Hocek group

Bioorganic & Medicinal Chemistry of Nucleic Acids
Joint laboratory of IOCB and Charles University

VÝZKUMNÉ CENTRUM ÚOCHB & GILEAD SCIENCES

GSRC

GILEAD SCIENCES & IOCB RESEARCH CENTRE

Od nukleosidů s modifikovanou bází k funkcionalizovaným nukleovým kyselinám

Doc. Ing. Michal HOCEK, CSc., DSc.

ÚOCHB AV ČR, v.v.i.
Flemingovo nám. 2
16610 Praha 6; Czech Republic
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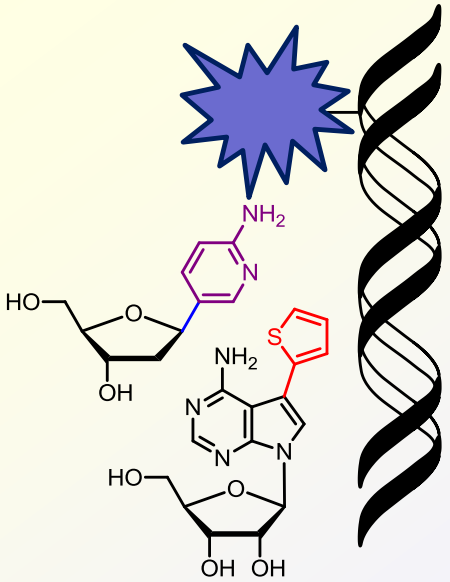
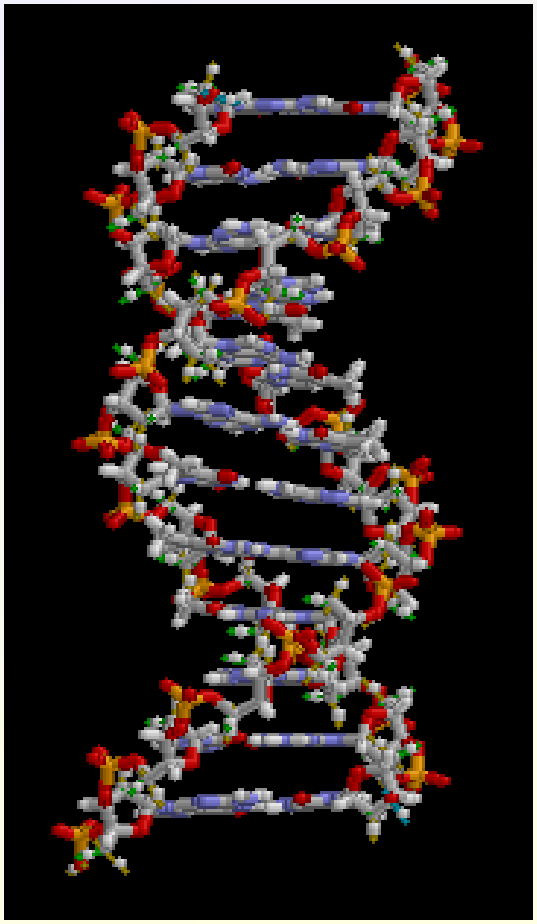
http://www.uochb.cas.cz/hocekgroup

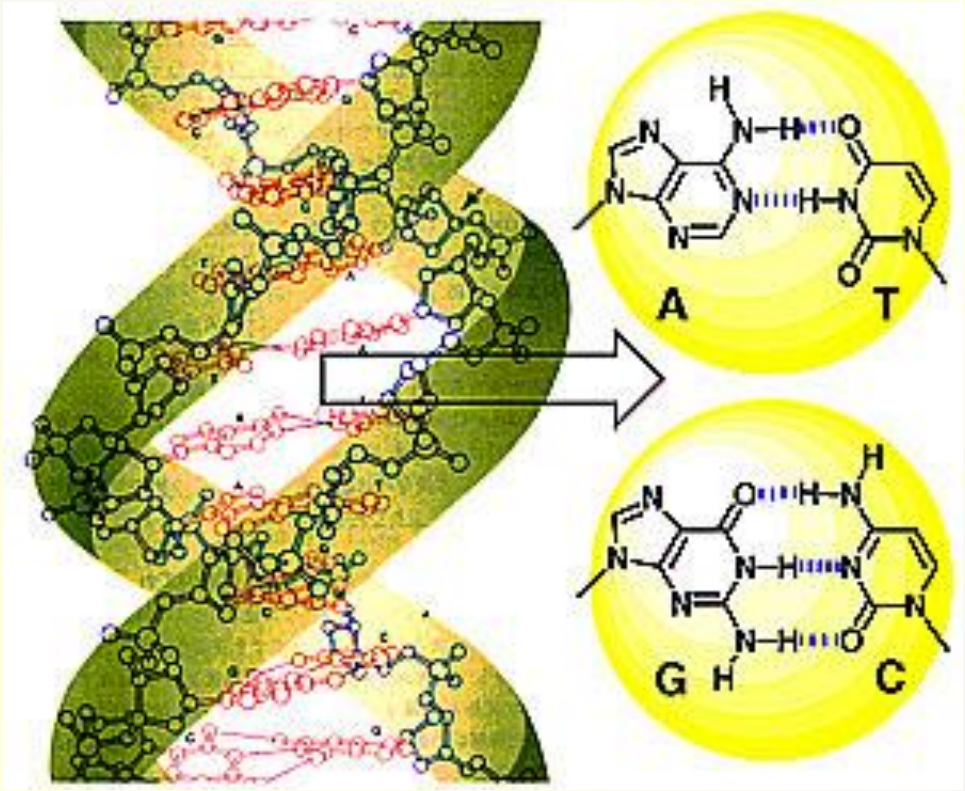
a

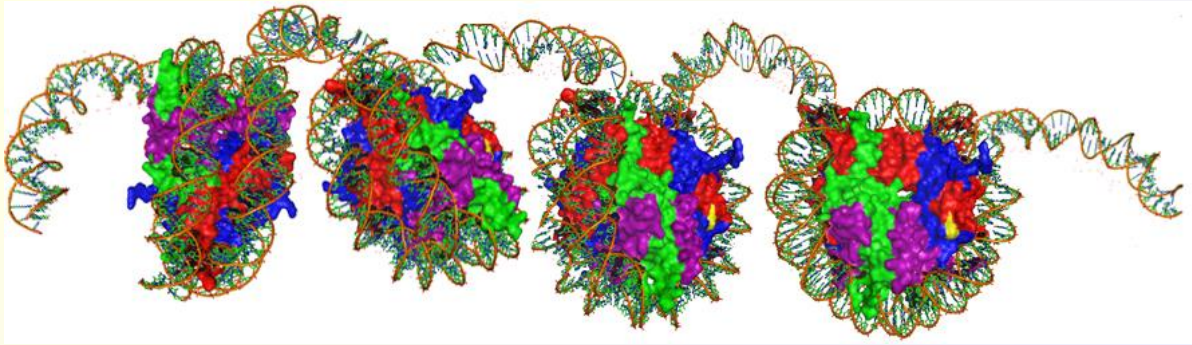
Katedra organické chemie, Přírodovědecká fakulta,
Univerzita Karlova v Praze



DNA

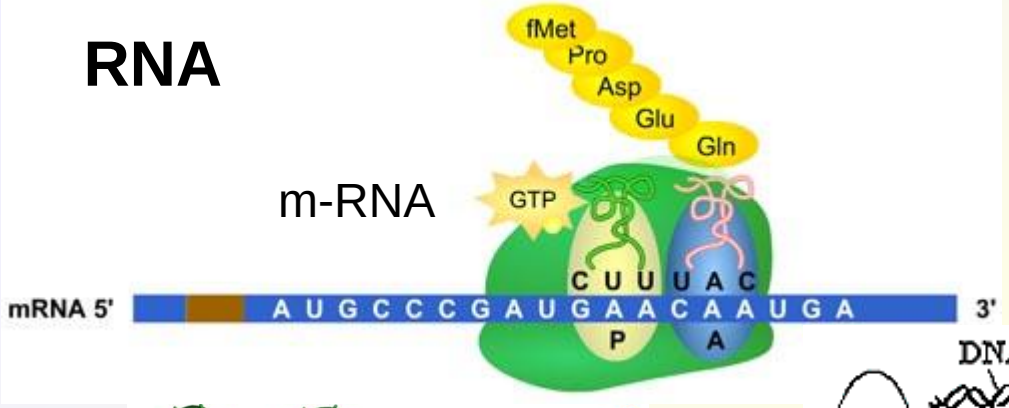


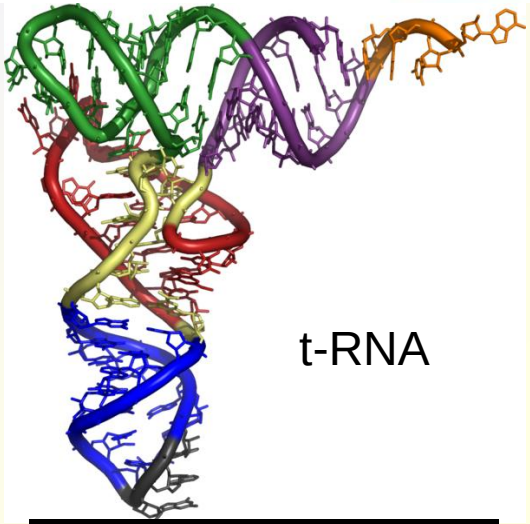




RNA

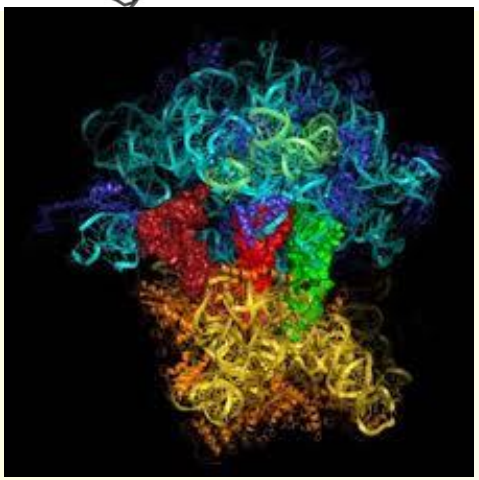
m-RNA

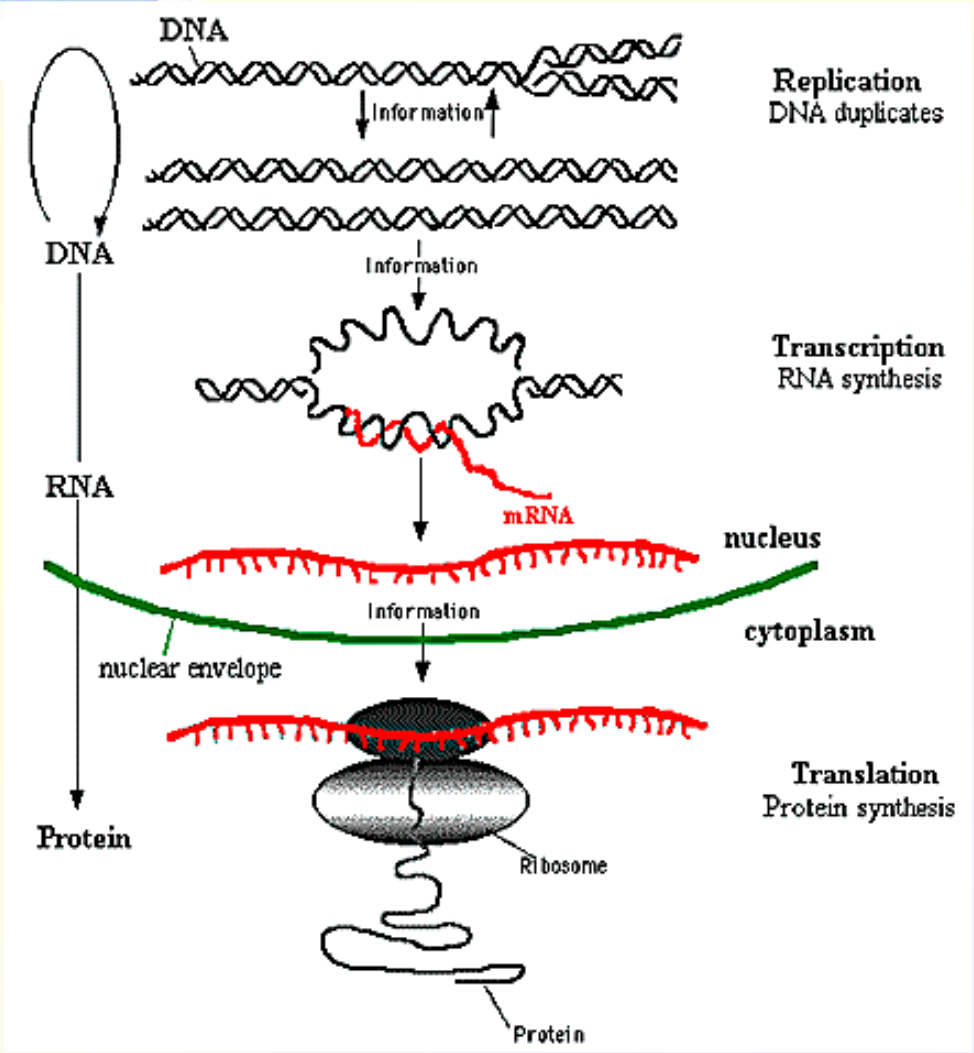




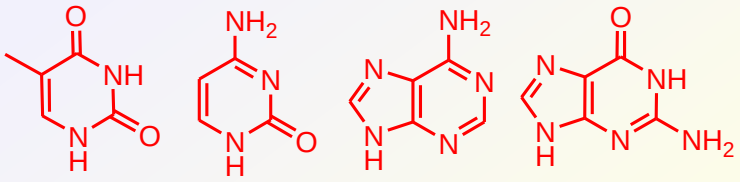
t-RNA

r-RNA

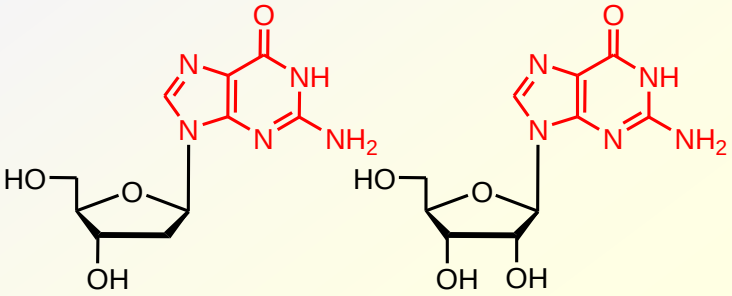




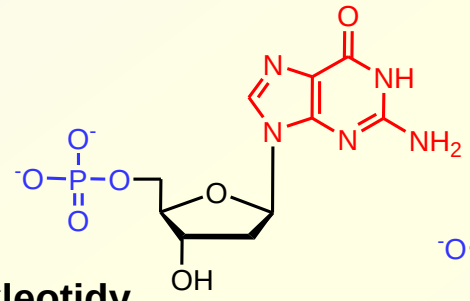
Složky nukleových kyselin



nukleobáze



nukleosidy



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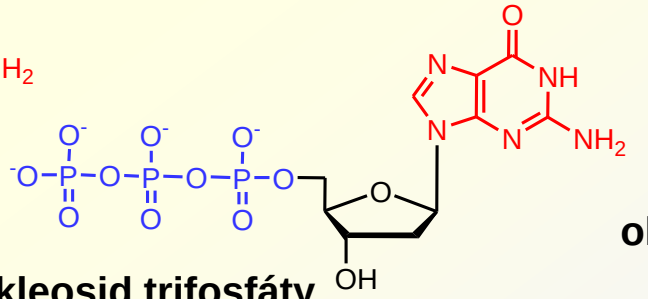
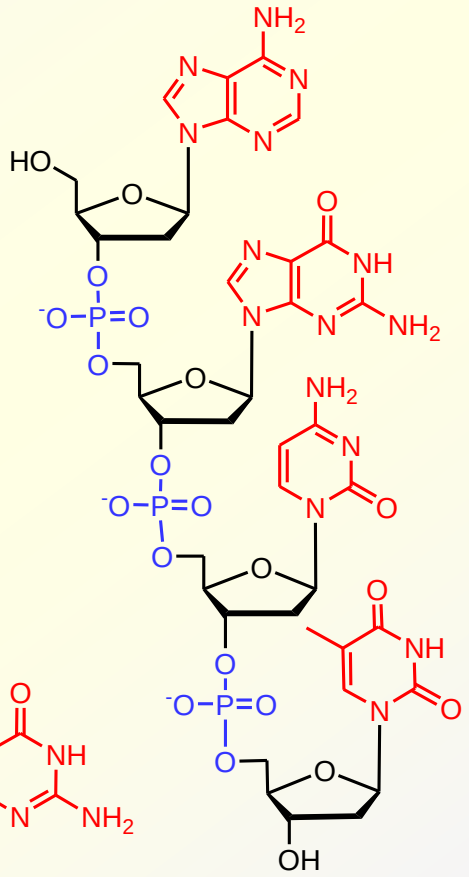
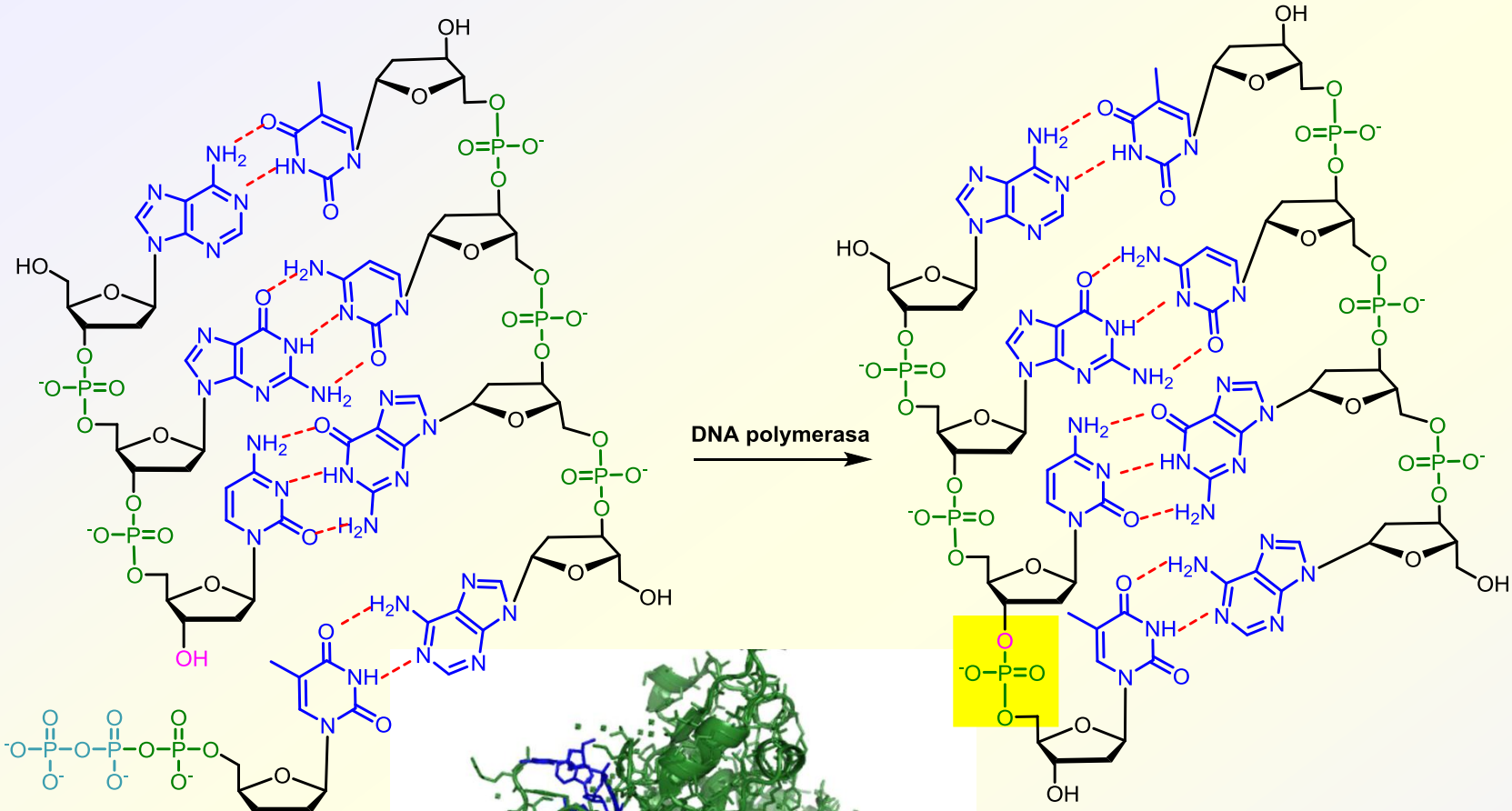


nukleosid trifosfáty (NTP)



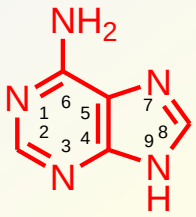
oligonukleotidy

Biosyntéza DNA – DNA polymerasa

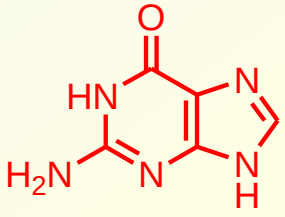


nukleosid trifosfáty

PURINY

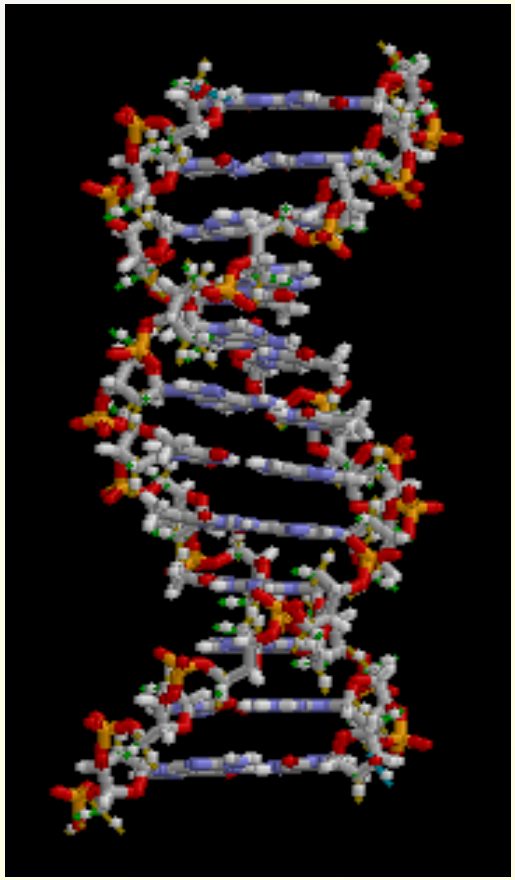


Adenin

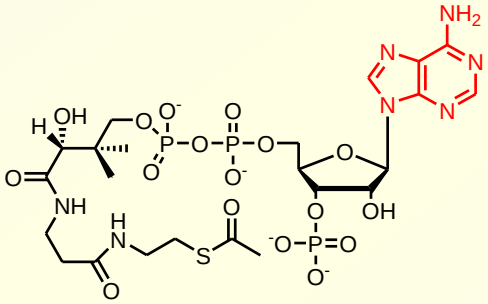


Guanin

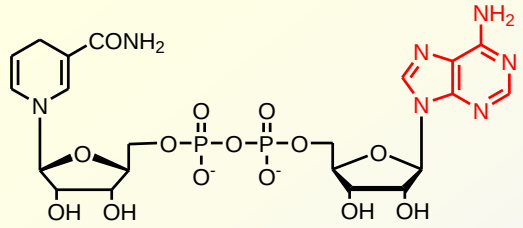
Složky nukleových kyselin



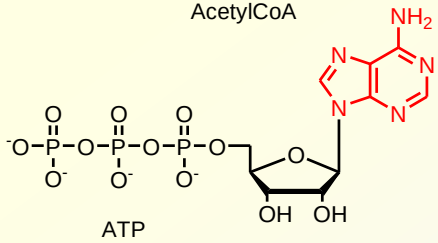
Kofaktory enzymů



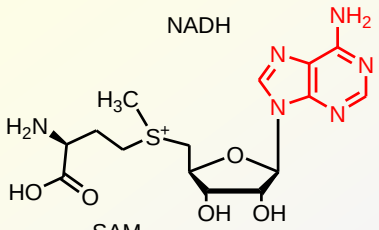
AcetylCoA



NADH

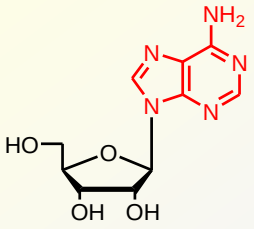


ATP

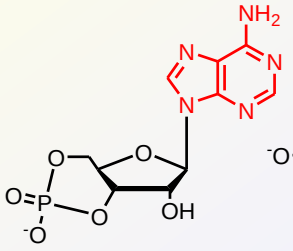


SAM

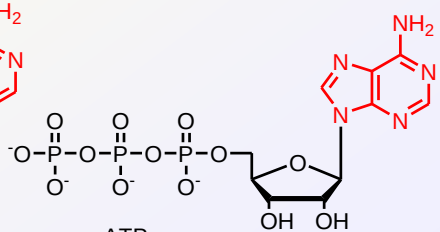
Signalní molekuly



A



c-AMP

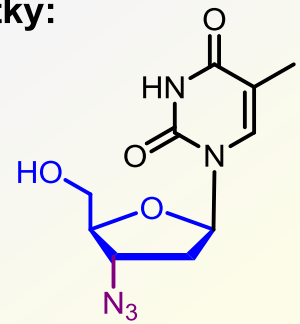


ATP

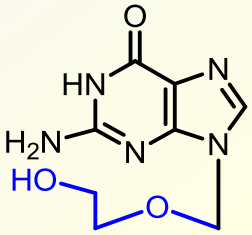
Klinicky užívaná nukleosidová a nukleotidová léčiva

protivirové látky:

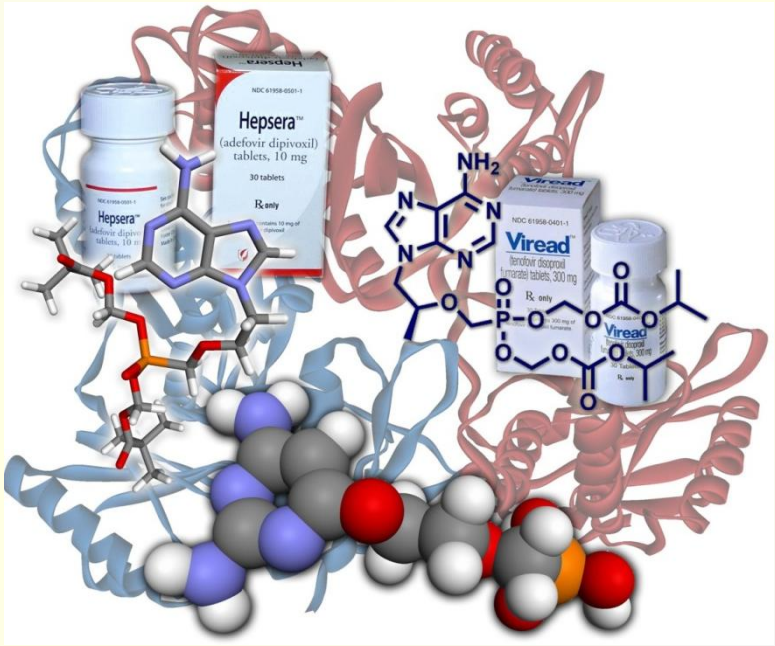
Didanosine, ddl



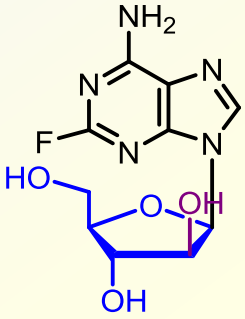
Zidovudine, AZT



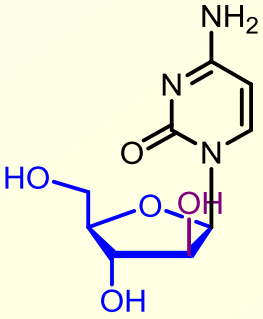
Acyclovir



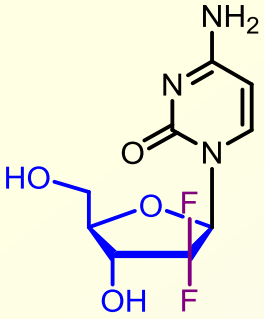
cytostatika (protinádorové) látky:



Fludarabine



AraC



Gemcitabine

Prof. RNDr. Antonín Holý, DrSc., Dr.h.c.

Deriváty a analogy nukleosidů a nukleotidů s modifikovanou bází

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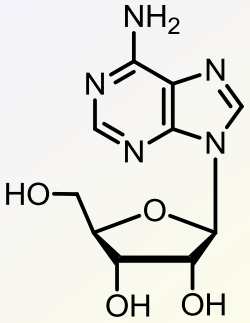
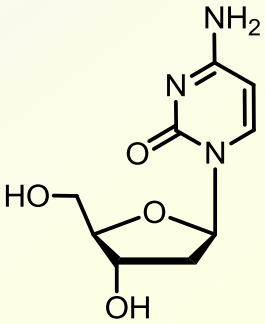


adenosine

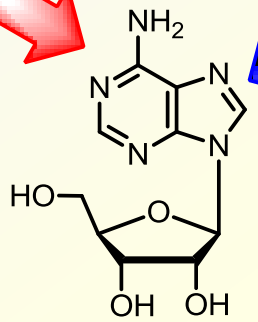


2'-deoxycytidine

efekt na párování bází

zachovává párování bází

modifikuje velký zlábek



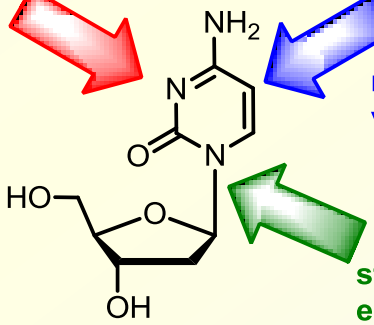
adenosin

efekt na párování bází

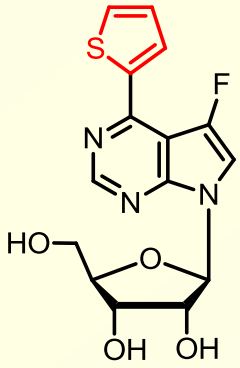
zachovává párování bází

modifikuje velký zlábek

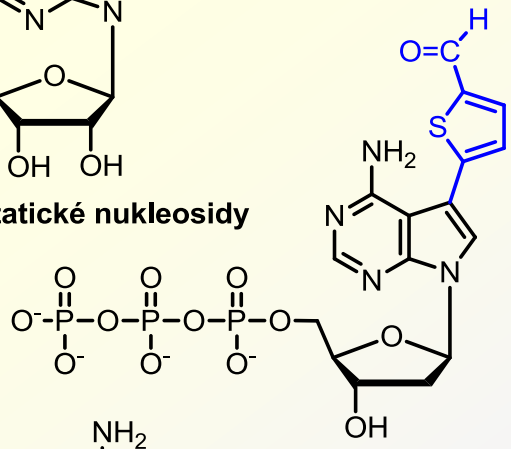
stabilia vuci enzymatickému stepení



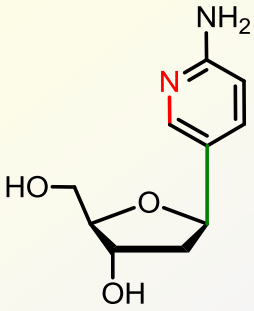
2'-deoxycytidin



cytostatické nukleosidy



modifikované dNTPs



C-nukleosidy

Organická syntéza

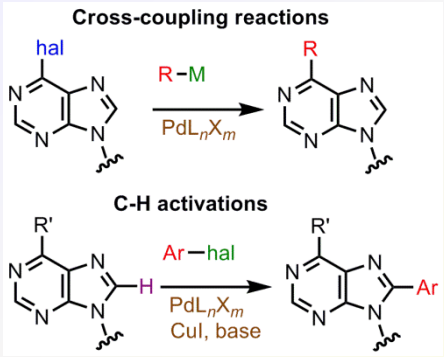
Farmakochemie (medicinal chemistry)

Chemická biologie

Hlavní oblasti výzkumu

Hocek group
Bioorganic & Medicinal Chemistry of Nucleic Acids
Joint laboratory of IOCB and Charles University

1. Syntetická metodika



Chem. Commun. **2007**, 4729
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J. Org. Chem. **2008**, *73*, 9048
Org. Biomol. Chem. **2009**, *7*, 866
J. Org. Chem. **2010**, *75*, 2302
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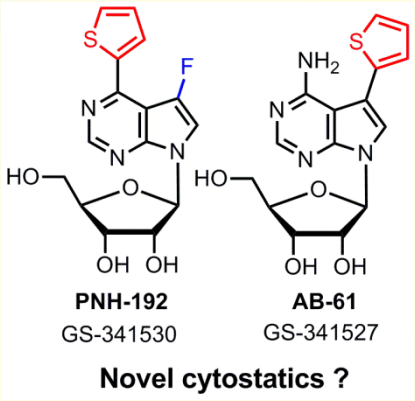
Synlett **2012**, *23*, 1305

2. Farmakochemie



J. Med. Chem. **2005**, *48*, 5869
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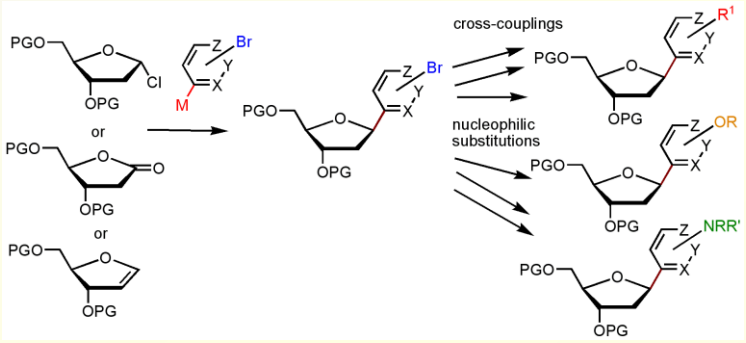
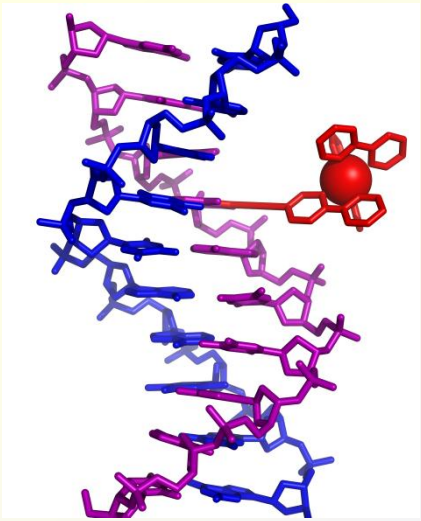
Bioorg. Med. Chem. **2008**, *16*, 2329
J. Med. Chem. **2010**, *53*, 460
ChemMedChem **2010**, *5*, 1386
Bioorg. Med. Chem. **2011**, *19*, 229
J. Med. Chem. **2011**, *54*, 5498
Bioorg. Med. Chem. **2012**, *20*, 5202
Bioorg. Med. Chem. **2012**, *20*, 6123
US8093226(B), WO2009089804(A1)
WO2010121576(A2)

3. C-nukleosidy pro chemickou biologii



J. Org. Chem. **2006**, *71*, 7322
J. Org. Chem. **2007**, *72*, 6797
J. Org. Chem. **2008**, *73*, 3798
ChemBioChem **2008**, *9*, 2796
Tetrahedron **2009**, *65*, 4471
Biochemistry **2009**, *48*, 10866
Chem. Rev. **2009**, *109*, 6729
J. Org. Chem. **2010**, *75*, 442
Biochemistry **2010**, *49*, 727
Eur. J. Org. Chem. **2010**, 2666
Eur. J. Org. Chem. **2010**, 5432
J. Org. Chem. **2011**, *76*, 6619
Biochemistry **2011**, *50*, 7243
J. Org. Chem. **2011**, *76*, 7781
Synthesis **2012**, *44*, 953
Eur. J. Org. Chem. **2012**, 1759

4. Funkcionalizované nukleové kyseliny pro bioanalýzu nebo chemickou biologii



Chem. Eur. J. **2007**, *13*, 6196
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Angew. Chem. Int. Ed. **2008**, *47*, 2059
Org. Biomol. Chem. **2008**, *6*, 3657
Chem. Eur. J. **2009**, *15*, 1144
Nucleic Acids Res. **2009**, *37*, 7612
Angew. Chem. Int. Ed. **2010**, *49*, 1064
J. Org. Chem. **2011**, *76*, 3457
ChemBioChem **2011**, *12*, 431
Chem. Eur. J. **2011**, *17*, 5833
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Angew. Chem. Int. Ed. **2011**, *50*, 8727
Chem. Soc. Rev. **2011**, *40*, 5802
J. Org. Chem. **2012**, *77*, 1026
Org. Biomol. Chem. **2012**, *10*, 49
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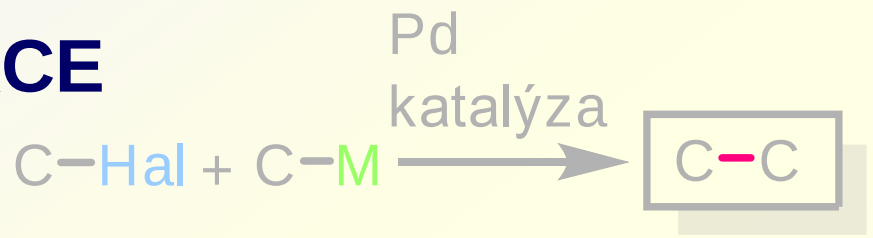
Chem. Commun. **2012**, *48*, 6921
Chem. Sci. **2012**, *3*, 2797

CROSS-COUPLING REAKCE



KUMADA

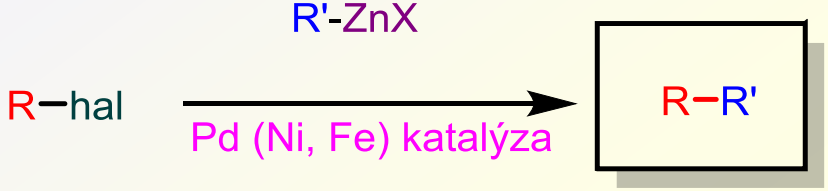
R'-MgX

SUZUKI-MIYAURA

R'-$B(OH)_2$

NEGISHI

R'-ZnX



STILLE

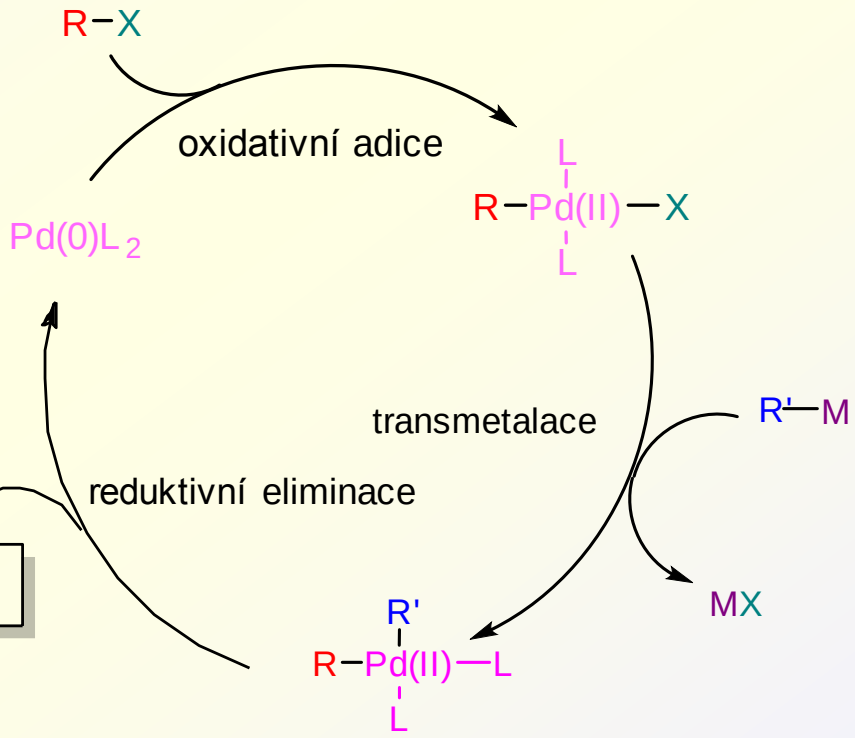
R'-$SnBu_3$

HIYAMA

R'-SiR_3, F^-

SONOGASHIRA

R'-C≡CH

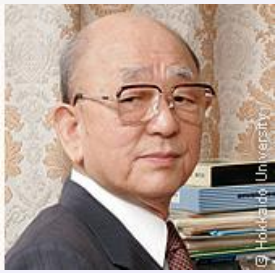


Nobelova cena za chemii 2010

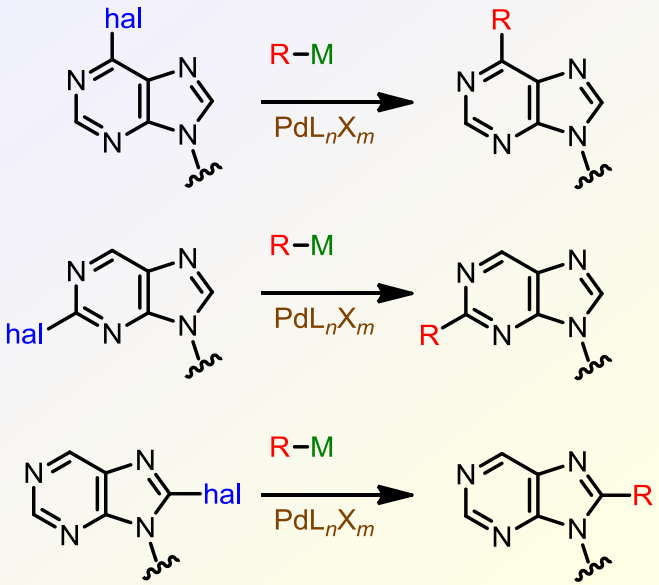
Richard Heck

Ei-ichi Negishi

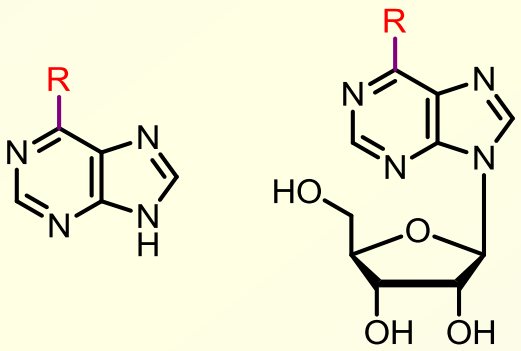
Akira Suzuki



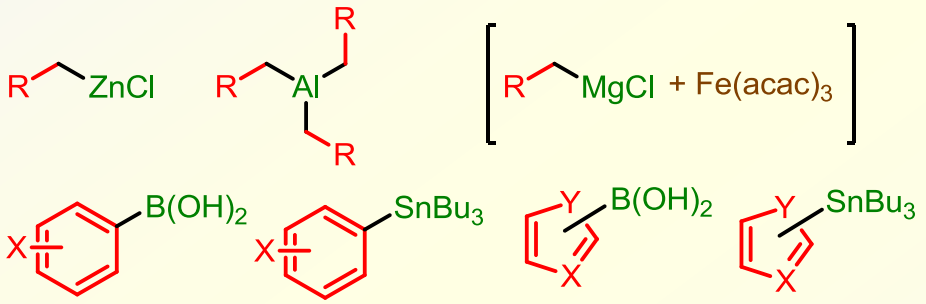
Cross-coupling reakce pro modifikace nukleobází



review: Hocek, M. *Eur. J. Org. Chem* **2003**, 245-254.

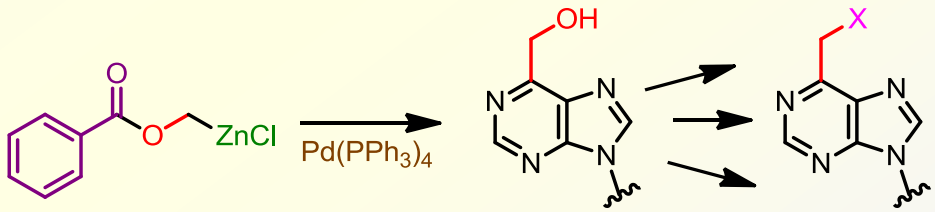


knihovna >2000 derivátu

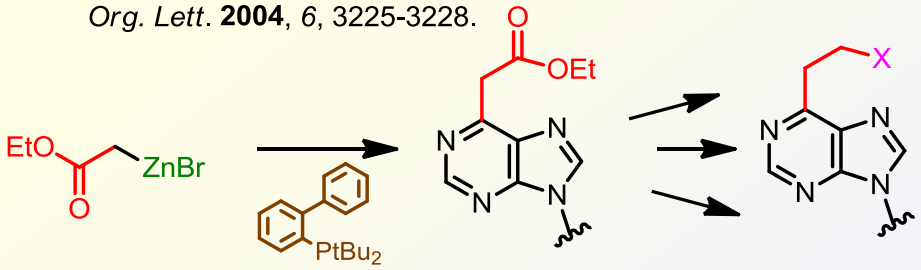


Catalysis: $Pd(PPh_3)_4$, $Pd(PPh_3)_2Cl_2$

Cross-coupling reakce s funkcionalizovanymi organokovy

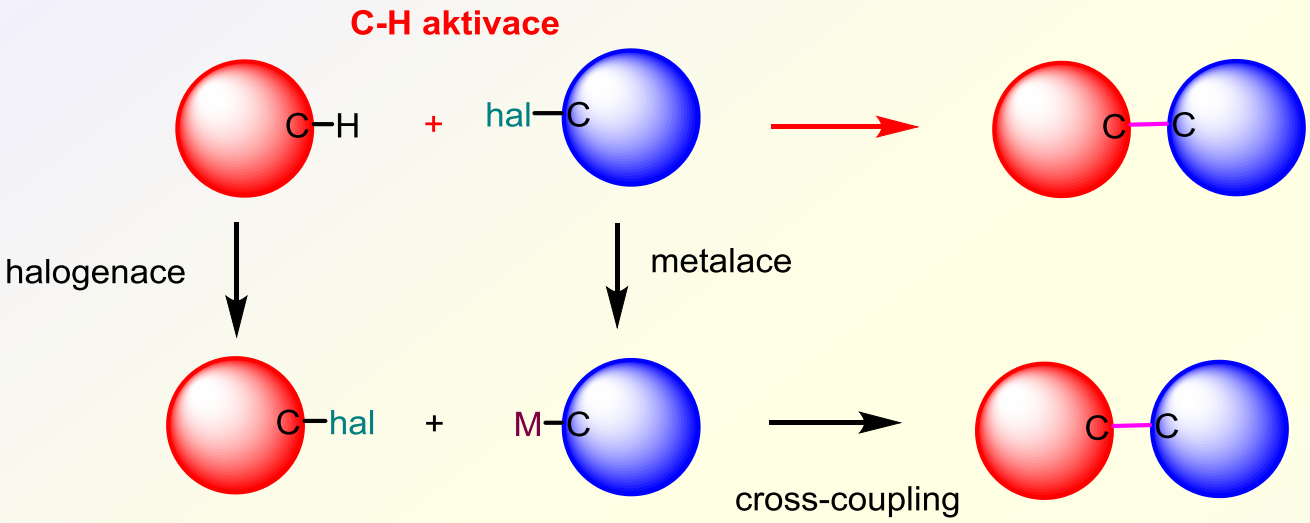


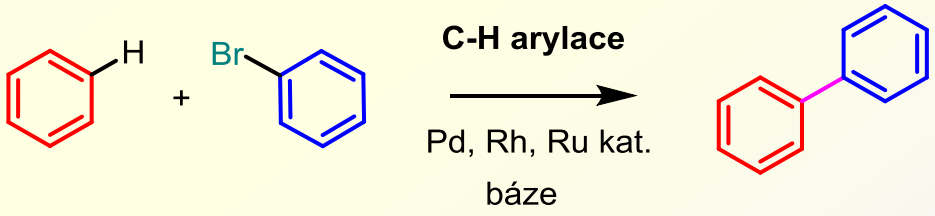
Org. Lett. **2004**, *6*, 3225-3228.

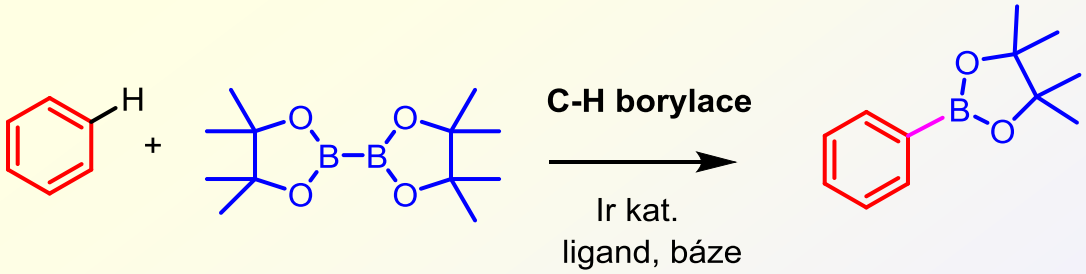


Tetrahedron Lett. **2007**,*48,* 5589-5592.

C-H AKTIVACE

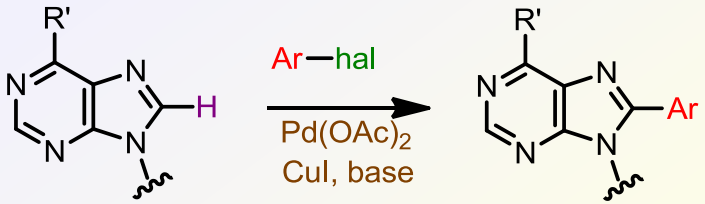

C-H aktivace
C–H
+
hal–C
C
C
halogenace
metalace
C–hal
+
M–C
C
C
cross-coupling


H
+
Br
C-H arylace
Pd, Rh, Ru kat.
báze

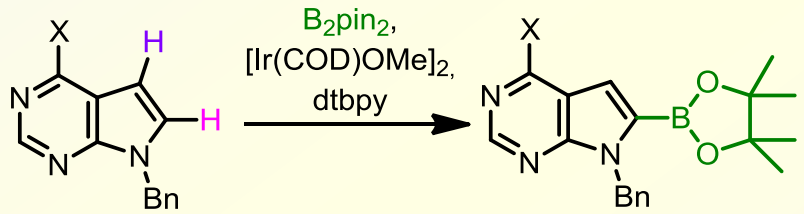

H
+
O
B–B
O
O
O
C-H borylace
Ir kat.
ligand, báze
O
B
O

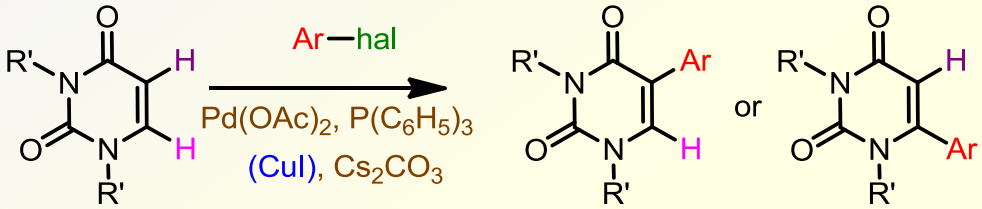
C-H aktivační reakce pro modifikace nukleobází

C-H arylace

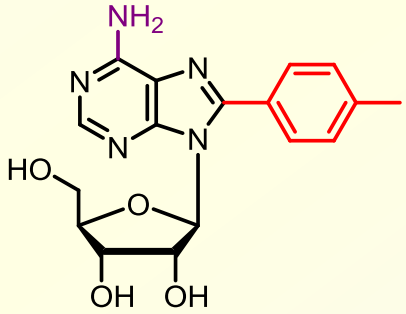


C-H borylace

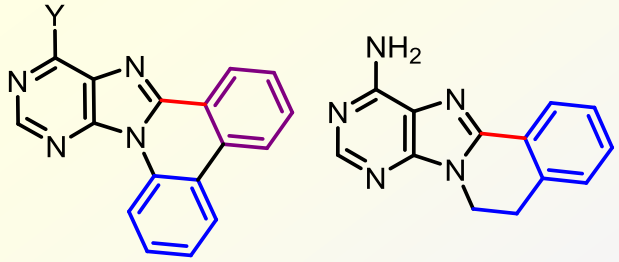




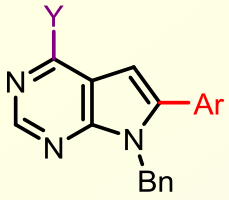
Příklady modifikovaných nukleobazí připravených cross-coupling reakcemi nebo/a C-H aktivacemi:



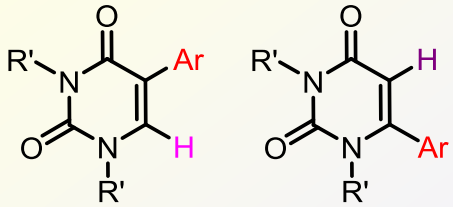
Chem. Commun. **2007**, 4729



J. Org. Chem. **2010**, *75*, 2302

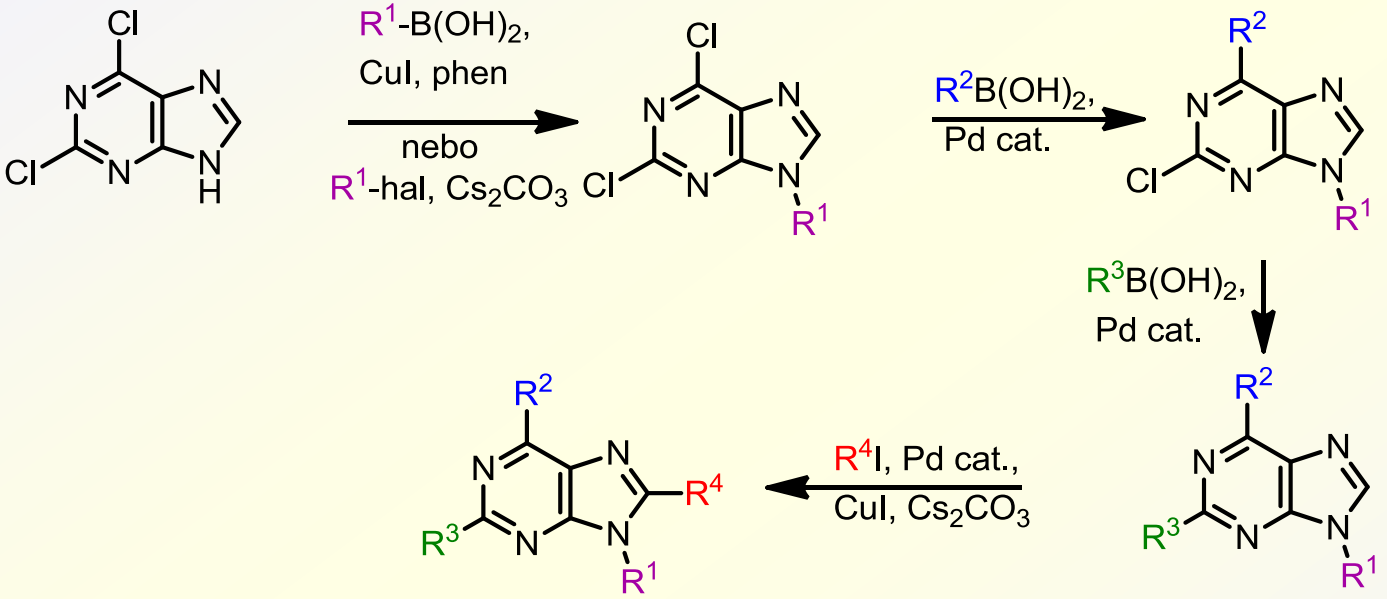


Org. Biomol. Chem. **2009**, *7*, 866



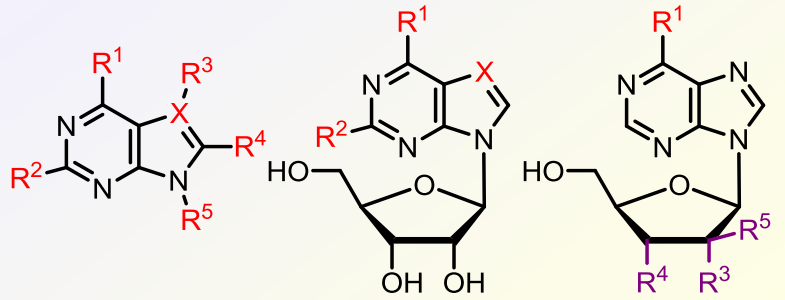
Eur. J. Org. Chem. **2009**, 3698
J. Org. Chem. **2011**, *76*, 5309

Obecná metoda přípravy 2,6,8,9-tetrasubstituovaných purinů kombinací chemo-regioselektivních N-arylací/alkylací, cross-coupling reakcí a C-H arylací



Čerňa, I.; Pohl, R.; Klepetářová, B.; Hocek, M. *J. Org. Chem.* **2008**, *73*, 9048-9054.

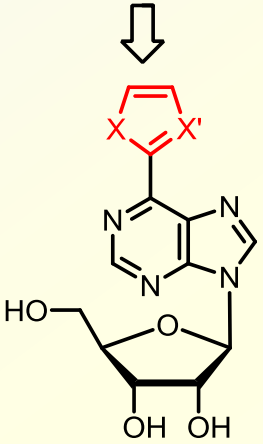
Farmakochemie



modifikované nukleobáze a nukleosidy

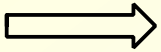
– knihovna >2000 derivátů

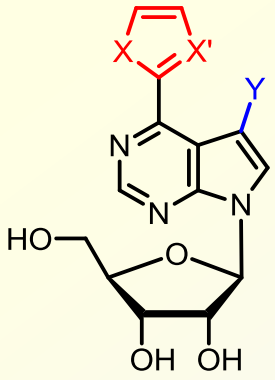
Testovány na cytostatickou a protivirovou aktivitu a inhibici vybraných enzymů.



cytostatická,
anti-HCV aktivita

J. Med. Chem. **2005**, *48*, 5869





X, X' = O, S, N/NH

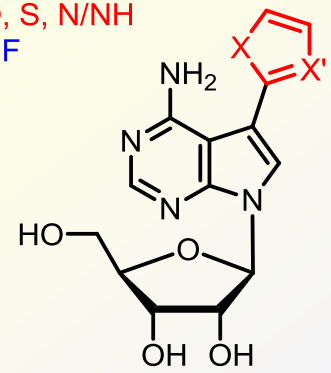
Y = H or F

nanomolární cytostatika

J. Med. Chem. **2010**, *53*, 460

US 8,093,226 B2

WO2009089804 (A1)



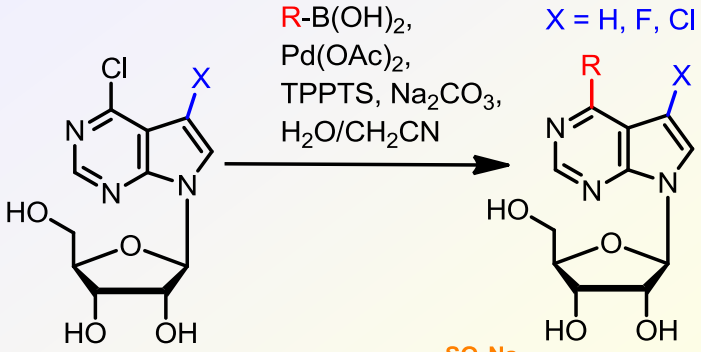
nanomolární cytostatika

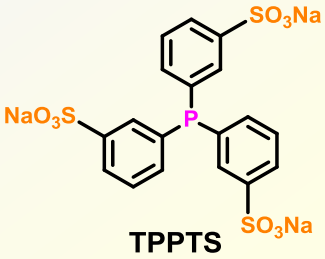
J. Med. Chem. **2011**, *54*, 5498

WO2010121576 (A2)

preklinické *in vivo* testování and vývoj

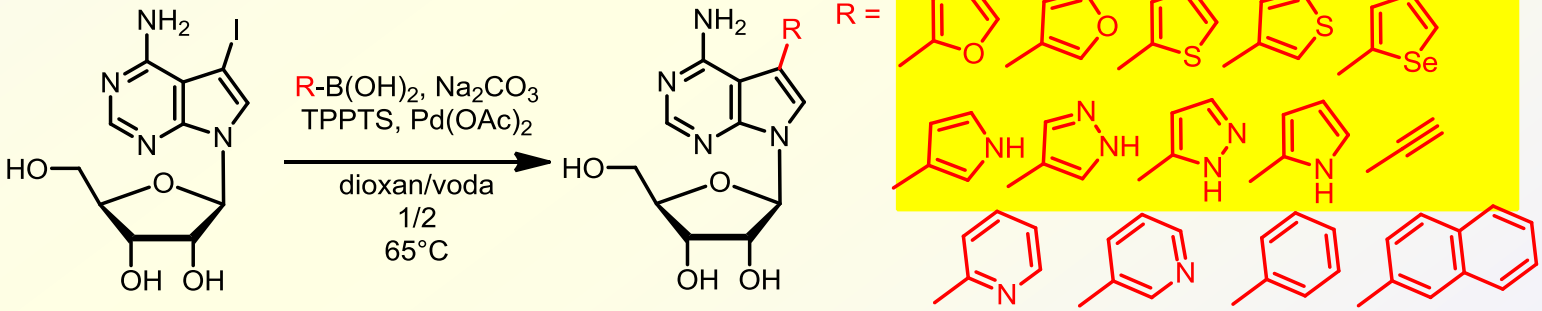
Syntéza deazapurinových ribonukleosidů





Nauš, P.; Pohl, R.; Votruba, I.; Dzubák, P.; Hajdúch, M.; Ameral, R.; Birkuš, G.; Wang, T.; Ray, A.; Mackman, R.; Cihlar, T.; Hocek, M.
J. Med. Chem. **2010**, *53*, 460-470.

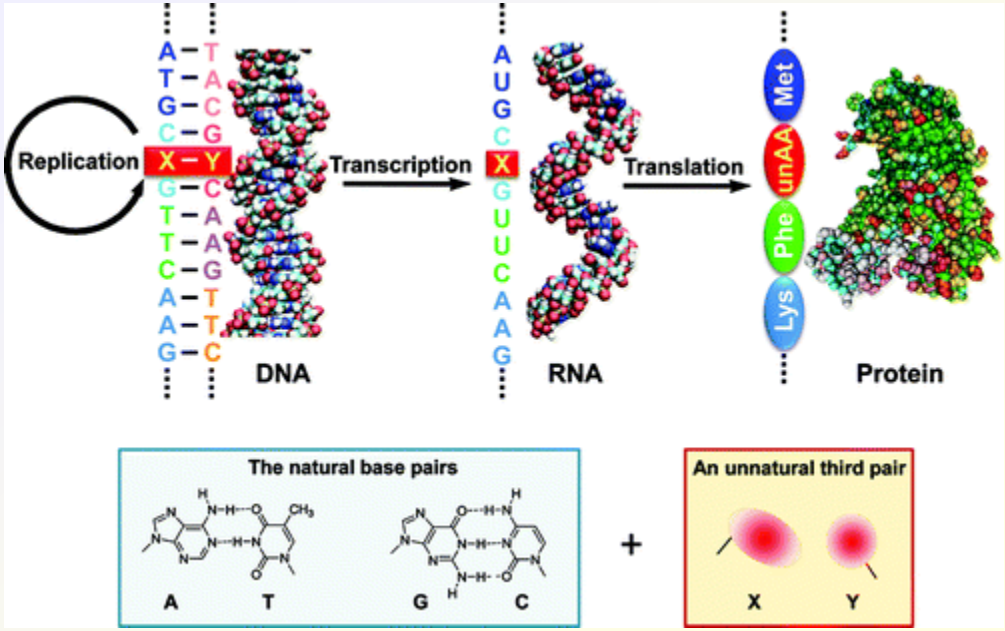
Hocek M., Nauš P., US 8,093,226 B2, WO2009089804 (A1), JP2011509949 (A), EP2231689 (A1), CA2711384 (A1).

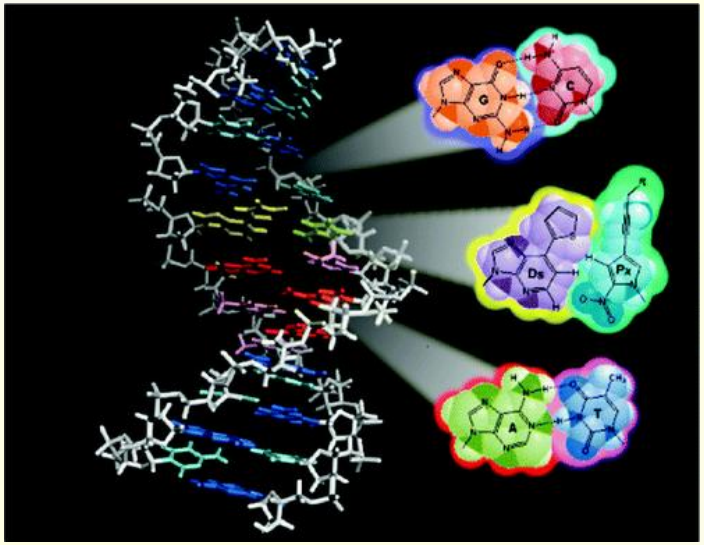


Bourderioux, A.; Nauš, P.; Perlíková, P.; Pohl, R.; Pichová, I.; Votruba, I.; Džubák, P.; Konečný, P.; Hajdúch, M.; Stray, K. M.; Wang, T.; Ray, A. S.; Feng, J. Y.; Birkus, G.; Cihlar, T.; Hocek, M.
J. Med. Chem. **2011**, *54*, 5498-5507.

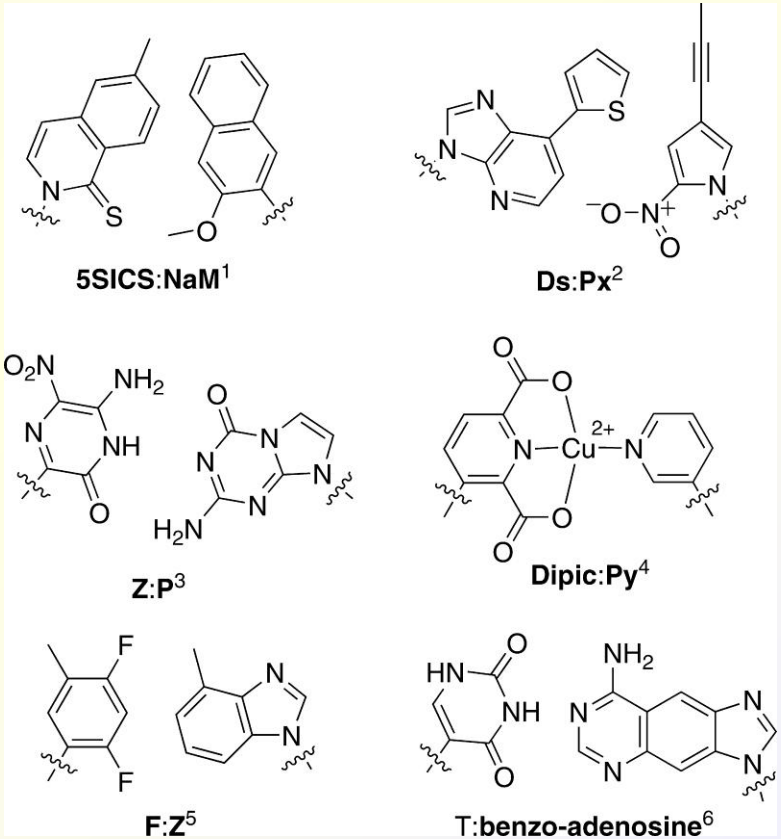
Bourderioux, A.; Naus, P.; Hocek, M. PCT/CZ2010/000050, WO2010121576 (A2)

Rozšíření genetické abecedy



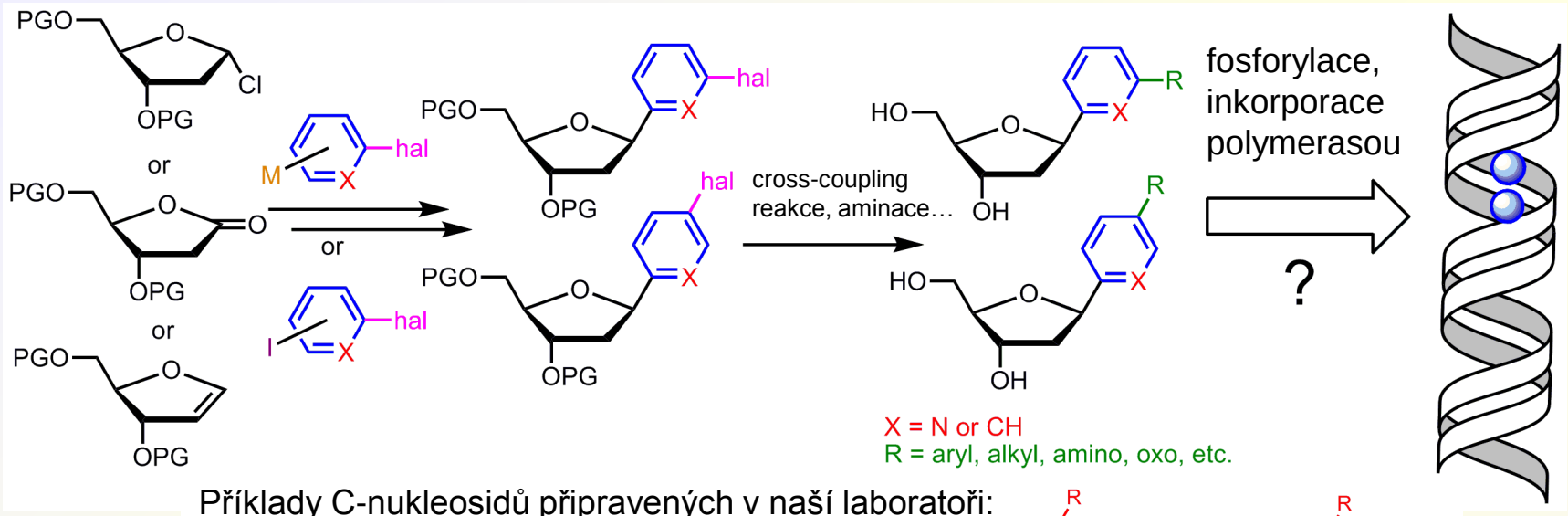


Nové umělé páry bází
(Kool, Hirao, Benner, Romesberg…)

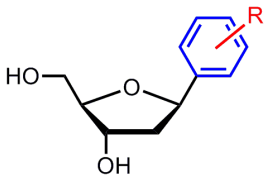


Použity obrázky z: Hirao et al. *Acc. Chem. Res.* **2012**, 45, 2055-2065.

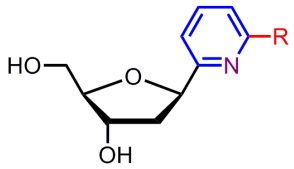
Modulární syntéza C-nukleosidů



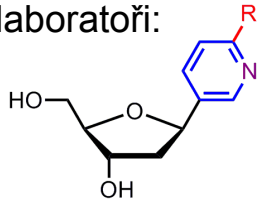
Příklady C-nukleosidů připravených v naší laboratoři:



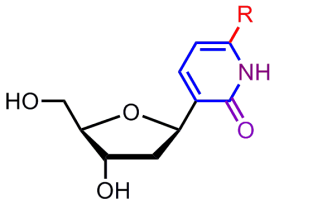
Eur. J. Org. Chem. **2005**, 4525
Synthesis **2008**, 1918



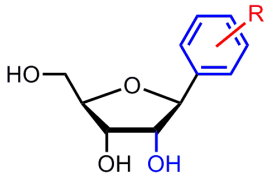
J. Org. Chem. **2006**, *71*, 7322
biochemical studies:
ChemBioChem **2008**, *9*, 2796



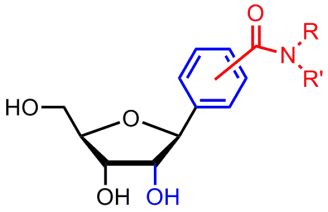
J. Org. Chem. **2007**, *72*, 6797
biochemical studies:
Biochemistry **2009**, *48*, 10866
Biochemistry **2010**, *49*, 727
Biochemistry **2011**, *50*, 7243



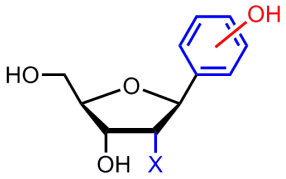
Eur. J. Org. Chem. **2012**, 1759



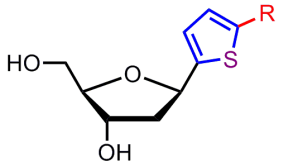
Eur. J. Org. Chem. **2008**, 1689



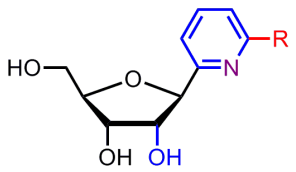
Tetrahedron **2009**, *65*, 4471



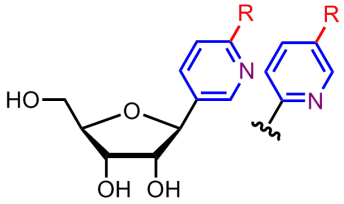
Synthesis **2010**, 4199



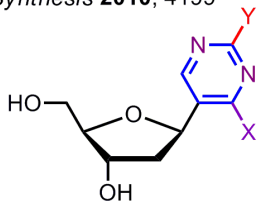
J. Org. Chem. **2008**, *73*, 3798



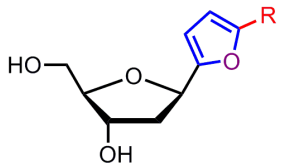
J. Org. Chem. **2010**, *75*, 442



J. Org. Chem. **2011**, *76*, 6619



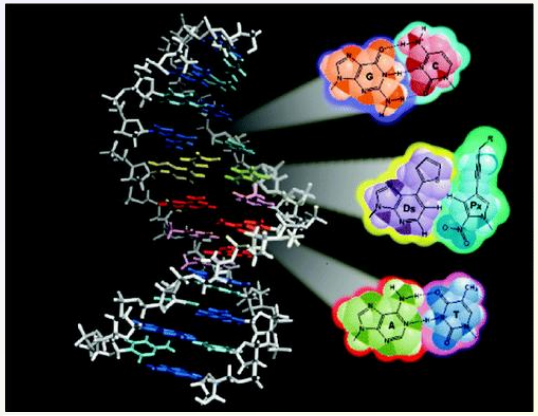
Eur. J. Org. Chem. **2010**, 2666
Synthesis **2012**, *44*, 953

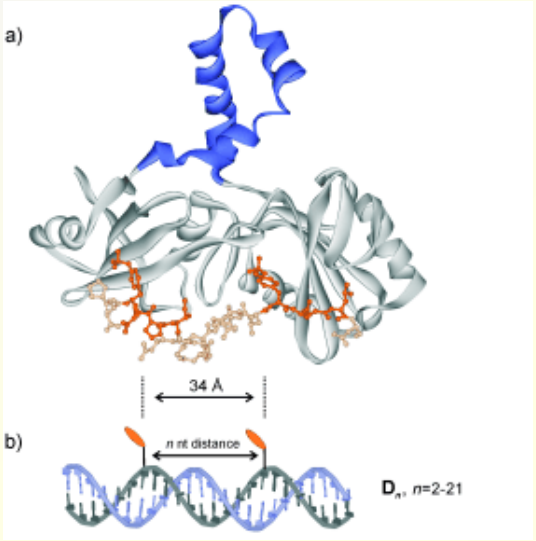


Eur. J. Org. Chem. **2010**, 5432

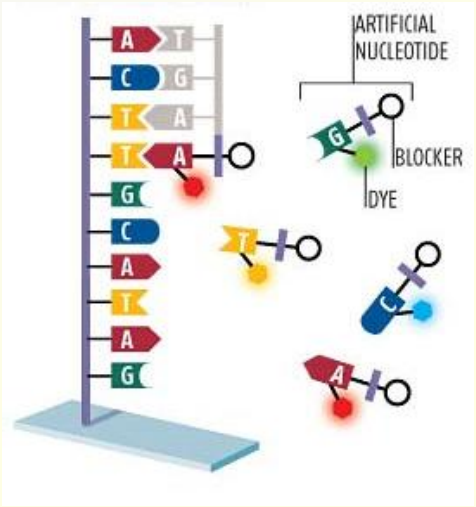
DNA s modifikovanými nukleobázemi - aplikace

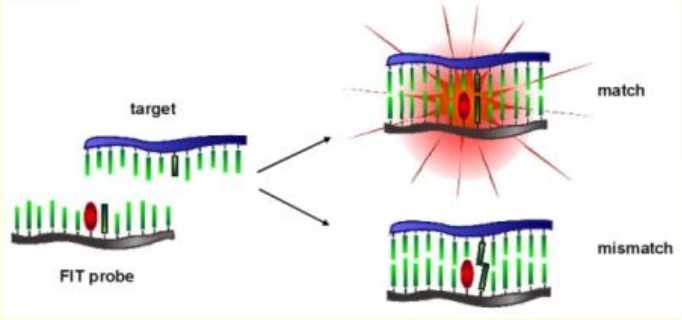
Chemická biologie

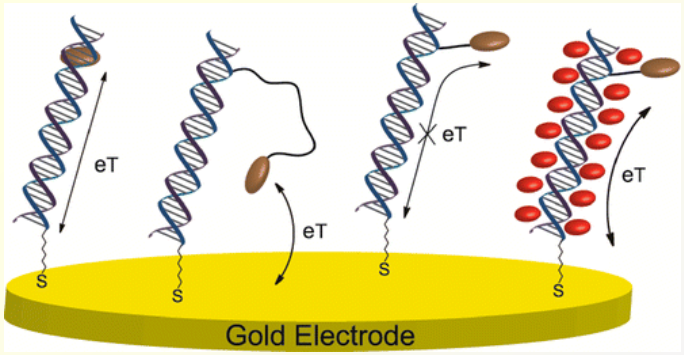




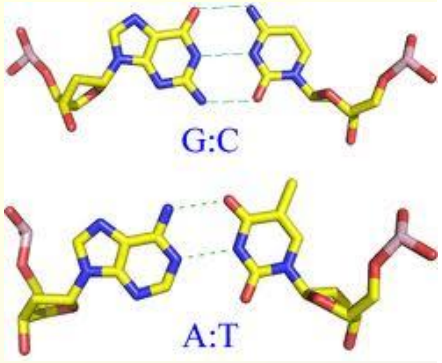
Bioanalýza/diagnostika

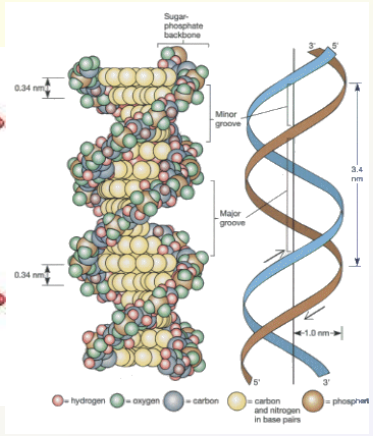


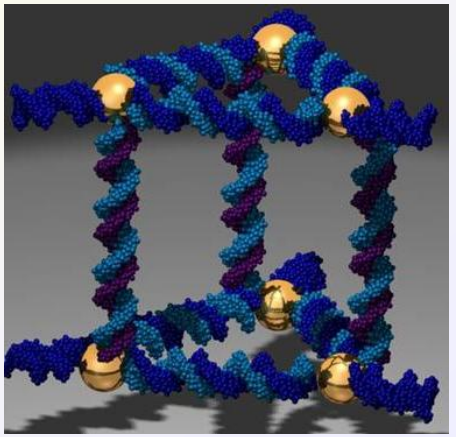




Materiálové aplikace







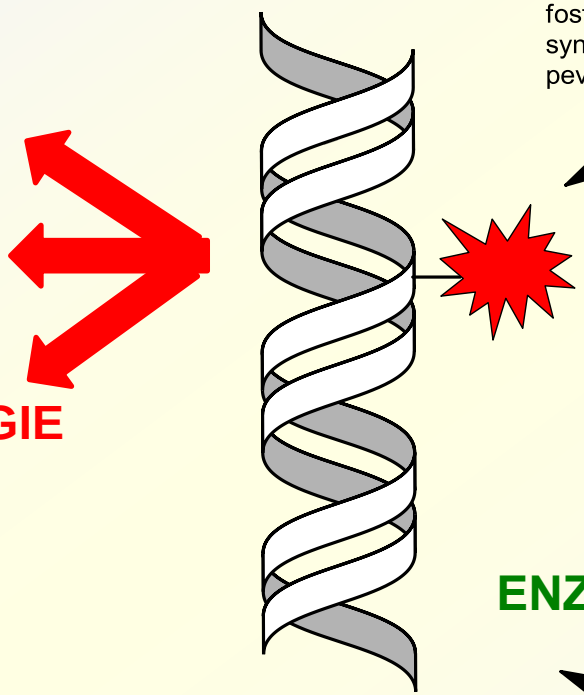
Functionalizované ON a DNA s modifikovanými bázemi

Aplikace:

DIAGNOSTIKA

CHEMICKÁ BIOLOGIE

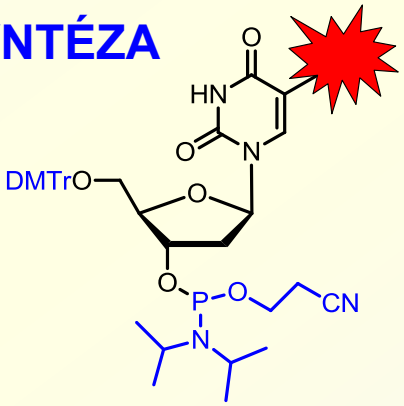
NANOTECHNOLOGIE



= různé funkční skupiny

CHEMICKÁ SYNTÉZA

fosforamiditová syntéza na pevném fázi

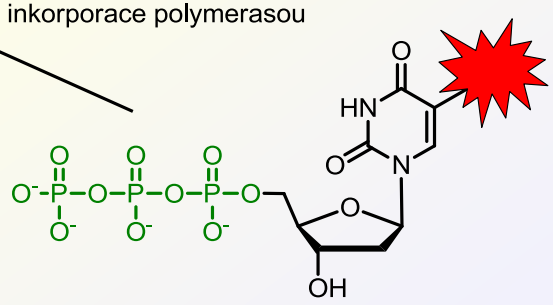


Problémy:

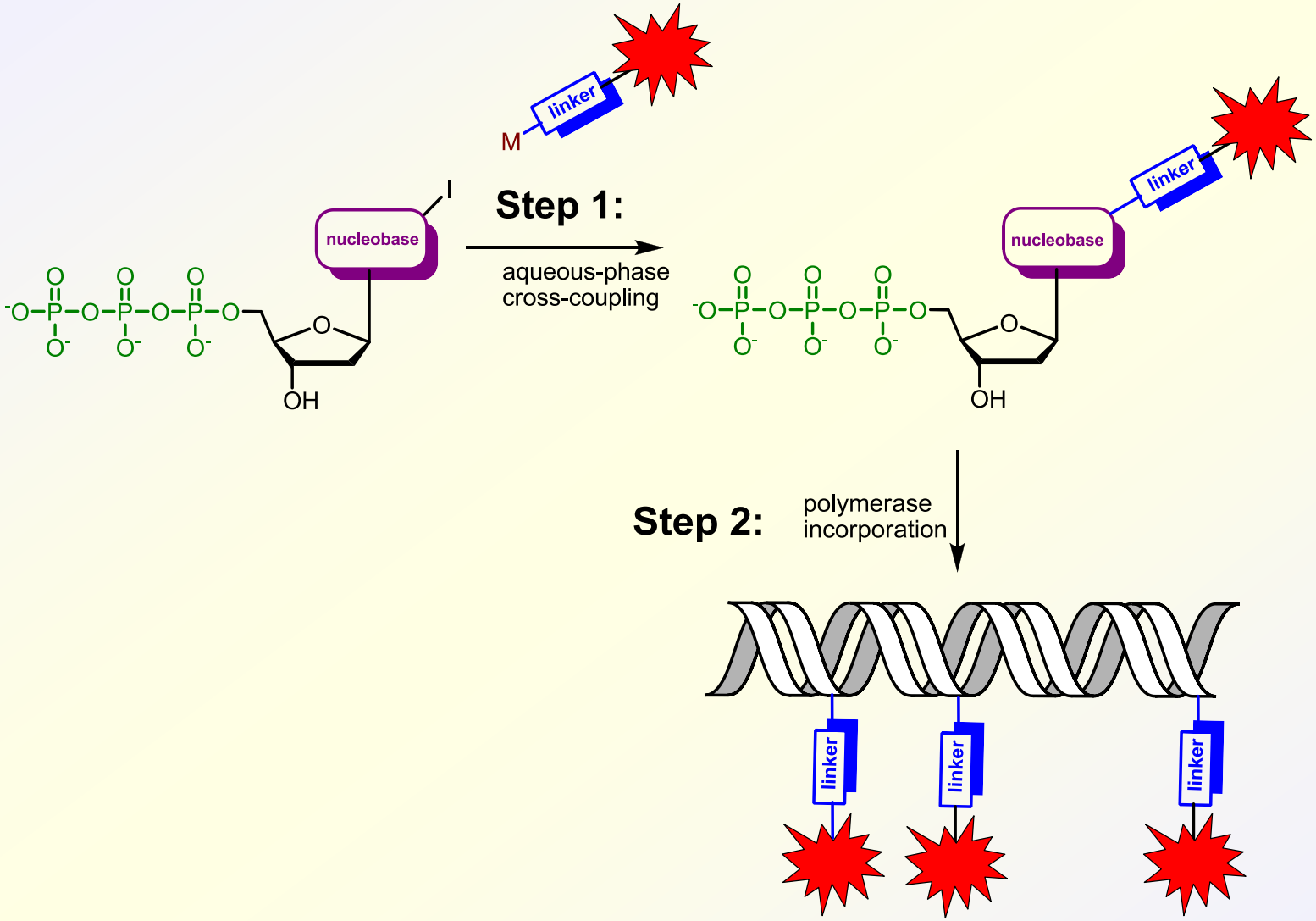
- pracná mnohakroková syntéza modifikovaných fosforamiditů
- omezená kompatibilita s fosforamiditovou syntézou (I_2, NH_3 atd.)
- nízké výtěžky pro dlouhé ON

ENZYMATICKÁ SYNTÉZA

inkorporace polymerasou

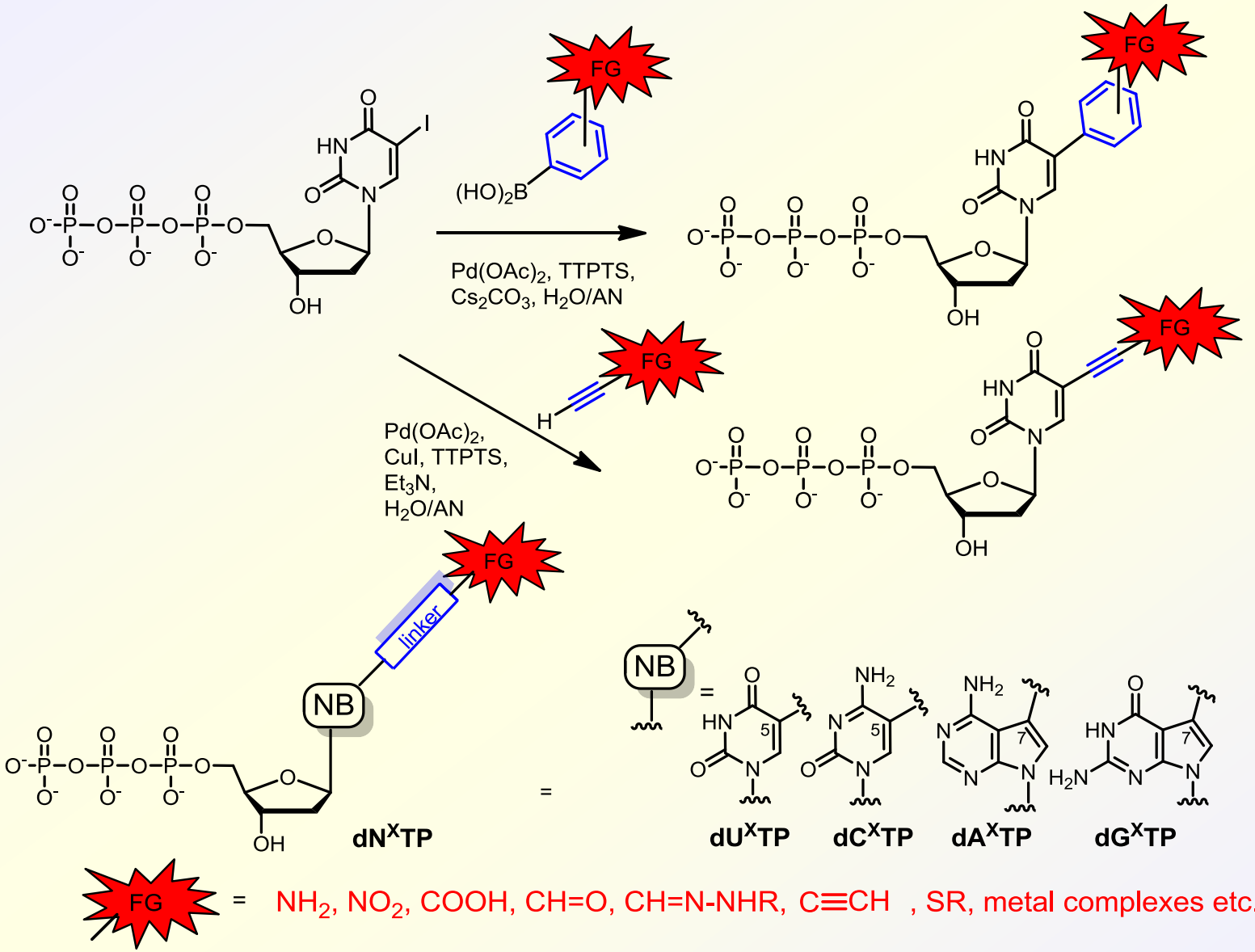


Nová dvoukroková metodika syntézy modifikovaných nukleových kyselin



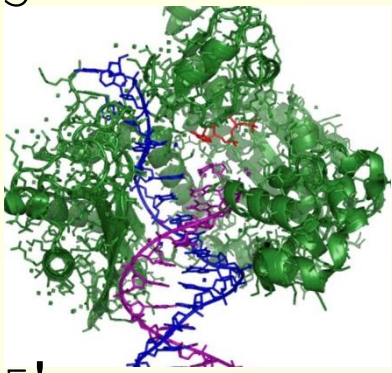
Reviews: Hocek, M.; Fojta, M. *Org. Biomol. Chem.* **2008**, *6*, 2233–2241.
Hocek, M.; Fojta, M. *Chem. Soc. Rev.* **2011**, *40*, 5802-5814.

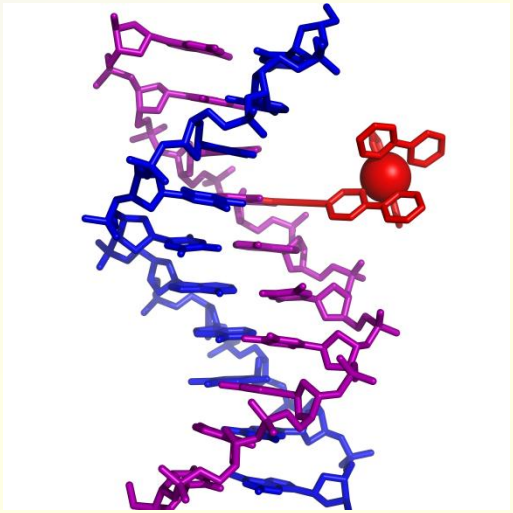
Cross-coupling reakce dNTPs ve vodných roztocích

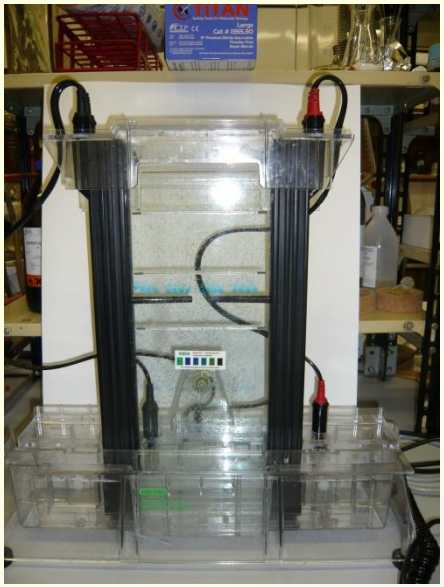


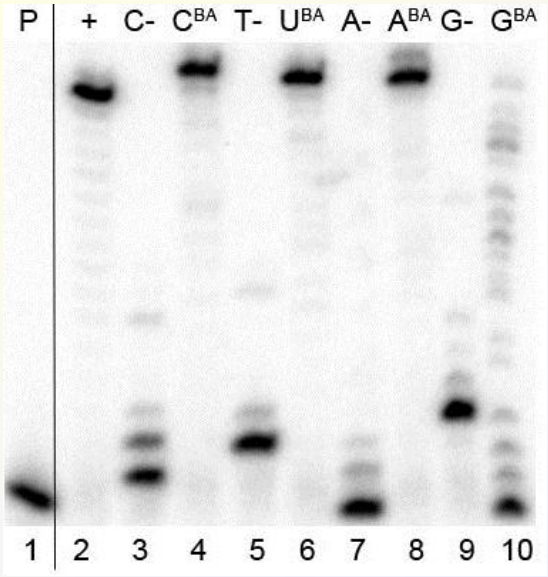
Reviews: Hocek, M.; Fojta, M. *Org. Biomol. Chem.* **2008**, *6*, 2233–2241.
Hocek, M.; Fojta, M. *Chem. Soc. Rev.* **2011**, *40*, 5802-5814.

Primer Extension (PEX) inkorporace modifikovaných dNTP









PCR inkorporace modifikovaných dNTPs

5'-GACATCATGAGAGACATCGCCTCTGGGCTAATAGGACTACTTCTAATCTGTAAGAGCAGATCCCTGGACAGGCAAGGAATACAGGTATTTTGTCCTTG-3'

3'-TTCCTTATGTCCATAAAACAGGAAC-5'

5'-GACATCATGAGAGACATCGC-3'

+ 3 x natural dNTP + functionalized dNTP (A1-A4 or T1-T2)

PCR

functionalized A

5'-GACATCATGAGAGACATCGCCTCTGGGCTAATAGGACTACTTCTAATCTGTAAGAGCAGATCCCTGGACAGGCAAGGAATACAGGTATTTTGTCCTTG-3'

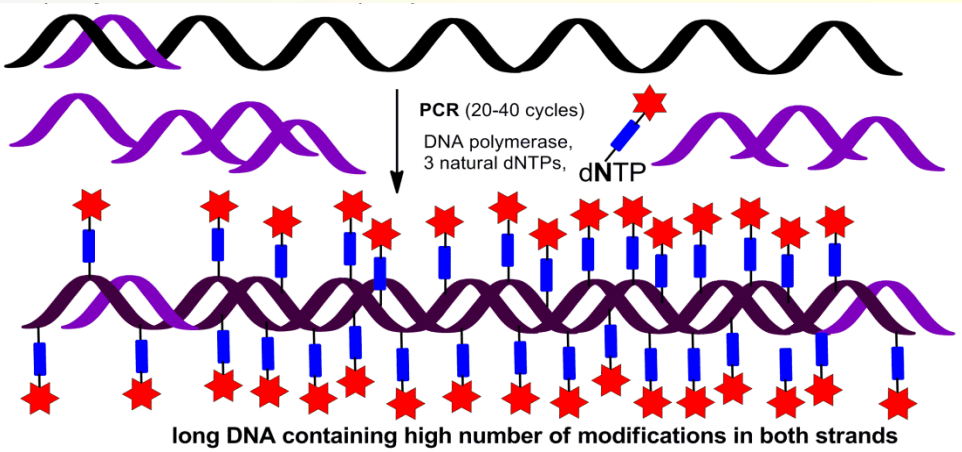
3'-CTGTAGTACTCTCTGTAGCGGAGACCCGATTATCCTGATGAAGATTAGACATTCTCGTCTAGGGACCTGTCCGTTCCTTATGTCCATAAAACAGGAAC-5'

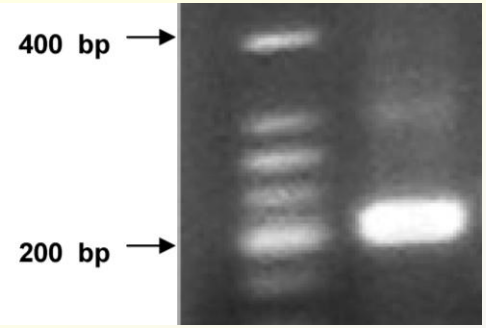
OR

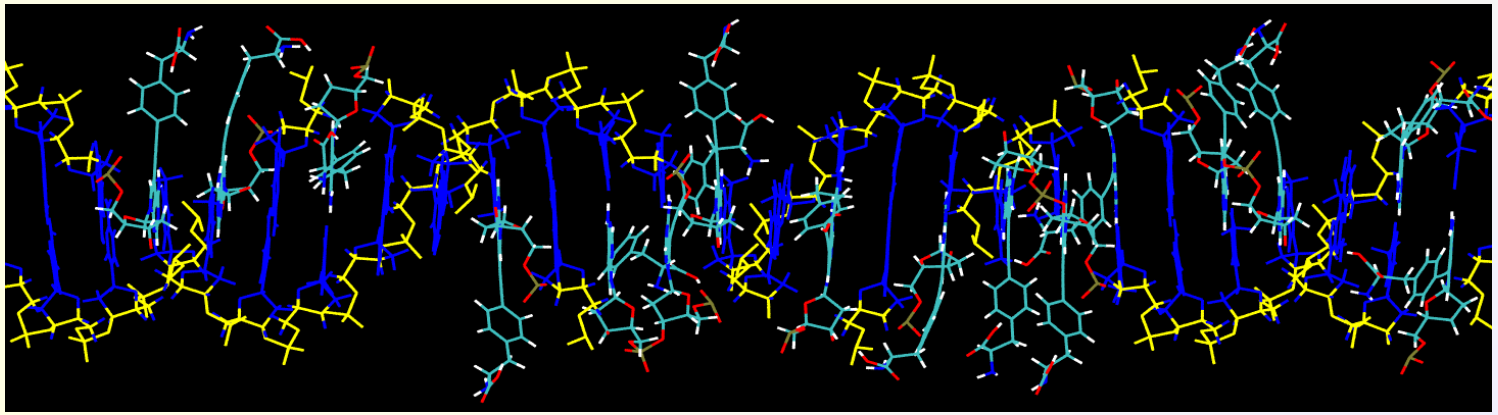
functionalized T

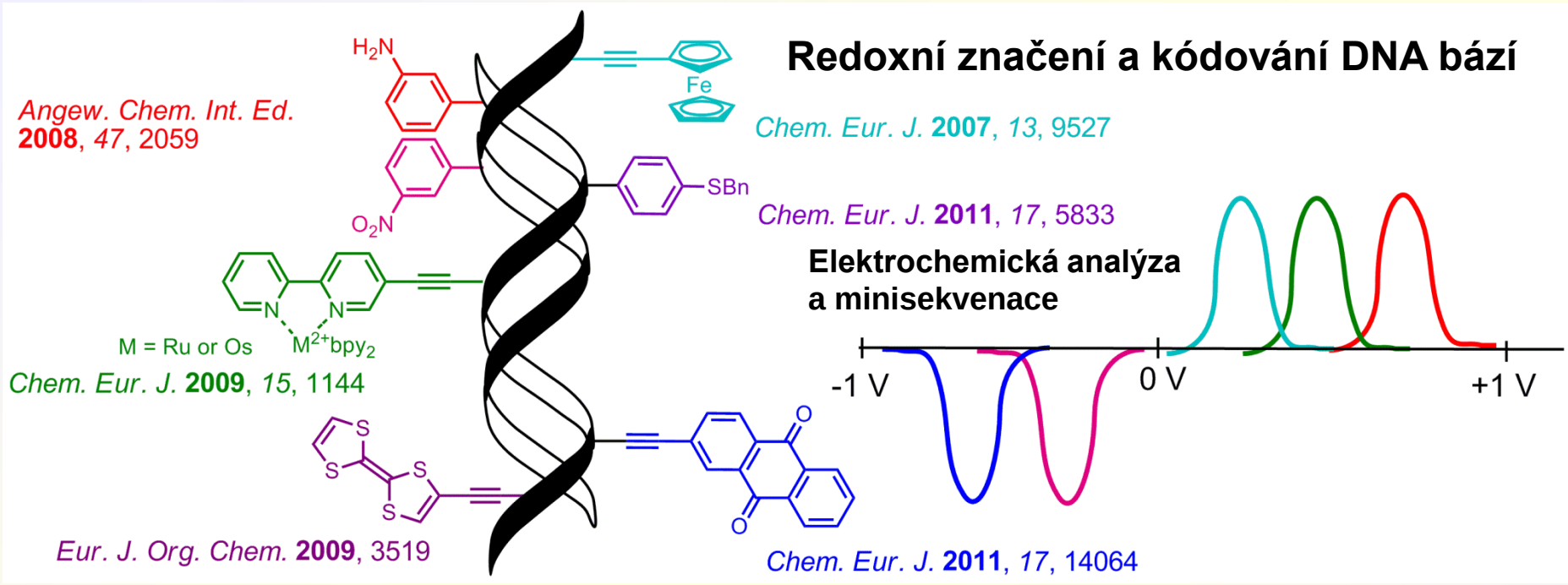
5'-GACATCATGAGAGACATCGCCTCTGGGCTAATAGGACTACTTCTAATCTGTAAGAGCAGATCCCTGGACAGGCAAGGAATACAGGTATTTTGTCCTTG-3'

3'-CTGTAGTACTCTCTGTAGCGGAGACCCGATTATCCTGATGAAGATTAGACATTCTCGTCTAGGGACCTGTCCGTTCCTTATGTCCATAAAACAGGAAC-5'





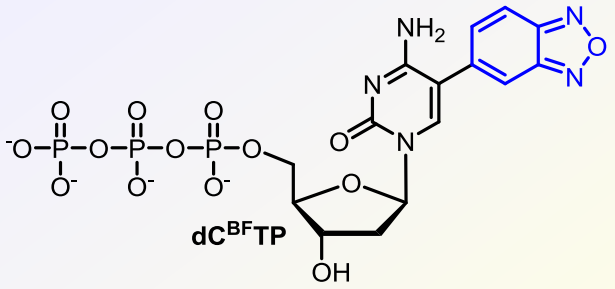


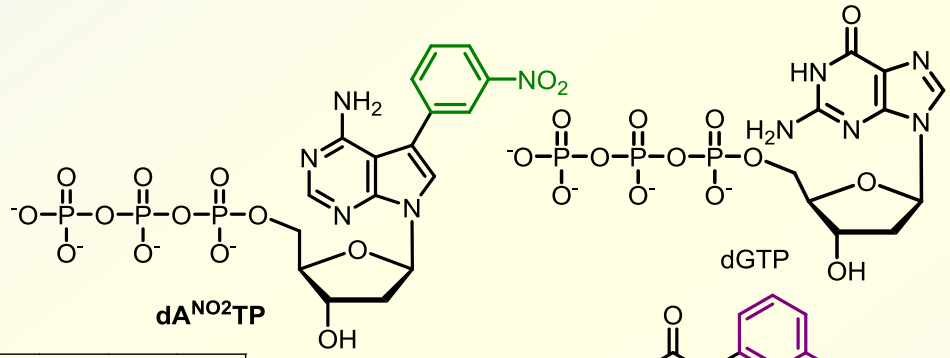


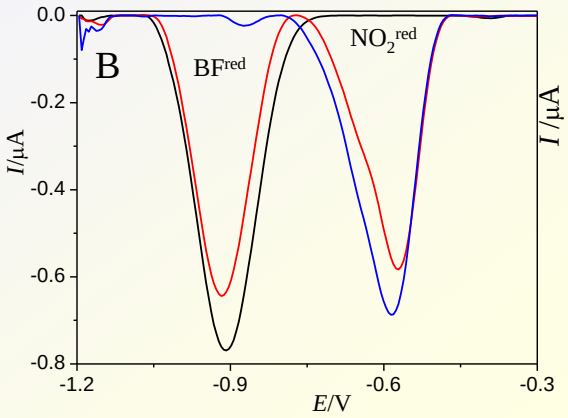
Aplikace v bioanalýze/diagnostice

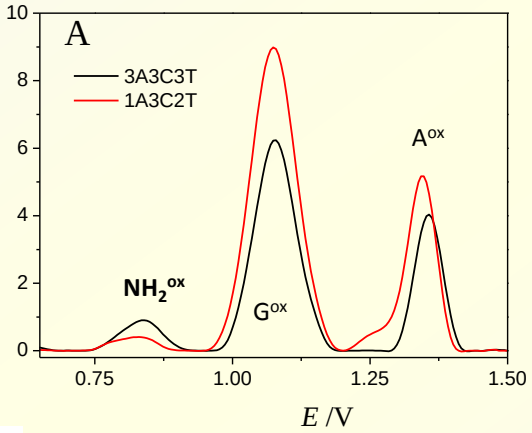
Spolupráce s Doc. M. Fojtou (BFU), Prof. J. Barkem (PřF UK) atd. – Centrum excelence grant (2012-2018)

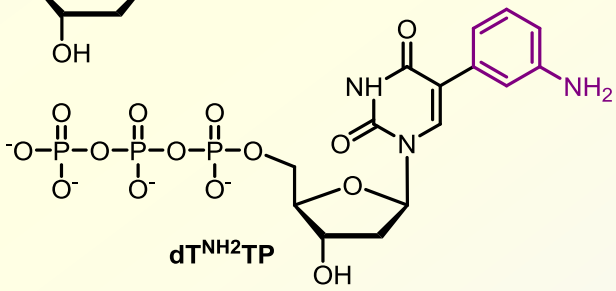
Multipotenciálové redoxní kódování nukleobází







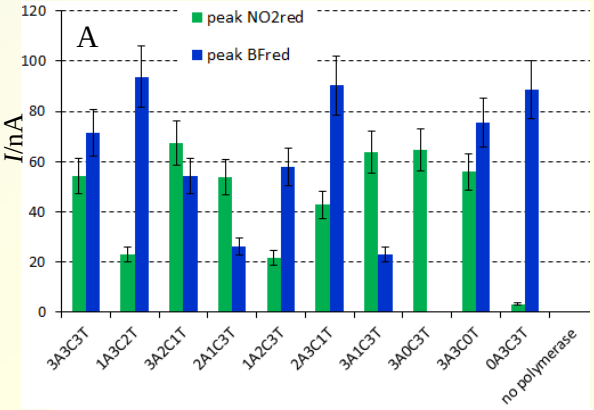


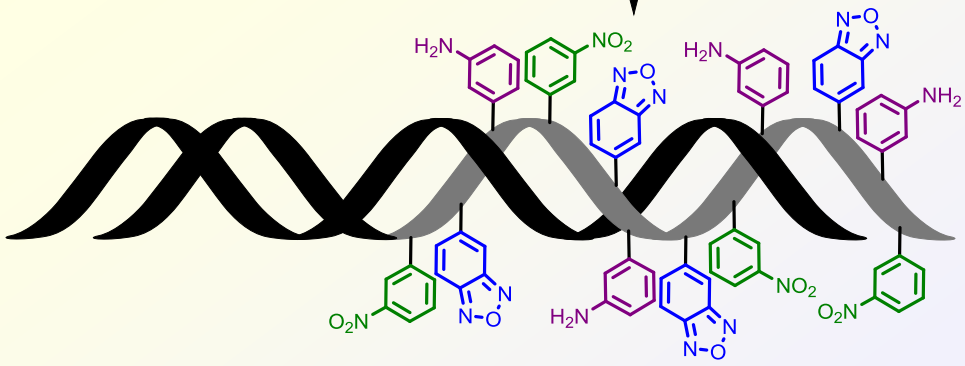


PEX

primer, template

DNA polymerase



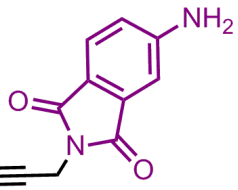


Balintová, J.; Plucnara, M.; Vidláková, P.; Pohl, R.; Havran, L.; Fojta, M.; Hocek, M. *Chem. Eur. J.* **2013**, *19*, 12720-12731.

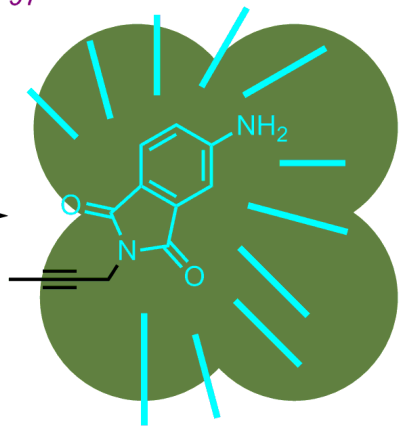
Nové fluorescenční značky pro DNA – studium interakcí DNA-protein

Chem. Sci. **2012**, *3*, 2797

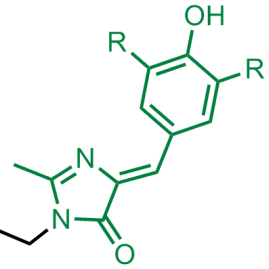
Sovatochromní



Protein

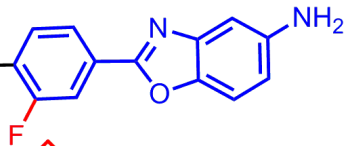


GFP-like - zvyšuje fluorescenci bráněním rotace



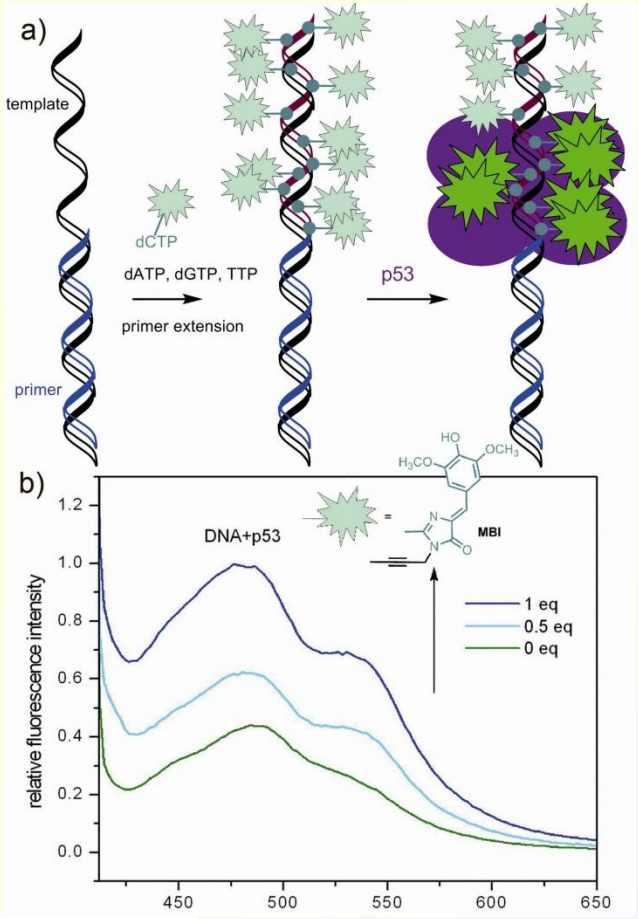
J. Org. Chem. **2012**, *77*, 8287

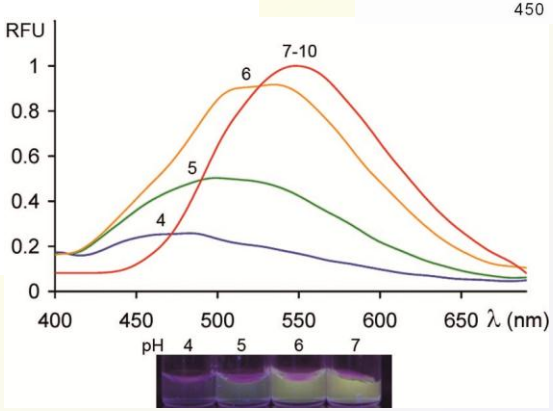
Sovatochromní a pH sensitivní

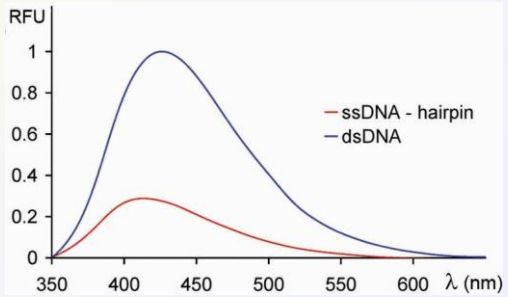


^{19}F NMR

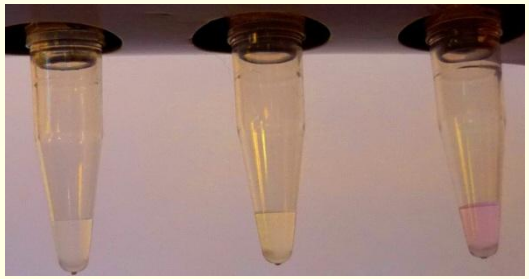
J. Org. Chem. **2012**, *77*, 1026

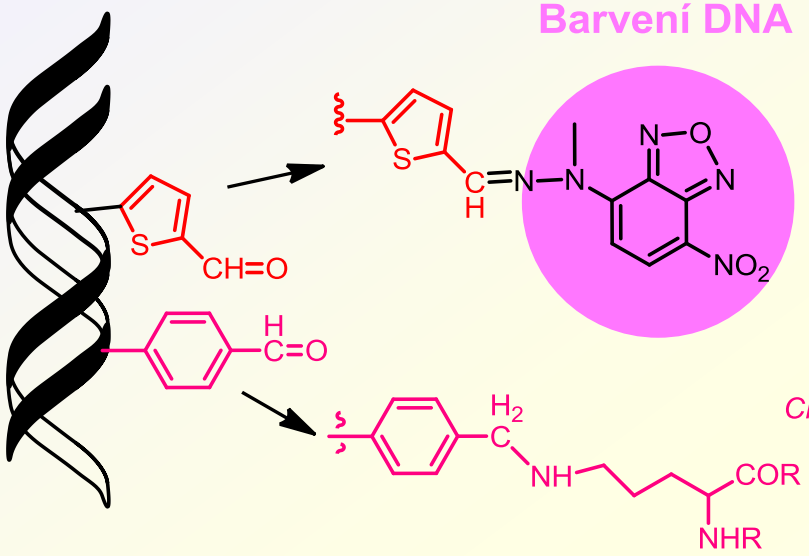






DNA modifikace reaktivními skupinami pro biokonjugace

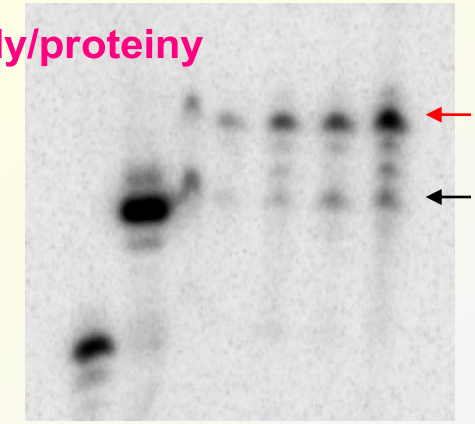


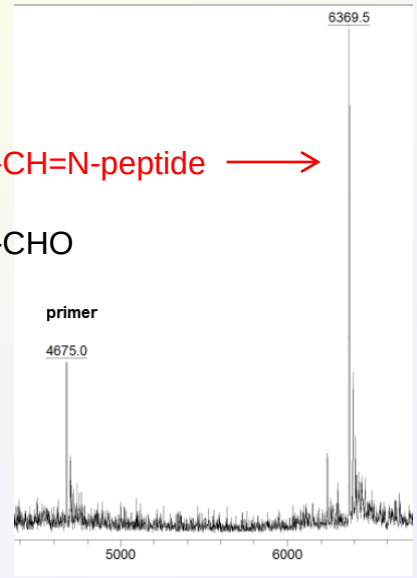


Angew. Chem. Int. Ed. **2010**, *49*, 1064

Chem. Eur. J. **2012**, *18*, 4080

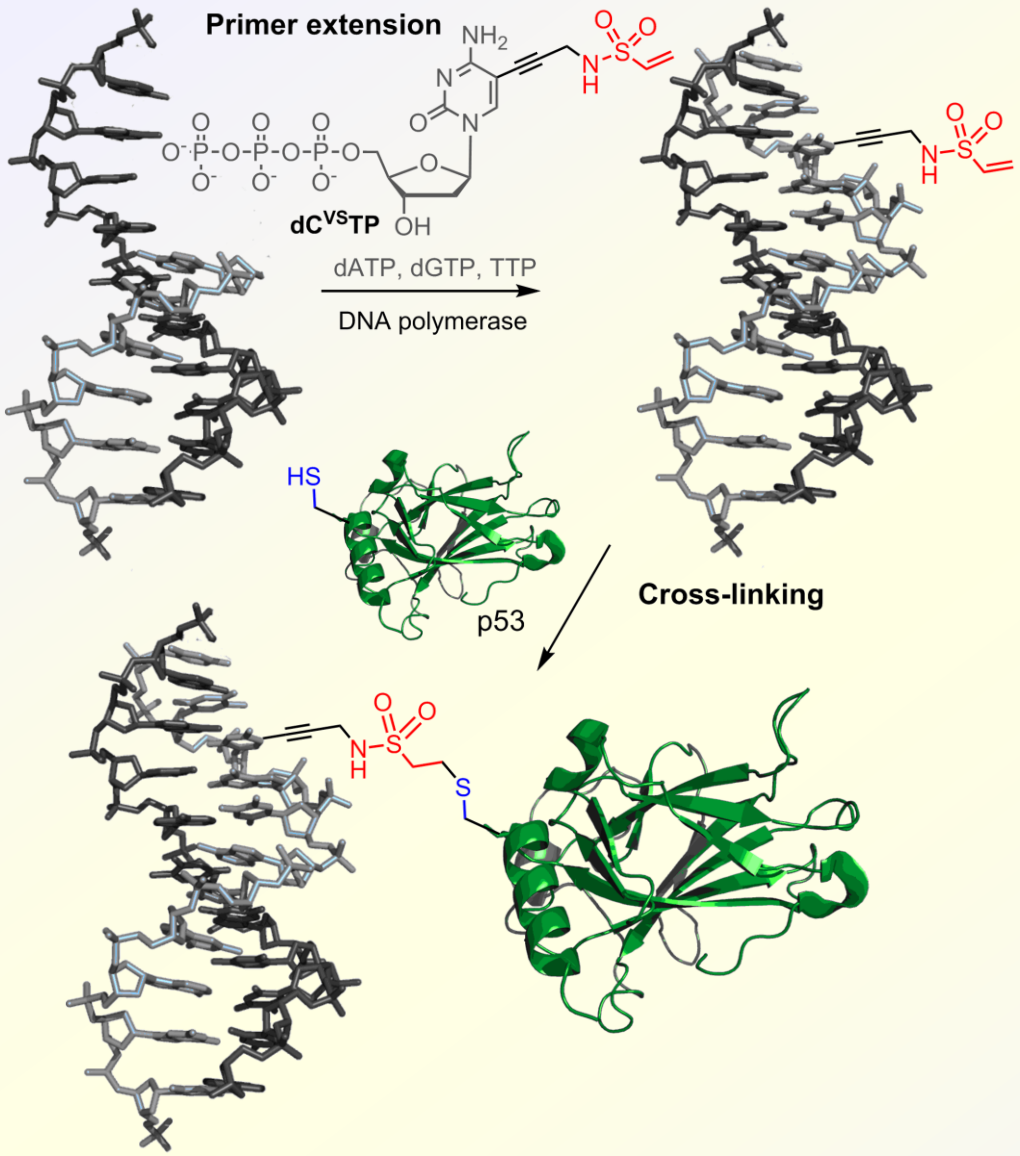
Konjugace s peptidy/proteiny obsahujícími Lysin

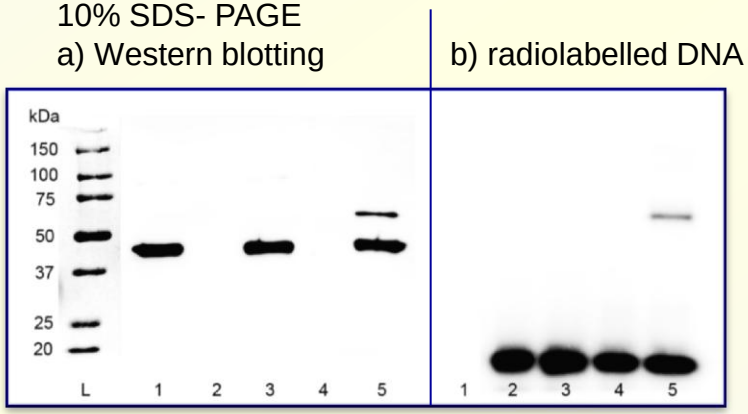


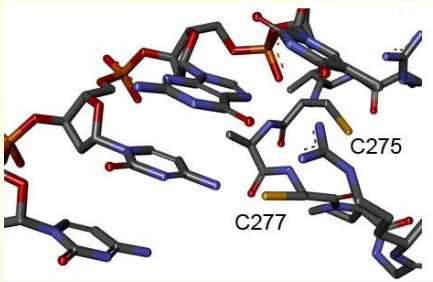


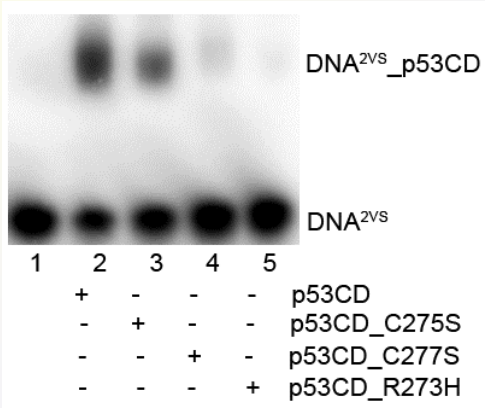
Vyvíjíme další bioortogonální reakce jiných reaktivních skupin

Bioorthogonální konjugace DNA s peptidy/proteiny - Michaelovy adice Cys



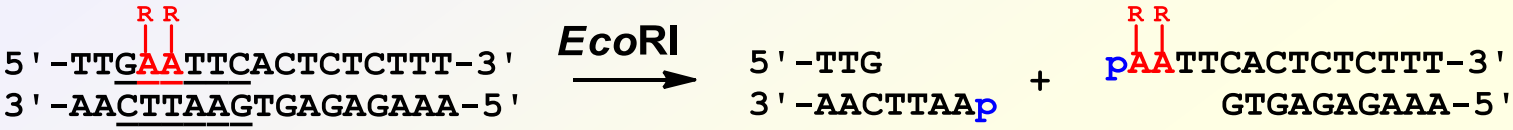


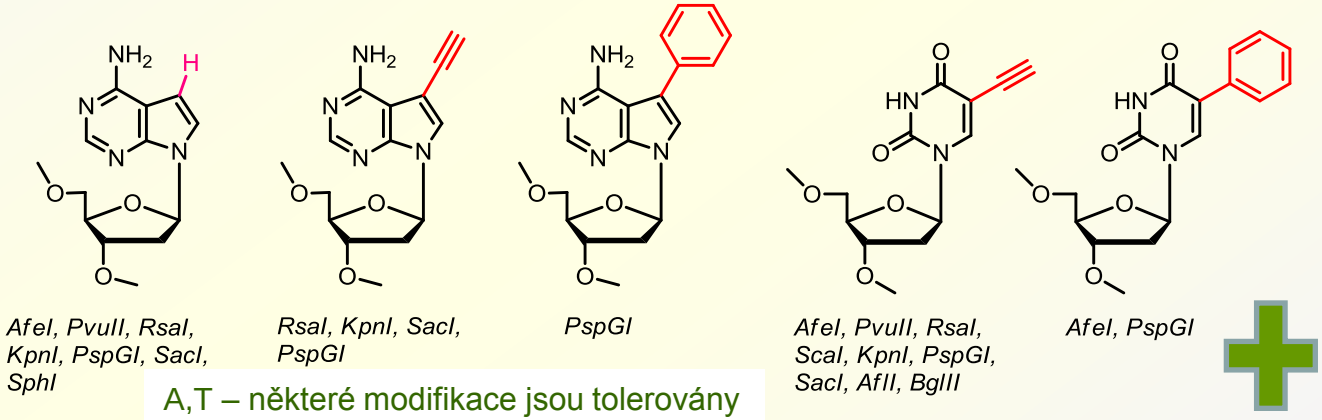




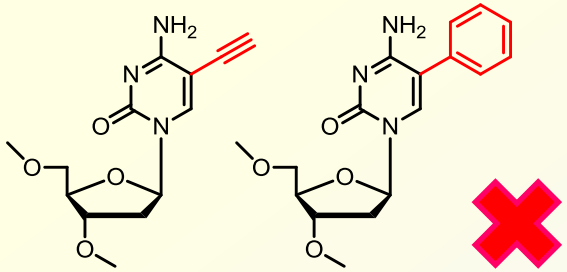
Dadová, J.; Orság, P.; Pohl, R.; Brázdová, M.; Fojta, M.; Hocek, M. *Angew. Chem. Int. Ed.* **2013**, *52*, 10515-10518.

Studium vlivu modifikací na štěpení DNA restrikčními endonukleasami

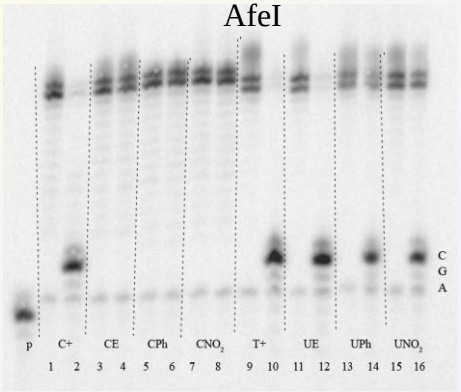




A,T – některé modifikace jsou tolerovány

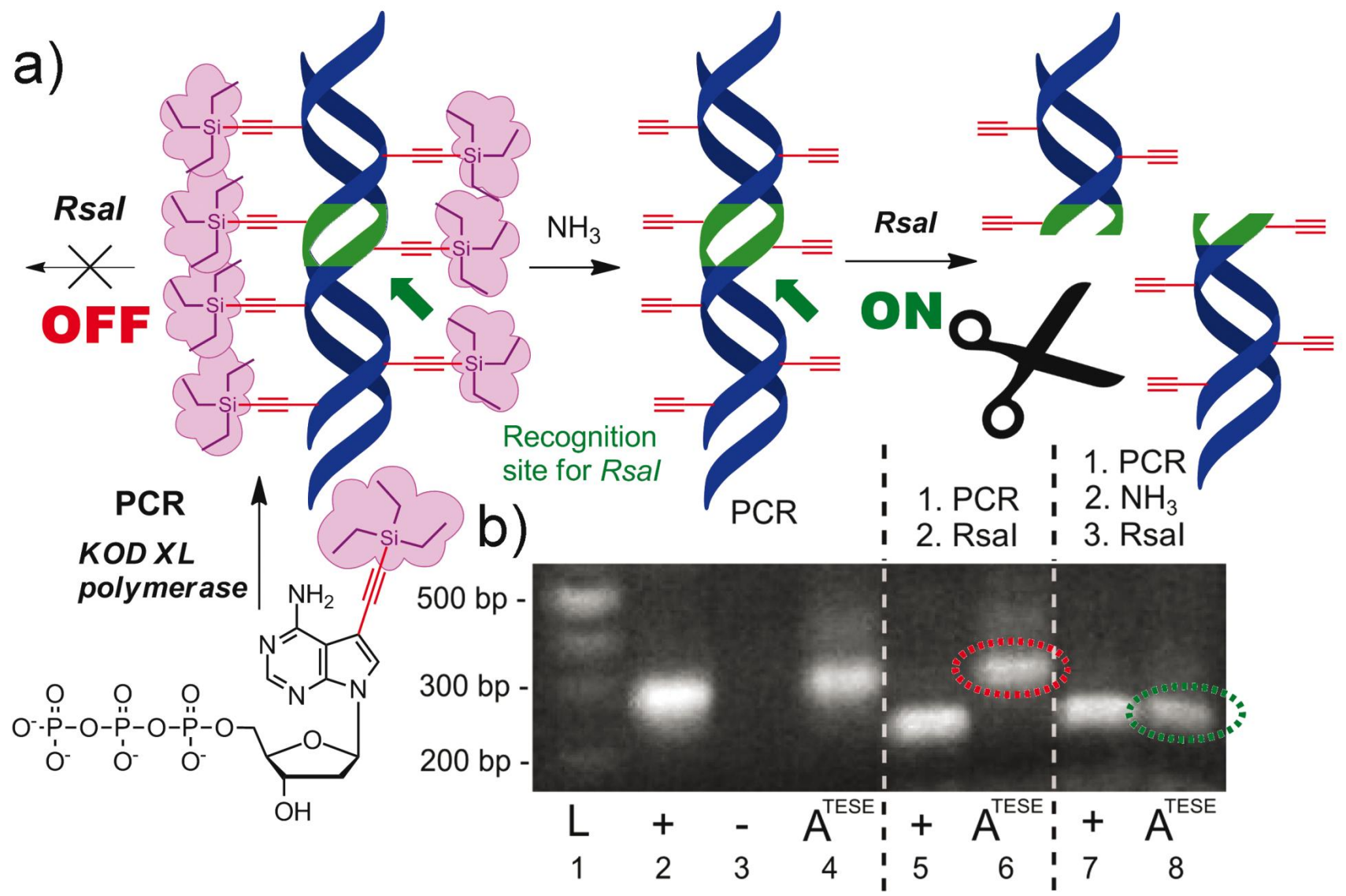


C – žádné modifikace nejsou tolerovány



Macíčková-Cahová, H.; Hocek, M., *Nucleic Acids Res.* **2009**, *37*, 7612-7622.
Macíčková-Cahová, H.; Pohl, R.; Hocek, M. *ChemBioChem* **2011**, *12*, 431-438.

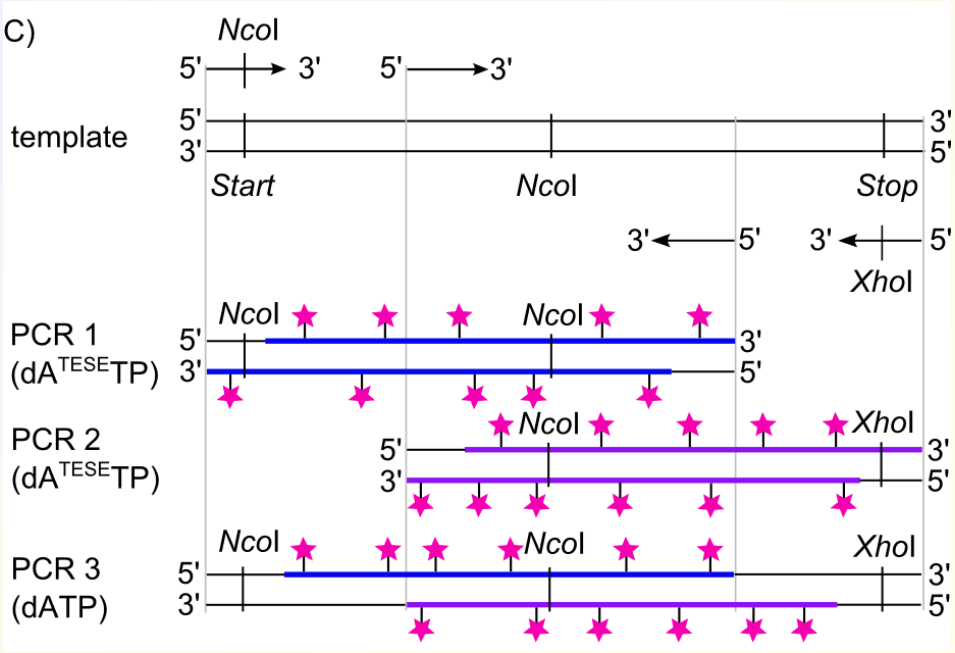
Přechodné chránění DNA proti štěpení RE

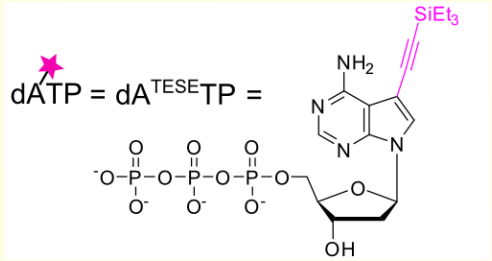


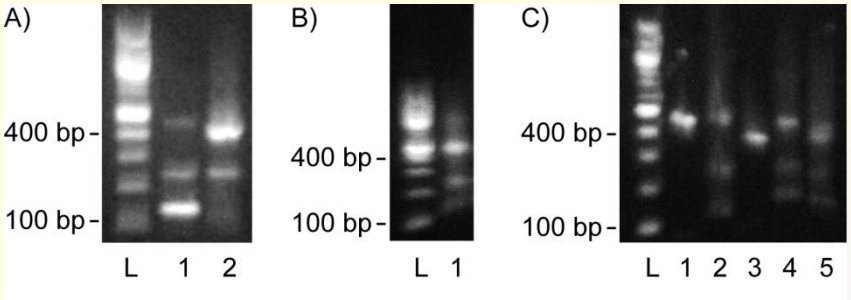
Kielkowski, P.; Macíčková-Cahová, H.; Pohl, R.; Hocek, M. *Angew. Chem. Int. Ed.* **2011**, *50*, 8727-8730.

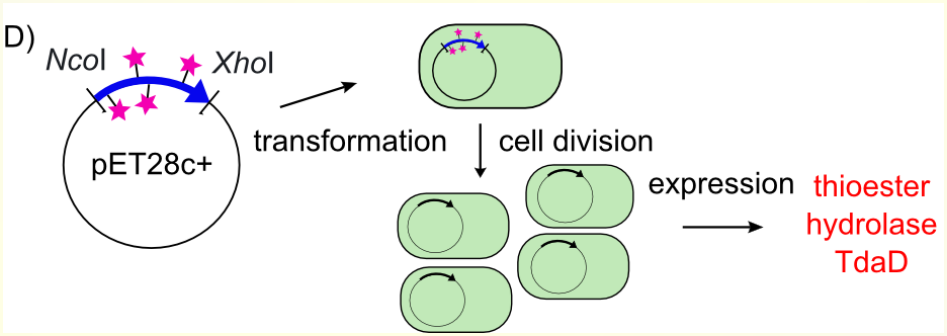
Nová protektivní strategie klonování a exprese genů

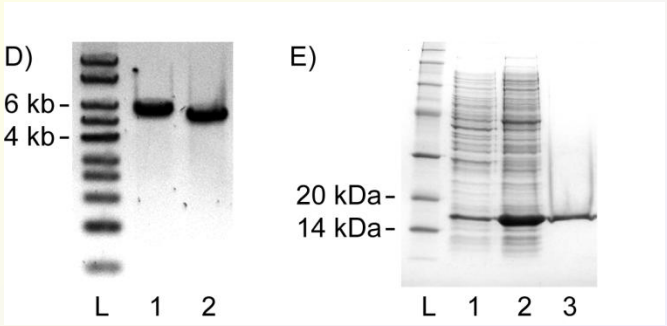
production of TdaD protein from *Phaeobacter gallaeciensis* using His_6-tag and pET28c+ plasmid





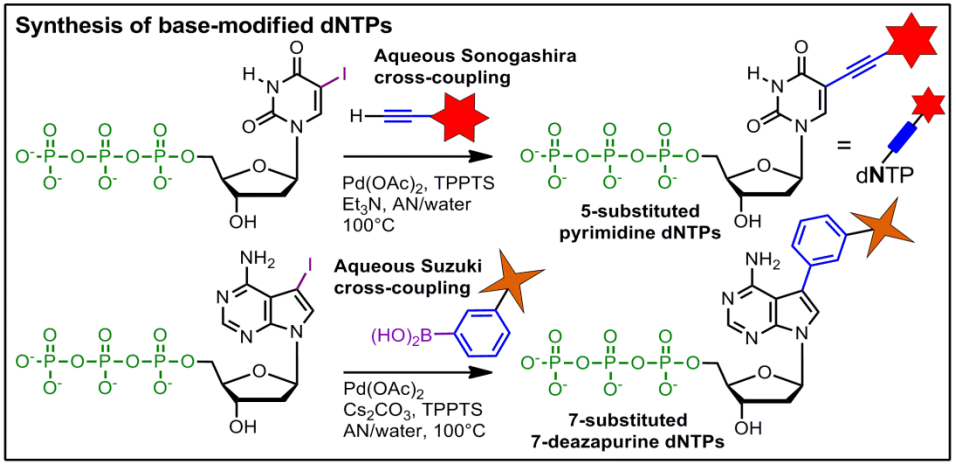




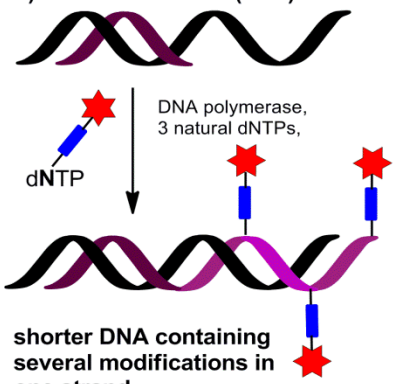


Kielkowski, P.; Brock, N. L.; Dickschat, J. S.; Hocek, M. *ChemBioChem* **2013**, *14*, 801-804.

Synthesis of base-modified dNTPs

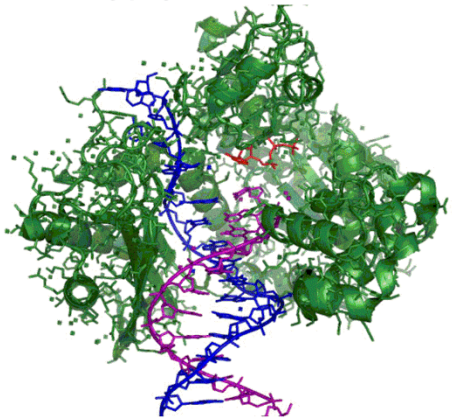


A) Primer extension (PEX)

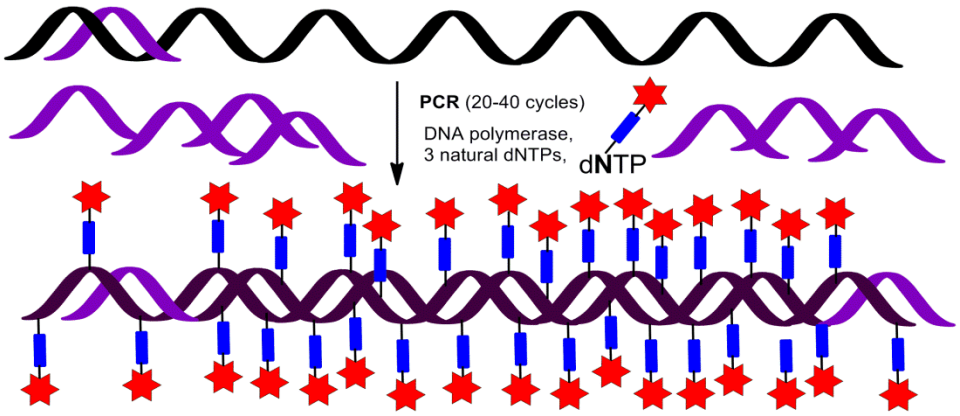


shorter DNA containing several modifications in one strand

Incorporation of the modified dNTPs to DNA by polymerase

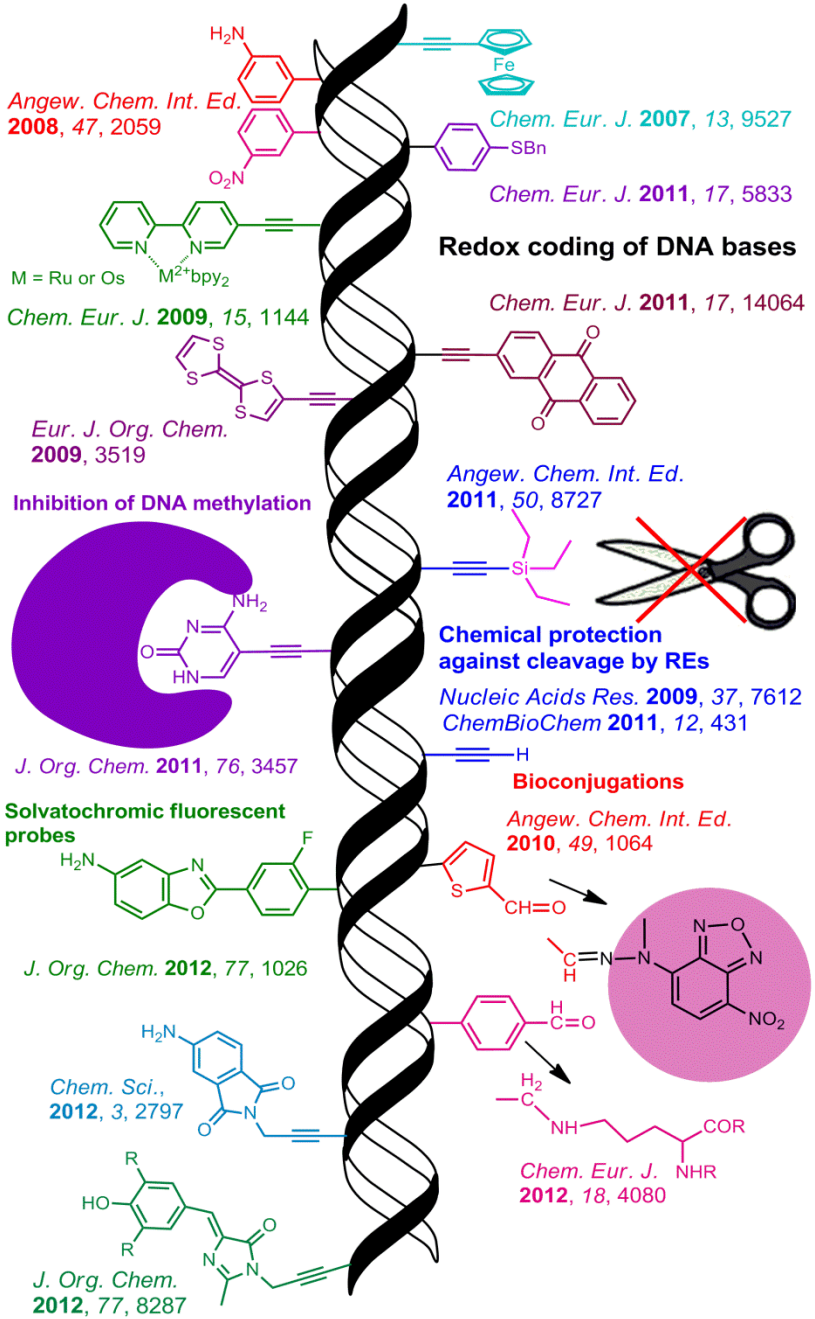


B) Polymerase chain reaction (PCR)



long DNA containing high number of modifications in both strands

Examples from the Hocek lab:



Hocek group

Bioorganic & Medicinal Chemistry of Nucleic Acids
Joint laboratory of IOCB and Charles University

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Technology Agency of the Czech Republic
... from ideas to applications

Petr Nauš, Pavla Perlíková, Michal Tichý, Martin Klečka, Pavel Kielkowski, Petra Ménová, Jitka Daďová, Michaela Mačková, Soňa Boháčová, Matouš Krömer **Jana Balintová, Zuzana Vaníková, Juraj Konč**
Vincent Maulnuit, **Michael Downey,** **Agata Olszewska,** **Nemanja Milisavljevic**
Chris Chambers, **Anna Tokarenko, Dmytro Dziuba, Nazarii Sabat,** **Anna Simonova**

Former members:
Petr Čapek, Milan Urban, Peter Šilhár, Milan Vrábel, Martin Kuchař, Nicolas Joubert, Aurelie Bourderioux, Zbyněk Hasník, Satu Ikonen, Robert Musiol, Hubert Chapuis, Igor Čerňa, Hana Cahová, Martin Štefko, Jan Riedl, Ludovic Eberlin, Jan Bárta, Lubica Kalachova, Veronika Raindlová, Tomáš Kubelka, Víťa Bambuch