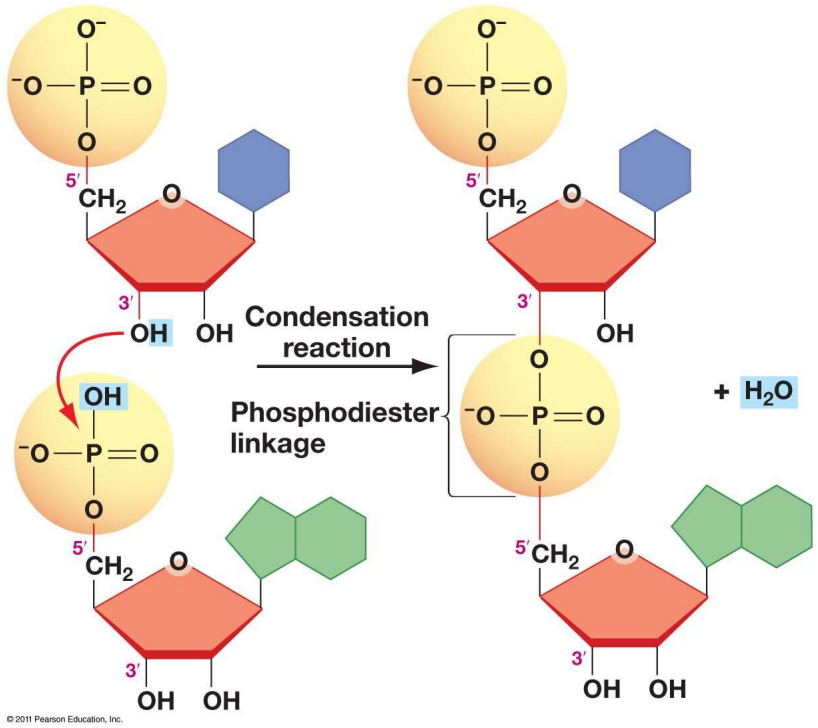
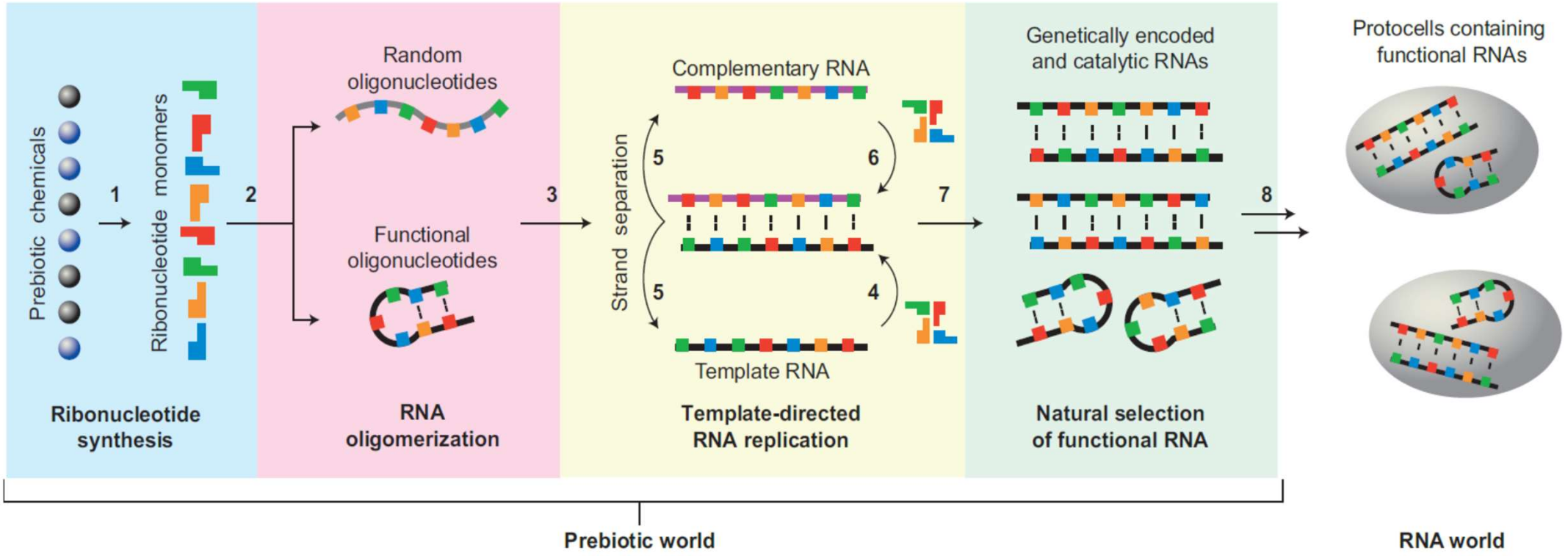
Nucleotide polymerization

Regioselective formation of 3’-5’ phosphodiester bonds between nucleotides

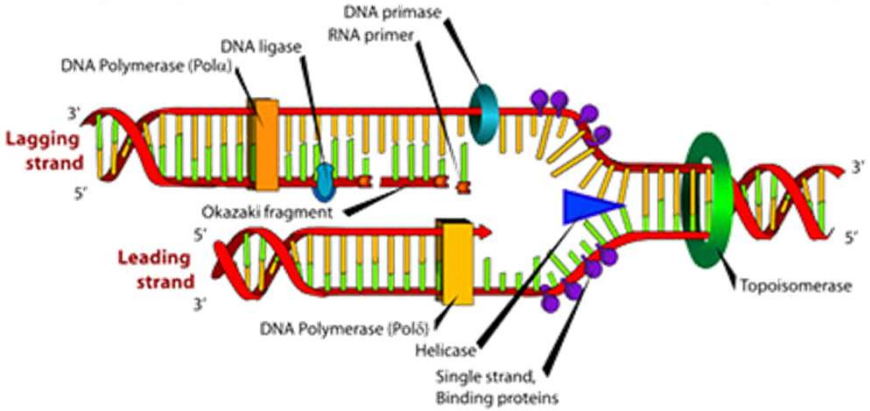


Nucleotide polymerization – sequence control

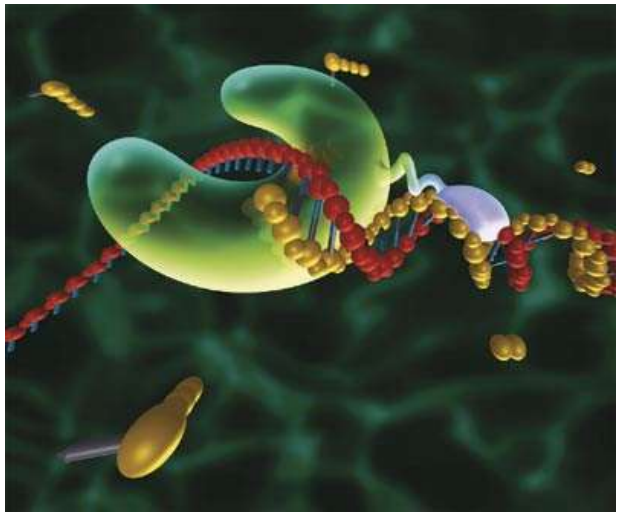


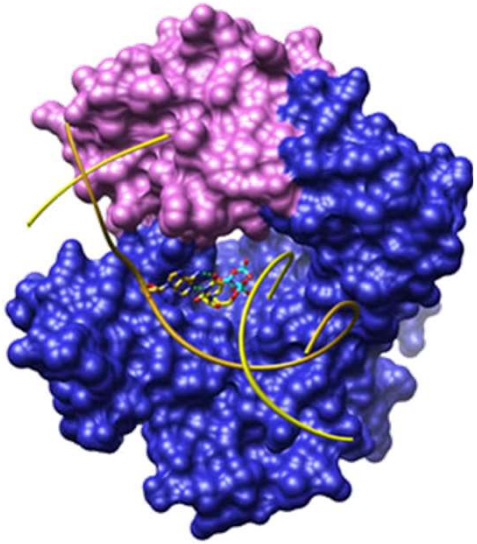
Vital chemical reactions

nucleotide polymerization → DNA/RNA polymerases

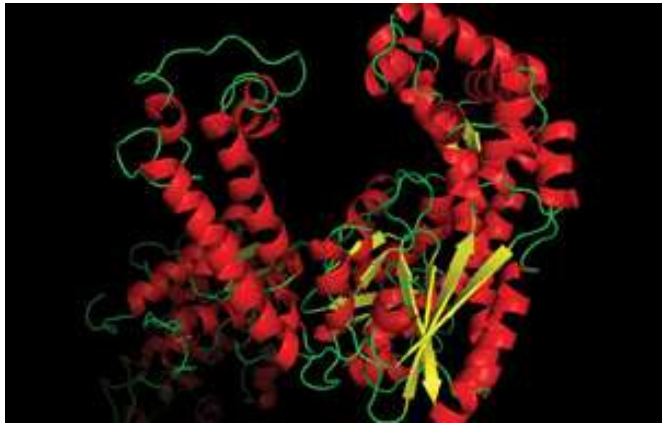


dxline.info/img/new_ail/dna-polymerase_1.jpg



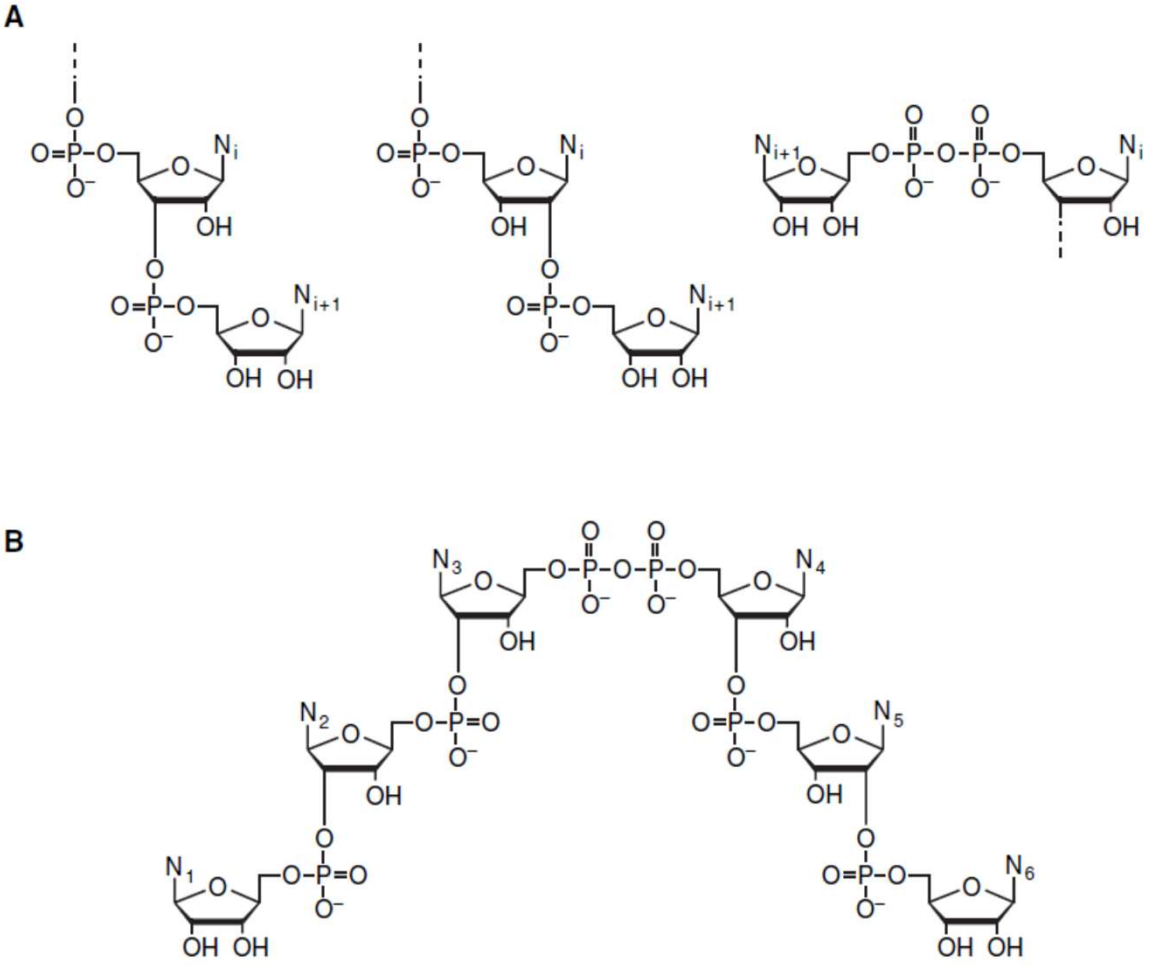


niehs.nih.gov



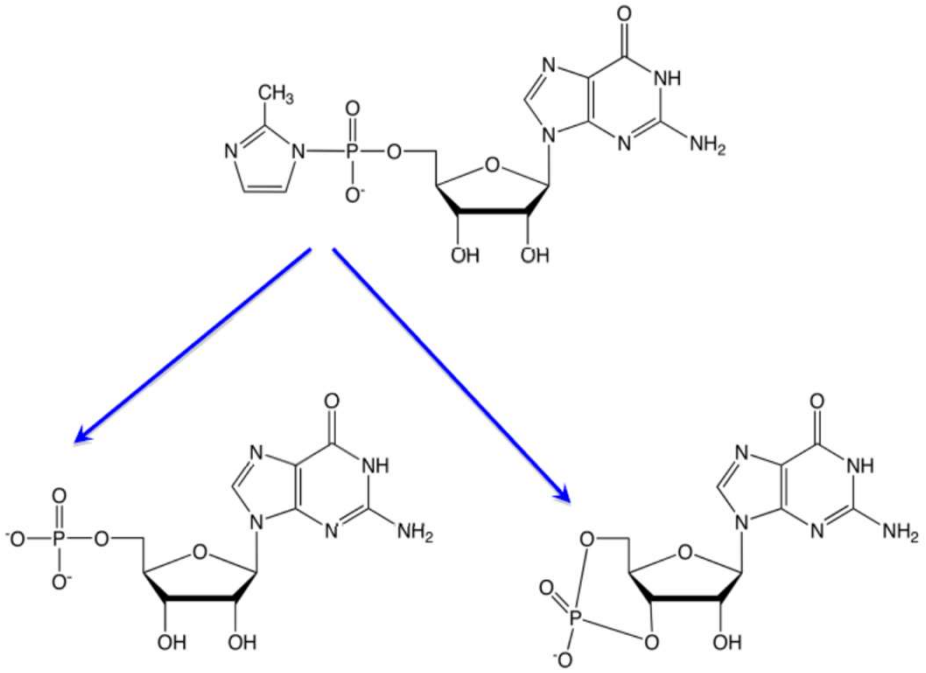
www.neb.com

Products of chemical condensation of nucleotides



(**A**) Reaction of an activated mononucleotide (N_{i+1}) with an oligonucleotide (N_1–N_i) to form a 3',5'-phosphodiester (left), 2',5'-phosphodiester (middle), or 5',5'-pyrophosphate linkage (right).
(**B**) Typical oligomeric product resulting from chemical condensation of activated mononucleotides

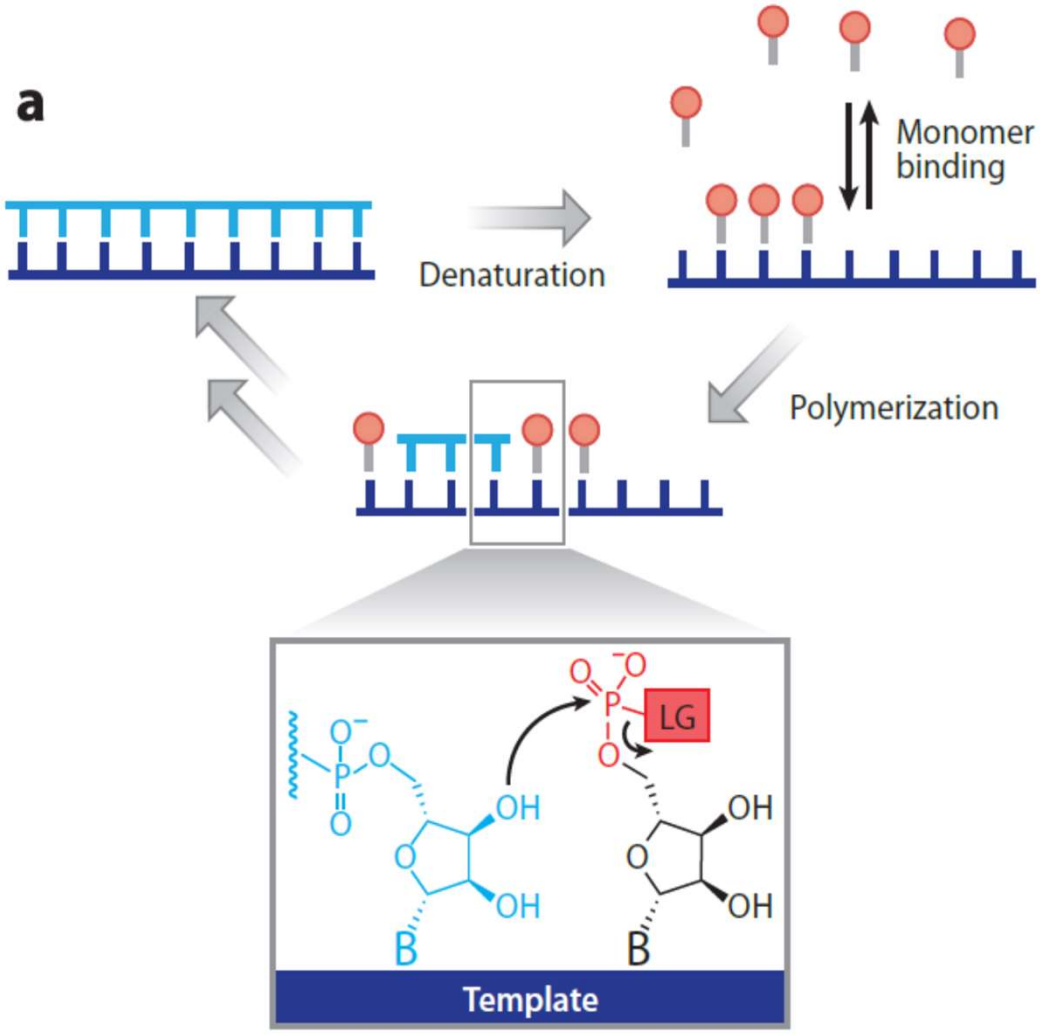
Degradation of activated nucleotides



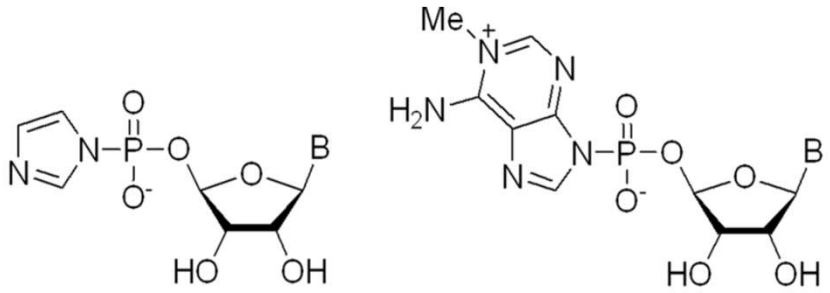
hydrolysis

3’,5’-cyclization

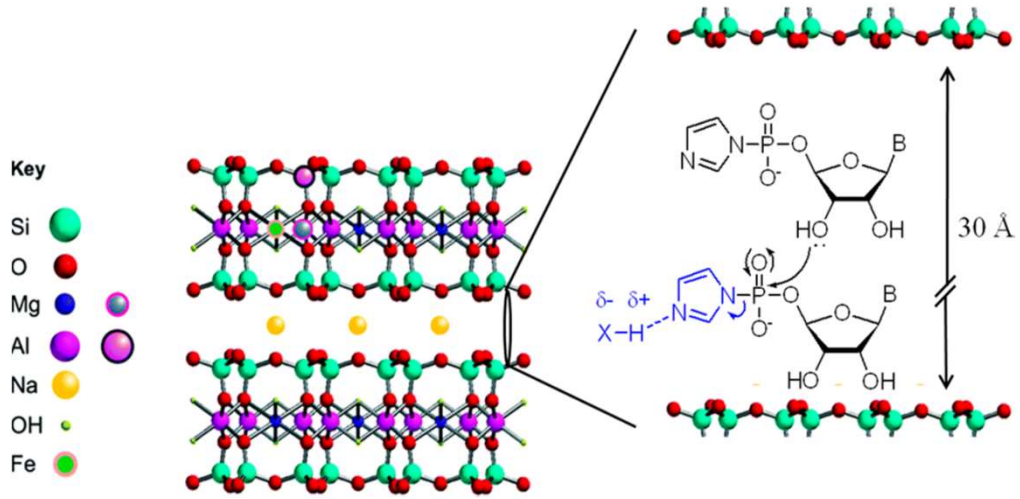
Template-directed synthesis



Montmorillonite



B = adenine, guanine, cytosine or uracil

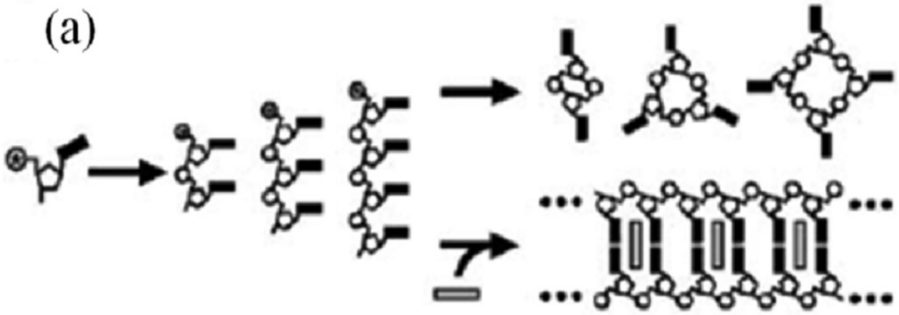


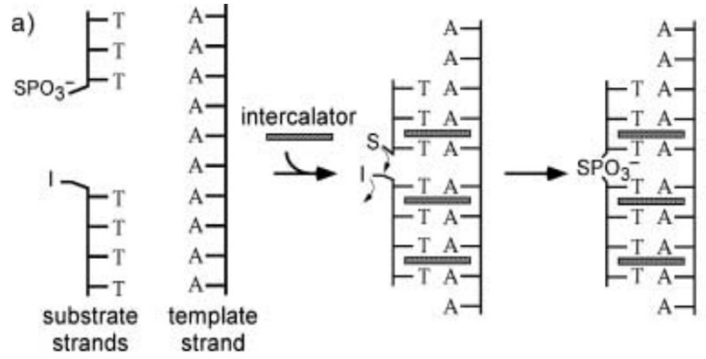
30-50 units successfully oligomerized

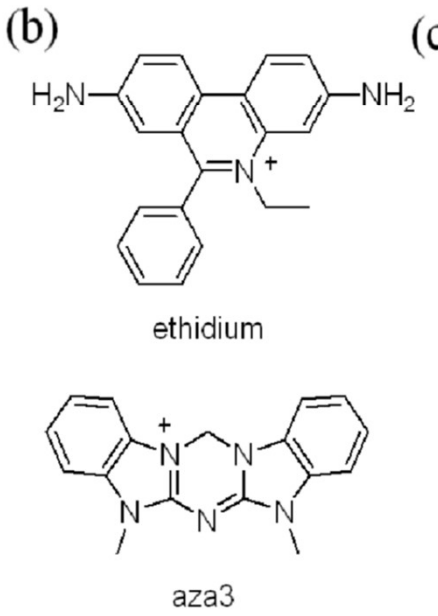
(Top) Structure of ribonucleotide 5′-phosphoimidazolides (left) and ribonucleotide 5′-phosphoro-1-methyladeninium (right). (Bottom) Unit cell of montmorillonite and phosphodiester bond formation within the clay interlayers, as proposed by Ferris and coworkers (right). XH, depicted in blue in the cartoon, is any undifferentiated protic species inside the clay galleries.

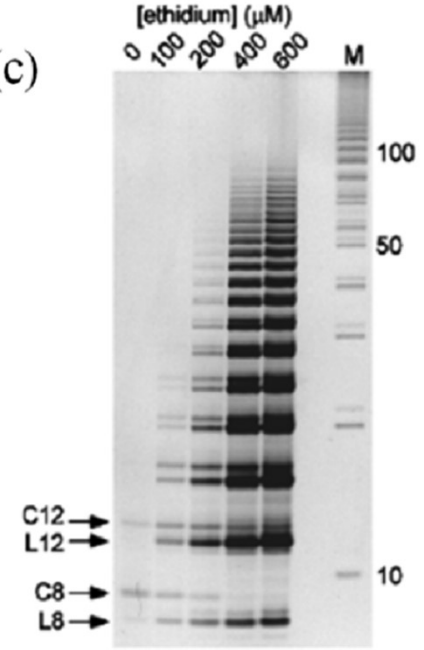
Joshi, P. C.; Aldersley, M. F.; Delano, J. W.; Ferris, J. P. *J. Am. Chem. Soc.* **2009**, *131*, 13369

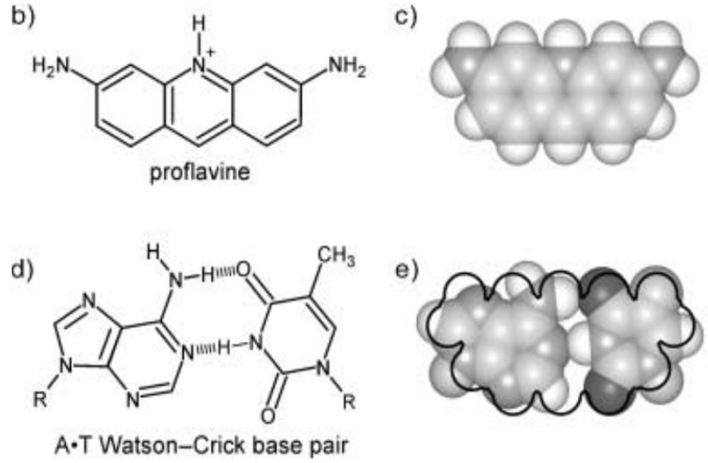
Intercalating agents









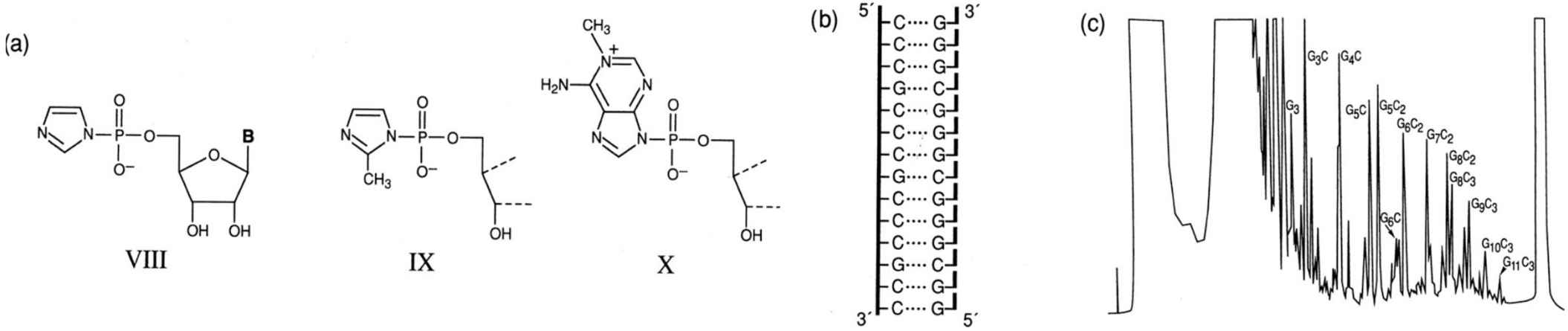


Rate increase by three orders of magnitude vs. ligation without proflavine

N. V. Hud *et al. Angew. Chem. Int. Ed.* **2004**, *43*, 2004 –2008

Template-directed synthesis

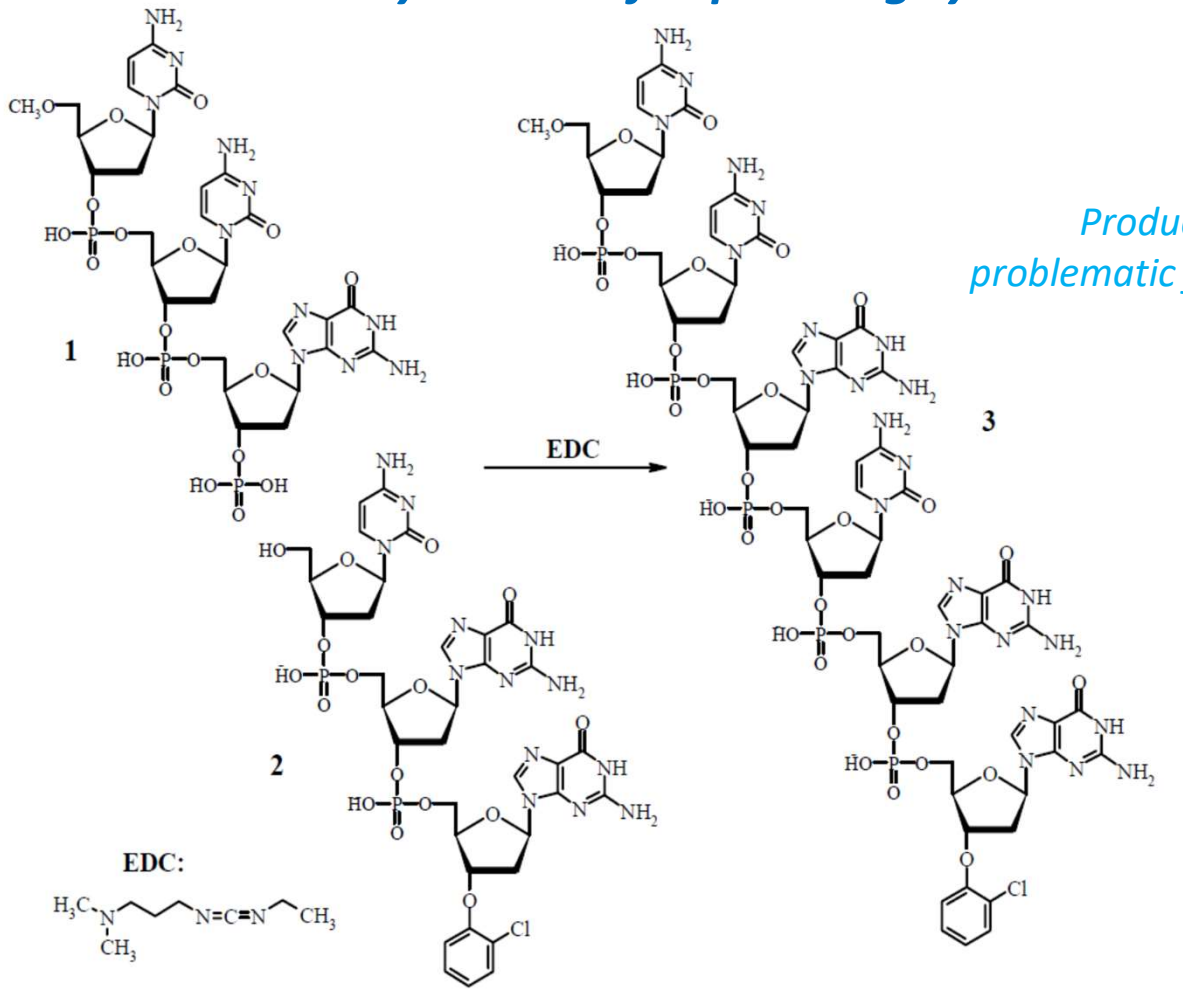
no example demonstrated yet, where single activated nucleotides would form a complementary strand on an RNA (or DNA) template without enzymatic support



Current experiments focus on ‚primer extention' or ‚filling abasic sites'– sequence-selective complementary nucleobase addition to a pre-existing strand (or between two pre-existing strands) already hybridized on a template. Here, pre-organization provided by the existing base-pairing network supports selection of the correct nucleoside to be joined.

Complementary approaches are regioselective ligation reactions of short oligonucleotides on templates, or dynamic covalent chemistry, where nucleobase-containing components would be added sequence-specifically to a pre-existing ‚empty' backbone on a template

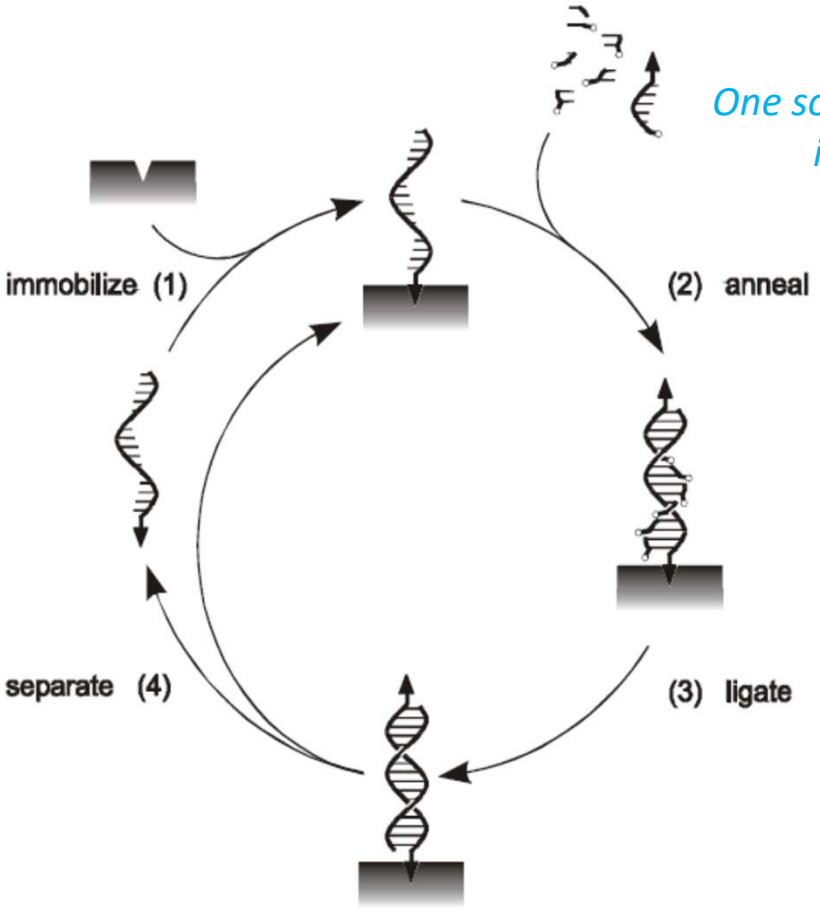
First non-enzymatic self-replicating system



Product inhibition –
problematic for longer templates

V. Patzke, G. von Kiedrowski *ARKIVOC* **2007** 293-310
D. Sievers, G. von Kiedrowski *Nature* **1994** *369(6477)*, 221-224
G. von Kiedrowski *Angewandte Chemie* **1986** *98(10)*, 932-934

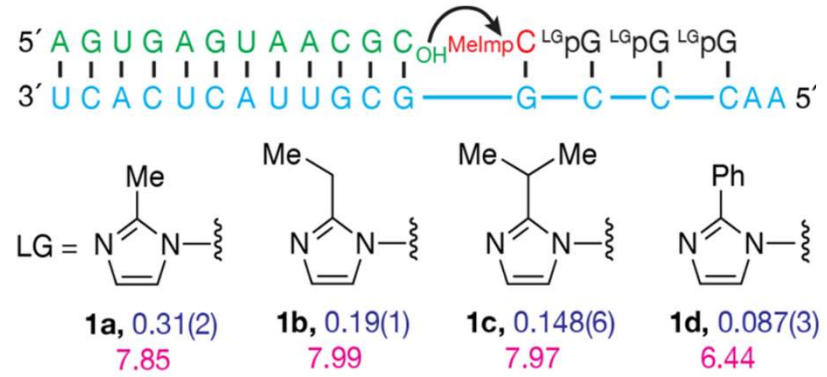
SPREAD – Surface-Promoted Replication and Exponential Amplification of DNA Analogues

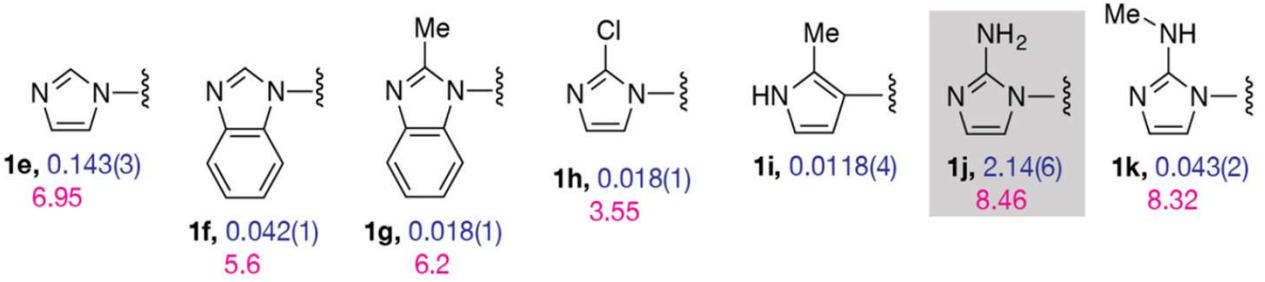


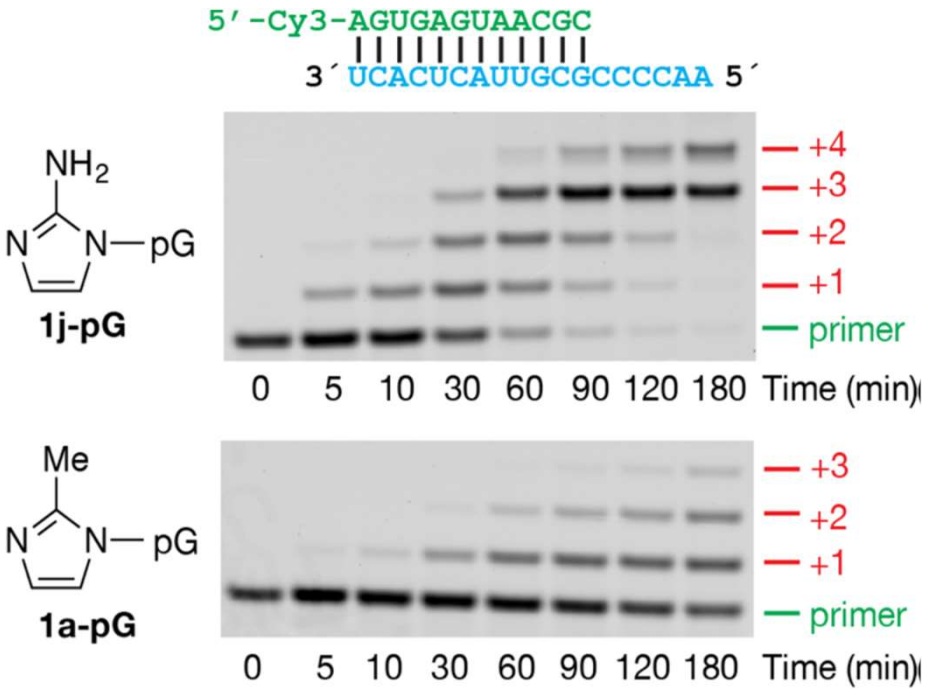
One solution for the product inhibition – immobilization of one strand

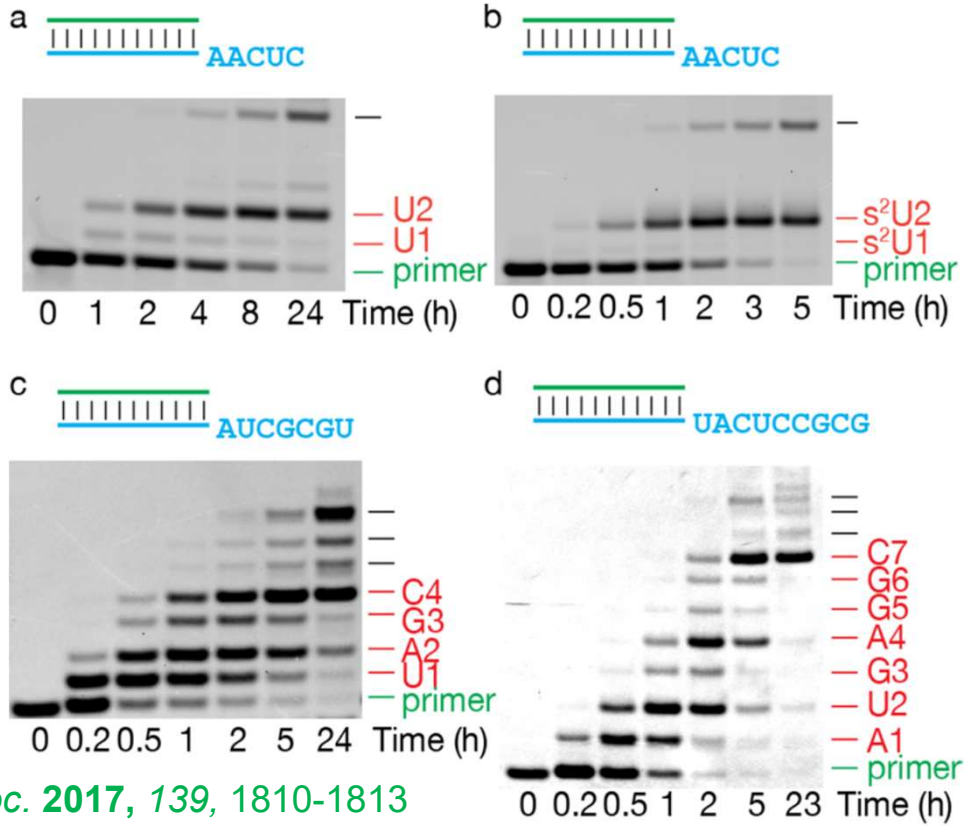
V. Patzke, G. von Kiedrowski *ARKIVOC* **2007** 293-310

Nonenzymatic primer extension



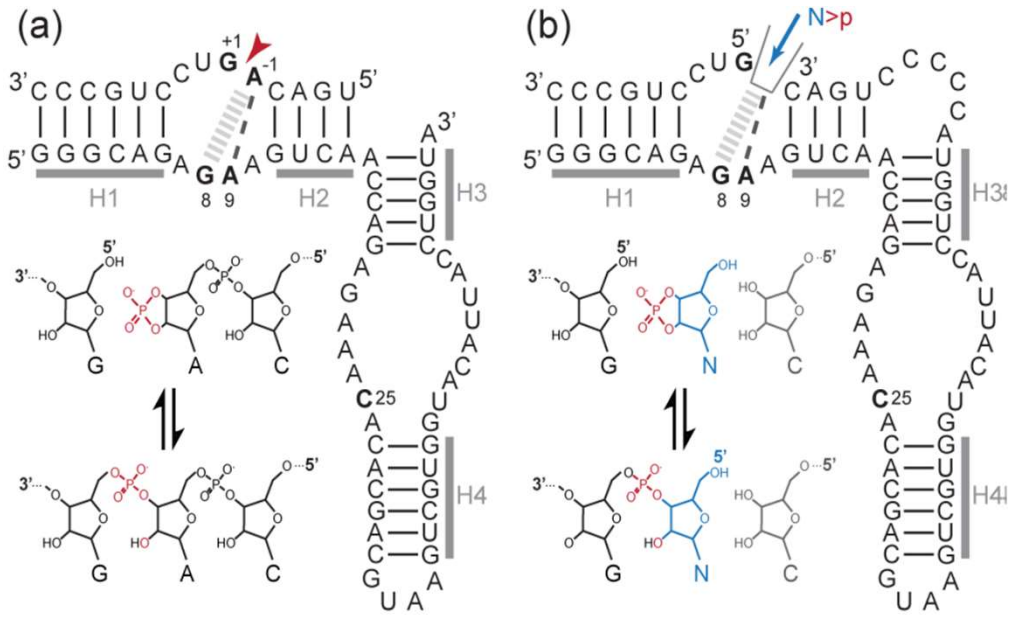


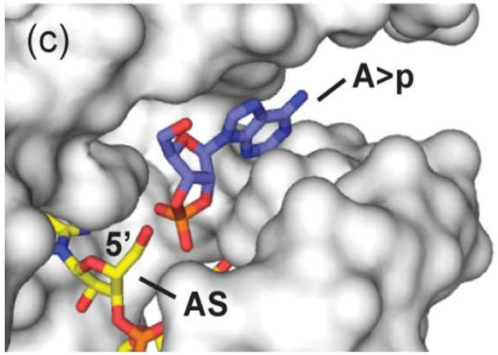


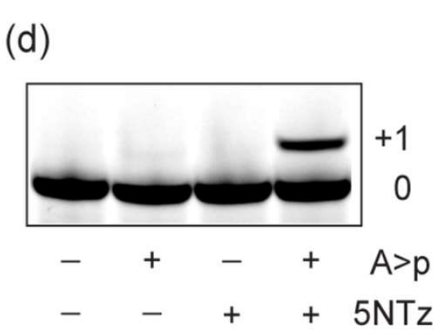


J. Szostak *et al. J. Am. Chem. Soc.* **2017,** *139,* 1810-1813

Ribozyme-catalyzed primer extension







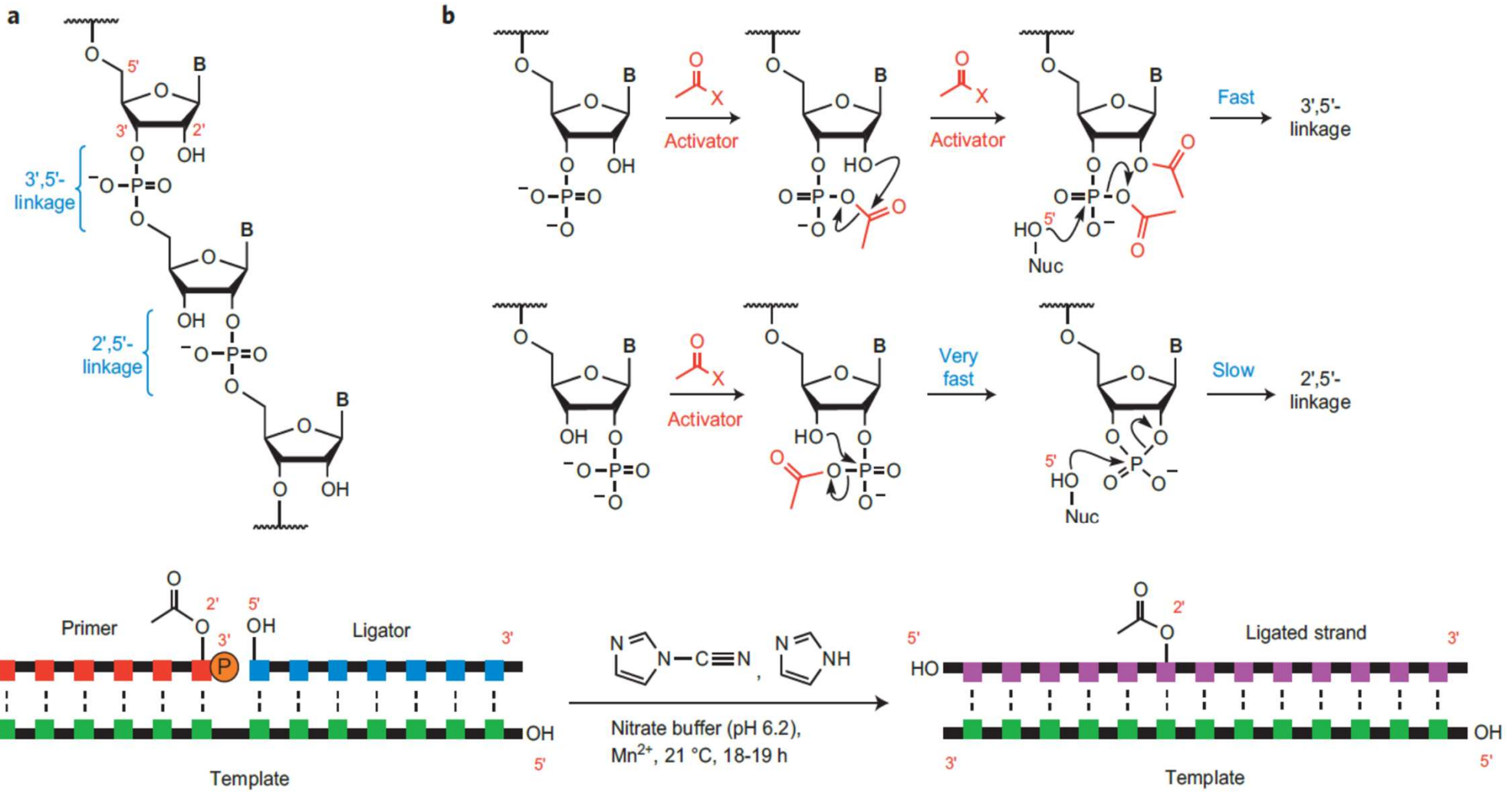
Design of a 5'-nucleotidyl transferase for N>p's.

(a) Two-way junction HPz (small hairpin ribozyme), which catalyzes reversible RNA ligation using a 2′,3′-cyclic phosphate.
(b) Redesign of HPz into 5NTz (nucleotidyl transferase).
(c) Structural model of the substrate-binding pocket of 5NTz (based on PDB1M5V).
(d) 5NTz catalyzes 5′-adenylation in ice (2 mM A>p, 2 μM 5NTz, 1 μM 3′-FITC-labeled AS, 72 h in ice at −7 °C).

An engineered hairpin ribozyme catalyzes addition of all four N>p's (2′,3′-cyclic A-, G-, U-, and CMP) to the 5′-hydroxyl termini of RNA strands (eutectic ice phase formation at −7 °C). 5′ addition of 2′,3′-cyclic phosphate-activated β-nicotinamide adenine dinucleotide (NAD>p), as well as ACA>p RNA trinucleotide, and multiple additions of GUCCA>p RNA pentamers was also observed.

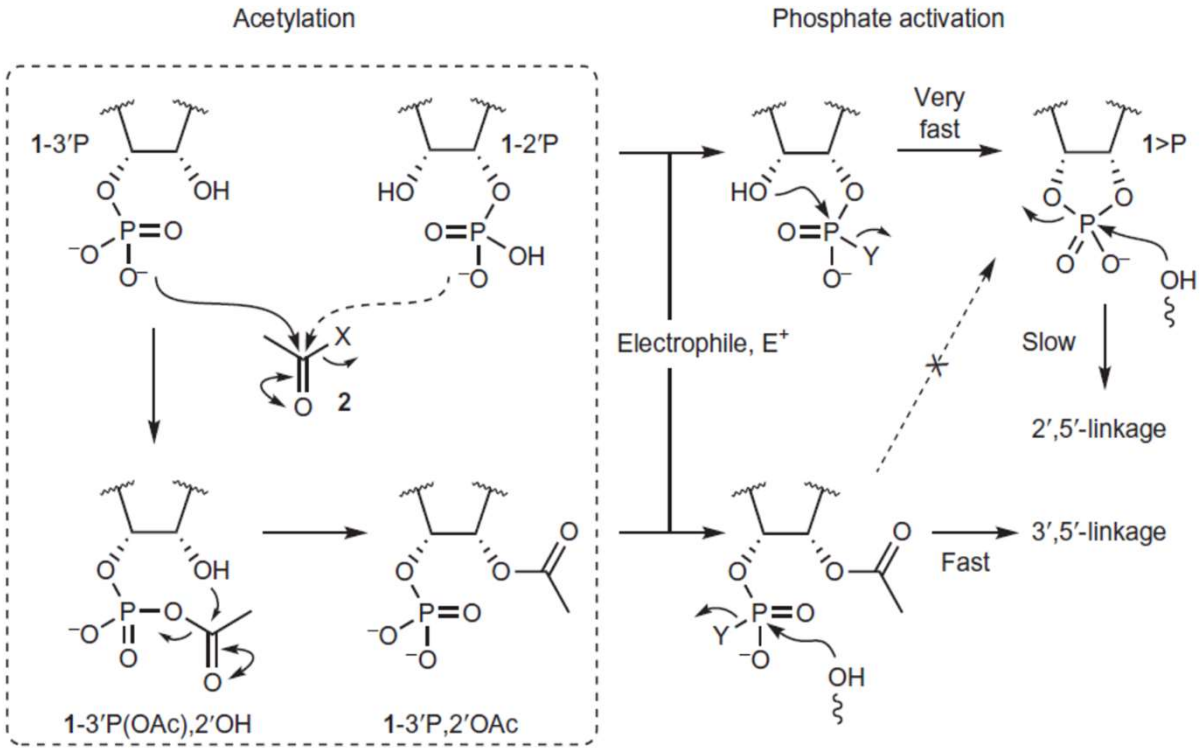
H Mutschler, P. Holliger *J. Am. Chem. Soc.* **2014,** *136,* 5193-5196

Regioselective ribonucleotide ligation



J. Sutherland *et al. Nature Chem.* **2013,** 383-389

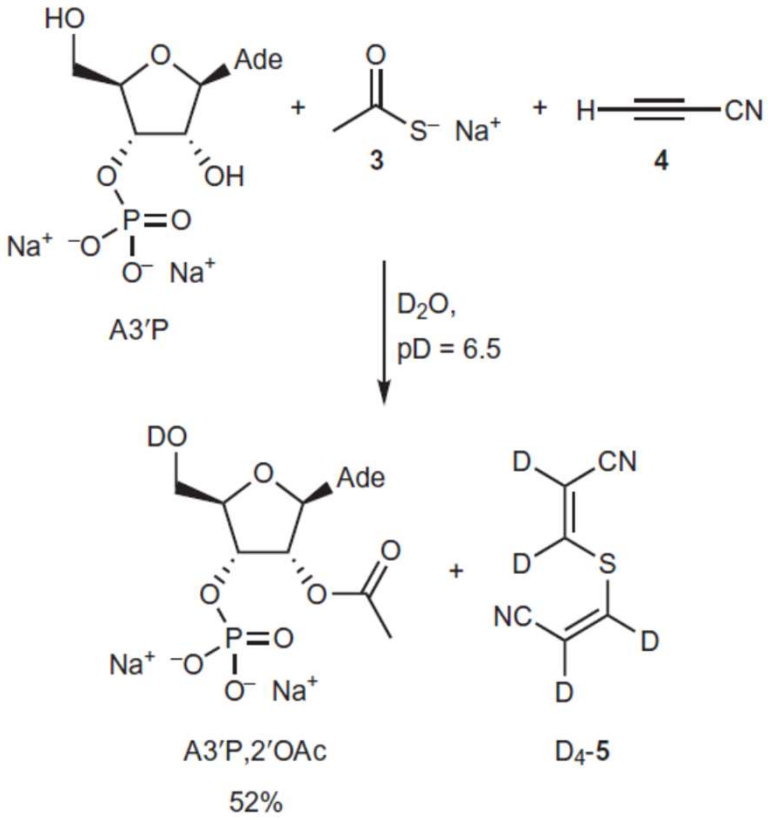
Regioselective ribonucleotide ligation



Protection of the 2’-OH group of **1**-3’P facilitates rapid template-directed 3’,5’-ligation after electrophilic phosphate activation. The 3’-OH group of **1**-2’P is protected to a lesser extent, such that **1**>P is the major product of phosphate activation and slow template-directed 2’,5’-ligation follows.
X = leaving group, Y = leaving group generated by electrophilic activation of phosphate oxygen with or without a subsequent nucleophilic displacement

J. Sutherland *et al. Nature Chem.* **2013,** 383-389

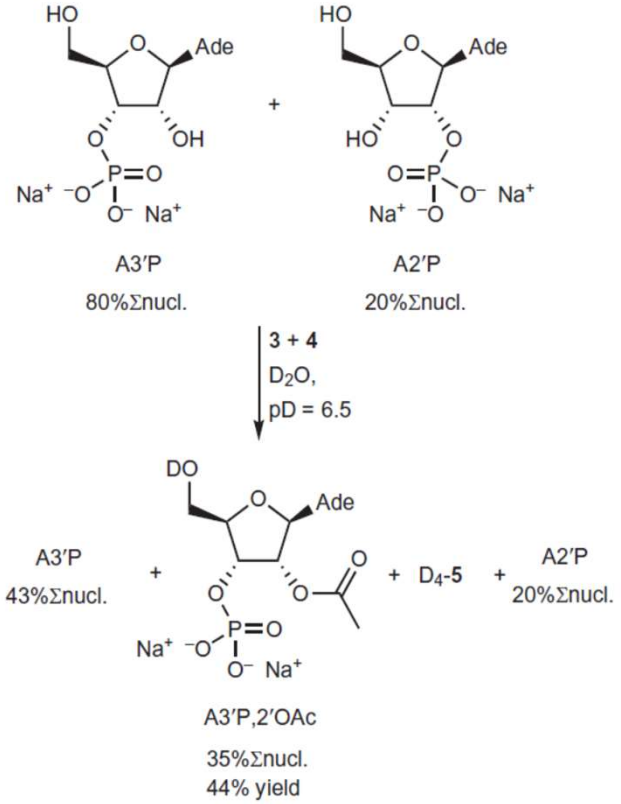
Regioselective ribonucleotide ligation

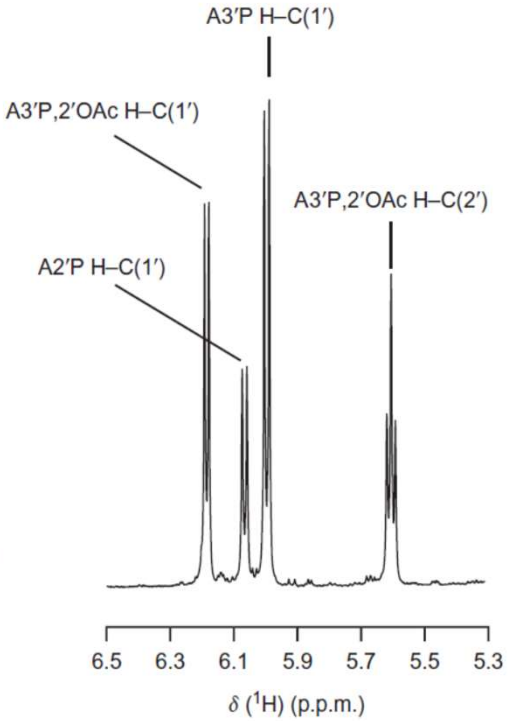


Treatment of adenosine-3'phosphate (A3'P) (100 mM) with sodium thioacetate 3 (100 mM) and cyanoacetylene 4 (200 mM) in D_2O at neutral pD for 24 hours results in selective acetylation of the 2-OH group.

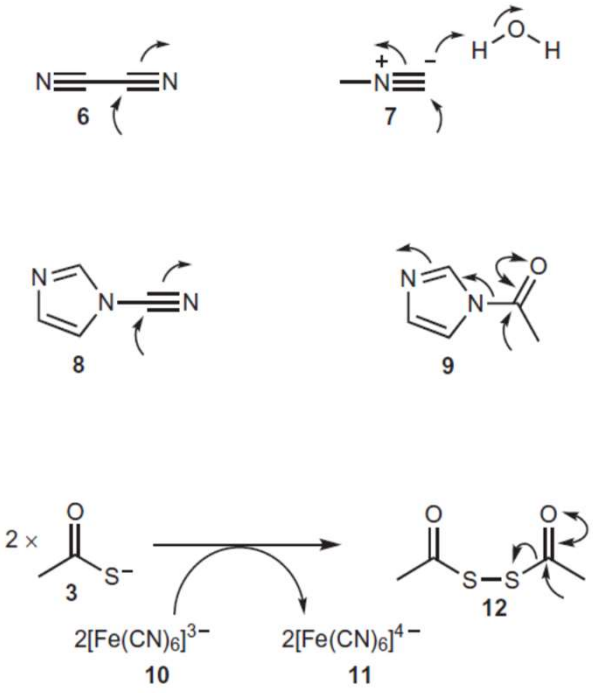
J. Sutherland *et al. Nature Chem.* **2013,** 383-389

Regioselective ribonucleotide ligation





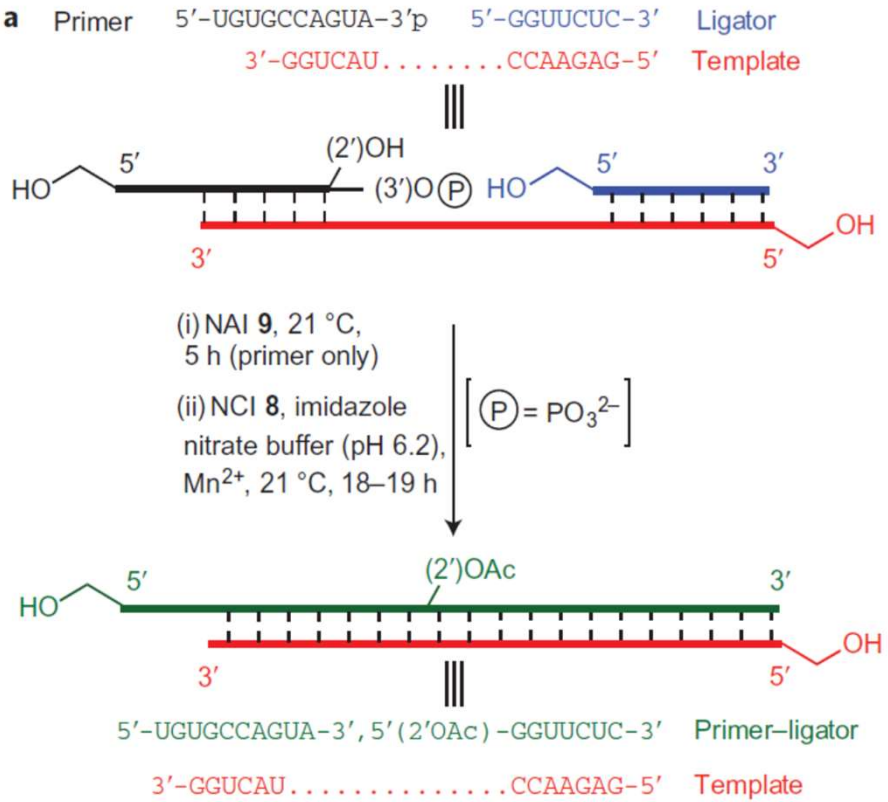
Treatment of A3'P (80 mM) and A2'P (20 mM) as given before results in the exclusive 2-acetylation of the former nucleotide. Partial ^{1}H NMR spectrum of the reaction products.



Additional electrophiles 6–8 shown to drive the acetylation of ribonucleotides with thioacetate 3. Direct acetylation with 9 is also possible, as is oxidative activation of 3 with ferricyanide 10 to afford ferrocyanide 11 and a dimeric acetylating agent 12. Curly arrows indicate electrophilic activation/acetylation steps.

Regioselective ribonucleotide ligation

Chemoselective acetylation of 3'P-oligoribonucleotides expedites templated ligation



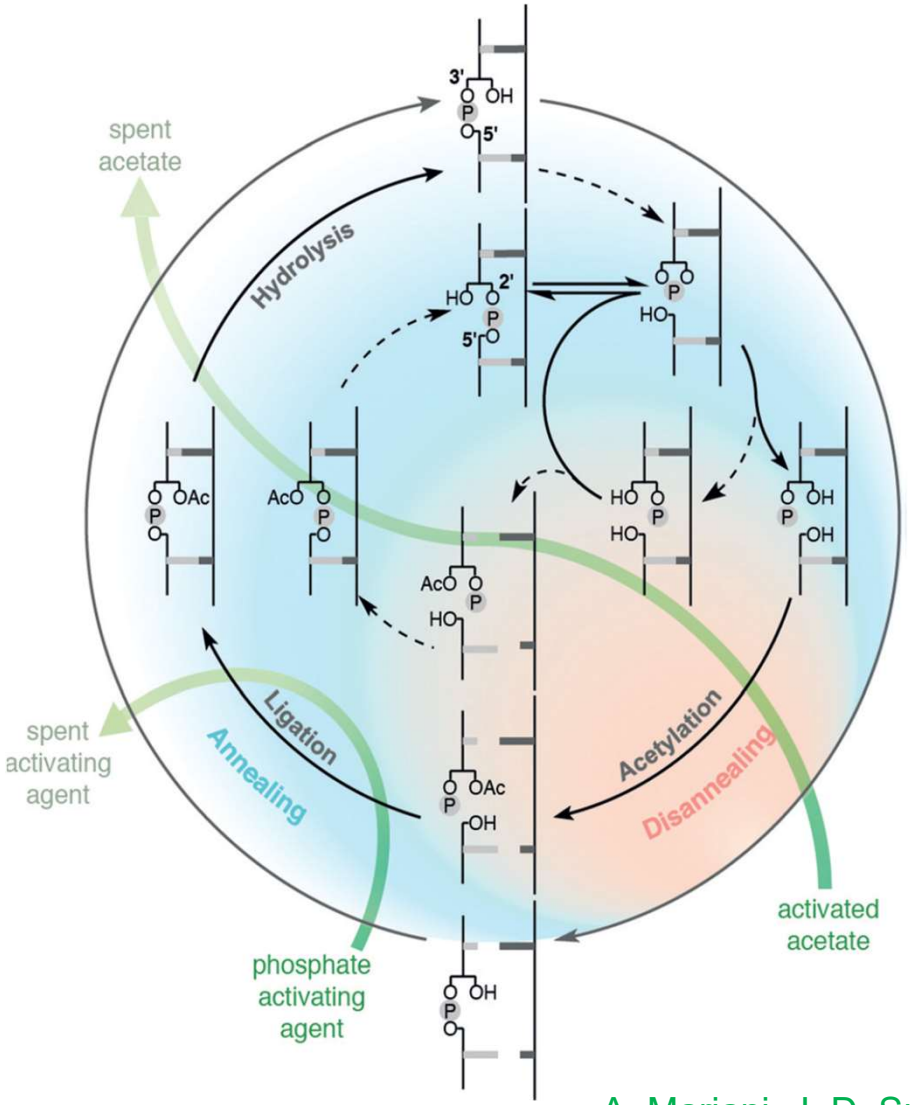
NAI – *N*-acetylimidazole

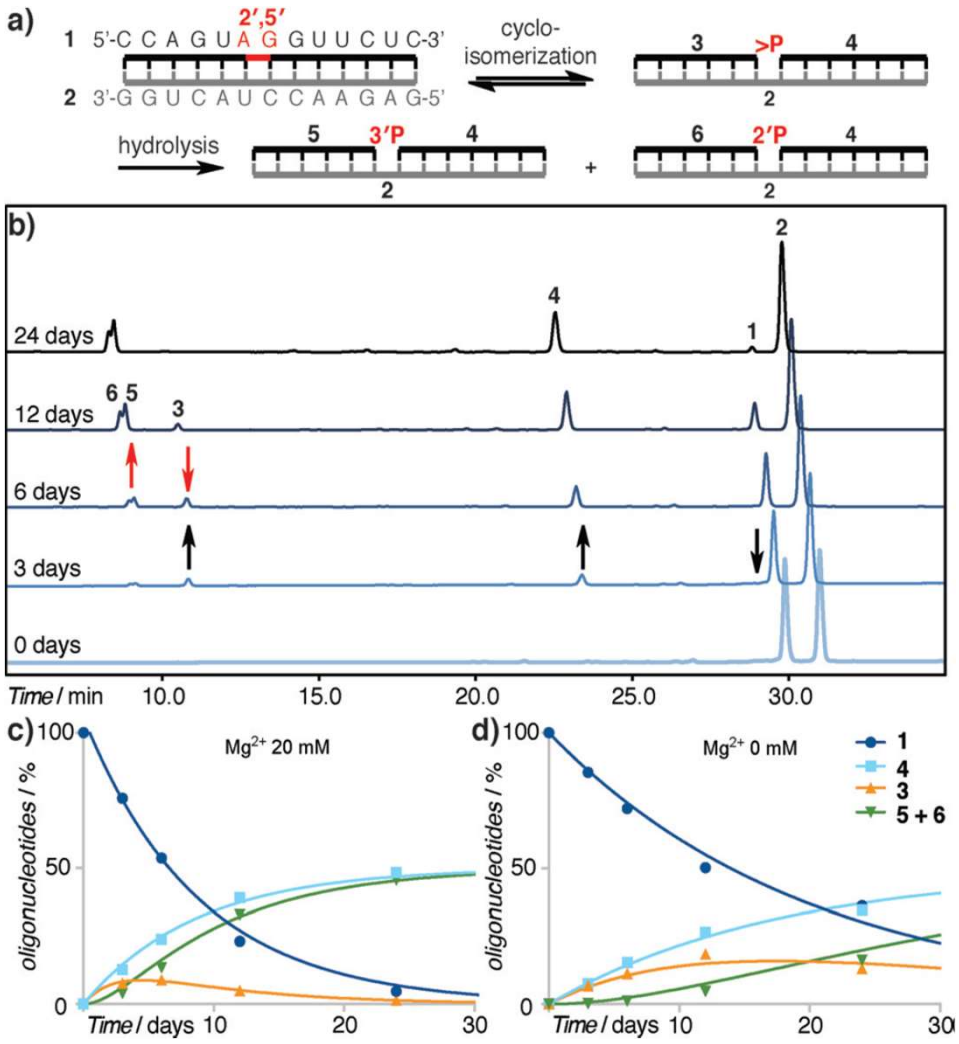
NCI – *N*-cyanoimidazole

Sequences and reaction conditions employed for acetylation (i) and subsequent templated ligation (ii). The acetylation mixture contained 80 mM primer and 50 mM NAI; the ligation mixture contained 4 mM primer from the acetylation reaction, 25 mM template, 30 mM ligator, 200 mM imidazole nitrate buffer (pH 6.2), 10 mM $MnCl_2$ and 100 mM NCI.

J. Sutherland *et al. Nature Chem.* **2013,** 383-389

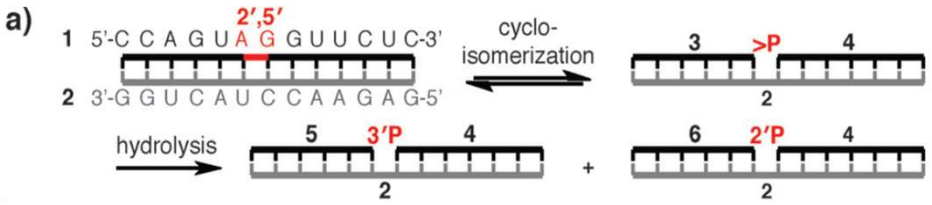
Correction mechanism 2'-5' → 3',5'



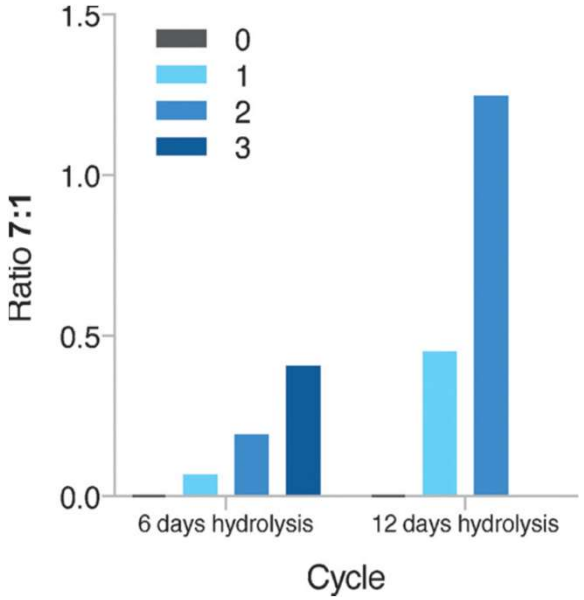


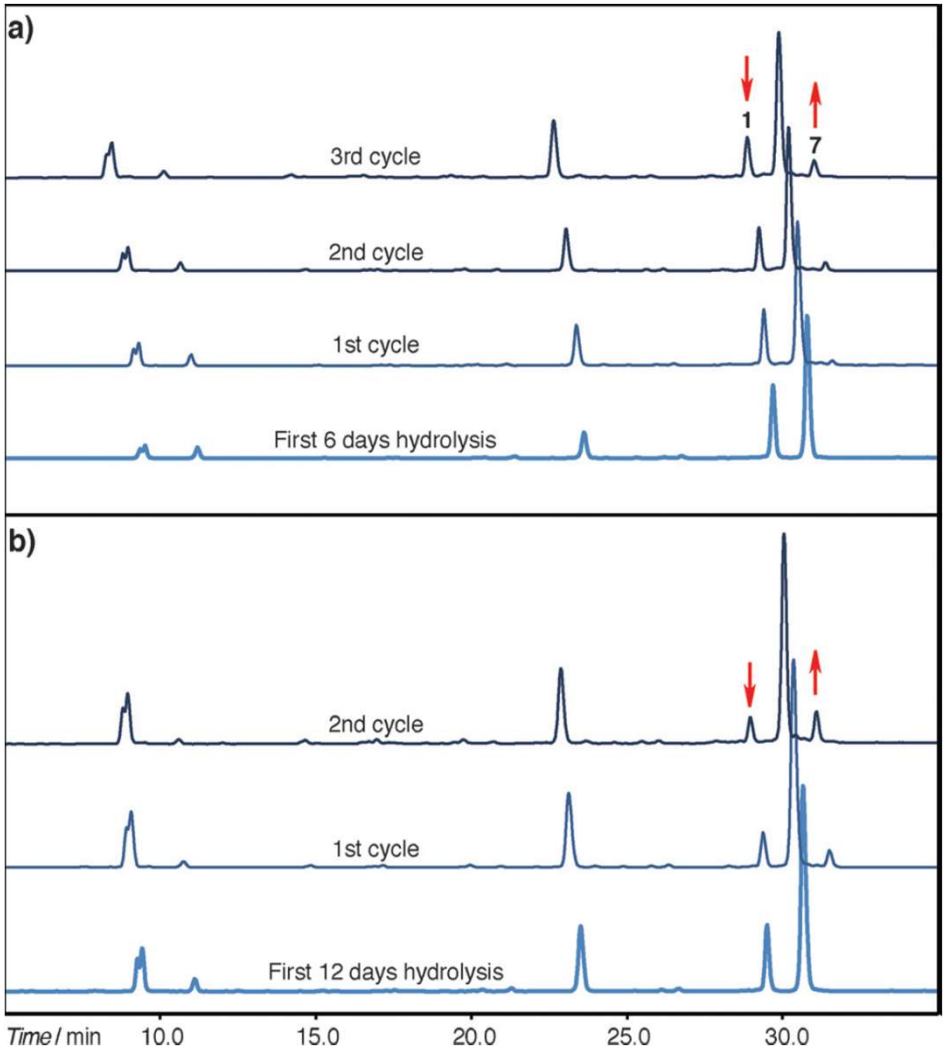
A. Mariani, J. D. Sutherland *Angew. Chem. Int. Ed.* **2017**, *56,* 6563-6566

Correction mechanism 2'-5' → 3',5'



1: full 2',5' link
7: full 3'5' link





A. Mariani, J. D. Sutherland *Angew. Chem. Int. Ed.* **2017**, *56,* 6563-6566