

Nukleové kyseliny

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Doporučená literatura:

- 1) Neidle, S.: Principles of Nucleic Acid Structure, Elsevier, AP, 2008
- 2) Calladine, C.R., Drew, H.R., Luisi, B.F., Travers, A.A.: Understanding DNA – The Molecule and How It Works, 3rd Ed., Elsevier Academic Press, 2004
- 3) Bates, A.D., Maxwell, A.: DNA Topology, Oxford Bioscience, 2005
- 4) Cotterill, R.: Biophysics: An Introduction, John Wiley & Sons, Ltd. 2002
- 5) Voet, D, and Voetová, J.G.: Biochemie, Victoria Publishing
- 6) Murray, R.K., Granner, D. K., Mayes, P., A., Rodwell, V., W.: Harper's Illustrated Biochemistry, Lange Medical Books, 2003
- 7) Schuenemann, V.: Biophysik: Eine Einfuehrung, Springer, 2005
- 8) Garrett, R.H., Grisham, C.M.: Biochemistry, 2nd ed., 1999
- 9) Bergethon, P.R., The Physical Basis of Biochemistry: The Foundations of Molecular Biophysics, 2nd ed., Springer, 2010

Nukleové kyseliny

-DNA

-RNA

-PNA

Základní stavební kameny nukleových kyselin:

1) Báze

- i) Purinové – menší, číslování arom. kruhu protisměru hod. r., **adenin (A)**, **guanin (G)**, obecně **P**
- ii) Pyrimidinové – větší, číslování ve směru hod. r., **cytosin (C)**, **uracil (U)**, **thymin (T)**, obecně **P**

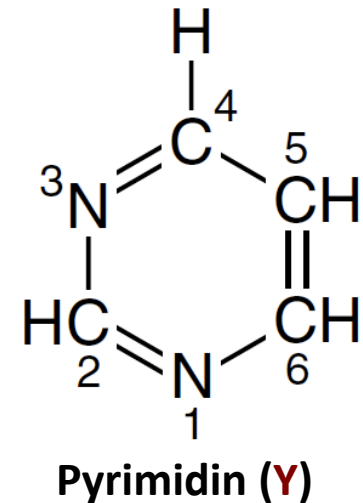
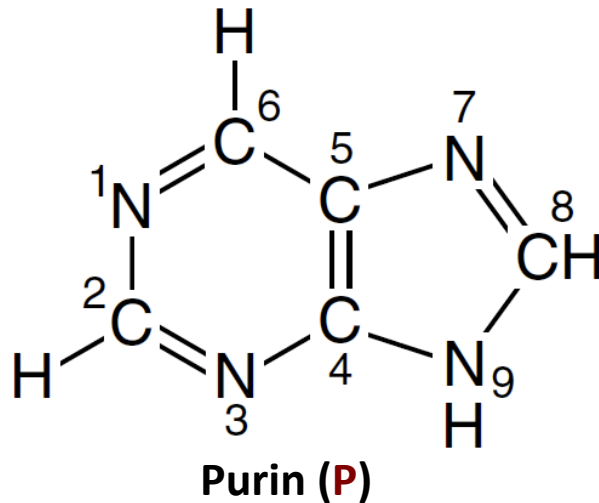
2) Cukr – 2'-deoxyribóza (**DNA**), ribóza (**RNA**)

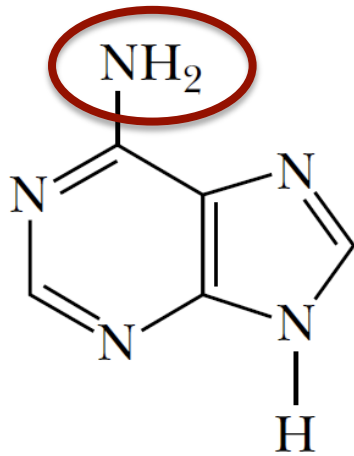
3) Fosfát

Báze:

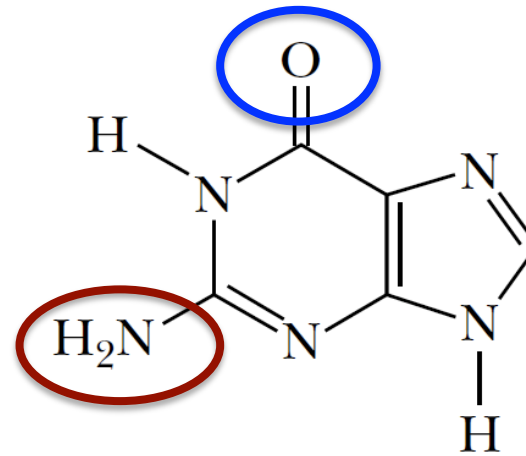
1) Standardní

2) Modifikované: N6-methyl-dA, 5-methyl-dC, xanthin, hypoxanthin, uric acid, 7-methylguanine, dimethylaminoadenin

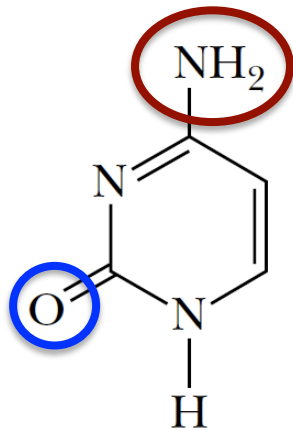




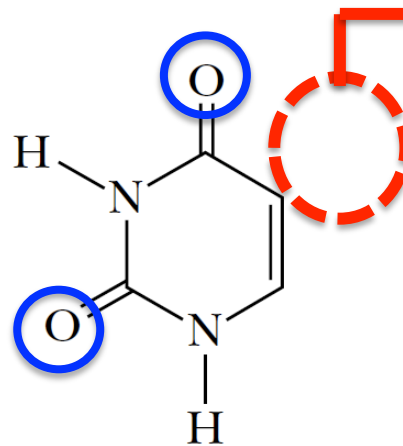
Adenine
(6-amino purine)



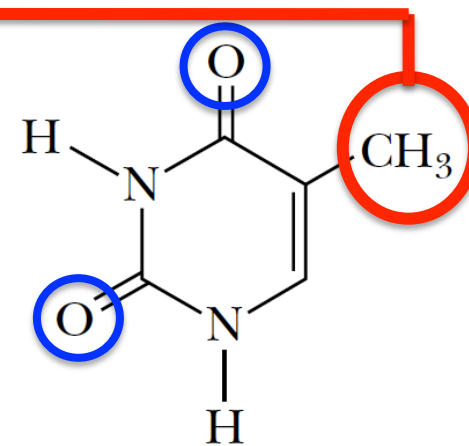
Guanine
(2-amino-6-oxy purine)



Cytosine
(2-oxy-4-amino
pyrimidine)

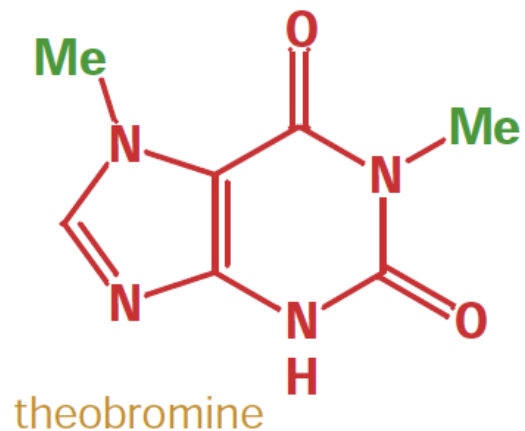
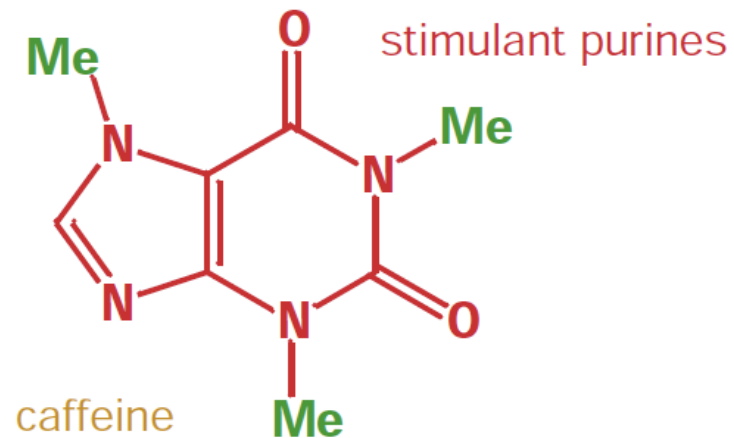
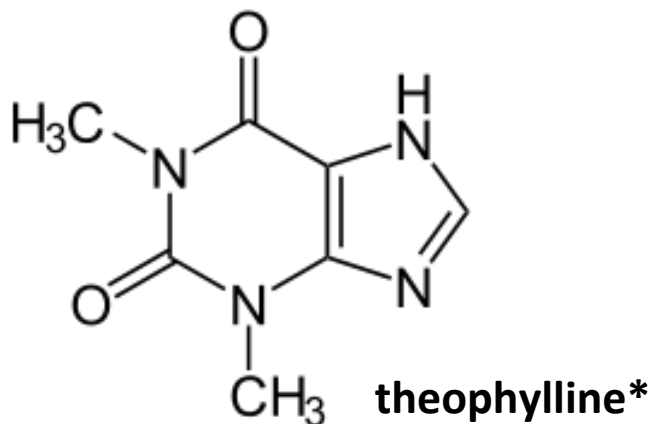


Uracil
(2-oxy-4-oxy
pyrimidine)



Thymine
(2-oxy-4-oxy
5-methyl pyrimidine)

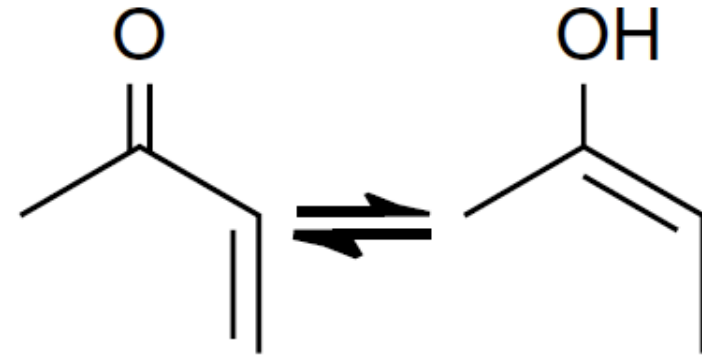
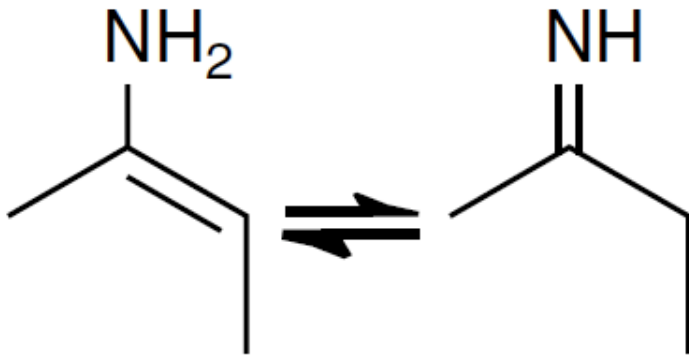
Stimulanty obsažené v kávě , čaji a čokoládě jsou metylované puriny



Me=CH₃

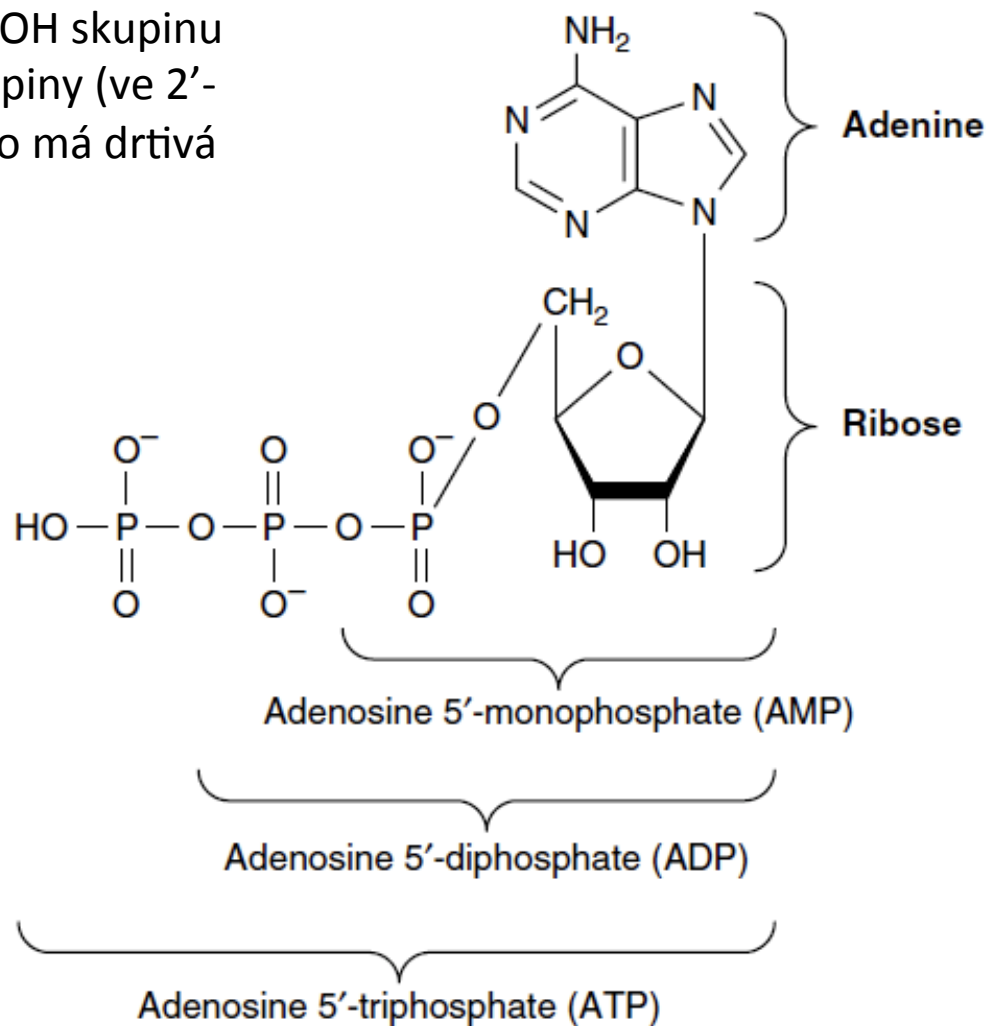
* Obsažen v čaji, ale není stimulantem

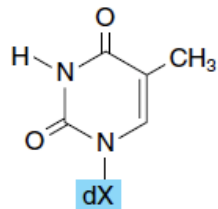
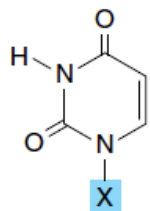
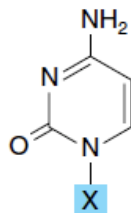
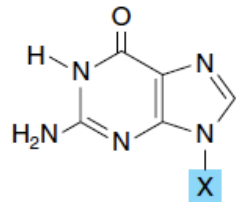
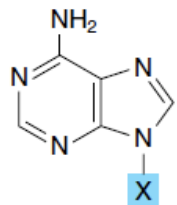
Tautomerie amino (amin \leftrightarrow imin) a keto (keto \leftrightarrow enol forma) skupin purinů a pyrimidinů, fyziologické podmínky favorizují amino a keto formy



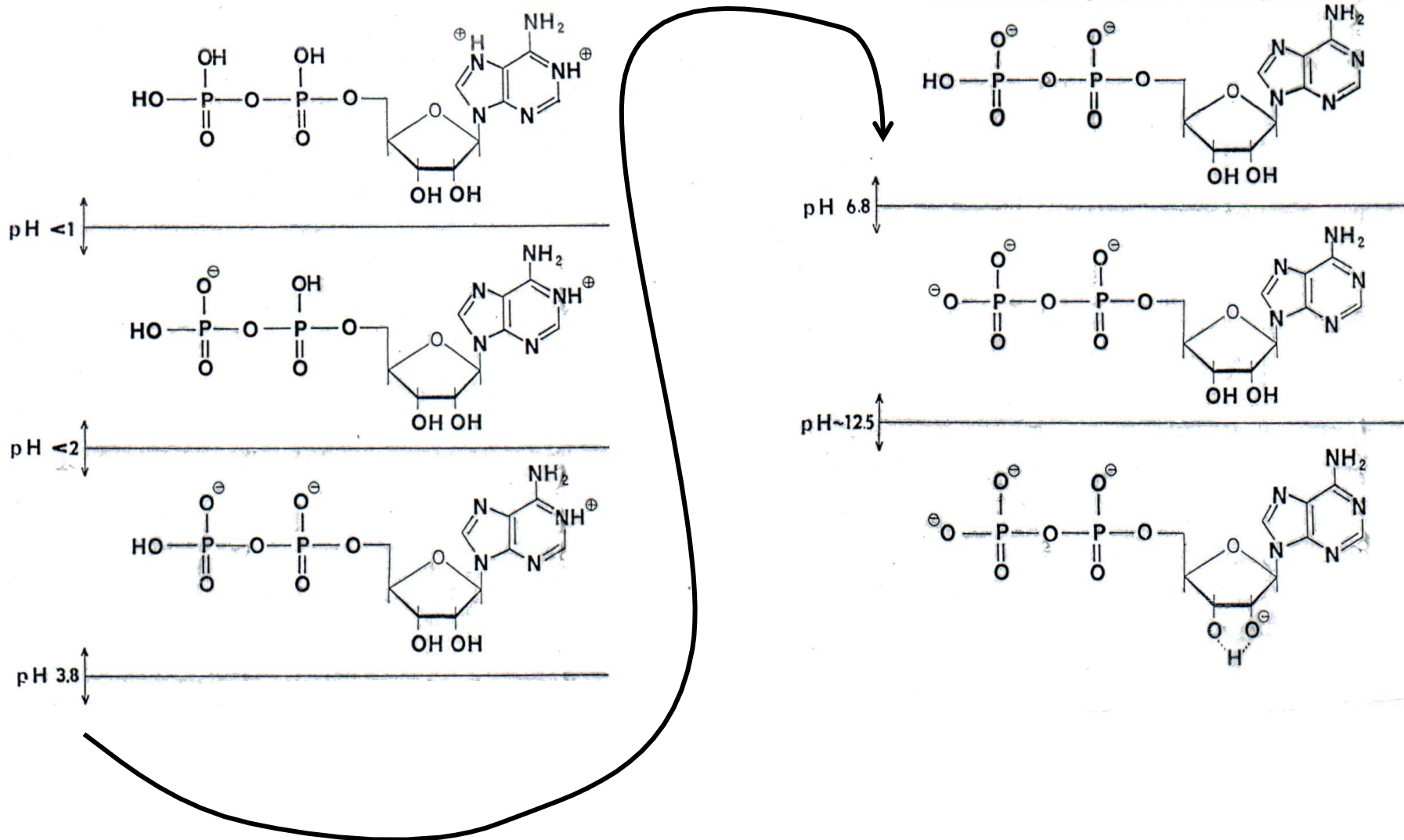
Nukleosidy a nukleotidy

Nukleotid vzniká esterifikací fosforu na –OH skupinu nukleosidu. V riboze lze esterifikovat 3 skupiny (ve 2'-deoxy-riboze pouze 2 –OH skupiny), přesto má drtivá většina ribonukleotidů fosfát v poloze 5'.

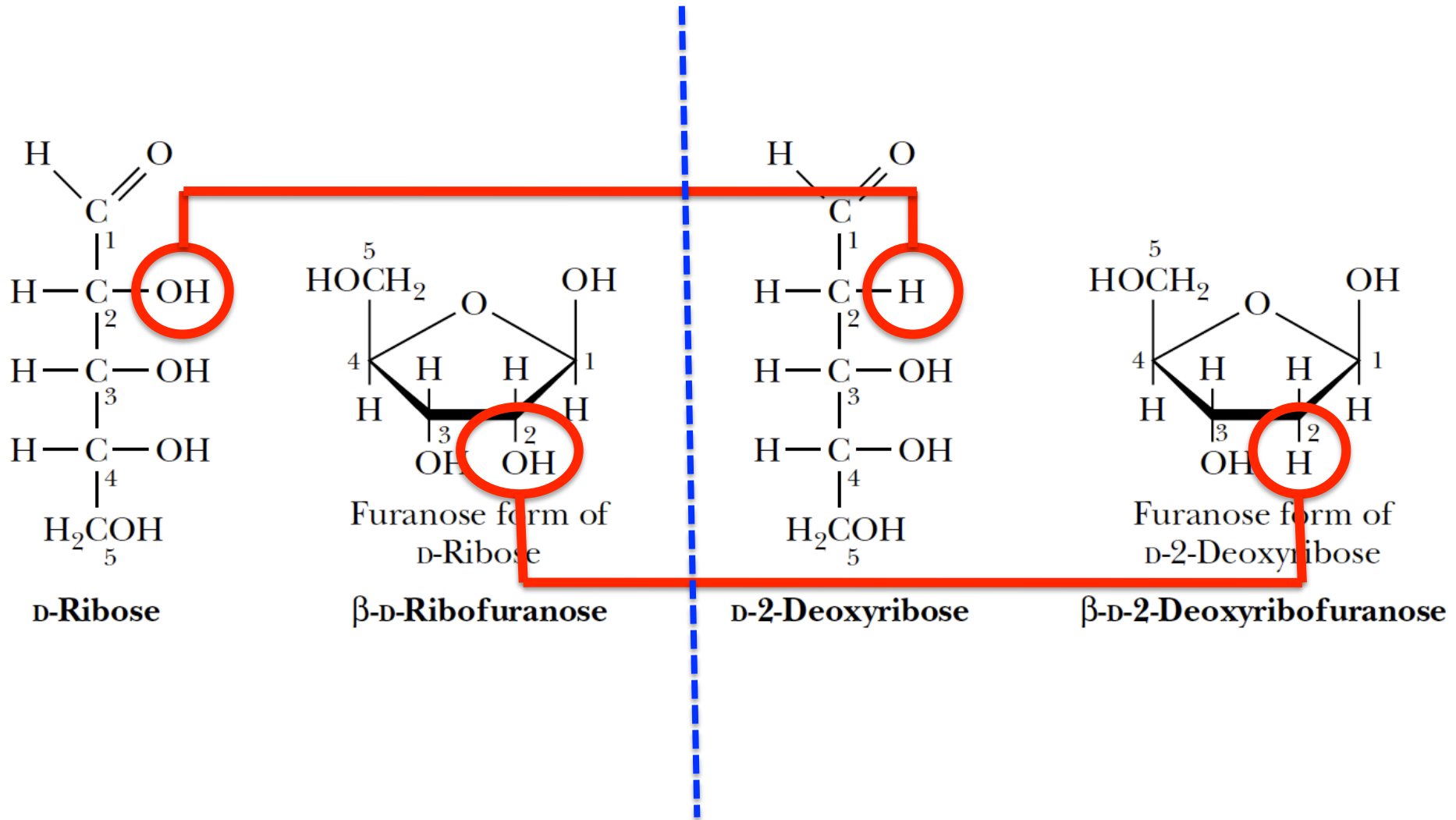


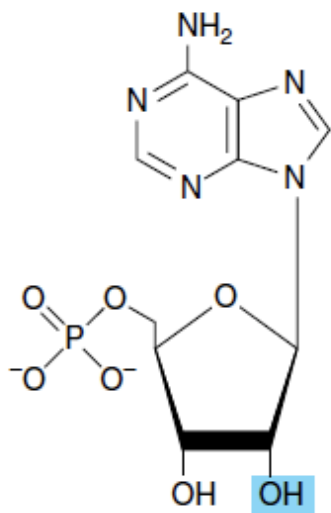


Název báze, X=H	Nukleosid, X=(deoxy)ribóza	Nukleotid, X= fosfo.ribóza
Adenin	Adenosin	Adenosinmonofosfát AMP
Guanin	Guanosin	Guanosinmonofosfát GMP
Cytosin	Cytidin	Cytidinmonofosfát CMP
Uracil	Uridin	Uridinmonofosfát UMP
Thymin	Thymidin	Thymidinmonofosfát TMP

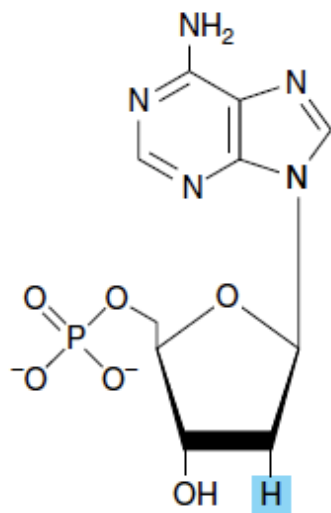


Cukr

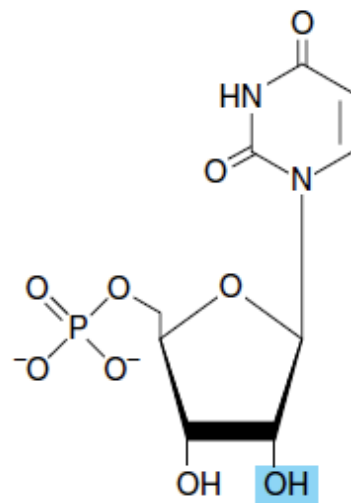




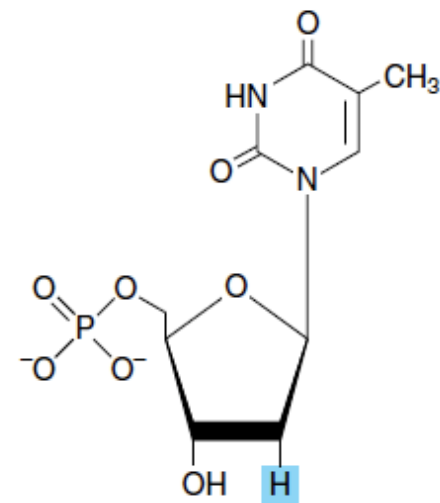
AMP



dAMP

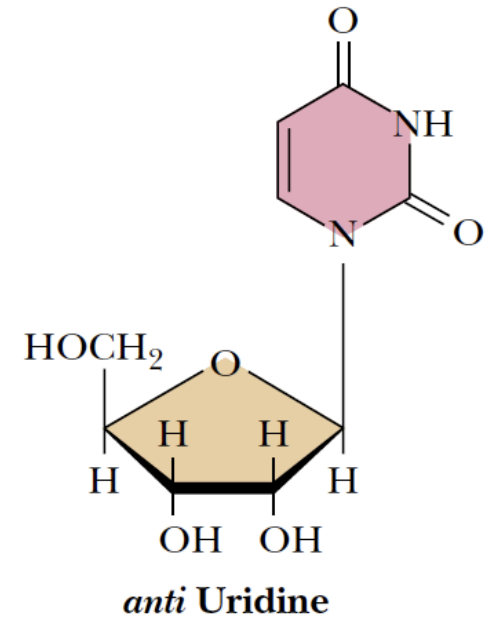
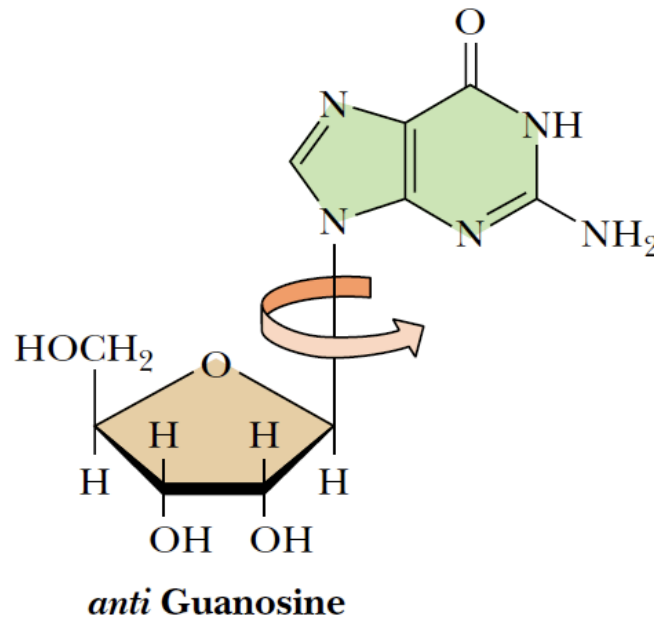
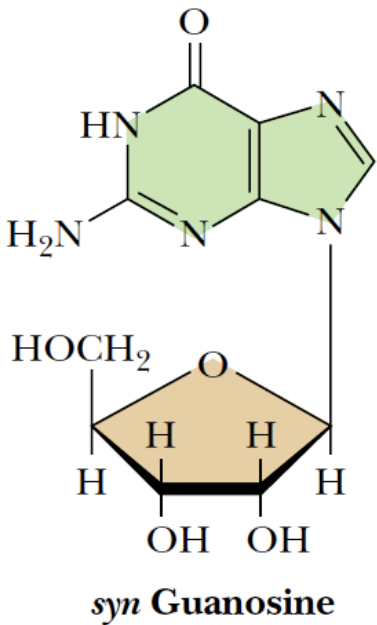


UMP

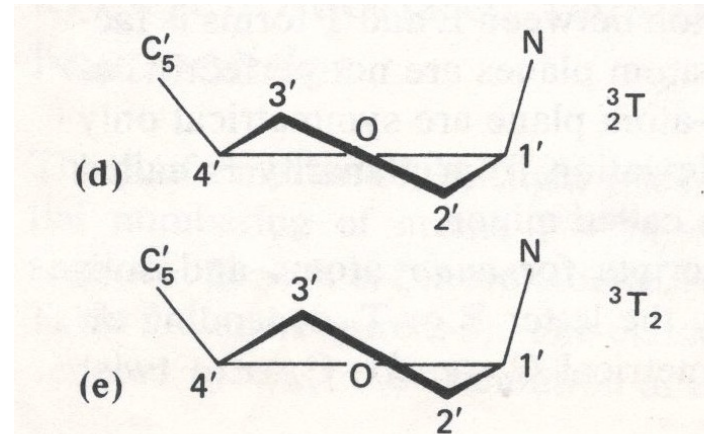
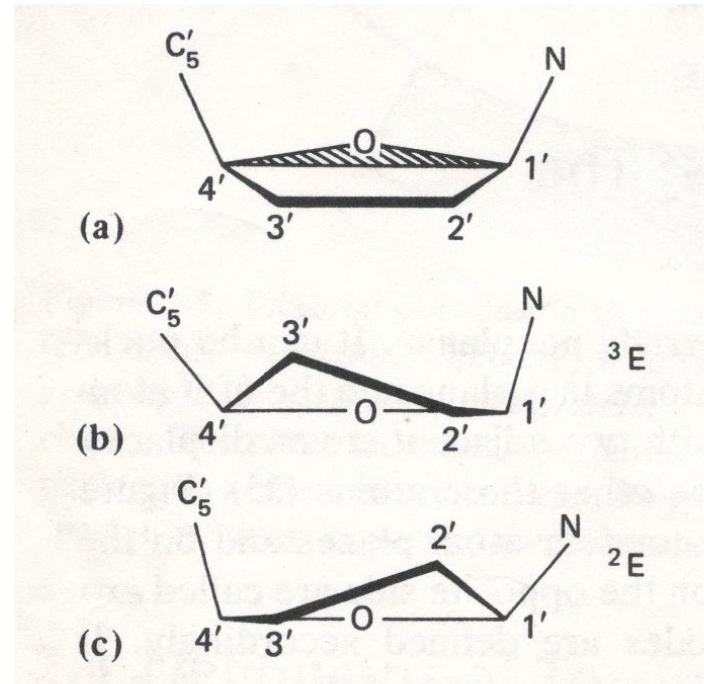
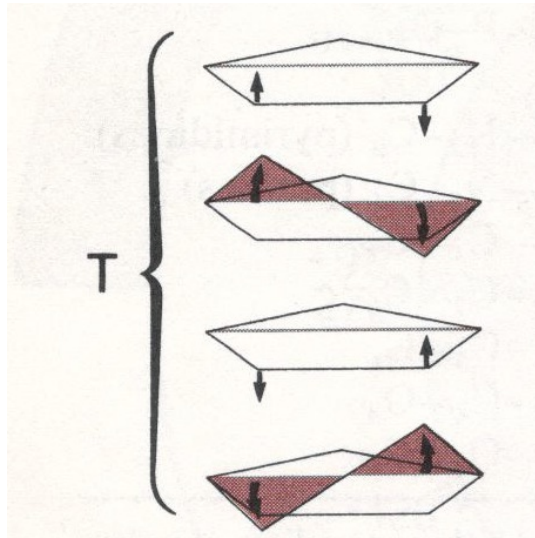
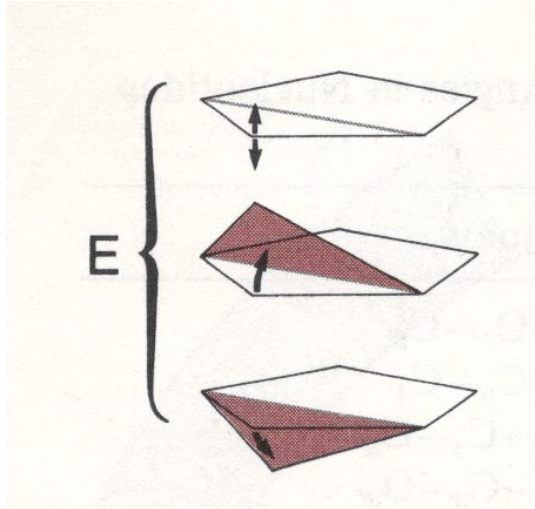


dTMP

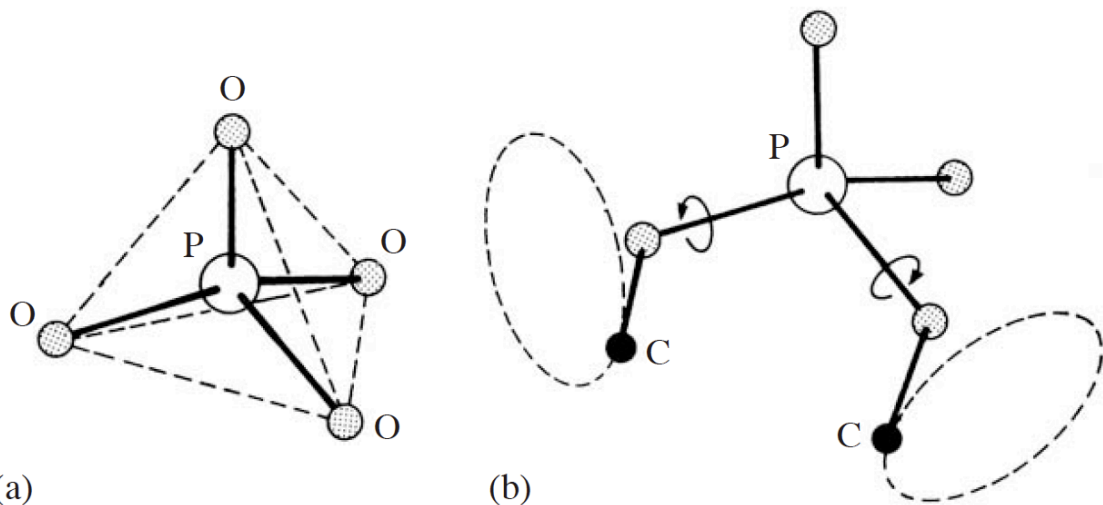
Vlivem stérického bránění báze se vyskytují dvě konformační uspořádání báze-cukr: *syn/anti*
Oba způsoby uspořádání se vyskytují v přírodě, přičemž **ANTI** převažuje



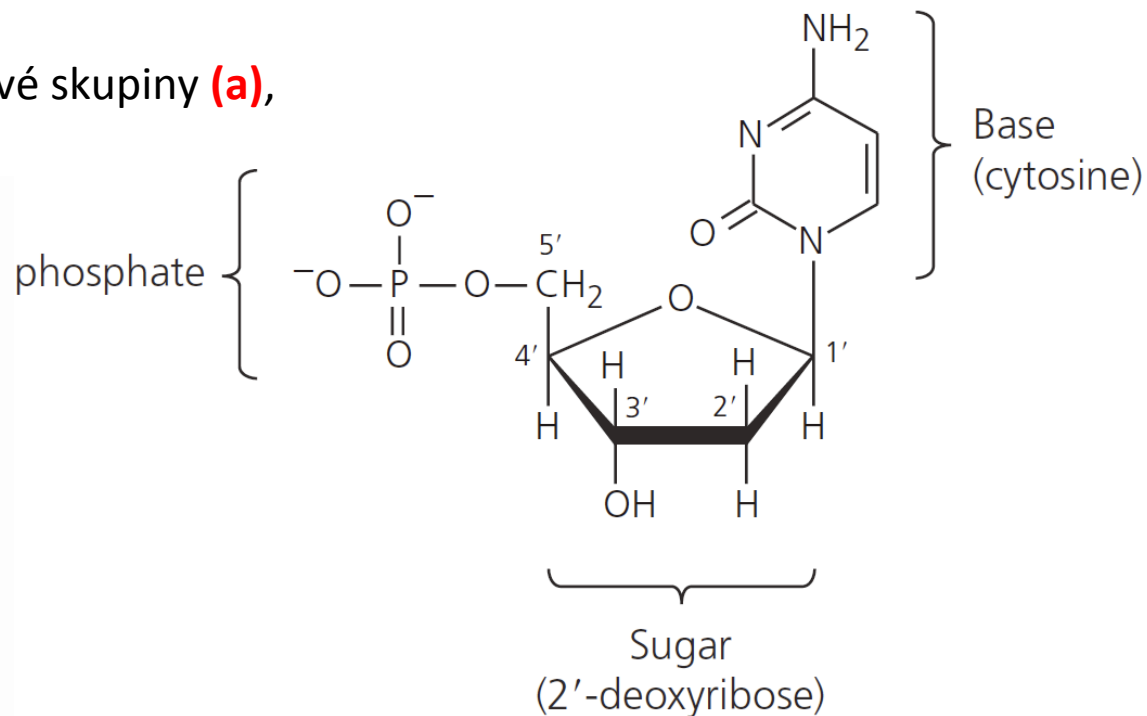
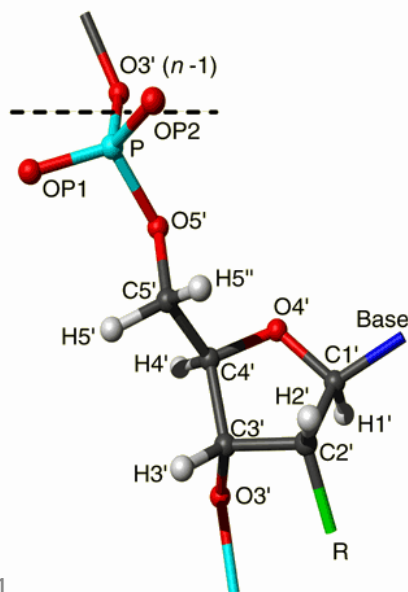
Konformace cukru - Sugar pucker



Fosfát; páteř nukleové kyseliny

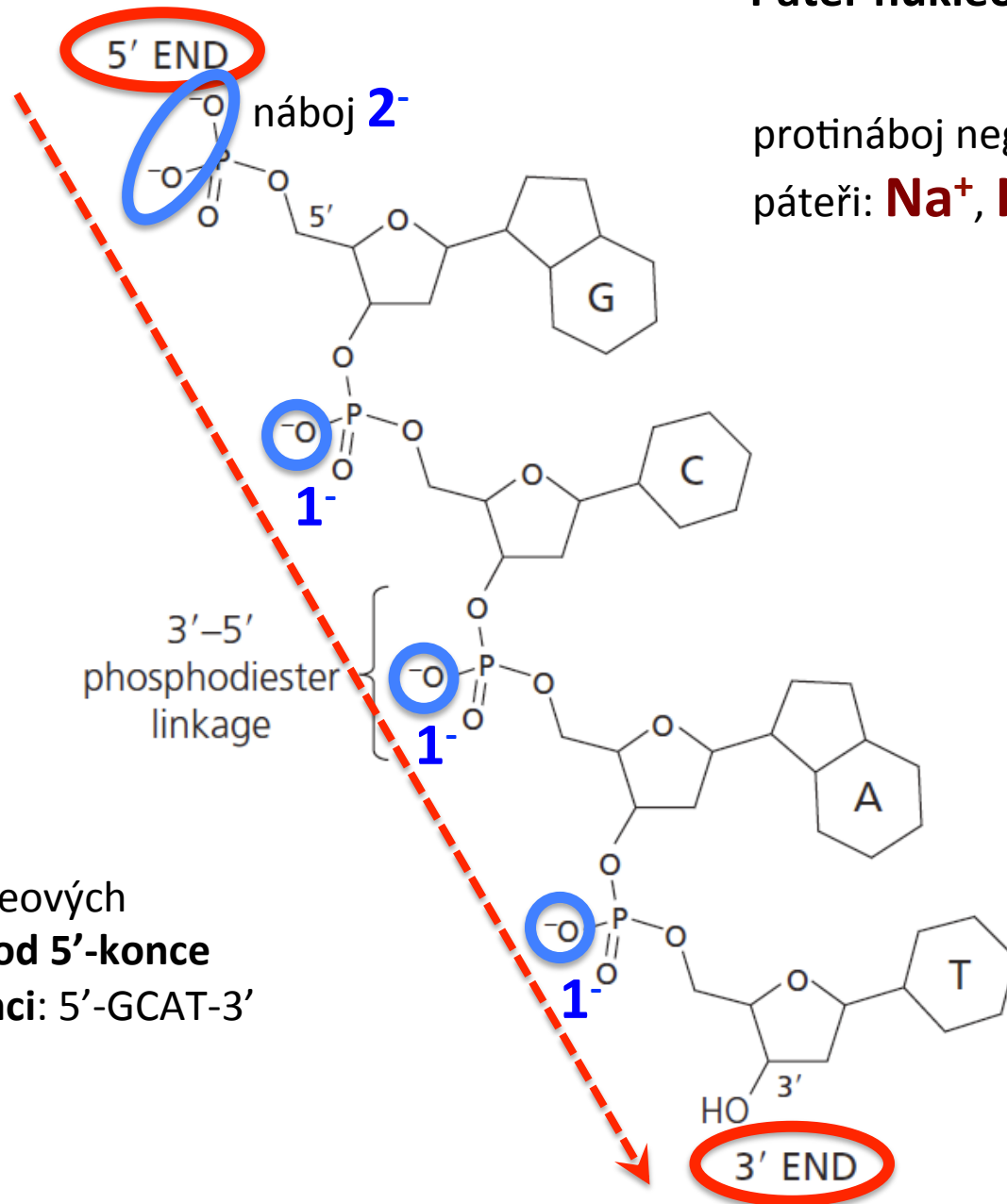


Tetraedrání uspořádání fosfátové skupiny **(a)**,
volnost rotace **(b)**



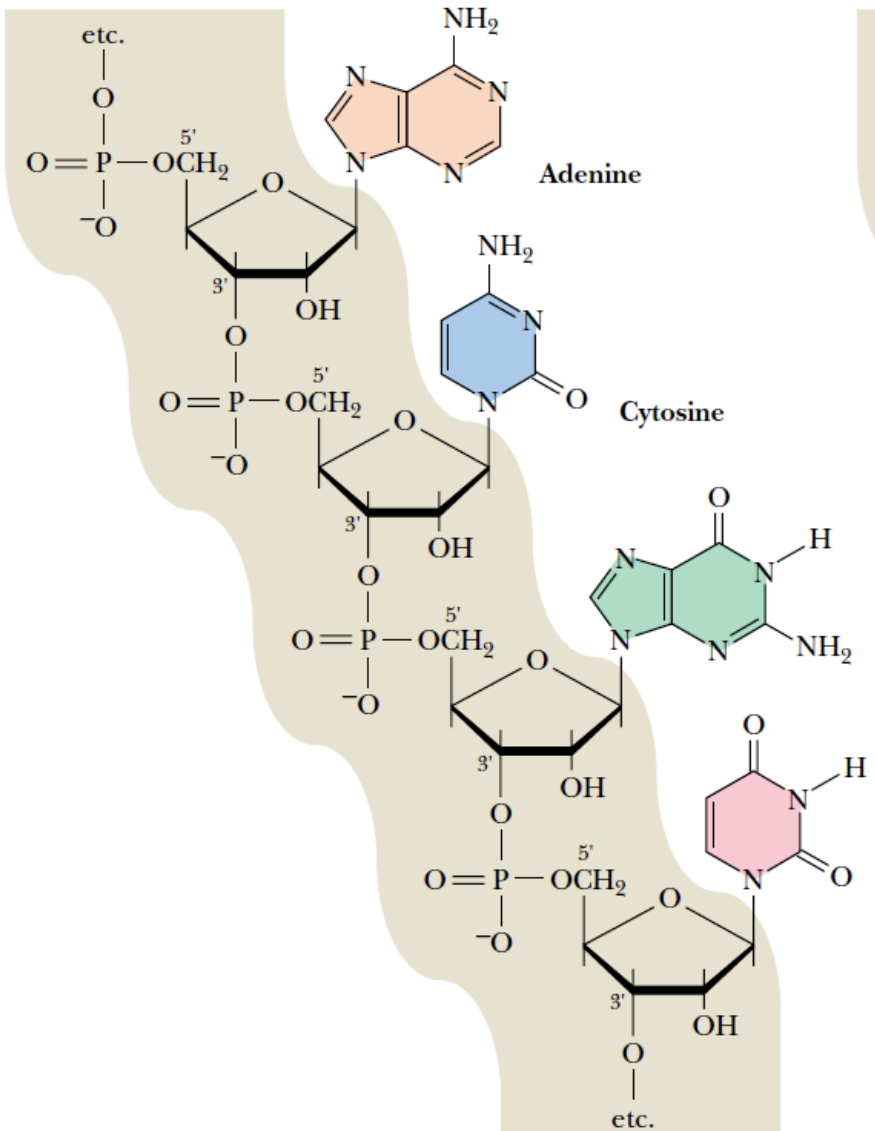
Páteř nukleové kyseliny

protináboj negativně nabitě
páteři: **Na⁺**, **K⁺**

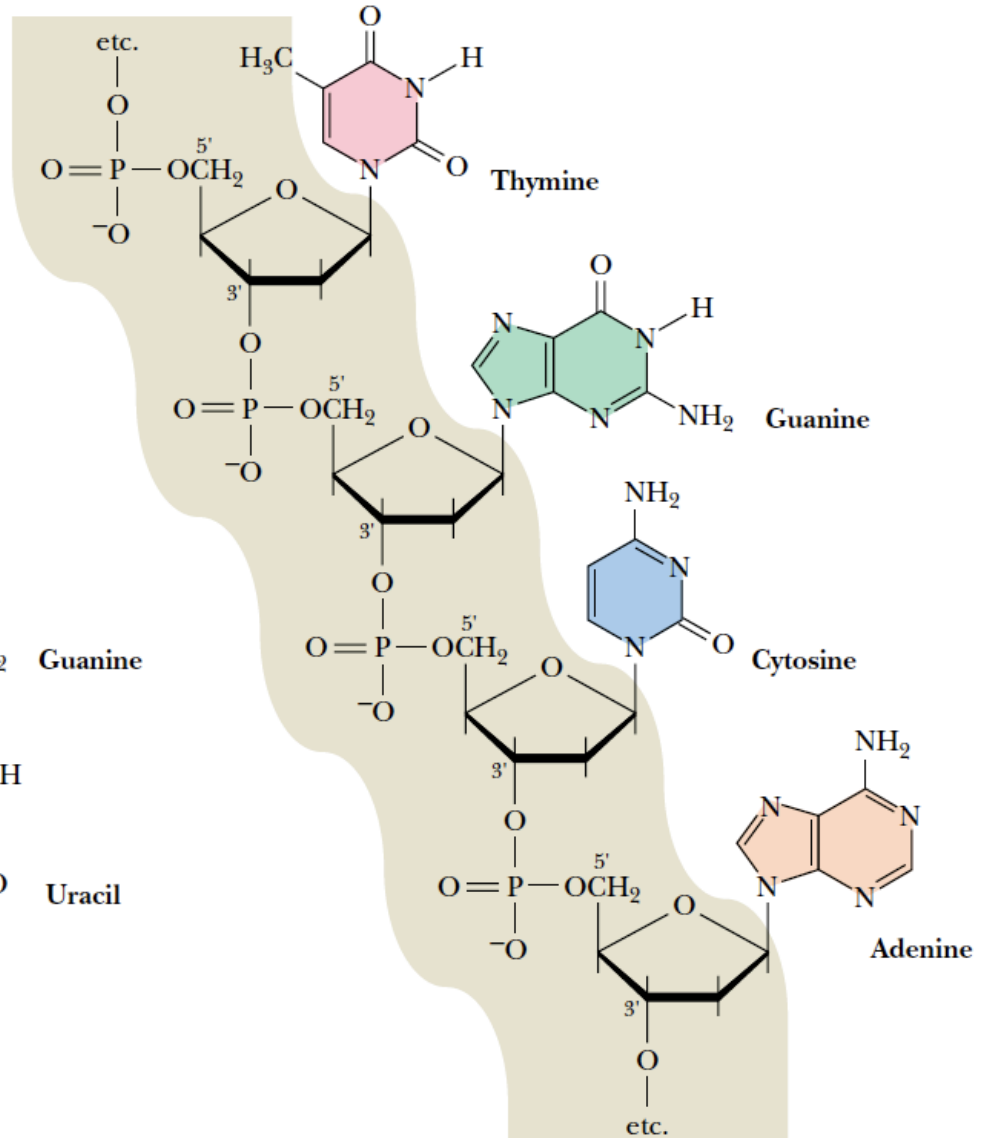


Báze v páteři nukleových
kyselin číslujeme **od 5'-konce**
směrem **ke 3'-konci**: 5'-GCAT-3'

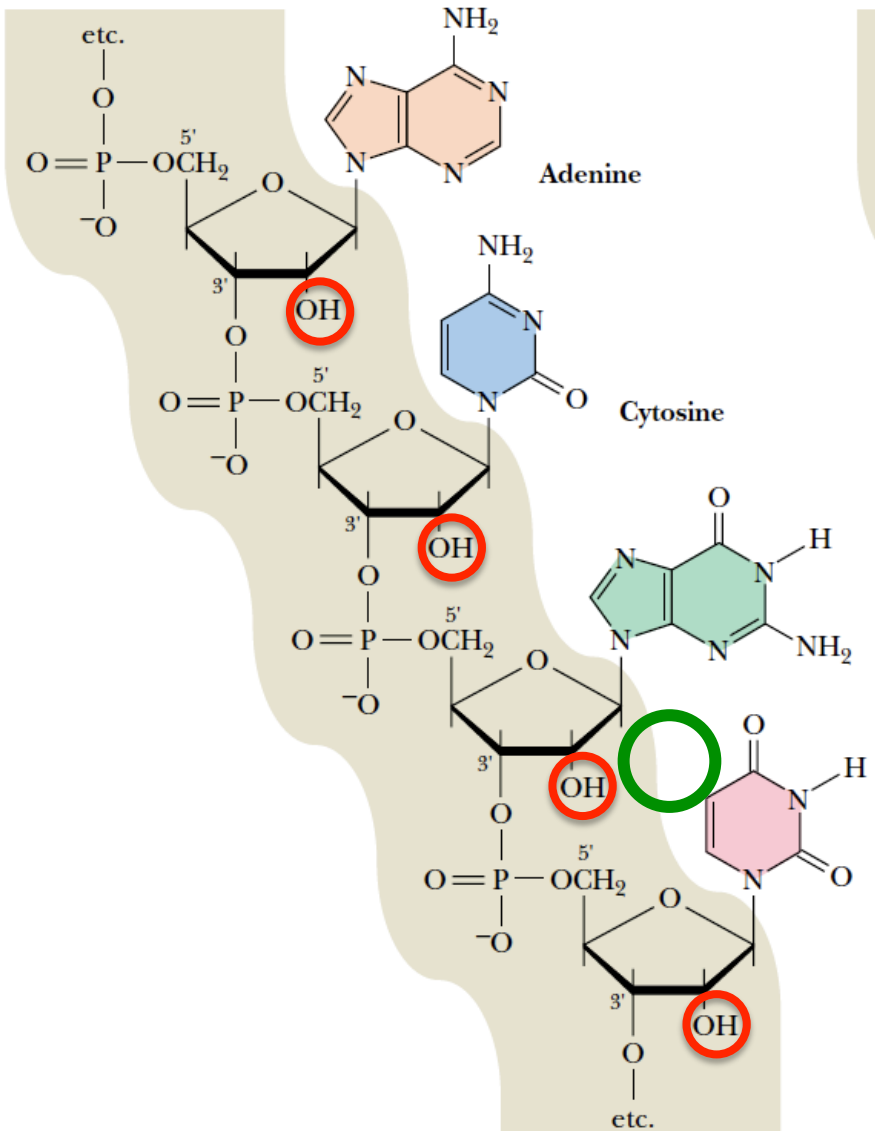
RNA



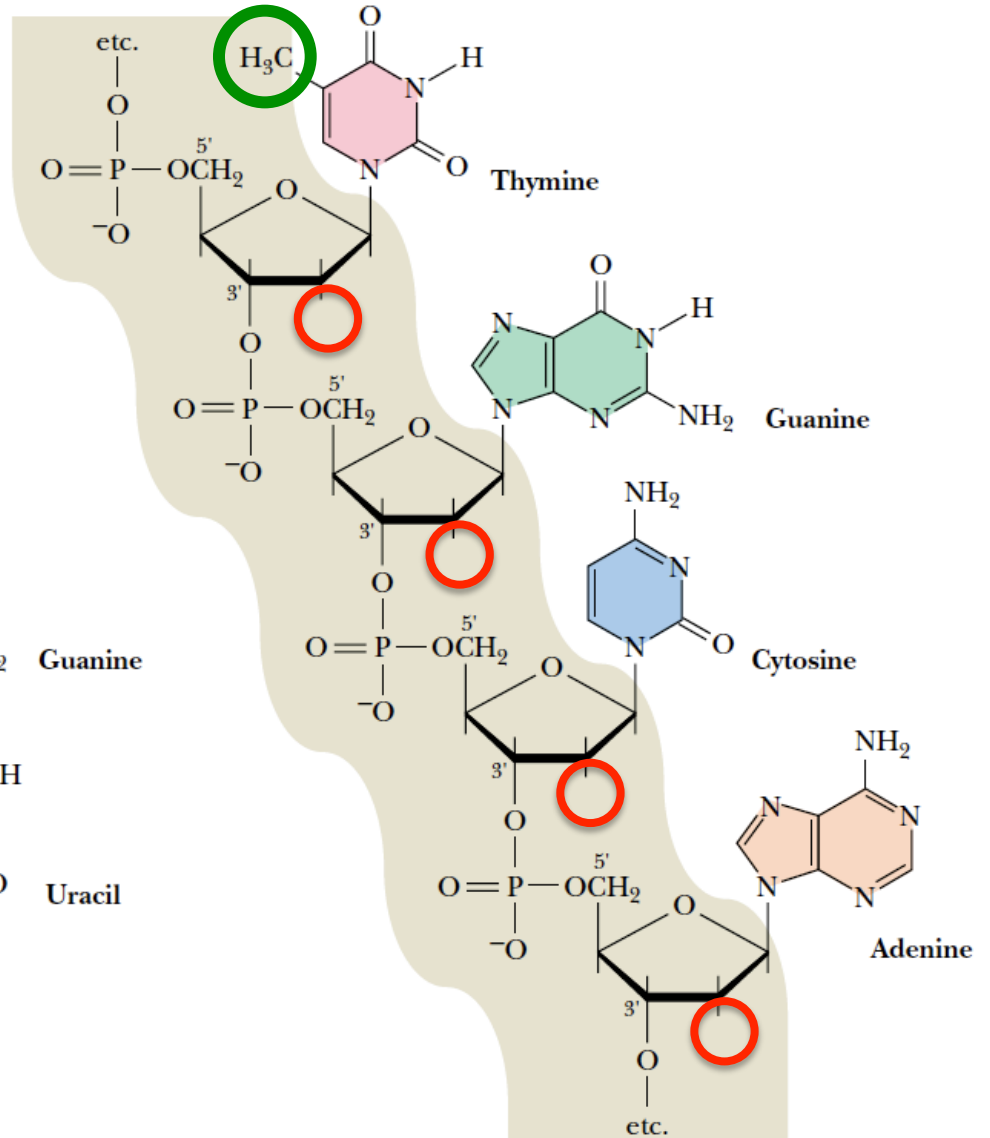
DNA



RNA



DNA

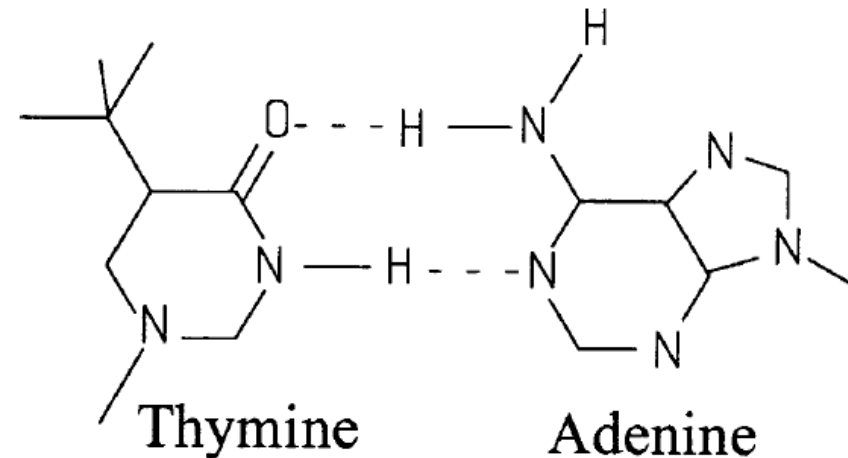
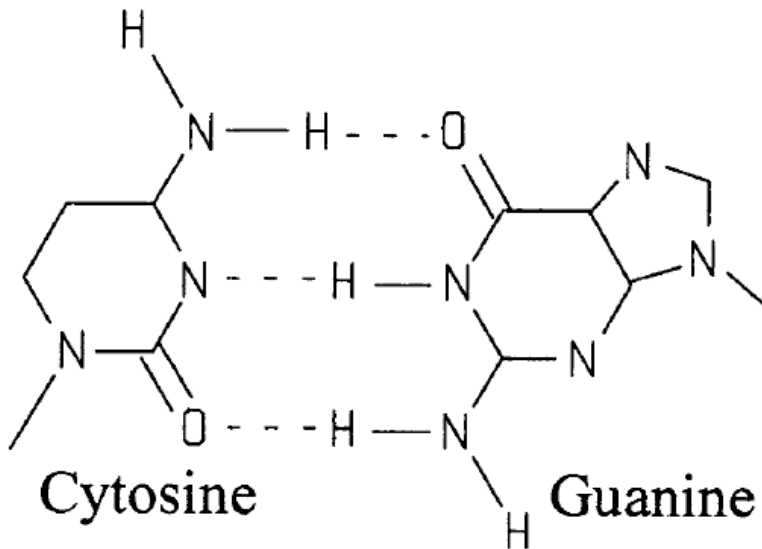


Párování bází NK: Watson-Crick, NC za medicínu a fyziologii 1962 [Watson(*1928)]

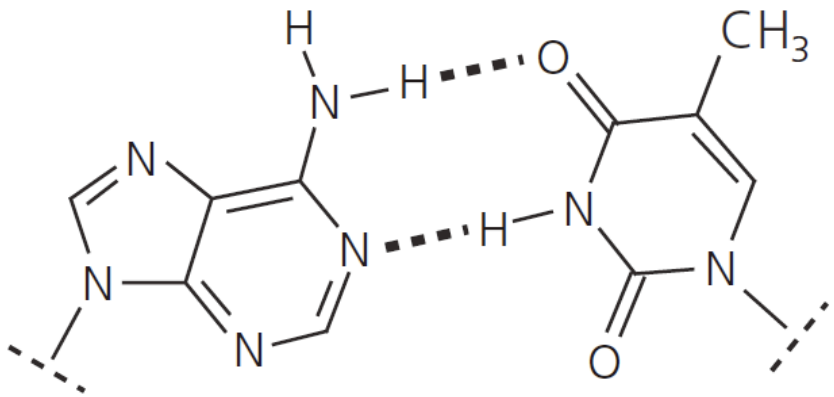


Vodíkové vazby

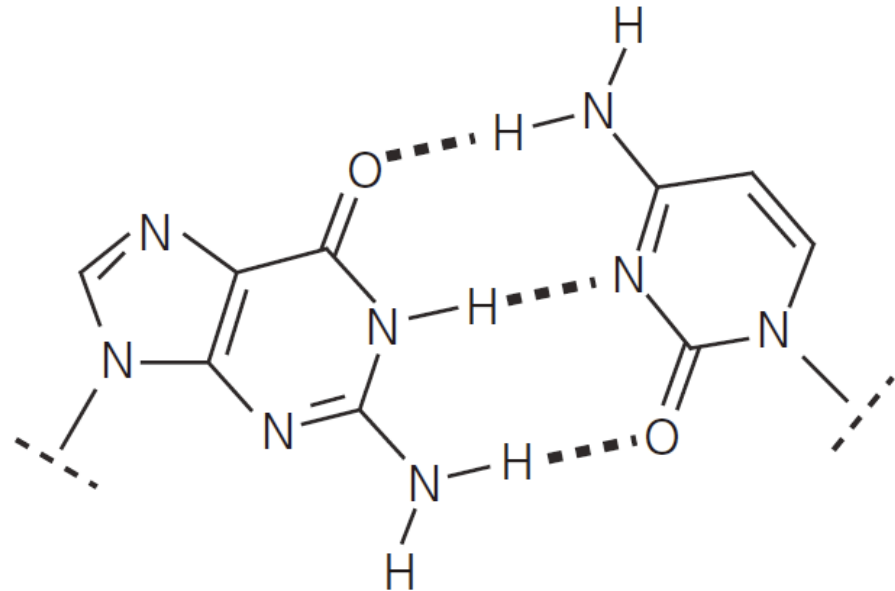
Jednotlivé řetězce dvojité šroubovice drží díky complementárními vazbami **N•••H-N** a **=O•••H-N**, které vznikají mezi **Cytosinem** a **Guaninem** a **Thyminem** a **Adeninem**.



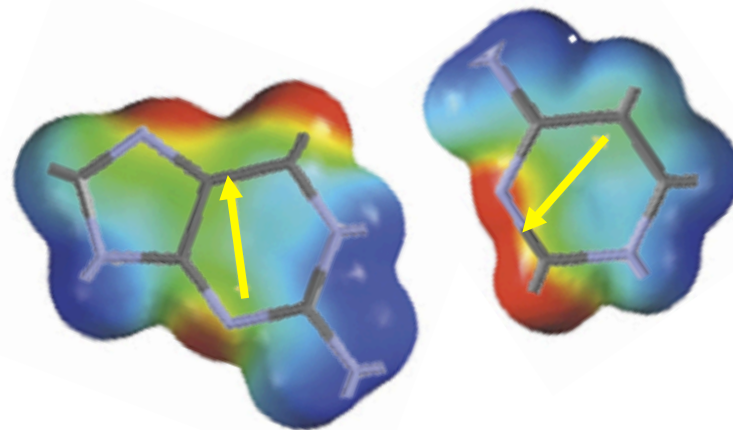
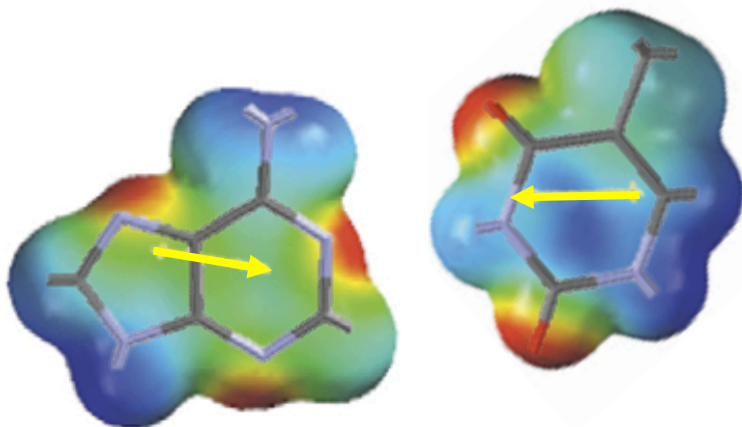
Každá vodíková vazba přispívá cca **20 kJ/mol** ke stabilizaci dvojšroubovice



adenine : thymine

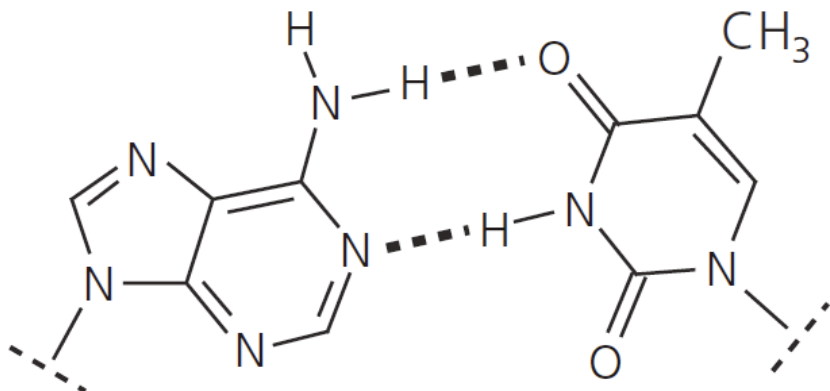


guanaine : cytosine

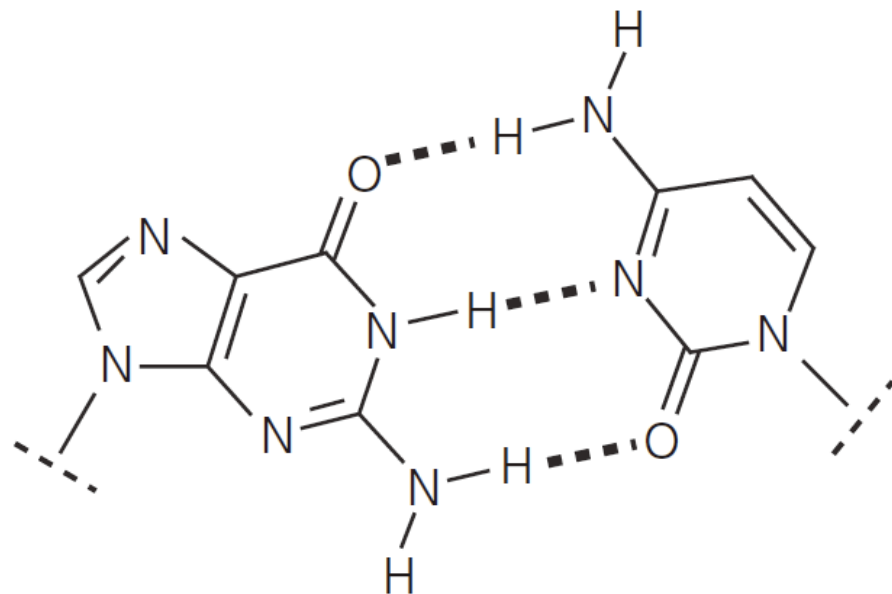


rozložení náboje v nukleobázích + 0 -, šipky označují dipólový moment

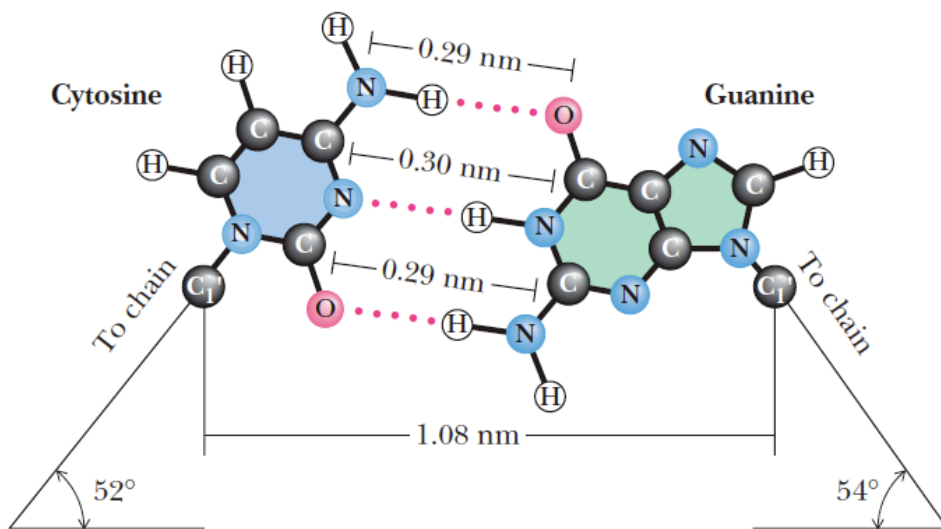
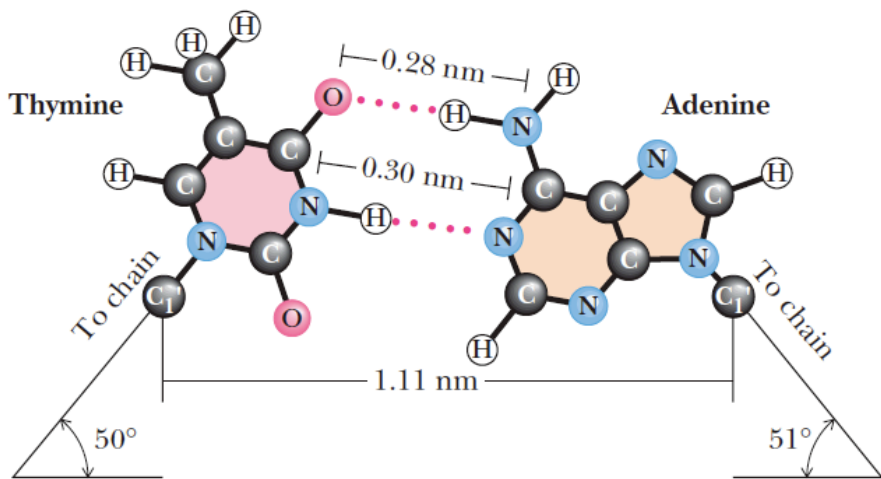
Watson-Crickovské párování bází

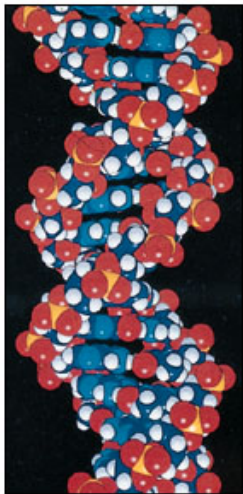


adenine : thymine



guanine : cytosine



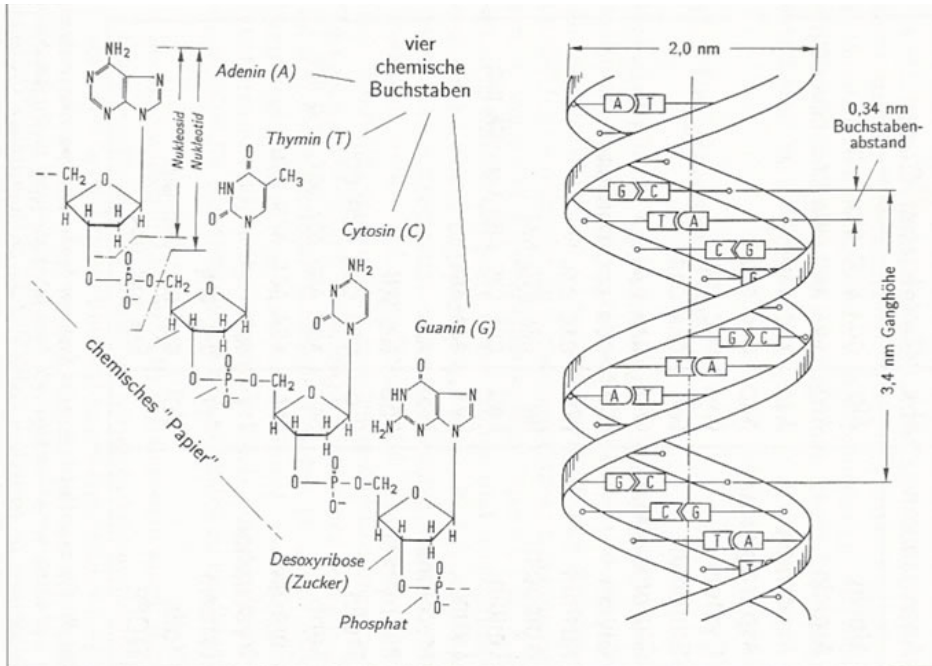
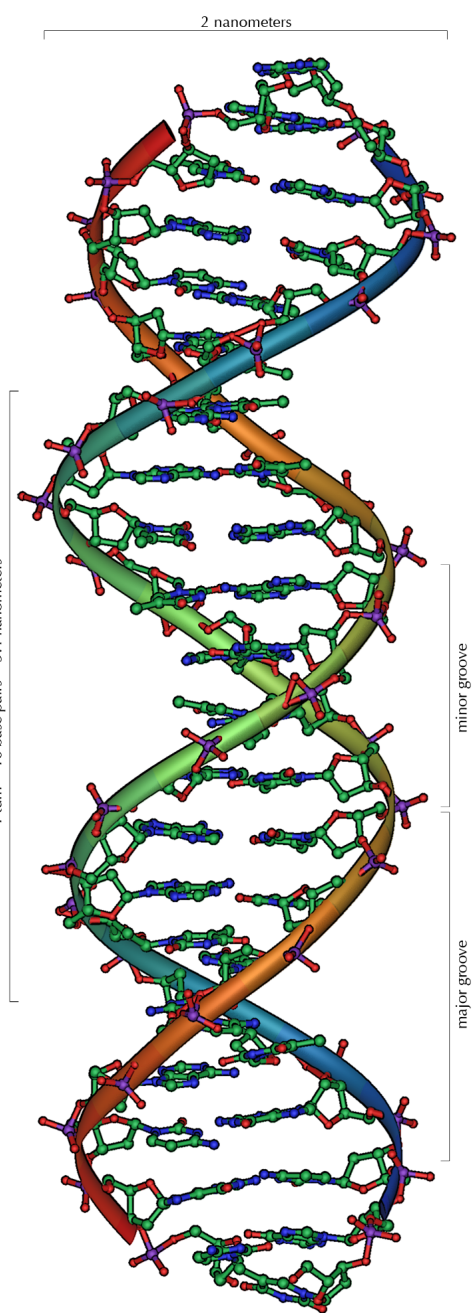
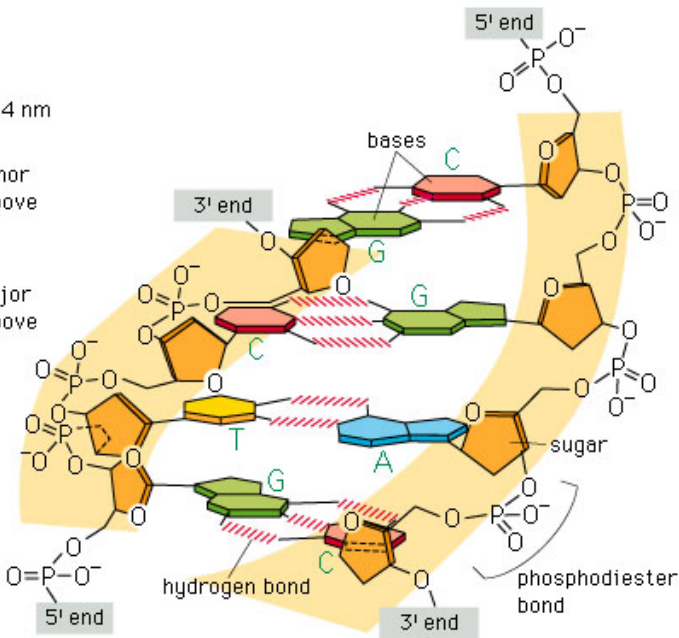


2 nm

0.34 nm

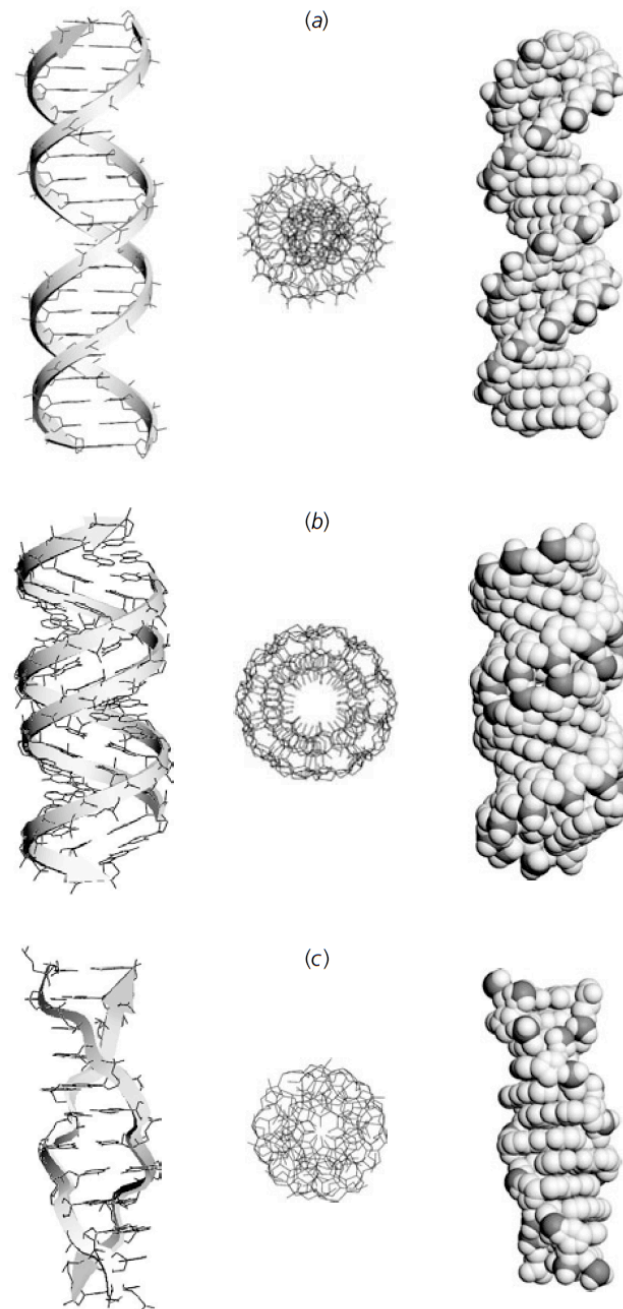
minor groove

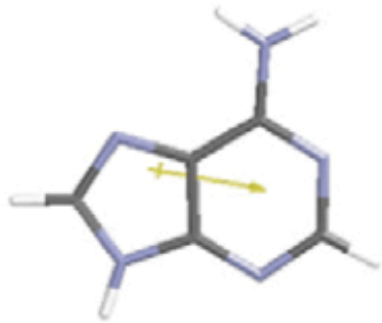
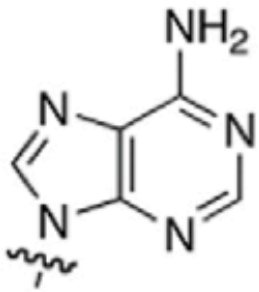
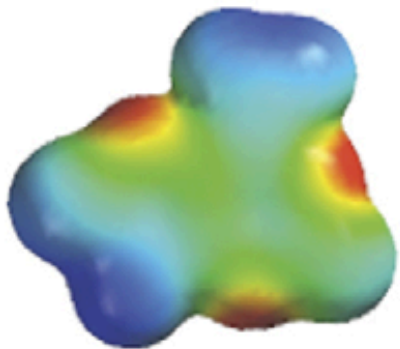
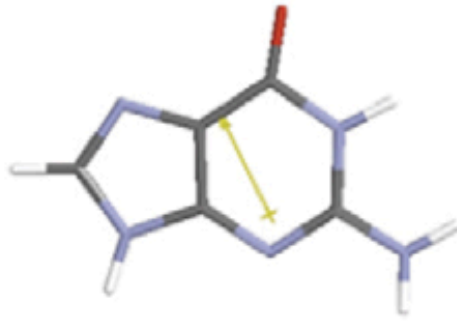
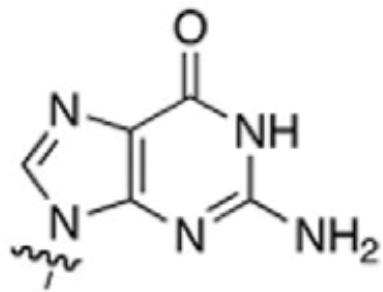
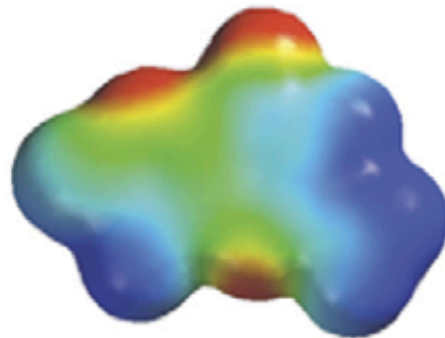
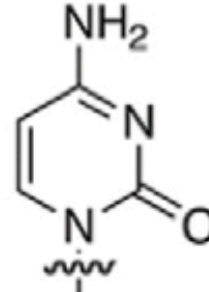
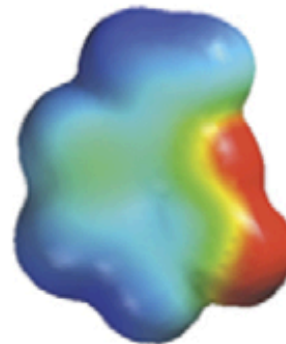
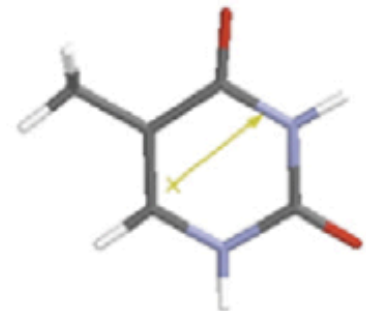
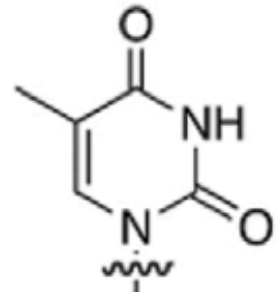
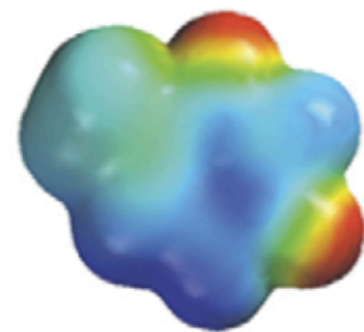
major groove



Nejběžnější typy DNA: **B-DNA** (a), **A-DNA** (b), **Z-DNA** (c)

DNA konformace	B	A	Z
Směr vinutí	pravotočivá	pravotočivá	levotočivá
Počet parů bazí na otáčku	10.5	11.0	12.0
Průměr šroubovice	~2.0 nm	~2.6 nm	~1.8 nm
Konformace cukru	C2'- <i>endo</i>	C3'- <i>endo</i>	C2'- <i>endo</i> (pyr) C3'- <i>endo</i> (pur)
Velký žlábek <i>Major groove</i>	široký, hluboký	úzký, hluboký	plochý
Malý žlábek <i>Minor groove</i>	úzký, hluboký	široký, mělký	úzký, hluboký



A**2.2 debye****G****5.9 debye****C****6.1 debye****T****4.0 debye**

Stabilita DNA dvojšroubovice:

1) Vodíkové můstky

2) Londonovy disperzní síly (LDF), dipól-dipólové interakce

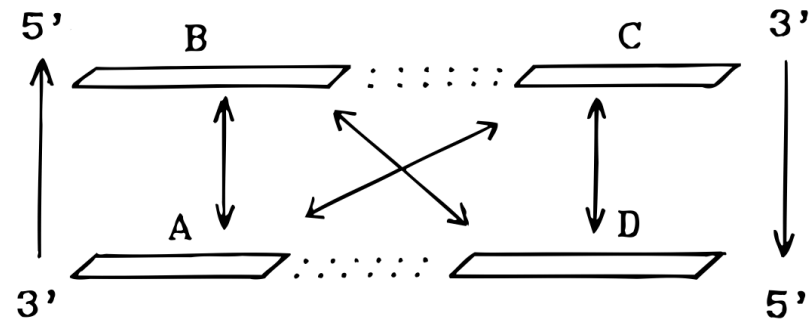
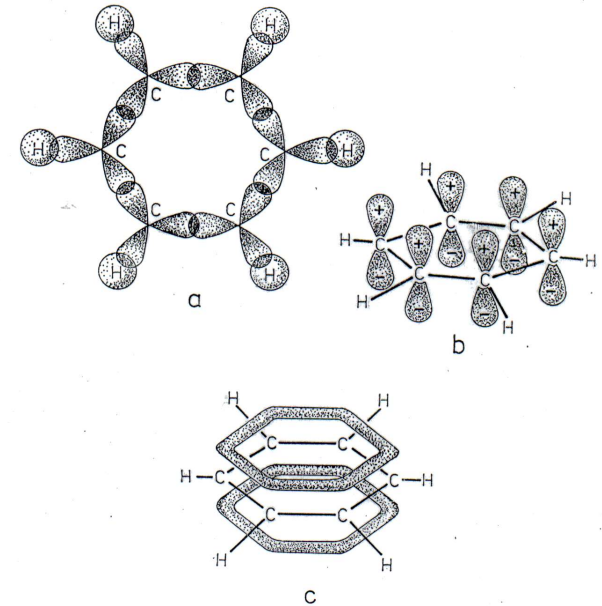
Nukleopár	Vodíkové vazby	LDF	Celková E
A:T	-26	+1.0	-25.0
G:C	-40	-16.3	-56.3

Energie v kJ/mol

A:T – opačné dipóly, G:C – působí atraktivně

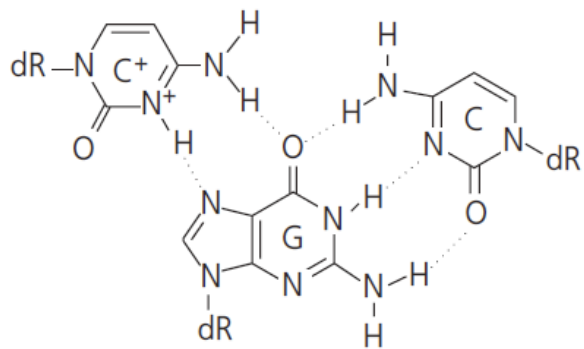
3) Patrové interakce – stacking

Působí mezi jednotlivými patry nukleopárů, stabilizují dvojšroubovici díky **elektronovým korelacím**, **van der Waalsovým** a **Coulombickým interakcím**

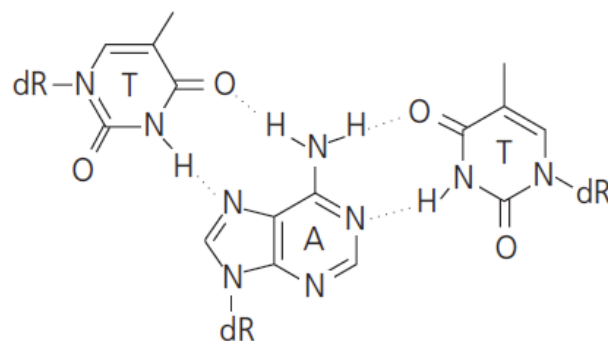


Non-Watson-Crickové (Hoogsteenovo – Karsten Hoogsteen) párování bazí

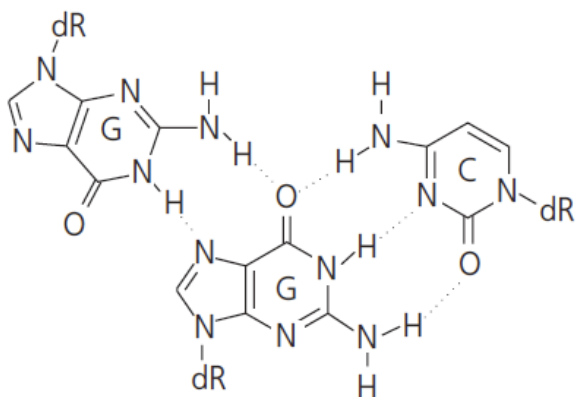
Triplexové struktury



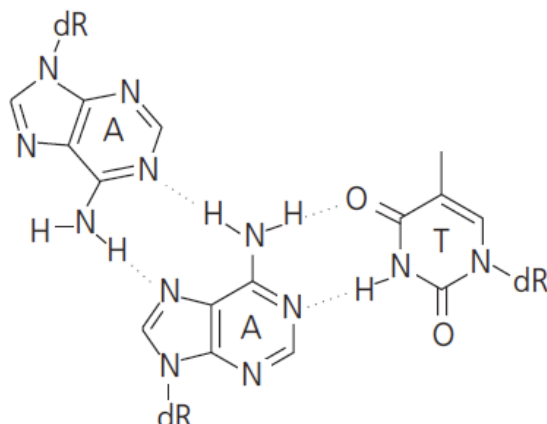
C⁺·GC



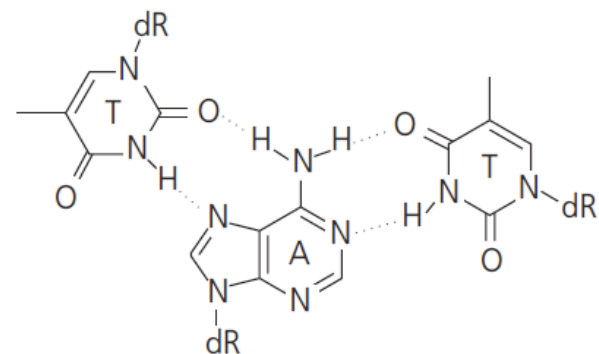
T·AT



G·GC

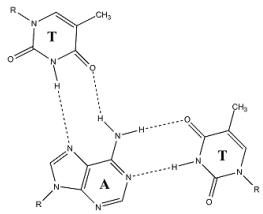


A·AT

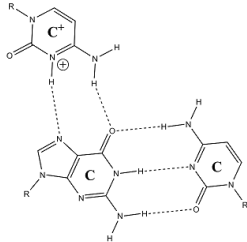


T·AT

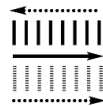
Triplexové struktury



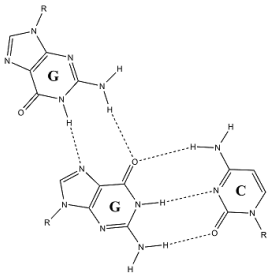
TA*T



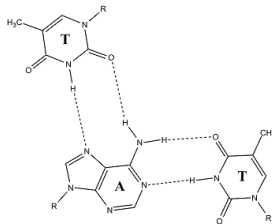
CG*C+



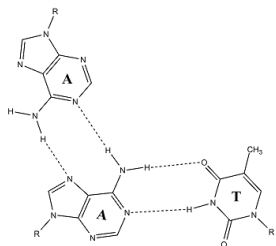
YR*Y



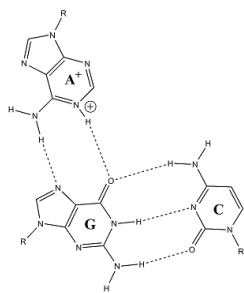
CG*G



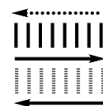
TA*A



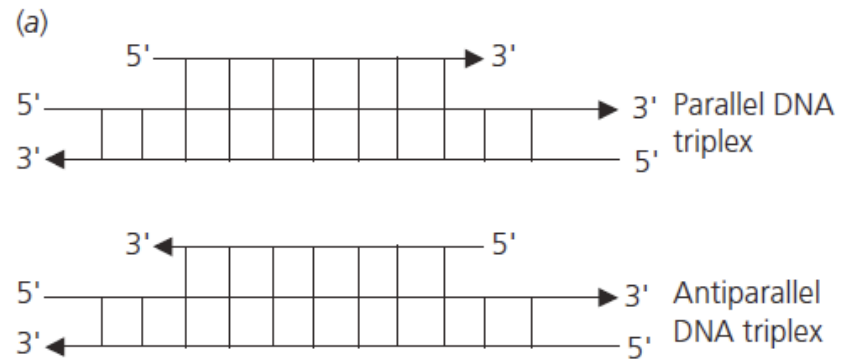
TA*T



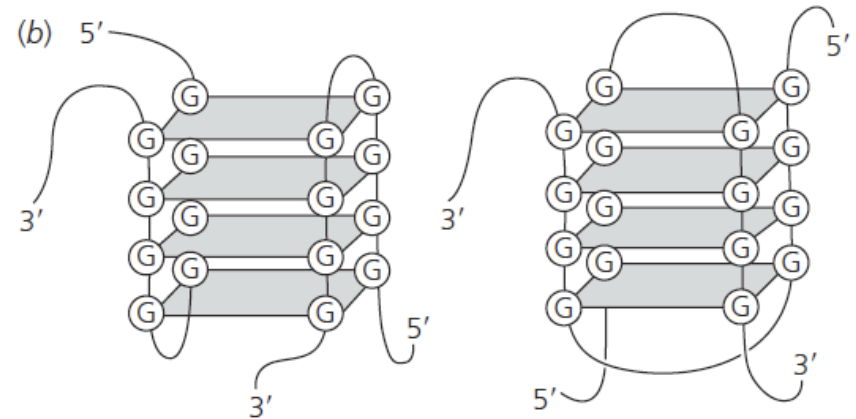
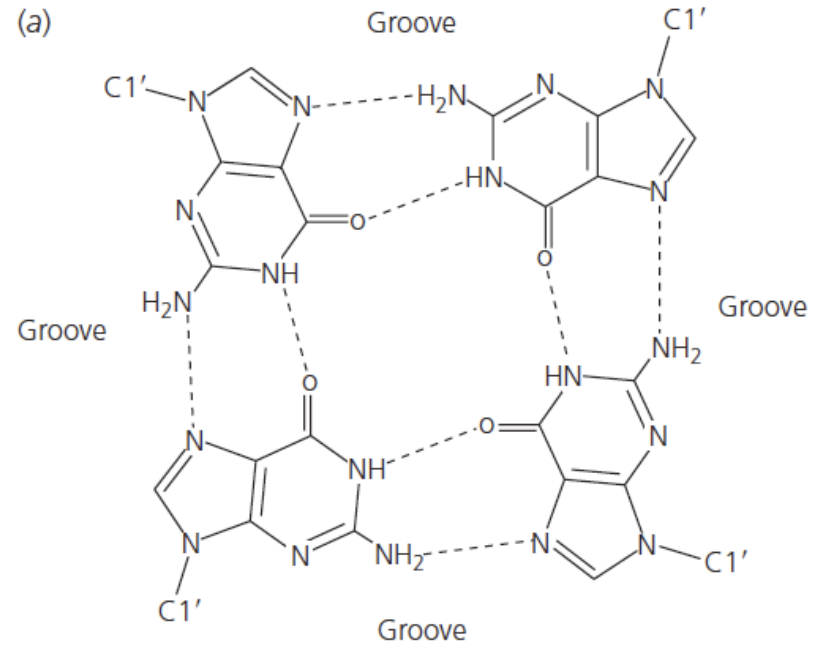
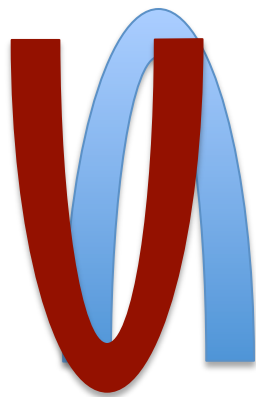
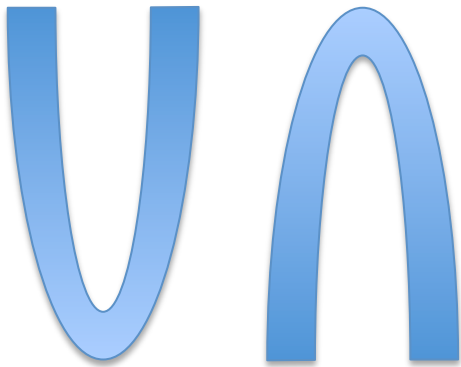
CG*A+

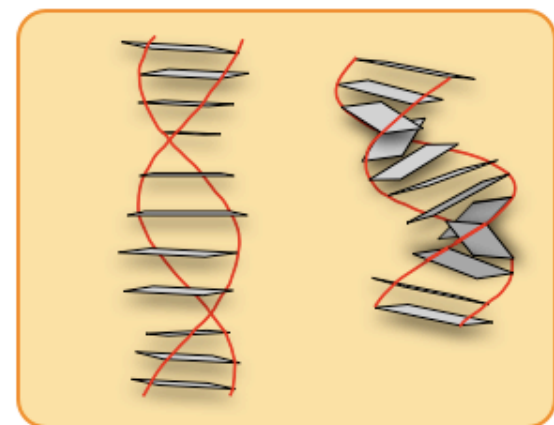
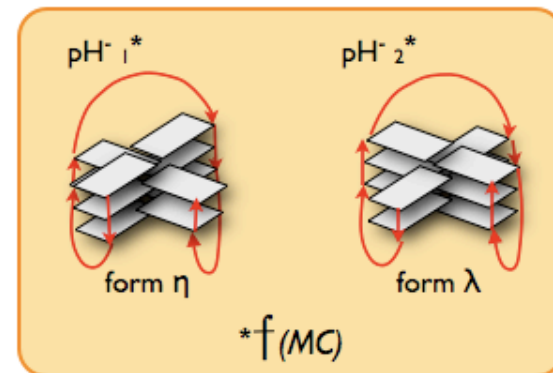
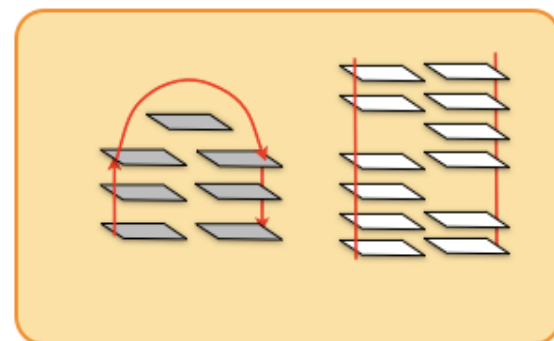
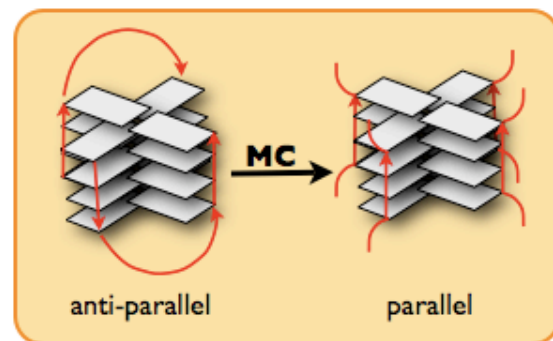
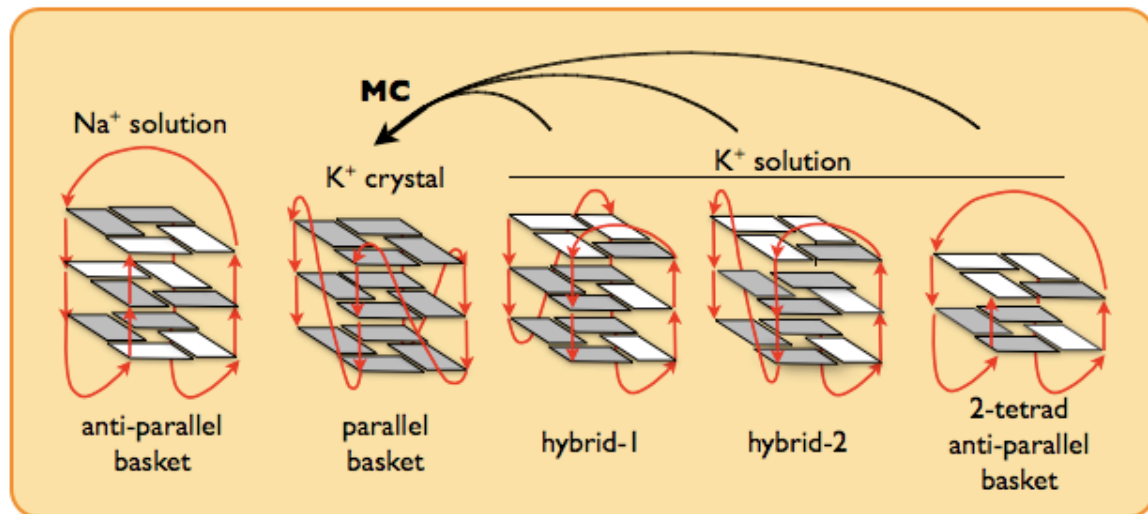


YR*R



Quadruplexové struktury





Polymorfie telomerických opakování *in vitro*

NMR

NMR

X-ray

Sekvenčně závislé

Na⁺

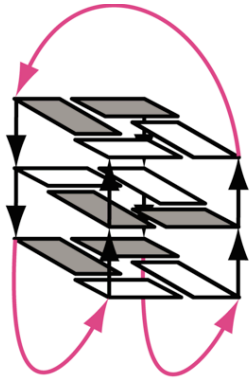
K⁺

K⁺

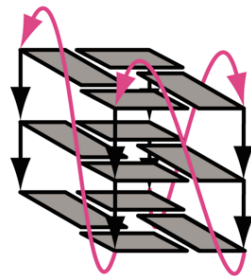
K⁺

K⁺

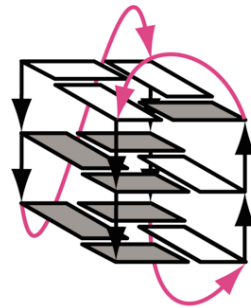
K⁺/PEG



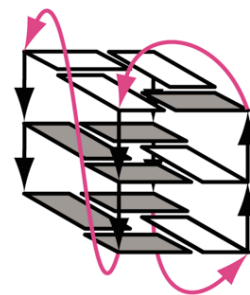
antiparallel
basket



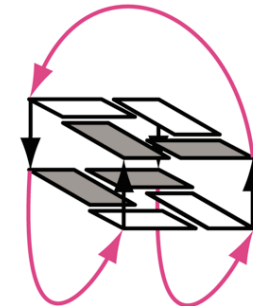
parallel
propeller



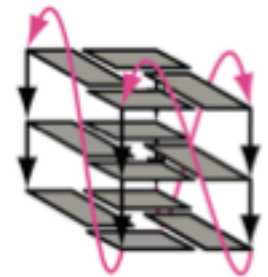
hybrid-1



hybrid-2



2-tetrad
antiparallel
basket



parallel
propeller

Wang et al. *Structure* (1993)

Parkinson et al. *Nature* (2002)

Amrus et al. *Nucleic Acids Res.* (2006)

Dai et al. *Nucleic Acids Res.* (2007)

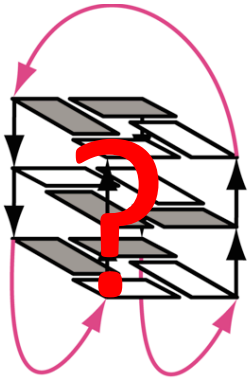
Lim et al. *J Am Chem Soc.* (2009)

Heddi et al. *J Am Chem Soc.* (2011)

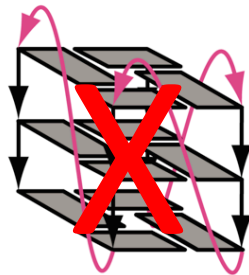
Polymorfie telomerických opakování *in vivo*

In-cell NMR

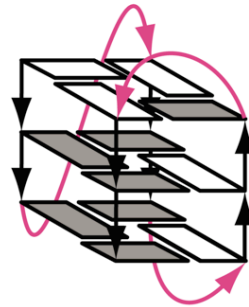
Sekvenčně závislé



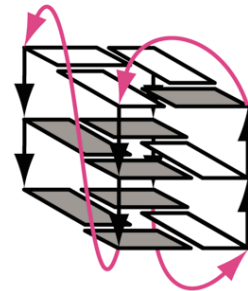
**antiparallel
basket**



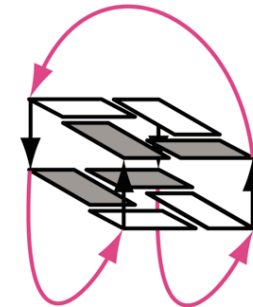
**parallel
propeller**



hybrid-1



hybrid-2



**2-tetrad
antiparallel
basket**

Hansel et al. Nucl Acids Res (2011)

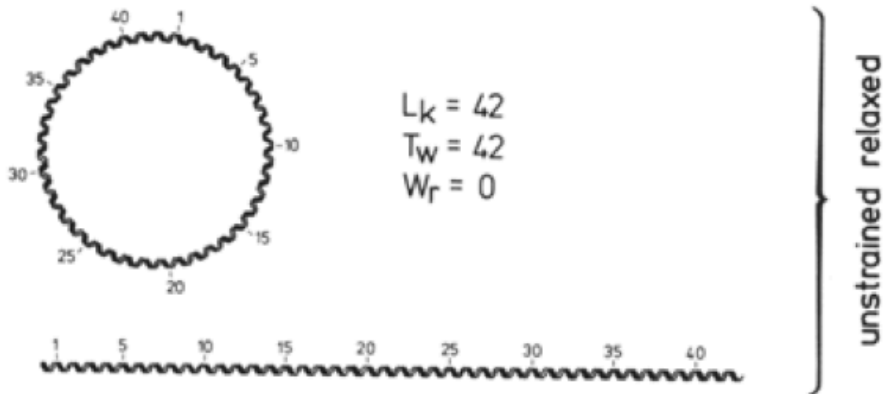
Superhelikální cirkulární DNA

$$L_k = W_r + T_w$$

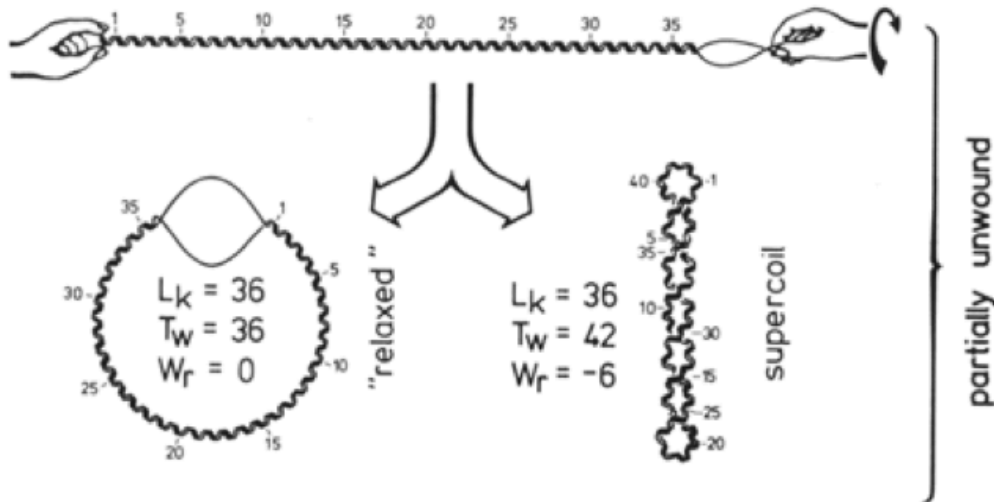
L_k - *linking* () – topologická vlastnost cirkulární DNA, udává, kolikrát je jeden řetězec DNA obtočen kolem druhého v pravotočivém směru (vzhledem k tomu, že referenční je B-DNA). **L_k** zůstává pro danou cirkulární DNA konstantní (neboť “konce” jsou zafixované a nemůže docházet k rozvinutí). **L_k** nabývá vždy celočíselných hodnot (konce DNA dvoušroubovice na sebe musí “pasovat”, aby došlo k uzavření kruhu).

T_w - *twisting* (otočení) – v relaxovaném stavu se $T_w = L_k$. **T_w** udává počet 360° otoček, které jsou na dvojšroubovici podél celé kružnice. Vzhledem k tomu, že pro B-DNA připadá cca 10 párů bazí na jednu otočku, **T_w** je přibližně rovno počtu párů bazí / 10. Pro pravotočivé otáčky je **T_w** kladné.

W_r - *writhing* (skřížení) – z důvodu strukturních “potřeb” DNA různé hodnoty **L_k** kružnice mohou způsobit nikoliv změnu v otáčkách (**T_w**), ale vznik superhelixu (superšroubovice). Vznik superhelixu je definován číslem **W_r**. Pro pravotočivé superšroubovice je **W_r** záporné!!!

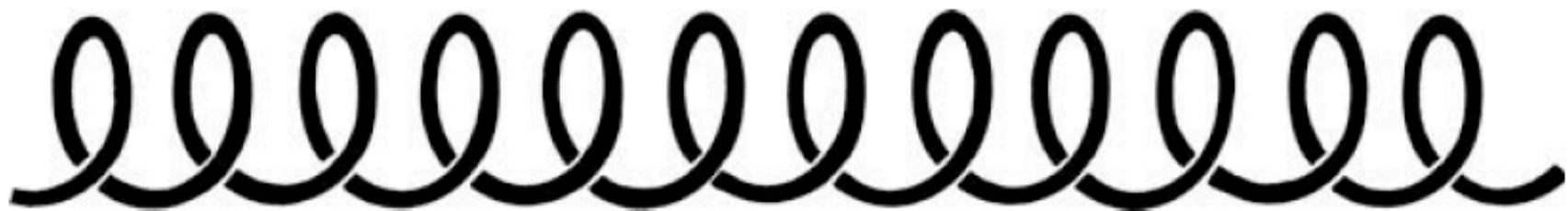


unwind by six
right handed turns
 $\Delta L_k = -6$

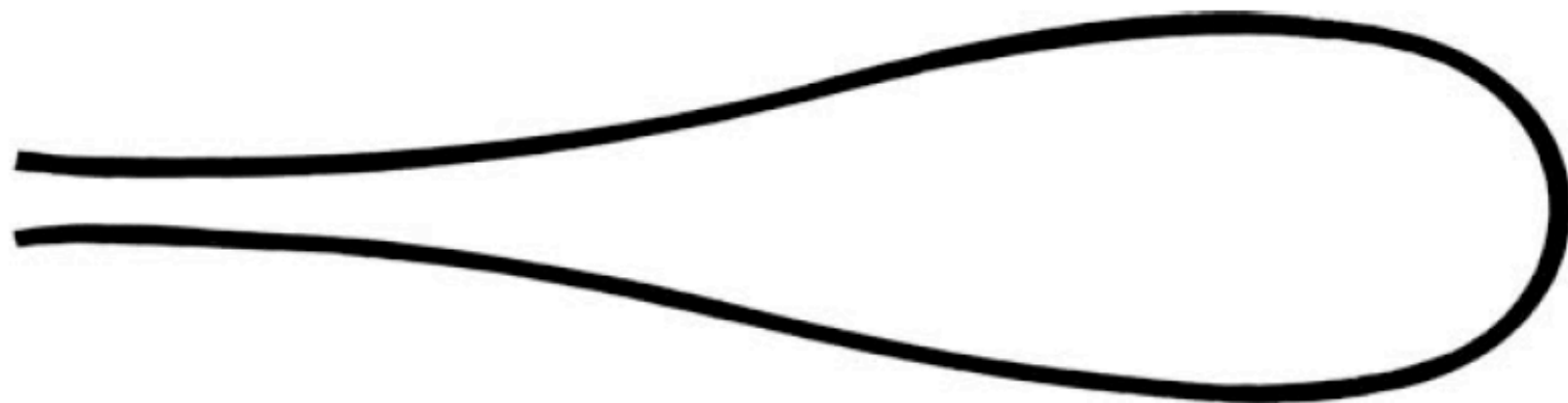


Vzhledem k tomu, že má DNA tendenci udržet B-DNA topologii, T_w se zvýší zpět na 42. L_k je ovšem topologické číslo, které **MUSÍ** zůstat konstantní, tedy 36 a k zachování rovnice $L_k = W_r + T_w$ W_r musí nabýt hodnoty **$W_r = -6$** .

(a)



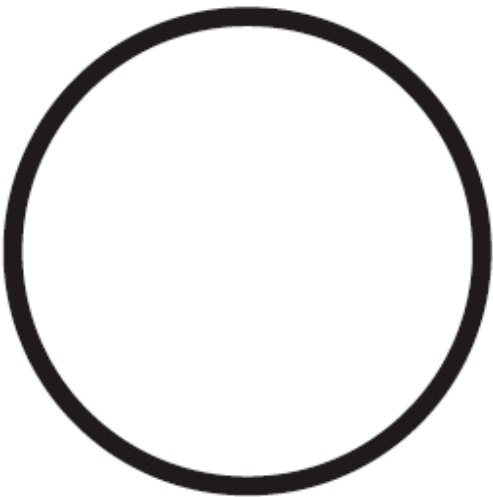
(b)



(c)



$$\text{Tw} = 0$$
$$\text{Wr} = 0$$



$$\text{Lk} = 0$$

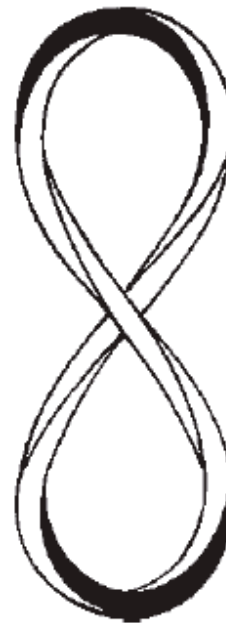
(a)

$$\text{Tw} = +3$$
$$\text{Wr} = 0$$



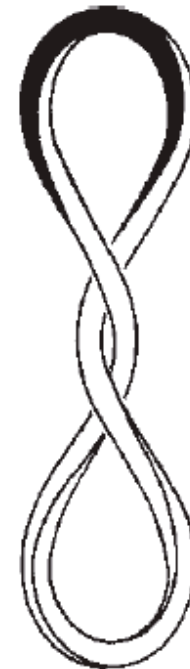
(b)

$$\text{Tw} = +2$$
$$\text{Wr} = +1$$



(c)

$$\text{Tw} = +1$$
$$\text{Wr} = +2$$



(d)

$$\text{Tw} = 0$$
$$\text{Wr} = +3$$



(e)



$$\text{Lk} = +3$$

