efficiency. The two fluorine atoms in F2Pmp may be able to interact with active site residues in the enzyme in a fashion analogous to that involving the phenolic oxygen and side chains in the active site of PTP1. K_i measurements for a simple phosphonic acid, Pmp-(Asp-Ala-Asp-Glu-pTyr-Leu) and F2Pmp-containing peptides suggest that although the principal recognition element is F2Pmp itself, the surrounding amino acids are required for high affinity binding. The binding of a high affinity ligand, Ac-Asp-Ala-Asp-Glu-F(2)Pmp-Leu-NH(2), to PTP1B (K(d) = 0.24 μM) is favored by both enthalpic and entropic contributions.[31]

A series of peptide analogs (Gly-pX-Tyr-Val-Pro-Met-Leu) was assessed for their ability to bind the C-terminal SH-domain of phosphatidylinositol-3-kinase and were found to bind in the following order; CHOH < CH_2 < CHF < CF_2 = O where the CF_2-phosphonate was the optimal phosphate mimic in this case. Different biological systems respond differently to the CHF- and CF_2-phosphonate analogs and clearly each has to be assessed individually.[32]

Tripeptide Bz-Glu-F(2)Pmp-F(2)Pmp-NH2 is also a low nanomolar inhibitor of PTP-1B that shows selectivity over several protein tyrosine phosphatases (IC_{50} of 40 nM).[33] The tripeptide having the sequence Glu-Phe(CF2P)-Phe(CF2P) is a potent and selective inhibitor of PTP1B. The crystal structure of PTP-1B in complex with BzN-EJJ-amide indicate that the high inhibitory potency is due to interactions of several of its chemical groups with specific protein residues and also revealed an unexpected binding orientation for a bisphosphonate inhibitor on PTP-1B with the participation of the second difluorophosphonomethyl phenylalanine (F(2)PMP) moiety.

A novel series of inhibitors of non-peptide structure that contain an aryl α,α-difluoro-β-ketophosphonate group has



Scheme 5/Shema 5

been synthesized and evaluated against protein tyrosine phosphatase 1B.[34] These compounds exhibit strong inhibitory activity, the best of which has a K_i value of 0.17 μM. These results demonstrate that aryl α,α-difluoro-β-keto-phosphonates are powerful phosphotyrosine mimics for development of potent PTP inhibitors. Other non-peptide inhibitors with similar structure and difluoromethylenephosphonate residue are also the active inhibitors of PNP 1B in nanomolar concentration.[35] In addition, these compounds were orally bioavailable and active in the animal models of non-insulin dependent diabetes mellitus (NIDDM).[36] It is necessary to mark intensive studies in the area of non-peptide inhibitors by S. Taylor[37] and S. Shibuya groups.[38]

Analogs of Phosphonoenolpyruvate (PEP). 2-[(Dihydroxyphosphonyl)difluoromethyl]propenoic acid **20** in addition to being isopolar and isosteric with PEP, was envisioned to be a potential Michael acceptor which could bind irreversibly to an enzyme site for which PEP is a substrate. The synthesis of disodium salt of **21** is shown in Scheme 5.[39] Disodium salt of **20** showed irreversible time-dependent inhibition of EPSP synthase, which catalyzes the transfer of carboxyvinyl group from phosphoenol pyruvate (PEP) to 5-hydroxyl group of shikimate 3-phosphate to produce 5-enolpyruvoylshikimate 3-phosphate as an intermediate in the biosynthesis of essential aromatic amino acids. EPSP synthase is inhibited by herbicide Glyphosate – widely used in agriculture.

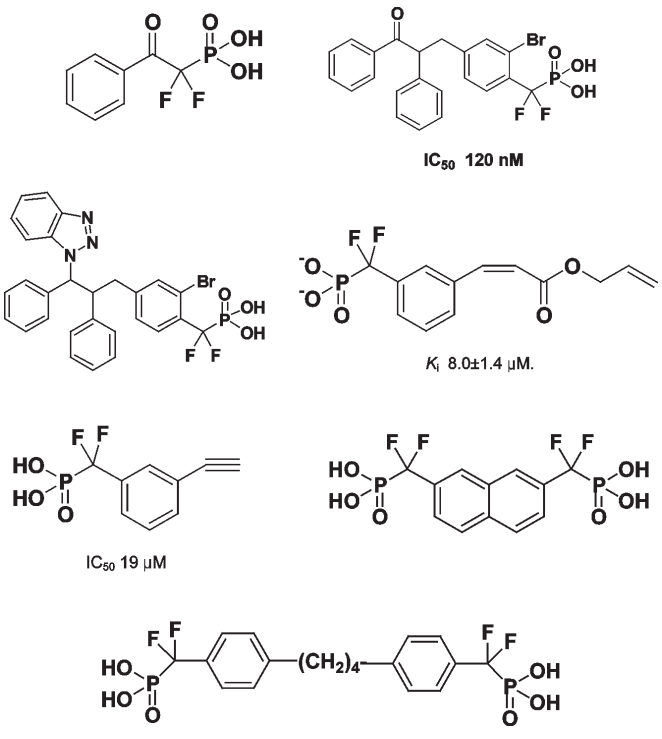
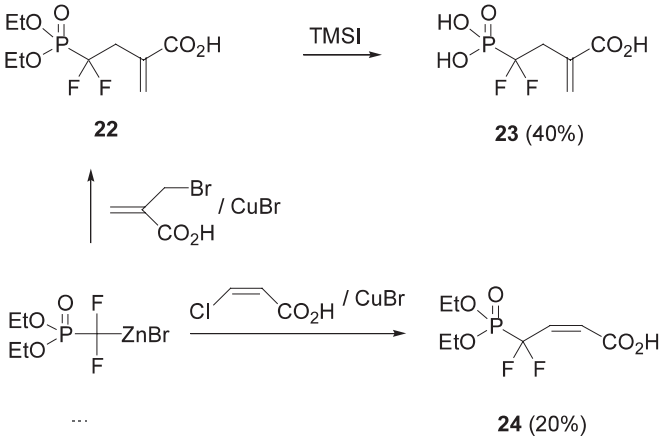


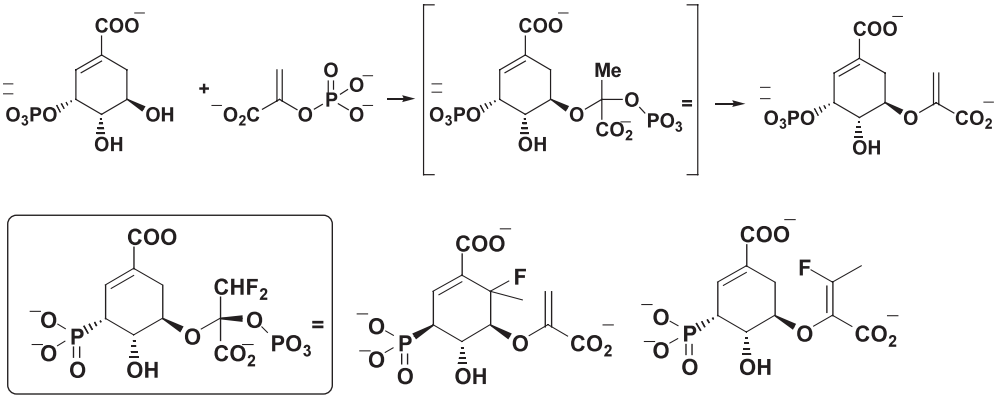
Fig. 12 – *Some non-peptide difluorophosphonate inhibitors*
Slika 12 – *Neki nepeptidni difluorfosfonatni inhibitori*

New types of phosphonate analogs of PEP have been developed by Kawamoto and Campbell (Scheme 6).[40] Diethyl 4,4-difluoro-4-(diethoxyphosphonyl)-2-methylenebutanoate **22** was prepared by the reaction of the zinc reagent with 2-(bromomethyl)acrylic acid in the presence of a catalytic amount of CuBr. Reaction of Zn-difluorophosphonate with *cis*-3-chloroacrylic acid afforded compound **24** in the same *Z*-configuration as the starting material. Compound **23** is expected to act as inhibitor of EPSP synthase, and compound **22** is expected to act as a potential inhibitor of prolidase.



Scheme 6/Shema 6

In 1992, P. Bartlett synthesized and studied analogs of tetrahedral intermediate in the process of EPSP biosynthesis, which contain CH_3, CHF_2 and CF_3 groups, in order to stabilize the labile ketal phosphate moiety. The most potent inhibitor of the enzyme was found to be (***R***)-difluoromethyl derivative with *Ki* 4 nM[41] (Scheme 7, compound in frame).

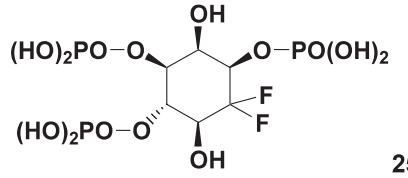


Scheme 7/Shema 7

Fluoro derivative EPSP is also an inhibitor of the enzyme with K_i 0.2 μM.[42]

The review R. Pongdee, H. Liu[43] ("Elucidation of enzyme mechanisms using fluorinated substrate analogues") presents numerous examples (Fig. 11) showing that the utilization of fluorinated substrates, mainly based on analogs of natural phosphates, played a key role in establishing the mechanistic course of an enzymatic reaction of isopentenyl pyrophosphate isomerase, glycosidases, UDP-N-acetylglucosamine enolpyruvyl transferase, thiamin phosphate synthase, chorismate synthase, etc.

Fluoro-analogs of D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] **25** that mobilize intracellular Ca^{2+} stores in SH-SY5Y neuroblastoma cells has been synthesized and investigated.[44] (–)-D-2,2-difluoro-2-deoxy-*myo*-Ins(1,4,5)P_3 [D--2,2-F_2-Ins(1,4,5)P_3] was a full agonist [EC_{50} 0.21 μM] and slightly less potent than D-Ins(1,4,5)P_3 [EC_{50} 0.13 μM]. D-2,2-F_2-Ins(1,4,5)P_3 mobilized Ca^{2+} with broadly similar kinetics to Ins(1,4,5)P_3 and was a substrate for Ins(1,4,5)P_3 3-kinase inhibiting Ins(1,4,5)P_3 phosphorylation (apparent K_i = 10.2 μM) but was recognized less well than Ins (1,4,5)P_3. L-2,2-F_2-Ins(1,4,5)P_3 was a potent competitive inhibitor of 3-kinase (K_i = 11.9 μM). Whereas D-2,2-F_2-Ins(1,4,5)P_3 was a good substrate for Ins(1,4,5)P_3 5-phosphatase, L-2,2-F_2Ins(1,4,5)P_3 was a relatively potent inhibitor (K_i = 19.0 μM).[44]



25

Phosphonocarbohydrates. Synthesis of fluorinated phosphonocarbohydrates appears attractive in order to prepare new classes of phosphate mimics of natural metabolites that retain high affinity for targeted enzymatic phosphate binding pockets, but are themselves resistant to phosphatase-mediated cleavage. Excellent reviews have recently been published on the synthesis and transformations of deoxy fluoro sugars and *C*-difluoromethylene-containing, *C*-trifluoromethyl and *C*-perfluoroalkyl carbohydrates.[45] As mentioned earlier, the most useful methods of grafting a $(RO)_2P(O)CF_2$ group on a carbohydrate are (i) nucleophilic displacement of primary triflates derived from monosaccharides (ii) nucleophilic addition-deoxygenation sequence; and (iii) radical addition of a dialkyl phosphite to the anomeric difluoromethylene compounds (Scheme 8).

Amino phosphonates with fluorine atoms. In contrast to the aminophosphonic and aminophosphinic acids area, actively developed in the last decade, only limited representatives of fluorine containing aminophosphonates are known at this moment (α-aminophosphonates[46] and β-aminophosphonates[2,14,47]).

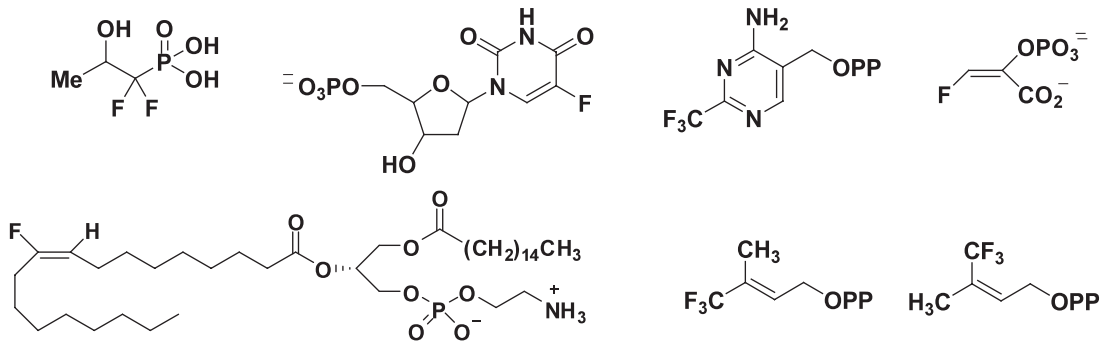
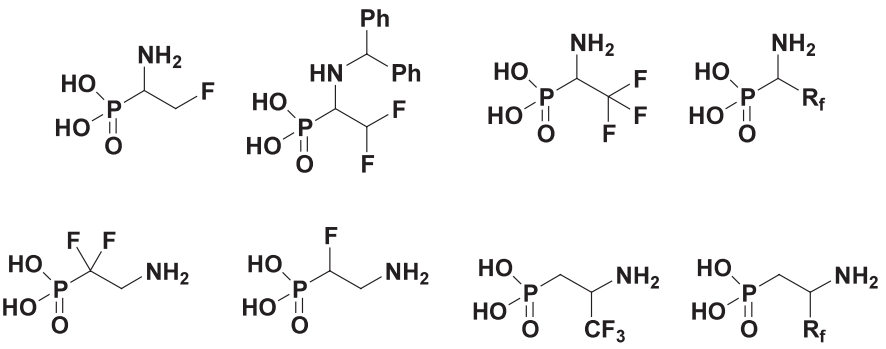
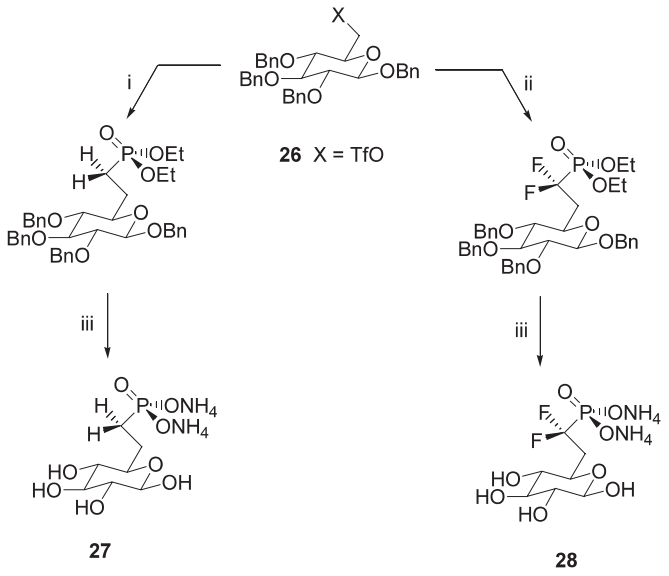


Fig. 13 – *Some fluorinated phosphates and phosphonates as substrates of enzyme*
Slika 13 – *Neki fluorirani fosfati i fosfonati kao enzimski supstrati*



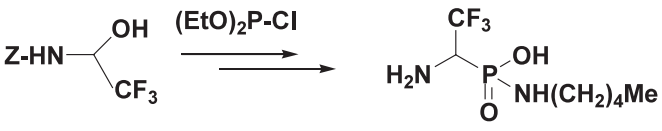
F i g. 14 – *Some examples α- and β-aminophosphonic acids*
S l i k a 14 – *Neki primjeri α- i β-aminofosfonskih kiselina*



Reagents: (i)$(EtO)_2P(O)Me$, BuLi; (ii) $(EtO)_2P(O)CF_2H$, LDA; (iii) TMSBr, CH_2Cl_2; then H_2, $Pd(OH)_2/C$, NH_4HCO_3

S c h e m e 8 / S h e m a 8

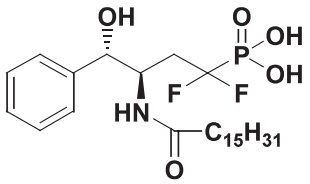
Aminophosphonic acids are used in many cases as good analogs of transition state of peptide bond hydrolysis and they are often potent inhibitors of peptidases. In order to increase stability of phosphonamides at physiological pH, recently French chemists synthesized α-trifluoromethyl-α-aminophosphonic acids from *O*-trifluoromethyl acetals and their amido esters (Scheme 9). The latter are perfectly stable at pH 4.7, and are expected to be antibiotics.[48]



S c h e m e 9 / S h e m a 9

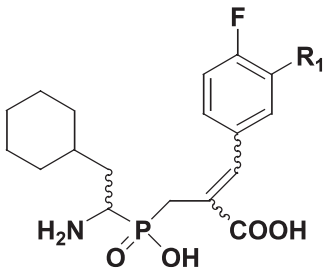
A series of short-chain analogs of *N*-palmitoylsphingosine-1-phosphate, modified by replacement of the phosphate and the long alkenyl side chain with hydrolytically stable difluoromethylene phosphonate and phenyl residue (Chart 4), were prepared to study the structure-activity relationship for inhibition of sphingomyelinase. The study revealed that inhibition is highly dependent upon the stereochemistry of the asymmetric centers of the acyl amino moiety, and resulted in identification of a non-competitive inhibitor with the same level of inhibitory activity of ***schyphostatin***,[49] the most potent of the few known small molecular inhibitors of sphingomyelinase (IC_{50} 3.3 μM, K_i 1.6 μM for Mg^{2+}-dependent N-SMase from bovine brain microsomes).

C h a r t 4 / D i j a g r a m 4



Renal dipeptidase (RDP) is an enzyme overexpressed in benign and malignant colorectal tumors. This enzyme is responsible for the hydrolytic scission of the lactam bond in carbapenems, potent broad-spectrum antibiotics that are resistant to the action of microbial β-lactamases. In an effort to identify potent inhibitors of this enzyme, a series of aminophosphinic acid derivatives were synthesized. Compounds in which the phenyl ring was *para* substituted with F and Br and olefin with *Z* geometry, showed high inhibitory activity against RDP enzyme (IC_{50} = 5–6 nM).[50]

C h a r t 5 / D i j a g r a m 5

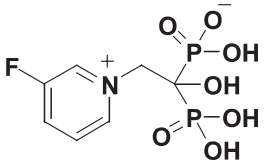


R= F, CF_3, Br

As mentioned earlier, nitrogen-containing bis-phosphonates are a modern important class of drugs used in a number of diseases. This year, a series of novel bisphosphonates –

pyridinyl-1-hydroxy-bisphosphonates have been designed and studied as inhibitors of FPPS (farnesyl diphosphate synthase) and bone resorption inhibition. The compound (Chart 6) with fluorine in pyridinium cycle has K_i 50 nM in FPPS assay and IC_{50} 75 nM in bone resorption assay. These results show that the compound is more active in the last case than currently used drugs.[6]

Chart 6/Dijagram 6

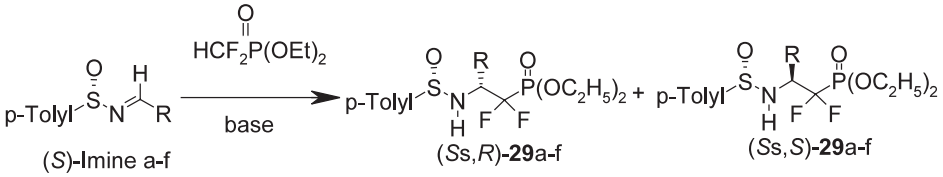


Current approaches to nonracemic fluorinated β-amino phosphonic acids have been largely based on coupling of phosphonodifluoromethyl organometallic reagents with a variety of electrophiles. The nucleophilic addition of methyl- and halomethylphosphonates anions to C=N double bond of enantiopure sulfinimines has proved to be effective for the asymmetric syntheses of β-aminophosphonic acids. We applied a similar strategy to the synthesis of α,α-difluoro-β-aminophosphonates and α,α-difluoro-β-aminophosphonic acids by the addition of phosphonodifluoromethyl carbanion to enantiomerically pure sulfinimines (Scheme 10).[51] In spite of relatively restricted nucleophilicity of phosphonodifluoromethyl carbanion *N*-sulfinyl-α,α-difluoro-β-aminophosphonate **29a** was obtained in good yield and diastereoselectivity. Due to its crystalline nature the major diastereomer of **29a** could be readily obtained in optically pure form by single crystallization of the crude reaction mixture.

The attractive feature of sulfinyl methodology is that the sulfinyl auxiliary can be simultaneously stereodirecting and protecting group and selectively removed from intermediate *N*-sulfinyl-β-aminophosphonate. The *N*-sulfinyl-α,α-difluoro-β-aminophosphonates (*S,s,R*)-**29** were *N*-desulfinylated by treatment with CF_3COOH in EtOH at room temperature. Under these conditions, the phosphonate group remained intact and α,α-difluoro-β-aminophosphonates (*R*)-**30** were isolated by flash chromatography in good yields. Then the amino group of (*R*)-**30** was reprotected with CbzCl, and hydrolysis of α,α-difluoro-β-aminophosphonates (*R*)-**30** in refluxing concentrated aqueous HCl (c = 10 mol dm^{-3}) results in α,α-difluoro-β-aminophosphonic acids (*R*)-**32** in high enantiomeric purity (Scheme 11).[51]

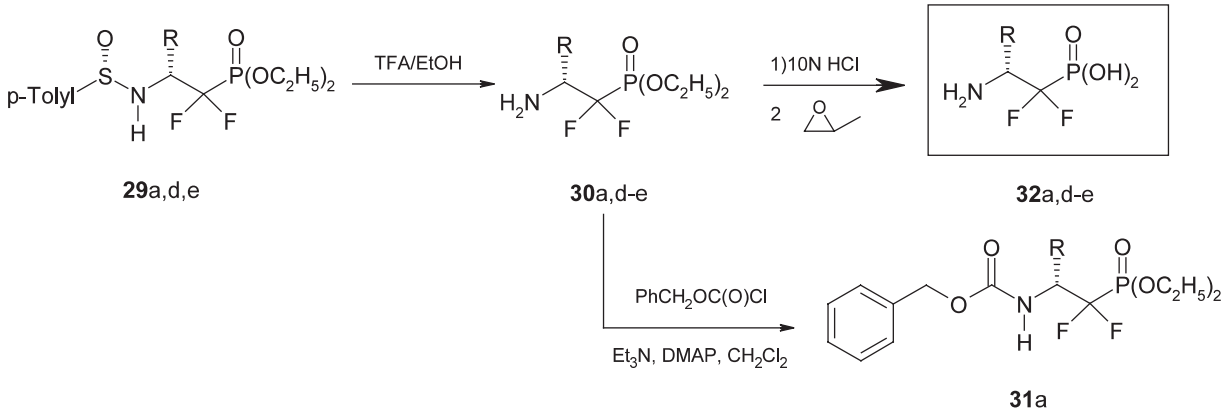
We believe that further use of amino phosphonates with fluorine near to as well as far from phosphorus has potential in the search for new inhibitors and future drugs. New data on activity aminophosphonic acids, especially – involved in peptide structures is a booster for these studies. For example, diphenyl phosphonate substrate **32** like peptides Z-D-Ser-Ala-Arg were described to be irreversible inhibitors for ***urokinase plasminogen activator*** (uPA).[52] Investigation of the structural requirements of a series of benzylphosphonic acid inhibitors of ***human prostatic acid phosphatase*** has led to the highly potent series of alpha-aminobenzylphosphonic acids. The alpha-benzylaminobenzylphosphonic acid **33**, with an IC_{50} = 4 nM, exhibited a 3500-fold improvement in potency over the carbon analog, alpha-phenylethyl.[53] (Chart 6)

Screening and optimization of a diverse set of bis-benzimidazoles (Chart 7) for inhibition of the hepatitis C virus (HCV) serine protease NS3/NS4A led to the identification of a potent Zn^{2+}-dependent inhibitor **34** under Zn^{2+} conditions (K_i=27 nM).[54]



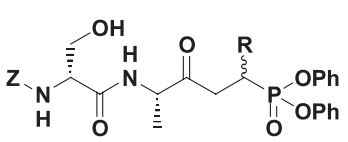
a: R=Ph; **b**: R=4-MeO-C_6H_4; **c**: R=4-CF_3-C_6H_4; **d**: R=2-thienyl; **e**: R=*n*-C_5H_{11}; **f**: R=*i*-Pr; **g**: R=*E*-PhCH=CH

Scheme 10/Shema 10



Scheme 11/Shema 11

Chart 7/Dijagram 7



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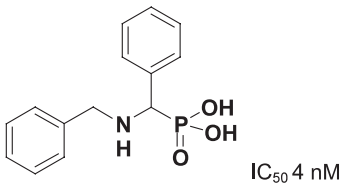
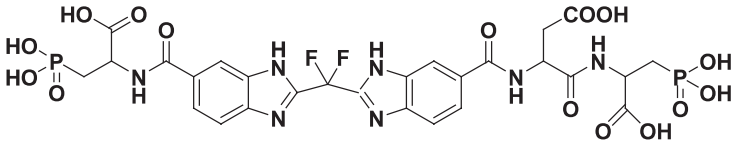


IC_{50} 4 nM

33

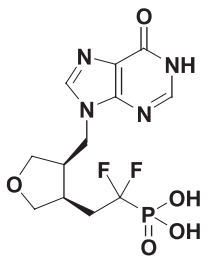
Chart 8/Dijagram 8



34

Fluorine-Containing Phosphonates – Nucleoside Analogs. Numerous publications demonstrate a growing interest in phosphorus-fluorine analogs of nucleosides. The first studies in the combination of fluorine and phosphorus for nucleosides were based on using fluorouracile – a very popular drug. Intensive investigations of new structures and targets have opened an extremely perspective direction in life science.

Purine-nucleoside phosphorylase (PNP) deficiency in humans leads to inhibition of the T-cell response. Potent membrane-permeable inhibitors of this enzyme are therefore considered to be potential immunosuppressive agents. 1,1-Difluoro-2-(tetrahydro-3-furanyl)ethylphosphonic acids possessing a *N*9-purinylmethyl functionality at the ring were synthesized by radical cyclization of allylic α,α- -difluorophosphonate and tested as "multi-substrate analogous" inhibitors for purine nucleoside phosphorylases. The IC_{50} values of *cis*-**35** and *trans*-**35** for human erythrocyte PNP-catalyzed phosphorylation of inosine were determined to be 88 and 320 nM, respectively. The stereochemistry of the inhibitors was found to affect significantly the inhibitory potency.[55] The *trans*-isomers **35** were 4-fold less potent than the corresponding *cis*-isomers. At an intracellular concentration of orthophosphate (1 mM), (+/–)-*cis*-**35**, the most potent compound of this series was shown to have IC_{50} and K_i values of 8.7 and 3.5 nM, respectively.[56] Phosphonate derivatives **36** which contain one or more fluorine atoms were found better PNP inhibitors than their non-fluorinated analogs, in all cases studied.[57]



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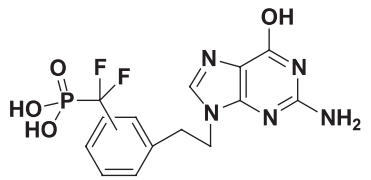
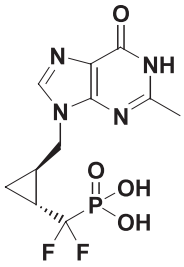


36

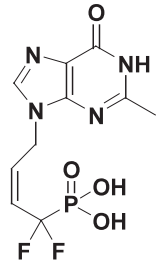
The binary complex of the trimeric calf spleen phosphorylase, which is highly homologous to human PNP, with the potent ground-state analogous inhibitor 9-(5,5-difluoro-5-phosphonopentyl)guanine (DFPP-G) was crystallized and analyzed by high-resolution X-ray diffraction. The crystal structure confirms that the inhibitor acts as a ***multisubstrate analog inhibitor as it binds to both nucleoside- and phosphate-binding sites***. The structure also provides the answers to some questions regarding the substrate specificity and molecular mechanism of trimeric PNPs.[58]

A series of 1,1-difluoro-5-(1*H*-9-purinyl)-2-pentenylphosphonic acid **38** as well as the related methano analogs **37** were prepared for evaluation of their PNP inhibitory activities. The cyclopropane ring and the hypoxanthine residue were found to increase the profile of inhibitory activity. All compounds were shown to be potent inhibitors of PNP purified from *Cellulomonas* sp. in nano-molar concentration[59] (Chart 8).

Chart 9/Dijagram 9

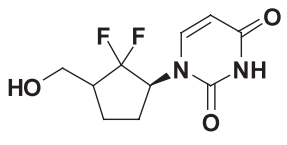


37, K_i = 28 nM



38, K_i = 20 nM

Synthesis of 2′,3′-dideoxy-6′,6′-difluorocarbocyclic nucleosides opens possibilities to search phosphonates and phosphates as new inhibitors and structural units for nucleotides (for example **39**).[60] Chinese chemists synthesized acyclic fluorine-containing nucleosides with phosphonate residue by electrophilic fluorination of 1-*tert*-butyldimethylsiloxy-2-(diethoxyphosphoryl)methoxy]-ethane and 3-*O*-benzyl-2-*O*-[(diethoxyphosphoryl)methyl]-1-*O*-(*tert*-butyldimethylsiloxy)-glycerol. The synthesized fluorinated acyclic nucleoside phosphonates **40** and **41** were tested against herpes viruses, respiratory viruses, hepatitis B virus and HIV. The monoammonium salt of the ethyl ester of F-PMEA was found to be active against human cytomegalovirus (HCMV), Epstein–Barr virus and measles with EC_{50} values from 5.6 to 32 mg/ml.[61] It is necessary to note that acyclonucleosides have also perspectives in the search for new drugs for smallpox infection and other DNA viruses.[62]



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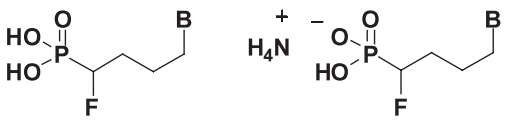
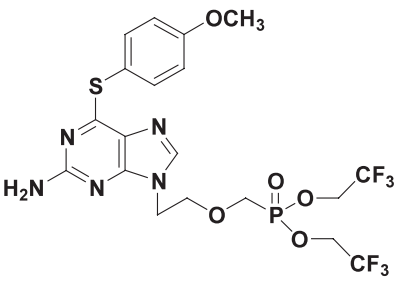


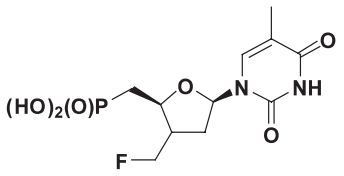
40 **41**

Scheme 12/Shema 12

2-Amino-6-(4-methoxyphenylthio)-9-[2-phosphonomethoxy)ethylpurine bis-(2,2,2-trifluoroethyl) ester **42** (ABE) is novel HBV-specific antiviral reagent and show anti-HBV activity in vitro (IC_{50} 0.003 μM, might be suitable for hepatitis B chemotherapy.[63]



42



43

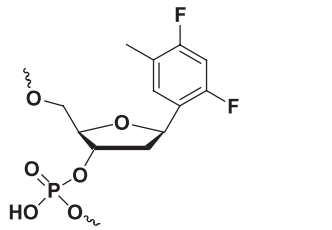
Scheme 13/Shema 13

Thymidine monophosphate kinase (TMPK) of *Mycobacterium tuberculosis* (TMPKmt) represents an attractive target for blocking the bacterial DNA synthesis. In an attempt to find high-affinity inhibitors of TMPKmt, various 3′-C-branched chain substituted nucleotides in the 2′-deoxyribo- and ribo-series were synthesized from one key intermediate. 2′-Deoxy analogue 3′-CH_2F **43** (Scheme 13) proved to be potent inhibitors of TMPKmt with K_i 15 μM. This series of inhibitors holds promise for the development of a new class of antituberculosis.[64]

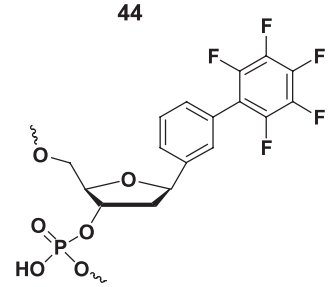
Recent publications demonstrate that fluorine-containing nucleosides **44**-**45** can be an important tool in DNA and RNA studies of double helices structure and hydrogen bonding, and stacking especially (Scheme 14). The main idea of these works is based on evidence of quadrupolar interactions of fluorohydrocarbons and formation C–F···H hydrogen bonds. In both cases the stacking increase and fluorinated duplex has higher thermodynamic stability.[65]

It is known that natural nucleotide and nucleoside conformations are distributed between "N" (North) and "S" (South) conformers (Scheme 15).

Gautier group realized a conformation analysis of the impact of CF_2- substitution of O atom on N/S equilibrium in model nucleotides. For uridine 3-monophosphate **46** (as model of natural nucleotides) in water N form occupies 57 %. By contrast modified CF_2-nucleoside **47** occurs nearly exclusively in N form (98 %) that was explained by the absence of S-driving gauche effect due to electronegativity of the group compared with phosphate. These data show that the introduction of difluorophosphonate at 3-position of nucleosides strongly effects the conformation dis- tribution and has a more fine influence than "simple" mimic.[66] Recently Gautier presented the synthesis of antisense modified nucleotides with P=S and CF_2-units on 3′-position of furanose (Chart 9) suitable for further preparation of desired oligonucleotides and testing on translation process as well as other properties of these modified oligonucleotides.[67]



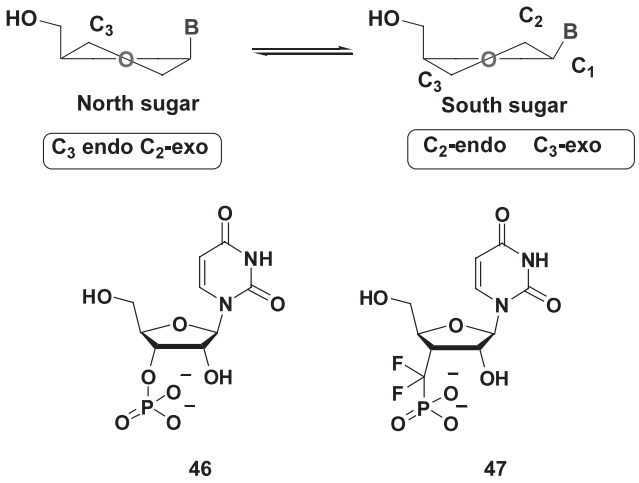
44



45

For example: Duplex
d(GATGAC**(X)**$_n$GCTAG)-d(CTAGC**(Y)**$_n$GTCATC) n= 1-4

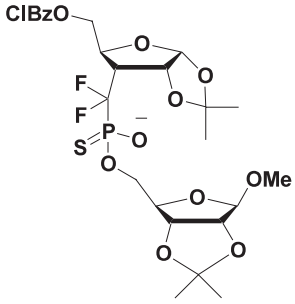
Scheme 14/Shema 14



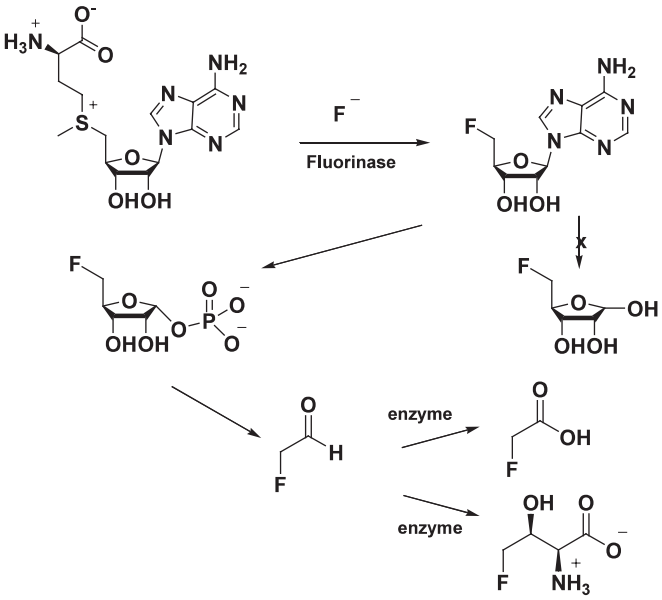
46 **47**

Scheme 15/Shema 15

Chart 10/Dijagram 10



And finally, **the union of fluorine and phosphorus has natural origin**! D. O'Hagan identified the first enzyme which mediates a reaction between *S*-adenosyl-L-methionine (SAM) and fluoride ion to generate 5'-fluoro-5'-deoxyadenosine (5'-FDA) as the first committed step on the pathway of fluoroacetate formation in biosynthesis. Scheme 16 shows a general pathway and fluorophosphate as key compound in biotransformations cell free extract from *Streptomyces cattleya*.[68]



Scheme 16 / Shema 16

Conclusion and perspectives

Today, chemistry and biochemistry of fluorine-phosphorus bioactive molecules are concentrated mainly on tetracoordinated tetravalent phosphorus derivatives with P=O fragment. In our opinion, pentacoordinated phosphorane structures with high diversity of substituents and their positions, which are directly mimics of transition state of phosphate hydrolysis, may be also very useful for biochemistry as well as specially constructed tricoordinated phosphorus compounds stable to oxidation and hydrolysis in cell conditions.

Phosphorus- and fluorine-containing biomimics as well as other molecules of biochemical interest are also very important tools in using NMR technology in life science and medicine for non-invasive detection and anatomic mapping. MRS is an imaging technique with the potential to record human and animal biochemistry in vivo.[69]

In the report we tried to present the very highly efficient and useful fluorine- phosphorus combination in organic molecules for purposes of modern life science. In biochemistry, the "P-F-union" enables investigation of the enzyme structure and mechanism of action more correctly, as well as creation on new anti-body enzymes. Enhancing or regulation of inhibitor properties of these compounds, their stability or selectivity, allow creation of new drugs for treatment of numerous serious diseases, especially viral infections and cancer. We would also like to direct your attention to this very promising area of modern "**life chemistry**".

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SAŽETAK

Fosfor i fluor – kombinacija za bioregulatore

V. Kukhar i Vl. Romanenko

Pregled opisuje vrlo visoku djelotvornost i korisnost kombinacije fluor-fosfor u sintezi organskih molekula za potrebe suvremenih prirodnih znanosti. U biokemiji npr. kombinacija P-F u biomolekulama omogućuje istraživanja građe enzima i mehanizme djelovanja puno točnije, ali i stvaranje novih enzimskih antitijela. Poboljšanje ili podešavanje inhibitorskih svojstava tih spojeva, njihove stabilnosti ili selektivnosti omogućuje stvaranje novih lijekova za brojne teške bolesti, osobito virusne infekcije i bolest rak.

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