Contents:

Summary

The thesis consists of a collection of 32 papers dealing with the synthesis of novel types of purine derivatives (bases and nucleosides) bearing C-substituent(s) attached to carbon atom(s) of the purine moiety. The original methodology of their synthesis is mostly based on cross-coupling reactions of halopurines with diverse organometallics catalyzed by complexes of Pd, Ni or Fe. The target compounds are used for biological activity screening and/or for applications in chemical biology or bioanalysis.

Novel methodologies of cross-coupling reactions include perfluoroalkylation of halopurines by perfluoroalkyl silanes in presence of KF and CuI and elaboration of the Suzuki-Miyaura reactions of halopurines with diverse arylboronic acids. The latter method was used for the synthesis of large series of 6-arylpurine nucleosides that possessed cytostatic and anti-HCV activity.

Regioselectivity of cross-coupling reactions of 2,6- and 6,8-dihalopurines was systematically studied. In dichloropurines, the reaction proceeds preferentially in the position 6, while in chloroiodopurines the iodine is displaced more easily. This approach was applied to the synthesis of many types of di and trisubstituted purine bases and nucleosides.

Novel methodologies of cross-coupling of halopurines with protected functionalized organometallics have been developed in order to prepare purines bearing functionalized C-substituents. Hydroxymethylation was achieved by the cross-coupling reactions of halopurines with (acyloxymethyl)zinc iodides followed by deprotection. 6-Hydroxymethylpurines were converted to 6-(fluoromethyl)- and 6-(difluoromethyl)purines. Conjugates of purines and amino acids were prepared by cross-couplings of halopurines with organometallics derived from amino acids.

Purine-purine and purine-pyrimidine conjugates connected by carbon linkers were designed and prepared as covalent base-pair analogues. The synthesis was based on cross-couplings, dimerizations and cyclotrimerizations of the corresponding alkynylpurines.

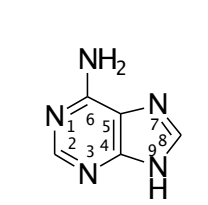
Several novel classes of biologically active purine bases and nucleosides were discovered: 6-aryl, 6-hydroxymethyl and 6-(mono-, di- or trifluoromethyl)purine ribonucleosides showed strong cytostatic activity, while 6-hetarylpurine ribonucleosides exerted high anti-HCV effect.

All these types of compounds are subject of further interdisciplinary studies.

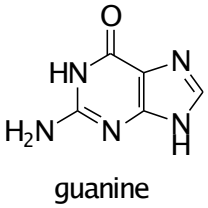
1. INTRODUCTION

Biogeneous purine derivatives (Chart 1) play a crucial role in most biological processes. Purine bases are constituents of nucleic acids as two letters of genetic alphabet forming Watson-Crick pairs with complementary pyrimidine bases. Number of enzymes of nucleotide and/or nucleic acids metabolism use purine bases, nucleosides and nucleotides as substrates. Furthermore, purines are also constituents of several cofactors (e.g. NADH, FAD, AcetylCoA, SAM, ATP etc.) used by many important classes of enzymes (oxidoreductases, transferases, ligases etc.). Therefore, most enzymes of nucleic acid metabolism and enzymes using nucleotide cofactors contain a purine (usually adenine) binding site. Moreover, purines and purine nucleosides and nucleotides participate in the signal transduction and regulation of many biological processes in cells and tissues as ligands of receptors (purinoceptors, adenosine receptors) and as second messengers (c-AMP). Therefore, purine bases, nucleosides and nucleotides were subject of extensive research and their structural modifications (derivatives and analogues) lead to discovery of thousands of biologically active compounds including many clinically used drugs (in particular antivirals and cytostatics). Furthermore, many modified purine derivatives were used in chemical biology for the study and modulation of biological processes at molecular level.

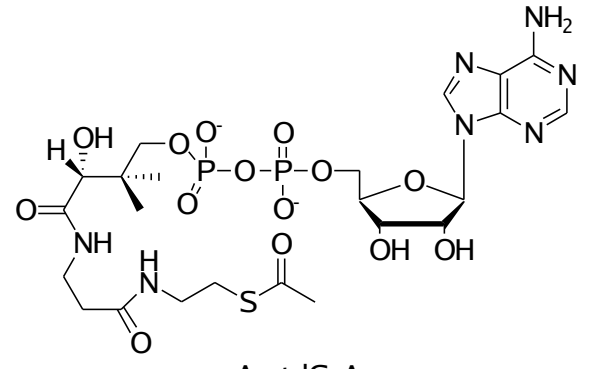
Chart 1 Structures of Biogeneous Purine Bases and Examples of Cofactors Containing Purine



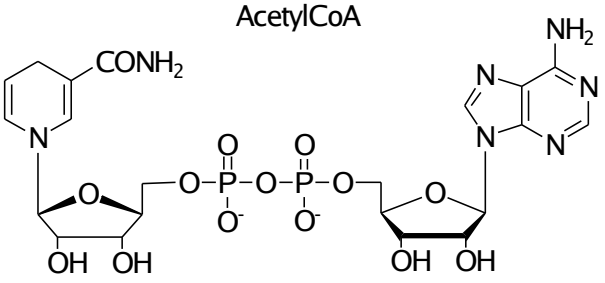
adenine



guanine



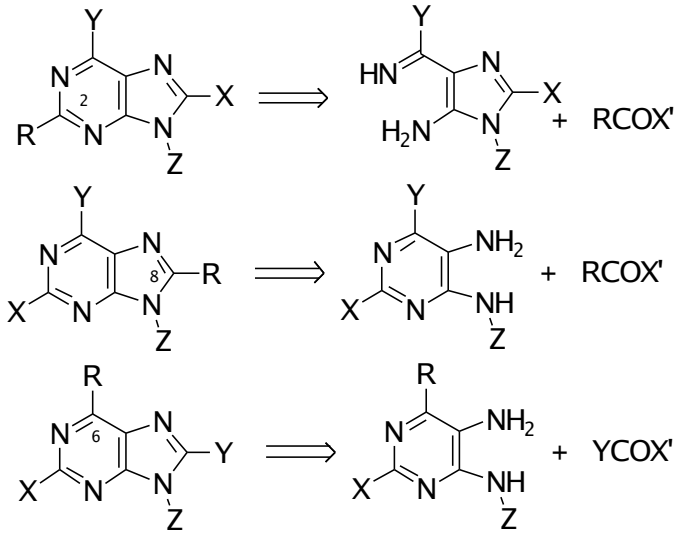
AcetylCoA



NADH

Purines bearing C-substituent(s) linked to carbon atoms of the purine moiety in the position(s) 2, 6 and/or 8 (further on quoted as C-C-purines for simplicity) have been only scarcely studied due to limited availability of these compounds by conventional chemistry. However, this subclass of purine derivatives may be of great interest. Introduction of a C-substituent to positions 2 and/or 6 would dramatically influence H-bonding ability to the complementary nucleobase or binding site of an enzyme or receptor. On the other hand, an introduction of a substituent to position 8 should preserve the pairing with pyrimidines and ability to form nucleic acid duplexes in which the substituent would point out to the major groove and thus modulate the formation of triplexes or interactions with proteins. Another important feature of C-C-purines due to the presence of very stable C-C bonds should be an increased stability towards enzymatic degradation (e.g. deamination by adenosine deaminase).

Scheme 1 Syntheses of Purines by Heterocyclizations



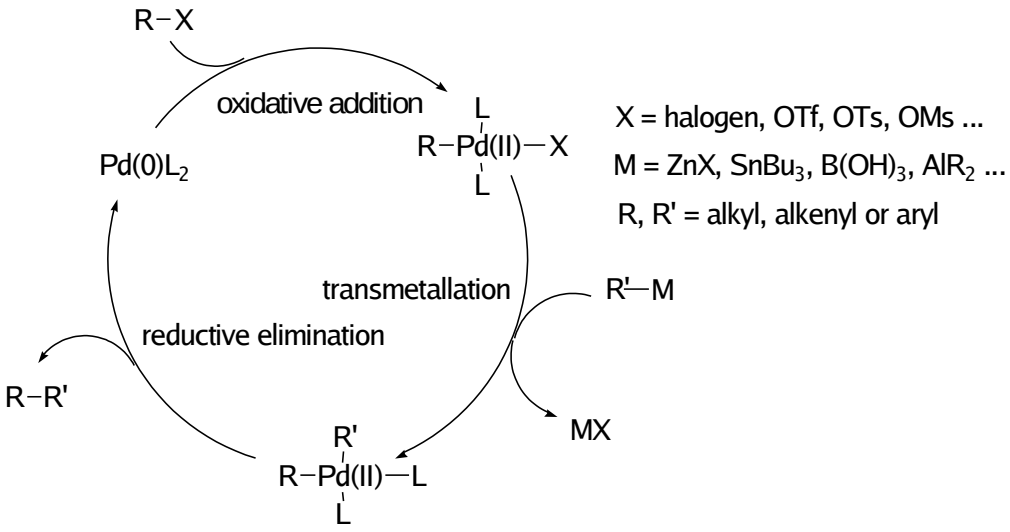
R = C-substituent
X, Y, Z = other substituents

Classical approach to the synthesis of C-C-purines is based on heterocyclizations (Scheme 1). Purines bearing C-substituents in position 2 are available by cyclizations of 4-aminoimidazole-5-carboxamides or nitriles with derivatives of carboxylic acids (esters, orthoesters etc.), while purines bearing C-substituents in position 8 are prepared by analogous cyclizations from 5,6-diaminopyrimidines. On the other hand, synthesis of 6-substituted purines is much more difficult and is based on multistep construction and cyclization of 4-substituted 5,6-diaminopyrimidines. In general the syntheses of C-C-purines by heterocyclizations usually

consist in multistep sequences with rather low yields and limited applicability to wider range of derivatives.

Other possible approaches to the C-C-purines are radical[1] or nucleophilic[2] substitutions or generation of carbanions or organometallics (Li, Mg or Zn) on purine skeleton followed by reaction[3] with electrophiles. All these reactions are of quite limited synthetic applicability. The first truly efficient and general approach to C-C-purines is based on cross-coupling reactions (Schema 2)[4]. The development of methodology of these reactions on purines and their applications in the synthesis of biologically relevant derivatives for biological activity screening and use in chemical biology and bioanalysis were the major directions of my independent research in the last 7 years and are the subject o this DSc. thesis.

Scheme 2 General Mechanism of Cross-coupling Reactions



2. SYNTHESES OF PURINE DERIVATIVES BY CROSS-COUPLING REACTIONS

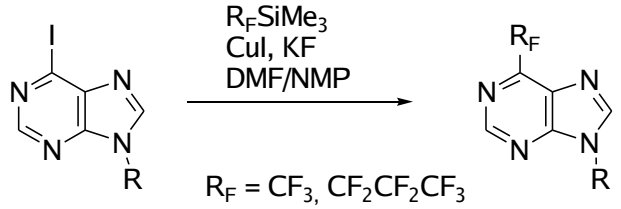
Applications of cross-coupling reactions in the synthesis of complex molecules started from 80-ties and very soon thereafter also their applications in chemistry of purines and nucleosides began (for detailed review, see ref.[5]). In the beginning, only rather scattered applications of reactions of halopurines with arylmagnesiumhalides, alkylcuprates and alkenylstannanes in special cases of particular syntheses[6] were reported. Systematic studies of cross-coupling reactions of 6-halogenpurines with aryl- a alkenyl(tributyl)stannanes and with aryl- a alkylzinc halides were performed from 90-ties in the laboratory of Prof. L.-L. Gundersen and resulted in the development of the first general methodology for the synthesis of 6-aryl-, 6-alkenyl- and partly also 6-alkylpurines[7].

In our group (partly in collaboration with Prof. Dalimil Dvořák, VŠCHT) we have studied cross-coupling reactions targeting three major directions. At first we have focused on the development of other alternative general methodologies of cross-coupling reactions with other types of organometallics (boronic acids, silanes etc.) either leading to novel types of derivatives hardly available by previously know chemistry or giving the corresponding (new or known) C-C-purines in a more practical, efficient and/or environmentally benign way. Further on, we have systematically studied regioselectivity of cross-coupling reactions of di- and trihalopurines in order to prepare di- or trisubstituted purine derivatives. Finally, we have developed new cross-coupling reactions of halopurines with protected functionalized organometallics giving a variety of novel purines bearing highly functionalized C-substituents. However, in all cases the new methodologies have been applied in the synthesis of novel purine bases, nucleosides and covalent analogues of base-pairs for biological activity screening and for specific uses in chemical biology.

2.1. Development of Basic Methodologies of Cross-coupling Reactions of Halopurines

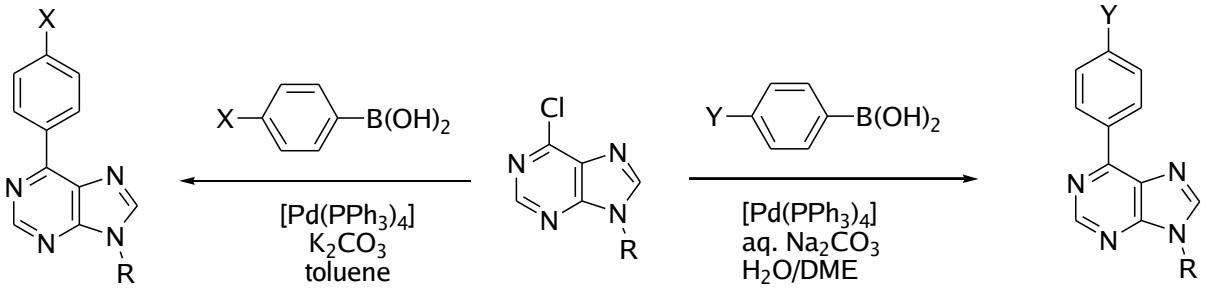
A new methodology of perfluoroalkylation of purines has been developed based on modified Hiyama reaction. Thus 6-iodopurines reacted with perfluoroalkylsilanes in the presence of KF and CuI to give 6-(perfluoroalkyl)purines in acceptable yields (Scheme 3)[8]. It was applied in the synthesis of 6-(perfluoroalkyl)purine bases and nucleosides and later also for acyclic nucleoside phosphonates[9]. An alternative approach to the synthesis of a series of 6-(trifluoromethyl)purine nucleosides consisted in a larger scale synthesis of 6-(trifluoromethyl)purine followed by alkylation or glycosidation reactions and deprotection[10].

Scheme 3. Synthesis of 6-(Perfluroalkyl)purines



In a close collaboration with the group of Prof. Dvořák we have developed a new practical and efficient methodology for the synthesis of arylpurines by the Suzuki-Miyaura reactions of halopurines with arylboronic acids under Pd catalysis (Scheme 4)[11,12]. Optimization of the reaction conditions resulted in two particular methods: (i) anhydrous conditions using solid K_2CO_3 in toluene were efficient for the reactions of electron-rich arylboronic acids, while (ii) aqueous Na_2CO_3 in a mixture of water and DME was suitable for electron-poor aryl- and alkenylboronic acids. Besides very good yields and simple work-up and isolation of products, the main advantage of the Suzuki reactions (as compared to the Stille reactions with toxic organostannanes) is the use of easily available (largely commercial), stable and non-toxic boronic acids. This is particularly important for the synthesis of compounds for biological activity screening where even trace amount of toxic contaminants may distort the results.

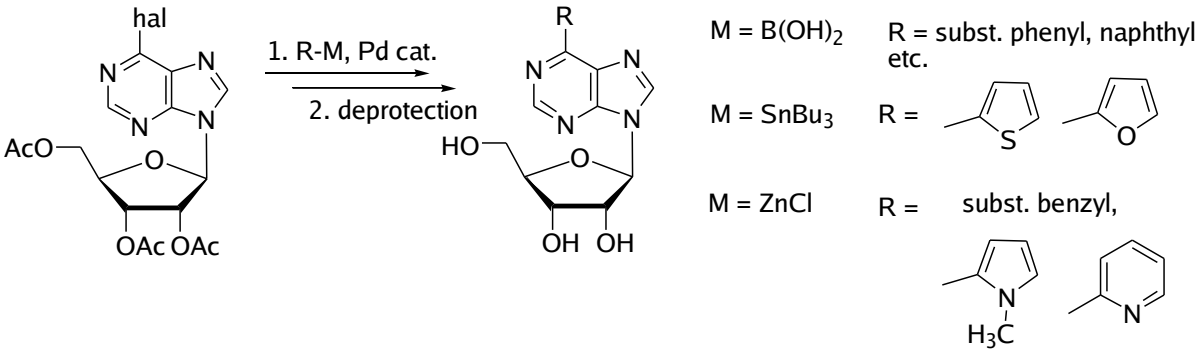
Scheme 4. Suzuki-Miyaura Reactions of 6-Chloropurines



Since some 6-phenylpurine ribonucleosides were found to show significant cytostatic effect (*vide infra*), a large series of related derivatives was prepared including sugar-modified nucleosides[13] and other purine ribonucleosides bearing diverse aryl[14], hetaryl[15] and benzyl groups in position 6 (Scheme 5). For the synthesis of some 6-hetaryl- and 6-benzylpurine derivatives we have developed and applied also cross-coupling reactions of other types of organometallics, i.e. organostannanes or organozinc halides. Later on, the series of 6-hetarylpurine ribonucleosides has been extended[16] to many other members prepared not only by

cross-coupling reactions but also by heterocyclizations of 6-ethynyl-, 6-cyano and 6-vinylpurine ribonucleosides.

Scheme 5. Syntheses of 6-Aryl- a 6-Benzylpurines by Cross-coupling Reactions



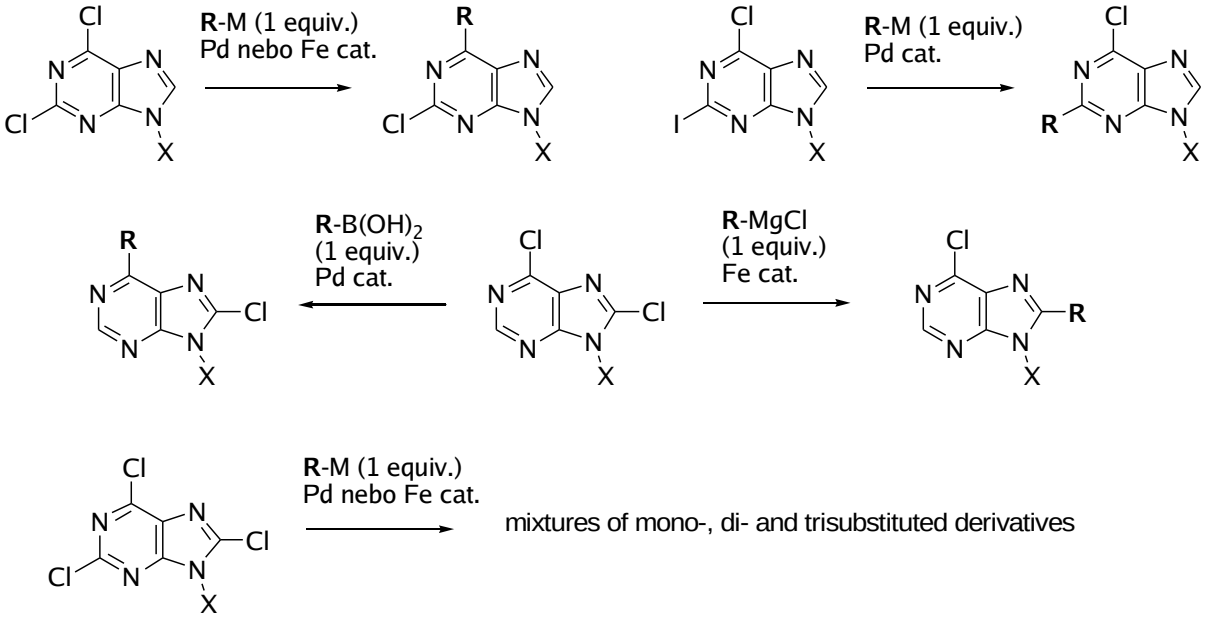
2.2. Regioselectivity of Cross-coupling Reactions of Di- a Trihalopurines

Within the framework of our methodology developments, also regioselectivity of cross-coupling reactions of 2,6- and 6,8-dihalopurines was studied. The only previously reported[17] model studies of regioselectivity in Pd-catalyzed reactions of 9-benzyl- 2,6- or 6,8-dihalopurines with organostannanes and organozinc reagents revealed that the 2,6- and 6,8-dichloropurines react preferentially in the position 6, while the chloro-iodopurines in the position of the better leaving group (iodine). However, due to the necessity to use excess of the organotin reagent, the regioselectivity was not complete and usually mixtures of products were obtained.

After our development of the Suzuki-Miyaura cross-coupling reactions of halopurines11, we have studied the regioselectivity of these reactions of phenylboronic acids with dihalopurines in order to prepare disubstituted derivatives12. This study showed that the reaction of 9-benzyl-2,6-dichloropurine with 1 equivalent of $PhB(OH)_2$ proceeds12 with complete regioselectivity to give exclusively 2-chloro-6-phenylpurine. On the other hand, analogous reaction of 9-benzyl-6-chloro-2-iodopurine led12 selectively to 6-chloro-2-phenylpurine. The remaining chloro-substituent can be used in another coupling or nucleophilic substitution reaction. Having this facile and selective method in hands, it was applied in the synthesis of 2- and 8-substituted 6-phenylpurine ribonucleosides. The former were prepared[18] from 2,6-dichloropurine nucleoside by first regioselective coupling in the position 6 followed by second coupling in the position 2. The latter were prepared[19] from protected 6-iodo-8-bromopurine nucleoside that also reacted preferentially in the position 6 followed by another reaction (cross-coupling or nucleophilic substitution) in the position 8. Later on, an analogous approach was used[20] in the synthesis of carba analogues of Myoseverin. Reactions of 2,6-dichloro-9-isopropylpurine with one

equivalent of 4-methoxyphenylboronic acid or 4-methoxybenzylzinc chloride proceeded in the position 6 and were followed by another reaction in the position 2.

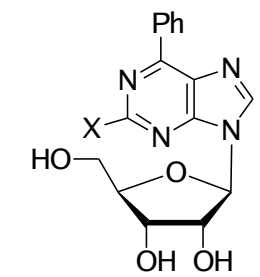
Scheme 6. Regioselectivity of Cross-coupling Reactions of Di- a Trihalopurines



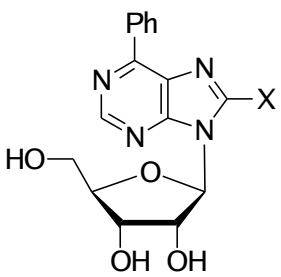
Recently, a new Fe-catalyzed cross-coupling of alkylmagnesium halides has been developed[21] and turned out to be a suitable method for the introduction of alkyl substituents. Therefore, we have also studied its regioselectivity in purines in order to prepare substituted derivatives of biologically active 6-methylpurines. Reactions of 2,6-dichloropurines with one equivalent of MeMgCl in presence of $Fe(acac)_3$ gave[22] selectively 2-chloro-6-methylpurines. On the other hand, the same reaction with trimethylaluminium under Pd-catalysis gave 2,6-dimethylprurines. This reaction was very recently used[23] in the synthesis of highly methylated purines and purinium salts as analogues of Heteromines.

Then we have studied regioselectivity of cross-coupling reactions in 6,8-dichloro-9-THP-purine[24]. In this case an interesting dichotomy has been found. While the Suzuki-Miyaura reaction with phenylboronic acid proceeded24 as expected in the position 6, the Fe-catalyzed reaction with methylmagnesium chloride occured24 in the position 8. In the both types of intermediates, the remaining chlorine was displaced by C-substituent via another cross-coupling or by O- or N-substituent via nucleoplilic substitution to get a series of 6,8-disubstituted purine bases after deprotection. Analogous reactions of 2,6,8-trichloro-9-THP-purine were[25] of only limited selectivity and gave separable mixtures of mono-, di- and trisubstituted derivatives that were used in further modifications to finally get 2,6,8-trisubstituted purines.

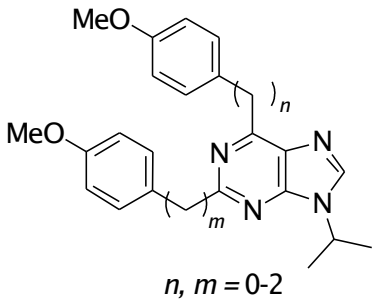
Chart 2: Typical Examples of Di- and Trisubstituted Purines Prepared:



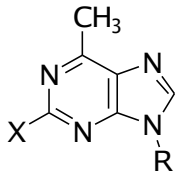
X = hal, NH_2, CH_3, CF_3 etc.



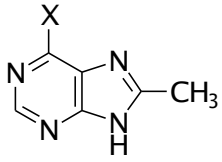
X = alkyl, aryl, NH_2, OR etc.



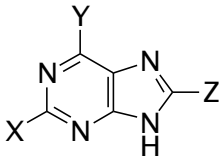
n, m = 0-2



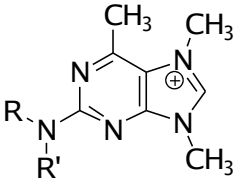
X = Cl, Me, Ph;
R = H, Rf, dRf



X = Me, Ph, NH_2 etc.



X, Y, Z = Cl, Me, Ph



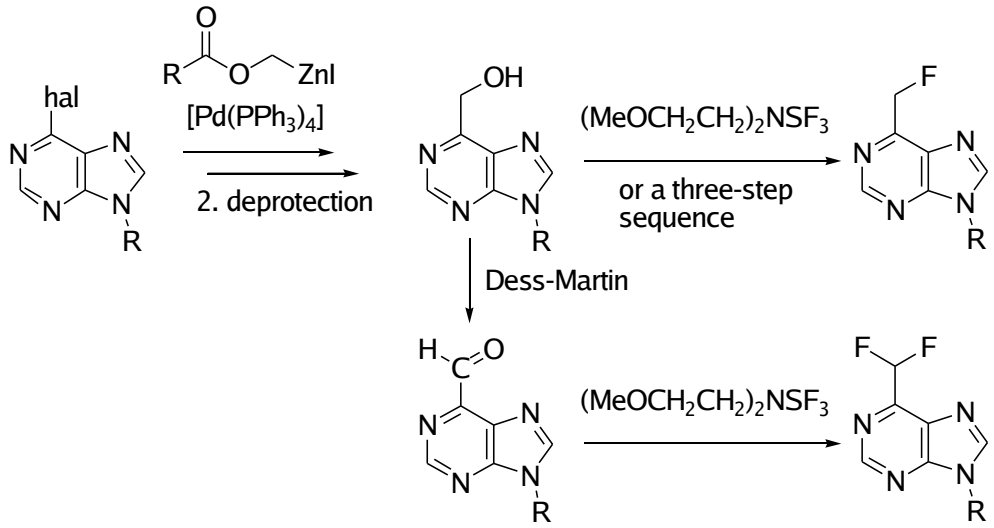
R, R' = H or CH_3

In conclusion, cross-coupling reactions of dihalopurines with organometallic reagents proceed with good regioselectivity and are applicable in the synthesis of disubstituted purine bases and nucleosides (Chart 2). On the other hand, the reactions of 2,6,8-trichloropurines are much less selective and therefore an alternative approach to the synthesis of trisubstituted purines based on a combination of cross-coupling reactions and C-H activation is under development in our group.

2.3. Cross-coupling Reactions of Halopurines with Functionalized Organometallics

Most of the previously described methods of cross-coupling reactions were only applicable for the synthesis of simple unfunctionalized alkyl, alkenyl, alkynyl or aryl substituents. However, in order to facilitate selective interactions with active sites of enzymes or receptors, purine derivatives bearing functionalized C-substituents (containing e.g. hydroxy, amino or carboxy groups) would be extremely attractive. Cross-coupling reactions of functionalized organometallics were quite rare in the past and now are of eminent interest of many laboratories. The problem is that these groups are quite polar and contain acidic hydrogens incompatible with highly reactive organometallics. Therefore the functionality must be protected by a suitable protecting group which must be easily introduced, survive the conditions of the cross-coupling reactions, and be easily cleavable at the end of the synthesis under mild conditions in order to prevent degradation of the relatively labile nucleosidic systems.

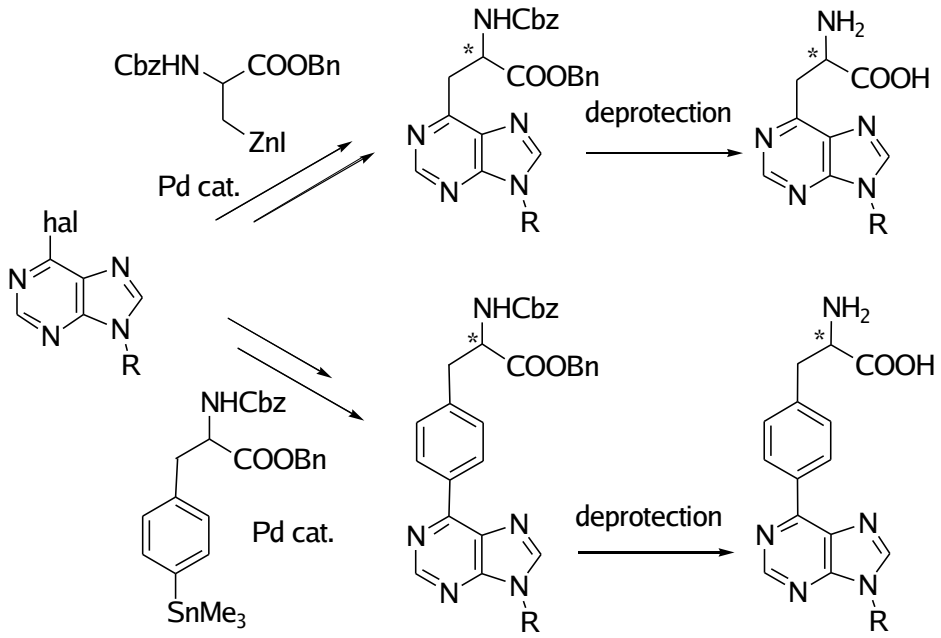
Scheme 7. Hydroxymethylation and Further Transformations



Thus our current efforts are strongly focused on the development of novel methodologies of cross-coupling reactions of halopurines with protected functionalized organometallics and on further transformations of the functional groups. An entry into the field of purines bearing functionalized C-substituents in position 6 is undoubtedly introduction of a hydroxymethyl group that is interesting itself and also suitable for further transformations. Thus a new method for cross-coupling hydroxymethylation of 6-chloro- or 6-iodopurines was developed. The reactions of the halopurines with acyloxymethytlzinc iodides (available by zincations of iodomethyl esters) catalyzed by $[Pd(PPh_3)_4]$ at room temperature generally gave[26] the coresponding 6-(acyloxymethyl)purines in very high yields. Mild deprotection of these intermediates under basic conditions afforded the desired 6-(hydroxymethyl)purines. The method was also applied[27] in the synthesis of 2-aminopurine derivatives (an amidine protection for NH_2 group was used) and regioselective hydroxymethylation of 2,6-dihalopurines. In order to further transform the hydroxy group, we needed sugar protected nucleosides with free hydroxymethyl group on purine. These intermediates were prepared[28] by selective deacetylation of 6-(acetyloxymethyl)purine nucleosides bearing toluoyl groups on the glycon part making use of aqueous ammonia in presence of $ZnCl_2$. The sugar-protected 6-(hydroxymethyl)purine nucleosides were converted28 to 6-(fluoromethyl)purines either by a single-step deoxyfluorination using DeoxoFluor reagent (moderate yields of ca 46-49%) or by a three-step sequence consisting in mesylation, Finkelstein reaction to 6-iodomethylpurines followed by nucleophilic substitution with AgF (total yields of 66-72% over three steps). A mild and

efficient oxidation of the 6-(hydroxymethyl)purines to 6-formylpurines was achieved making use of Dess-Martin periodinane reagent and the subsequent deoxofluorination of the aldehyde by DeoxoFluor gave 6-(difluoromethyl)purines in acceptable yields[29].

Scheme 8. Synthesis of Purinyl Amino Acids



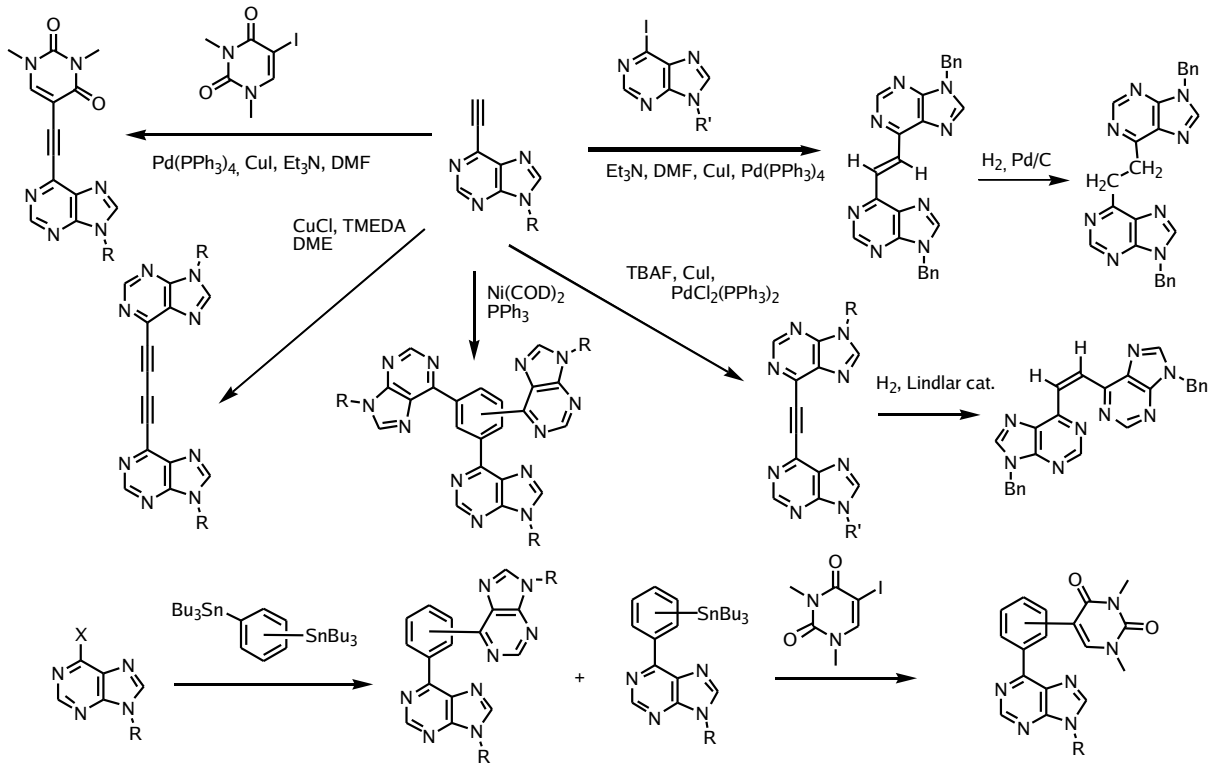
Another type of purines bearing functionalized C-substituents under study were C-C conjugates of purines and amino acids. Such compounds could in principle inteact both with binding sites for nucleosides and with binding sites for amino acids and also they are a promising scaffold for the construction of stable bioconjugates of oligonucleotides (or nucleic acids) with peptides (or proteins). The first type of amino acid moiety we have tried to attach to purine was glycine. Thus Pd-catalyzed -arylations of (difenylmethyliden)glycinate by 6-iodopurines gave the desired protected (purine-6-yl)glycine. However, any attempts to cleave the protecting groups under diverse conditions lead[30] to the destruction of the amino acid skeleton (deamination and/or decarboxylation). Therefore, we have turned our attention to other two types of amino acids: alanines and phenylalanines (Scheme 8). (Purin-6-yl)alanines were efficiently prepared[31] by Pd-catalyzed cross-coupling reactions of 6-iodopurines with protected iodozincalanines followed by a two-step deprotection. In this case there was no racemization of the amino acid moiety during the generation of the organozinc, cross-coupling and deprotection. On the other hand, the corresponding phenylalanines were prepared by two alternative methods[32]. Cross-coupling reactions of 6-halopurines with 4-boronophenylalanine proceeded32 very well and also the

deprotection was achieved under relatively mild conditions. However, the diastereomeric purity of the final products in nucleoside series was only ca. 80% indicating that a partial racemization/epimerization occurred during the synthesis. Therefore, we have elaborated an alternative method consisting in the cross-coupling reactions of analogous (trimethylstannyl)phenylalanines. Also in this case, suitable reactions conditions and catalytic system giving good yields of the desired products were found32 and, fortunately, the final products were optically and/or diastereomerically pure compounds. Later on, we have succeeded in the preparation of (adenosin-8-yl)phenylananines[33] by the cross-coupling reactions of unprotected 4-boronophenylalanine with unprotected 8-bromoadenosine nucleosides and nucleotides in aqueous solutions under conventional heating or under microwave conditions.

2.4. Syntheses of Covalent Analogues of Base-Pairs

Conjugates of two or three purine and/or pyrimidine bases connected by all-carbon linkers of diverse length and configuration were designed as covalent analogues of base-pairs. Such compounds themselves may intercalate to DNA, if incorporated into an oligonucleotide, they could be complementary to abasic sites of damaged DNA, and if incorporated to duplex, they should form permanent cross-links. Synthesis of these compounds (Scheme 9) was based on the Sonogashira cross-coupling reactions of 6-halo- and 6-ethynylpurines and/or 5-iodo- a 5-ethynylpyrimidines[34,35], the Stille cross-coupling reactions[36] of 6-chloropurines or 5-iodopyrimidines with phenylenebis(stannanes) and on Ni-catalyzed cyclotrimerizations[37,38] of ethynylnucleobases. Later on, optimalization of the conditions and variations of orthogonal protecting groups enabled us to apply this methodology also to the corresponding nucleoside derivatives of bis(purin-6-yl)acetylenes and –diacetylenes[39].

Scheme 9. Synthesis of Covalent Analogues of Base-Pairs and Triplets

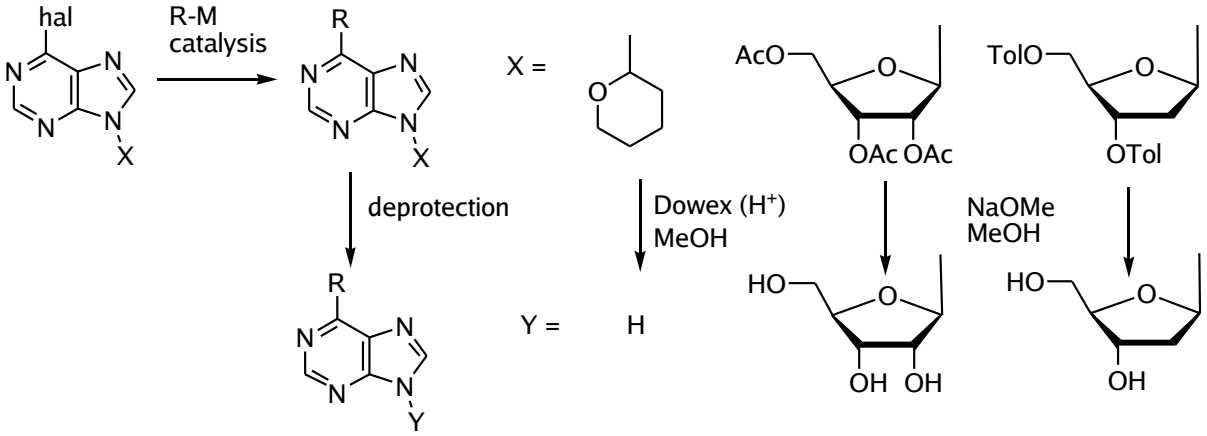


2.5. Syntheses of Purine Derivatives for Applications in Medicinal Chemistry, Chemical Biology and Bioanalysis

2.5.1. Syntheses and Biological Activity of Purine Bases and Nucleosides

All newly developed methodologies (as well as some adapted known ones) are applied in the syntheses of series of novel modified purine bases and nucleosides for biological activity screening. Suitable protecting groups were found both for nucleobases and for nucleosides (Scheme 10). For the synthesis of purine bases we use 9-(tetrahydropyran-2-yl) protected halopurine derivatives cleavable by acidic cation exchanger in methanol. Acyl protection (acetyl, benzoyl or 4-toluoyl) of the glycon part is used in the synthesis of nucleosides. These groups are easily removable under very mild conditions by a catalytic amount of NaOMe in methanol (acid labile groups are not suitable in such cases due to very limited stability of nucleosidic systems under acidic conditions).

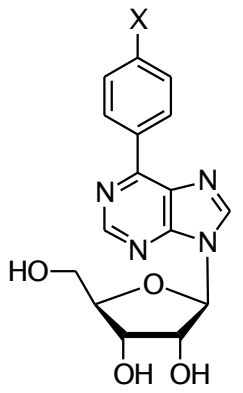
Scheme 10. Protecting Groups for Cross-coupling Reactions of Purines and Nucleosides



Large series (hundreds) of modified nucleobases and nucleosides were prepared in our laboratory during the last 8 years and all of them were screened for biological activity. Cytostatic activity (inhibition of cell-culture growth) was studied in collaboration with Dr. I. Votruba (IOCB) on the following cell-lines of leukemias and tumors: (HeLa S3, L1210, CCRF-CEM a HL-60). Antiviral activity screening (in particular Hepatitis C virus) was studied was in collaboration with Pharmasset, Inc. (Alabama). Dr. Votruba also studied inhibition of adenosine deaminase (ADA).

By the systematic screening of our compounds several new types of biologically active purines and nucleosides have been discovered (Chart 3). The first and most important lead structures were 6-(4-substituted phenyl)purine ribonucleosides14 showing very promising cytostatic effects (IC_{50} = 0.1-10 µM). Later on, also 6-hetaryl- and some 6-benzylpurine ribonucleosides15 were found to possess cytostatic activity in similar level. The structure of these nucleosides was then further modified to many types of derivatives and analogues, however, all the additional modifications on the glycon part (deoxyribonucleosides, acyclic analogues) or on the purine moiety (additional substituent in position 2 or 8) resulted in entirely inactive compounds. Therefore, the pharmacophor necessary for the cytostatic effect of this class of compounds could be formulated as purine ribonucleosides bearing either a 4-substituted phenyl or a small 6- or 5-membered heterocyclic ring in the position 6. Quite recently, we have also found that a subclass of these compounds (purine ribonucleosides bearing 5-membered heterocycles in position 6) exhibit a very high antiviral activity against HCV virus in the replicon assay (EC_{90} = 0.1-1 µM). This class of compounds is still extensively studied in order to find a mechanism of action and even more active compounds suitable for preclinical studies.

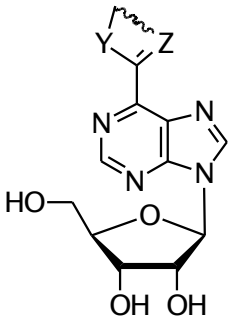
Chart 3. Examples of Biologically Active Purine Bases and Nucleosides from Our Laboratory



X = H, F, Cl, OMe, SMe

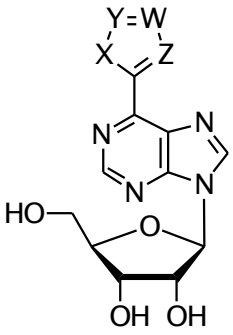
CYTOSTATIC ACTIVITY
IC_{50} = 0.1-10 μM

various 5- and 6-membered hetaryl



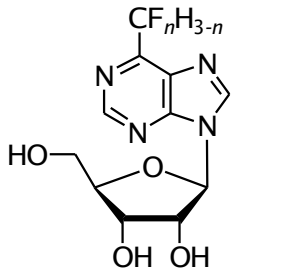
Y, Z = CH, O, S or N

5-membered hetaryl



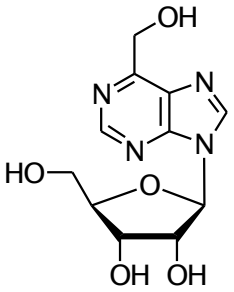
X, Y, W, Z = CH, O, S or N

Anti-HCV ACTIVITY
EC_{90} = 0.1-1 μM



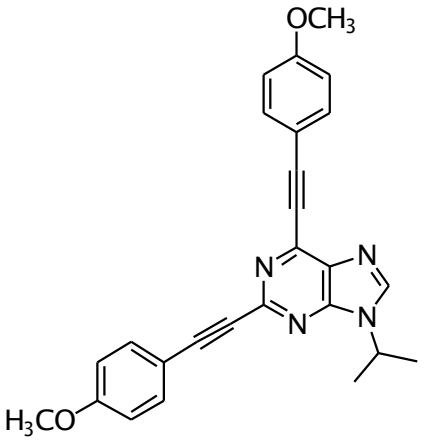
CF_nH_{3-n} = CF_3, CF_2H, CH_2F

CYTOSTATIC ACTIVITY
IC_{50} = 1 μM

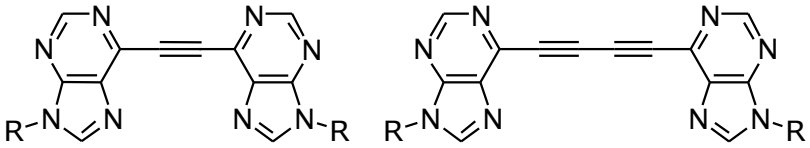


CYTOSTATIC ACTIVITY
IC_{50} = 0.01-0.1 μM

ADA inhibition
IC_{50} = 1.7 μM



CYTOSTATIC ACTIVITY
IC_{50} = 0.6-3 μM



CYTOSTATIC ACTIVITY
IC_{50} = 0.3-2 μM

Purine ribonucleosides bearing a substituted methyl group in position 6 were found to be another important class of biologically active compounds. 6-(Hydroxymethyl)-9-(-D-ribofuranosyl)purine26[27] exerts very high cytostatic activity (IC_{50} = 0.01-0.1 μM) against leukemia cell lines and moderate inhibition of adenosine deaminase. On the other hand 6-(trifluoromethyl)purine8, 6-(fluoromethyl)purine28 and 6-(difluoromethyl)purine29

ribonucleosides showed somewhat lower cytostatic effect (IC_{50} = ca. 1 μM) and did not inhibit ADA.

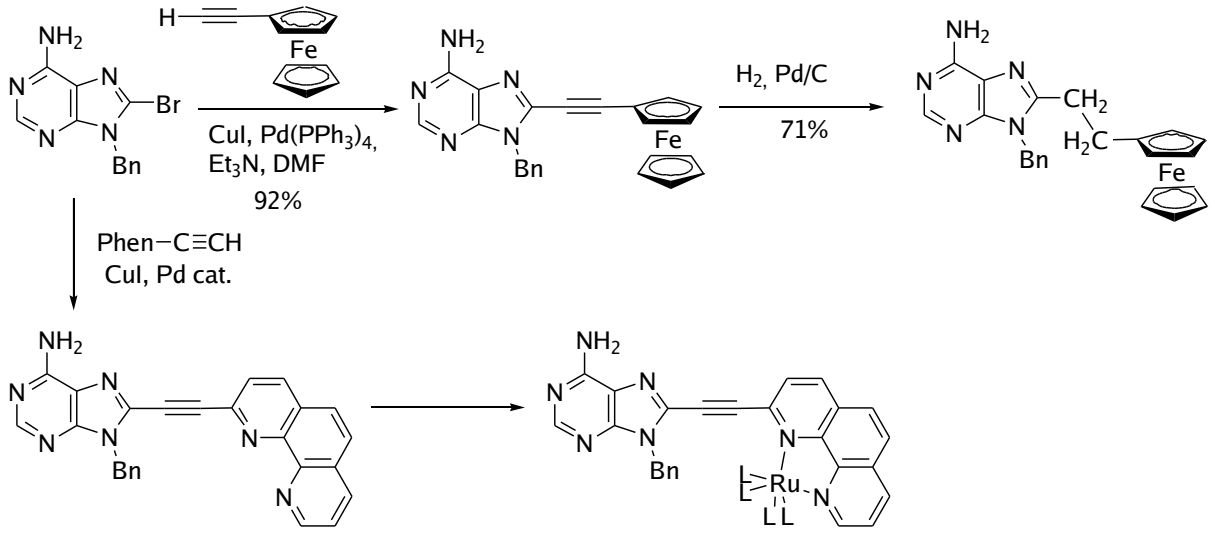
From substituted purine bases, interesting cytostatic activity was found in 2,6-bis(4-methoxyphenylethynyl)-9-isopropylpurine20, 9-benzyl-2-ferrocenylethynyladenine[40] and bis(purin-6-yl)acetylenes and -diacetylenes34[,39].

2.5.2. Syntheses of Purines for Applications in Chemical Biology and Bioanalysis

Several modified purine nucleosides bearing hydrophobic substituents (methyl, fluoromethyl, trifluoromethyl) in position 6 were phosphorylated and the triphosphates are currently under study as substrates for DNA polymerases by Prof. R. D. Kuchta (University of Colorado) in order to find new base pairs suitable for extension of the genetic alphabet. This project now continues by the studies of very simple model phenyl or pyridyl C-nucleosides.

Another direction of our current efforts targets to applications in bioanalysis. The goal is the construction of oligonucleotide probes bearing electroactive metal complexes connected to position 8 of purine bases via a conjugate linker. At first we have studied adenines bearing ferrocenylethynyl group as model compounds (Scheme 11) 40. Their electrochemical behavior was promising but we were unable to incorporate them into oligonucleotides due to limited stability of ferrocene to oxidation. Therefore, we are now preparing analogous building blocks containing metal complexes of bipyridine or phenanthroline.

Scheme 11. Synthesis of Purines Bearing Electrochemical Markers



3. CONCLUSIONS

This DSc. thesis consists of a collection of 32 papers covering our results in the development of cross-coupling methodology and of its applications in synthesis of novel modified purine bases and nucleosides for biological activity screening and applications in chemical biology and bioanalysis. During the last ca. 8 years we have prepared hundreds of new purine bases, nucleosides and base-pair analogues and discovered several types of promising biologically active compounds. Purine ribonucleosides bearing aromatic or hetaryl group were found to possess strong cytostatic effect and 6-hetarylpurine nucleosides also anti-HCV activity. Also hydroxymethyl and several fluoromethylpurine nucleosides showed interesting cytostatic activity. Further interdisciplinary studies are now under way searching for other novel types of biologically active compounds. Several types of our compounds are now also applied in chemical biology and bioanalysis.

Papers composing this thesis have acquired significant citation impact (nearly 300 independent citations). The projects have attracted a lot of collaborations with academic institutions (e.g. University of Utah, University of Colorado at Boulder, Technische Univesität München, BFU Brno etc.) and with chemical and pharmaceutical industry (financial support and collaboration from Sumitomo Chemical Inc., Osaka, Japan; collaboration with Pharmasset, Inc., Tucker AL, U. S. A.). The methodology of the cross-coupling reactions on purines and nucleosides is now widely used in many laboratories and also the new cytostatic and antiviral compounds are inspiring further design of new compounds with potential biological activity. The quality and impact of the results was also acknowledged by several invitations to give plenary lectures at important international conferences (e.g., *Gordon Research Conference on Purines, Pyrimidines and Related Substances,* Newport, U. S. A., 2003; *2nd Nucleic Acid Chemical Biology (NACB) Symposium,* Odense, Denmark, 2005; *4th International Symposium on Nucleic Acids Chemistry*, Fukuoka, Japan 2005, etc.).

4. REFERENCES

[1] Examples: (a) Nair, V.; Chamberlain, S. D. *J. Am. Chem. Soc.* **1985**, *107*, 2183-2185. (b) Nair, V.; Chamberlain, S. D. *J. Org. Chem.* **1985**, *50*, 5069-5070.

[2] Examples: (a) Yamane, A.; Matsuda, A.; Ueda, T. *Chem. Pharm. Bull.* **1980**, *28*, 150-156. (b) Hamamichi, N.; Miyasaka, T. *J. Heterocycl. Chem.* **1990**, *27*, 2011-2015. (c) Hamamichi, N.; Miyasaka, T. *Chem. Pharm. Bull.* **1992**, *40*, 843-845.

[3] Examples: (a) Kumamoto, H.; Tanaka, H.; Tsukioka, R.; Ishida, Y.; Nakamura, A.; Kimura, S.; Hayakawa, H.; Kato, K.; Miyasaka, T. *J. Org. Chem.* **1999**, *62*, 7773-7780. (b) Tobrman, T.; Dvořák; D. *Org. Lett.* **2003**, *5*, 4289-4291.

[4] Diederich F., Stang P. J. (Eds): *Metal-Catalyzed Cross-Coupling Reactions*, Wiley, New York **1998**.

[5] Hocek, M. *Eur. J. Org. Chem.* **2003**, 245-254.

[6] Examples: (a) Bergstrom, D. E.; Reddy, P. A. *Tetrahedron Lett.* **1982**, *23*, 4191-4194. (b) Sugimura, H.; Takei, H. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 664-666. (c) Nair, V.; Turner, G. A.; Chamberlain, S. D. *J. Am. Chem. Soc.* **1987**, *109*, 7223-7224. (d) Nair, V.; Buenger, G. S. *J. Am. Chem. Soc.* **1989**, *111*, 8502-8504. (e) Nair, V.; Purdy, D. F. *Tetrahedron* **1991**, 47365-382. (f) Mamos, P.; Van Aerschot, A. A.; Weyns, N. J.; Herdewijn, P. A. *Tetrahedron Lett.* **1992**, *33*, 2413-2416. (g) Van Aerschot, A. A.; Mamos, P.; Weyns, N. J.; Ikeda, S.; De Clercq, E.; Herdewijn, P. *J. Med. Chem.* **1993**, *36*, 2938-2942.

[7] (a) Gundersen, L. L.; Langli, G.; Rise, F. *Tetrahedron Lett.* **1995**, *36*, 1945-1948. (b) Gundersen, L. L.; Bakkestuen, A. K.; Aasen, A. J.; ∅ veras, H.; Rise, F. *Tetrahedron* **1994**, *50*, 9743-9756.

[8] Hocek, M.; Holý, A. *Collect. Czech. Chem. Commun.* **1999**, *64*, 229-241.

[9] Česnek, M; Hocek, M.; Holý, A. *Collect. Czech. Chem. Commun.* **2000**, *65*, 1357-1373.

[10] Hocková, D.; Hocek, M.; Dvořáková, H.; Votruba, I. *Tetrahedron* **1999**, *55*, 11109-11118.

[11] Havelková, M.; Hocek, M.; Česnek, M.; Dvořák, D. *Synlett* **1999**, 1145-1147.

[12] Havelková, M.; Dvořák, D.; Hocek, M. *Synthesis* **2001**, 1704-1710.

[13] Hocek, M.; Holý, A.; Votruba, I.; Dvořáková, H. *Collect. Czech. Chem. Commun.* **2000**, *65*, 1683-1697.

[14] Hocek, M.; Holý, A.; Votruba, I.; Dvořáková, H. *J. Med. Chem.* **2000**, *43*, 1817-1825.

[15] Hocek, M.; Holý, A.; Votruba, I.; Dvořáková, H. *Collect. Czech. Chem. Commun.* **2001**, *66*, 483-499.

[16] Hocek, M.; Nauš, P.; Pohl, R.; Votruba, I.; Furman, P. A.; Tharnish, P. M.; Otto, M. J. *J. Med. Chem.* **2005**, *48*, 5869-5873.

[17] (a) Langli, G.; Gundersen L.-L.; Rise. F. *Tetrahedron* **1996**, *52*, 5625-5638. (b) Nolsoe, J. M. J.; Gundersen, L.-L.; Rise, F. *Acta Chem. Scand.* **1999**, *53*, 366-372.

[18] Hocek, M.; Holý, A.; Dvořáková, H. *Collect. Czech. Chem. Commun.* **2002**, *67*, 325-335.

[19] Hocek, M.; Hocková, D.; Štambaský, J. *Collect. Czech. Chem. Commun.* **2003**, *68*, 837-848.

[20] Hocek, M.; Votruba, I.; Dvořáková, H. *Tetrahedron* **2003**, *59*, 607-611.

[21] (a) Fürstner, A.; Leitner, A. *Angew. Chem. Int. Ed.* **2002**, *41*, 609. (b) Fürstner, A.; Leitner, A.; Mendez, M.; Krause, H. *J. Am. Chem. Soc.* **2002**, *124*, 13856-13864.

[22] Hocek, M.; Dvořáková, H. *J. Org. Chem.* **2003**, *68*, 5773-5776.

[23] Hocek, M.; Pohl, R.; Císařová, I. *Eur. J. Org. Chem.* **2005**, 3026-3030.

[24] Hocek, M.; Hocková, D.; Dvořáková, D. *Synthesis* **2004**, 889-894.

[25] Hocek, M.; Pohl R. *Synthesis* **2004**, 2869-2876.

[26] Šilhár, P.; Pohl, R.; Votruba, I.; Hocek, M. *Org. Lett.* **2004**, *6*, 3225-3228.

[27] Šilhár, P.; Pohl, R.; Votruba, I.; Hocek, M. *Collect. Czech. Chem. Commun.* **2005**, *70*, 1669-1695.

[28] Šilhár, P.; Pohl, R.; Votruba, I.; Hocek, M. *Org. Biomol. Chem.* **2005**, *3*, 3001-3007.

[29] Šilhár, P.; Pohl, R.; Votruba, I.; Hocek, M. *Synthesis* **2006**, in press.

[30] Hocek, M. *Heterocycles* **2004**, *63*, 1673-1677.

[31] Čapek, P.; Pohl, R.; Hocek, M. *J. Org. Chem.* **2004**, *69*, 7985-7988.

[32] Čapek, P.; Pohl, R.; Hocek, M. *J. Org. Chem.* **2005**, *70*, 8001-8008.

[33] Čapek, P.; Hocek, M. *Synlett* **2005**, 3005-3007.

[34] Hocek, M.; Votruba, I. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1055-1057.

[35] Hocek, M.; Dvořáková, H.; Císařová, I. *Collect. Czech. Chem. Commun.* **2002**, *67*, 1560-1578.

[36] Havelková, M.; Dvořák, D.; Hocek, M. *Tetrahedron* **2002**, *58*, 7431-7435.

[37] Hocek, M.; Starý, I.; Stará, I. G.; Dvořáková, H. *Tetrahedron Lett.* **2001**, *42*, 519-521.

[38] Hocek, M.; Starý, I.; Stará, I. G.; Dvořáková, H. *Collect. Czech. Chem. Commun.* **2002**, *67*, 1223-1235.

[39] Nauš, P.; Votruba, I.; Hocek, M. *Collect. Czech. Chem. Commun.* **2004**, *69*, 1955-1970.

[40] Hocek, M.; Štěpnička, P.; Ludvík, J.; Císařová, I.; Votruba, I.; Řeha, D.; Hobza, P. *Chem. Eur. J.* **2004**, *10*, 2058-2066.

5. List of Papers Composing the Thesis:

1. **Hocek, M.**; Holý, A.: "Perfluoroalkylation of 6-Iodopurines by Trimethyl(perfluoroalkyl)silanes. Synthesis of 6-(Perfluoroalkyl)purine Bases, Nucleosides and Acyclic Nucleotide Analogues"

Collect. Czech. Chem. Commun. **1999**, *64*, 229-241.
2. Havelková, M.; **Hocek, M.**; Česnek, M.; Dvořák, D.: "The Suzuki-Miyaura Cross-Coupling Reactions of 6-Halopurines with Boronic Acids Leading to 6-Aryl and 6-Alkenylpurines" *Synlett* **1999**, 1145-1147.
3. Hocková, D.; **Hocek, M.**; Dvořáková, H.; Votruba, I.:"Synthesis and Cytostatic Activity of Nucleosides and Acyclic Nucleoside Analogues Derived from 6-(Trifluoromethyl)purines" *Tetrahedron* **1999**, *55*, 11109-11118.
4. **Hocek, M.**; Holý, A.; Votruba, I.; Dvořáková, H.: "Synthesis and Cytostatic Activity of Substituted 6-Phenylpurine Bases and Nucleosides: Application of the Suzuki-Miyaura Cross-Coupling Reactions of 6-Chloropurine Derivatives with Phenylboronic Acids" *J. Med. Chem.* **2000**, *43*, 1817-1825.
5. Česnek, M; **Hocek, M**; Holý, A.: "Synthesis of Acyclic Nucleotide Analogues Derived from 2-Amino-6-*C*-Substituted Purines via Cross-Coupling Reactions of 2-Amino-9-[2-(diisopropoxyphosphorylmethoxy)-ethyl]-6-halopurines with Diverse Organometallic Reagents" *Collect. Czech. Chem. Commun.* **2000**, *65*, 1357-1373.
6. **Hocek, M.**; Holý, A.; Votruba, I.; Dvořáková, H.: "Cytostatic 6-Arylpurine Nucleosides II. Synthesis of Sugar-Modified Derivatives: 9-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-, 9-(5-Deoxy-β-D-ribofuranosyl)- and 9-(2,3-dihydroxypropyl)-6-phenylpurines" *Collect. Czech. Chem. Commun.* **2000**, *65*, 1683-1697.
7. **Hocek, M.**; Starý, I.; Stará, I. G.; Dvořáková, H.: "Cyclotrimerization of 6-ethynylpurines. Synthesis of 1,2,4- and 1,3,5-tris(purin-6-yl)benzenes as novel Hoogsteen-triplet analogues." *Tetrahedron Lett.* **2001**, *42*, 519-521.
8. **Hocek, M.**; Holý, A.; Votruba, I.; Dvořáková, H.: "Cytostatic 6-Arylpurine Nucleosides III. Synthesis and Structure-Activity Relationship Study in Cytostatic Activity of 6-Aryl-, 6-Hetaryl- and 6-Benzylpurine Ribonucleosides" *Collect. Czech. Chem. Commun.* **2001**, *66*, 483-499.
9. Havelková, M.; Dvořák, D.; **Hocek, M.**: "The Suzuki-Miyaura Cross-Coupling Reactions of 2-, 6- or 8-Halopurines with Boronic Acids Leading to 2-, 6- or 8-Aryl- or -Alkenylpurine Derivatives" *Synthesis* **2001**, 1704-1710.
10. **Hocek, M.**; Votruba, I.: "Covalent Analogues of DNA Base-Pairs and Triplets II. Synthesis and Cytostatic Activity of is(purin-6-yl)acetylenes, -diacetylenes and Related Compounds" *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1055-1057.
11. **Hocek, M.**; Holý, A.; Dvořáková, H.: "Cytostatic 6-Arylpurine Nucleosides IV. Synthesis of 2-Substituted 6-Phenylpurine Ribonucleosides" *Collect. Czech. Chem. Commun.* **2002**, *67*, 325-335.
12. Havelková, M.; Dvořák, D.; **Hocek, M.** "Covalent Analogues of DNA Base-Pairs and Triplets III. Synthesis of 1,4- and 1,3-bis(purin-6-yl)benzenes and 1-(1,3-dimethyluracil-5-yl)-3 or 4-(purin-9-yl)benzenes" *Tetrahedron* **2002**, *58*, 7431-7435.
13. **Hocek, M.**; Starý, I.; Stará, I. G.; Dvořáková, H.: "Covalent Analogues of DNA Base-Pairs and Triplets IV. Synthesis of trisubstituted benzenes bearing purine and/or pyrimidine rings by cyclotrimerization of 6-ethynylpurines and/or 5-ethynyl-1,3-dimethyluracil" *Collect. Czech. Chem. Commun.* **2002**, *67*, 1223-1235.
14. **Hocek, M.**; Dvořáková, H.; Císařová, I.: "Covalent Analogues of DNA Base-Pairs and Triplets V. Synthesis of Purine-Purine and Purine-Pyrimidine Conjugates Connected By Diverse Types of Acyclic Carbon Linkages" *Collect. Czech. Chem. Commun.* **2002**, *67*, 1560-1578.
15. **Hocek, M.**: "Syntheses of Purines Bearing Carbon Substituents in Positions 2, 6 or 8 by Metal- or Organometallics-Mediated C-C Bond Forming Reactions" *Eur. J. Org. Chem.* **2003**, 245-254.
16. **Hocek, M.**; Votruba, I.; Dvořáková, H. "Synthesis of Carba-Analogues of Myoseverine by Regioselective Cross-coupling Reactions of 2,6-Dichloro-9-isopropylpurine" *Tetrahedron* **2003**, *59*, 607-611.
17. **Hocek, M.**; Hocková, D.; Štambaský, J.: "Cytostatic 6-Arylpurine Nucleosides V. Synthesis of 8-Substituted 6-Phenylpurine Ribonucleosides" *Collect. Czech. Chem. Commun.* **2003**, *68*, 837-

848.
18. **Hocek, M.**; Dvořáková, H.: " An Efficient Synthesis of 2-Substituted 6-Methylpurine Bases and Nucleosides by Fe- or Pd-Catalyzed Cross-Coupling Reactions of 2,6-Dichloropurines " *J. Org. Chem.* **2003**, *68*, 5773-5776.
19. **Hocek, M.**; Štěpnička, P.; Ludvík, J.; Císařová, I.; Votruba, I.; Řeha, D.; Hobza, P.:"Ferrocene-Modified Purines as Potential Electrochemical Markers: Synthesis, Crystal Structures, Electrochemistry and Cytostatic Activity of (Ferrocenylethynyl)- and (Ferrocenylethyl)purines" *Chem. Eur. J.* **2004**, *10*, 2058-2066.
20. **Hocek, M.**; Hocková, D.; Dvořáková, D.:"Dichotomy in Regioselective Cross-Coupling Reactions of 6,8-Dichloropurines with Phenylboronic Acid and Methylmagnesium Chloride: Synthesis of 6,8-Disubstituted Purines" *Synthesis* **2004**, 889-894.
21. **Hocek, M.**: "Synthesis of Protected (Purin-6-yl)glycines via Pd-Catalyzed -Arylation of Ethyl N-(Diphenylmethylidene)glycinate with 6-Iodopurines" *Heterocycles* **2004**, *63*, 1673-1677.
22. Šilhár, P.; Pohl, R.; Votruba, I.; **Hocek, M.**: "Facile and Efficient Synthesis of 6-(Hydroxymethyl)purines" *Org. Lett.* **2004**, *6*, 3225-3228.
23. Nauš, P.; Votruba, I.; **Hocek, M.**: "Covalent Analogues of DNA Base-Pairs and Triplets VII. Synthesis and Cytostatic Activity of Bis(purin-6-yl)acetylene and -diacetylene Nucleosides" *Collect. Czech. Chem. Commun.* **2004**, *69*, 1955-1970.
24. Čapek, P.; Pohl, R.; **Hocek, M.**: "A Facile and Efficient Synthesis of (Purin-6-yl)alanines" *J. Org. Chem.* **2004**, *69*, 7985-7988.
25. **Hocek, M.** ; Pohl R.: "Regioselectivity in Cross-Coupling Reactions of 2,6,8-Trichloro-9-(tetrahydropyran-2-yl)purine: Synthesis of 2,6,8-Trisubstituted Purine Bases" *Synthesis* **2004**, 2869-2876.
26. **Hocek, M.**; Pohl, R.; Císařová, I.: "Highly Methylated Purines and Purinium Salts as Analogues of Heteromines" *Eur. J. Org. Chem.* **2005**, 3026-3030.
27. Šilhár, P.; Pohl, R.; Votruba, I.; **Hocek, M.**: "The First Synthesis and Cytostatic Activity of Novel 6-(Fluoromethyl)purine Bases and Nucleosides" *Org. Biomol. Chem.* **2005**, *3*, 3001-3007.
28. **Hocek, M.**; Nauš, P.; Pohl, R.; Votruba, I.; Furman, P. A.; Tharnish, P. M. Otto, M. J.: "Cytostatic 6-arylpurine Nucleosides 6. SAR in Anti-HCV and Cytostatic Activity of Extended Series of 6-Hetarylpurine Ribonucleosides" *J. Med. Chem.* **2005**, *48*, 5869-5873.
29. Čapek, P.; Pohl, R.; **Hocek, M.**: "Synthesis of Enantiomerically Pure (Purin-6-yl)phenylalanines and Their Nucleosides, a Novel Type of Purine-Amino Acid Conjugates" *J. Org. Chem.* **2005**, *70*, 8001-8008.
30. Šilhár, P.; Pohl, R.; Votruba, I.; **Hocek, M.**: " Synthesis of 2-Substituted 6-(Hydroxymethyl)purine Bases and Nucleosides" *Collect. Czech. Chem. Commun.* **2005**, *70*, 1669-1695..
31. Čapek, P.; **Hocek, M.**: "Efficient One-Step Synthesis of Optically Pure (Adenin-8-yl)phenylalanine Nucleosides" *Synlett* **2005**, 3005-3007.
32. Šilhár, P.; Pohl; R.; Votruba, I.; **Hocek, M.**: "Synthesis and Cytostatic Activity of Novel 6-(Difluoromethyl)purine Bases and Nucleosides" *Synthesis* **2006**, in press.

Other papers by the author:

33. Böhm, S.; **Hocek, M.**; Nešpůrek, S.; Kuthan, J.: "Photocoloration of 2,4,4,6-Tetraaryl-1,4-dihydroppyridines; a Semiempirical Quantum Chemical Study." *Collect. Czech. Chem. Commun.* **1994**, *59*, 262-272.
34. Böhm, S.; **Hocek, M.**; Němeček, J.; Havlíček, V.; Kuthan, J.: "Preparation snd Spectroscopic Properties of Spirocyclic 1-Methyl-2,4,4,6-tetraaryl-1,4-dihydropyridines." *Collect. Czech. Chem. Commun.* **1994**, *59*, 1105-1114.
35. **Hocek, M.**; Masojídková, M.; Holý, A: "A Facile Synthesis of 2-(Aminomethyl)Purines" *Synthesis* **1994**, 1401-1402.
36. **Hocek, M.**; Masojídková, M.; Holý, A: "Synthesis of Acyclic Nucleotide Analogues Derived from 2-(Aminomethyl)adenine and 2-(Aminomethyl)hypoxanthine" *Collect. Czech. Chem. Commun.* **1995**, *60*, 875-882.

37. **Hocek, M.**; Holý, A: "A Facile Synthesis of 6-Cyanopurines" *Collect. Czech. Chem. Commun.* **1995**, *60*, 1386-1389.
38. **Hocek, M.**; Holý, A.: "Acyclic Nucleotide Analogues Derived from 6-(1-Aminoalkyl)purines" *Collect. Czech. Chem. Commun.* **1996**, *61 (Special Issue)*, S55-S58.
39. **Hocek, M.**; Masojídková, M.; Holý, A.; Andrei, G.; Snoeck, R.; Balzarini, J.; De Clercq, E.: "Synthesis and Antiviral Activity of Acyclic Nucleotide Analogues Derived from 6-(Aminomethyl)purines and Purine-6-carboxamidines" *Collect. Czech. Chem. Commun.* **1996**, *61*, 1525-1537.
40. **Hocek, M.**; Masojídková, M.; Holý, A.: "Synthesis of Acyclic Nucleotide Analogues Derived from *N*-Substituted 6-(1-Aminoethyl)purines *via* 6-Acetylpurine Derivatives" *Tetrahedron* **1997**, *53*, 2291-2302.
41. **Hocek, M.**; Masojídková, M.; Holý, A.: "Synthesis of Acyclic Nucleotide Analogues Derived from 6-Hetarylpurines *via* Cross-Coupling Reactions of 9-[2-(Diethoxyphosphonylmethoxy)ethyl]-6-iodopurine with Hetaryl Organometallic Reagents" *Collect. Czech. Chem. Commun.* **1997**, *62*, 136-146.
42. **Hocek, M.**: "Synthesis and Biological Activity of 2- and 6-*C*-Substituted Purine Bases, Nuceosides and Acyclic Nucleotide Analogues - Alfred Bader Award Lecture (in Czech)" *Chem. Listy* **2000**, *94*, 978-982.
43. Turek, P.; Kotora, M.; **Hocek, M.**; Císařová, I. "[2+2+2]-Co-cyclotrimerization of 6-Alkynylpurines with Diynes: a Method for Preparation 6-Arylpurines" *Tetrahedron Lett.* **2003**, *44*, 785-788.
44. **Hocek, M.**; Dvořák, D.; Havelková, M.: " Covalent Analogues of Nucleobase-Pairs " *Nucleosides, Nucleotides Nucleic Acids* **2003**, *22*, 775-777.
45. **Hocek, M.**: " C-C and C-X Bond Formation by Cross-coupling Reactions Catalyzed by Transition Metal Complexes (in Czech) " *Chem. Listy* **2003**, *97*, 1145-1150.
46. Sečkářová, P.; Marek, R.; Maliňáková, K.; Kolehmainen, E.; Hocková, D.; **Hocek, M.**; Sklenář, V.: "Direct Determination of Tautomerism in Purine Derivatives by Low-Temperature NMR Spectroscopy" *Tetrahedron Lett.* **2004**, *45*, 6259-6263.
47. Turek, P.; Kotora, M.; Tišlerová, I.; **Hocek, M.**; Votruba, I.; Císařová, I.: "Co-cyclotrimerization of 6-Alkynylpurines with , -Diynes as a Novel Approach to Biologically Active 6-Arylpurines" *J. Org. Chem.* **2004**, *69*, 9224-9233.
48. Turek, P.; Kotora, M.; **Hocek, M.**; Votruba, I.: "Novel Method for Preparation of Highly Substituted 6-Arylpurines by Reactions of 6-Alkynylpurines with Zirconacyclopentadienes" *Collect. Czech. Chem. Commun.* **2005**, *70*, 339-349.
49. **Hocek, M.**; Pohl, R.; Klepetářová, B.: "A New Modular and Practical Methodology for the Synthesis of 4- or 3-Substituted Phenyl C-Nucleosides" *Eur. J. Org. Chem.* **2005**, 4525-4528.
50. Řeha, D.; **Hocek, M.**; Hobza, P.: "Exceptional Thermodynamic Stability of DNA Duplex Modified by Nonpolar Base Analogues is Due to Increased Stacking Interaction and Favourable Solvation: Correlated *ab initio* Calculations and Molecular Dynamics Similation" *Chem. Eur. J.* **2005**, in press.