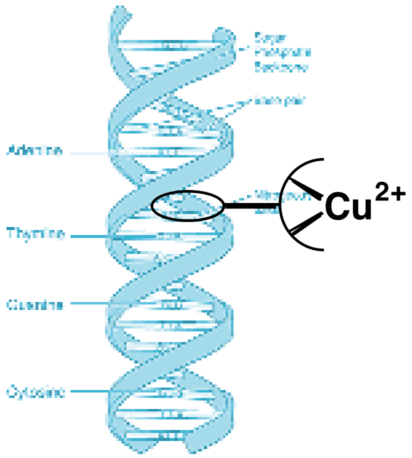
DNA-Based Asymmetric Catalysis



$$\mathbf{A} + \mathbf{B} \xrightarrow{\textit{Asymmetric Catalyst}} \mathbf{C}$$

Newly formed
chiral center(s)

Roelfes, G.; Feringa, B. L. *Angew. Chem., Int. Ed. Engl.* **2005**, *44*, 3230

Michel Grenon
June 25th, 2005

Presentation Outline

- ***Deoxyribozymes (DNAzymes): DNA Catalysts for Bioorganic Chemistry***
  - *Example of DNAzyme that cleaves RNA*
  - *In vitro selection approach to synthesize DNAzymes*
  - *Example of DNAzyme that ligates RNA*
  - *Other reactions catalyzed by DNAzymes*
  - *DNAzymes catalytic parameters, mechanism and structures*

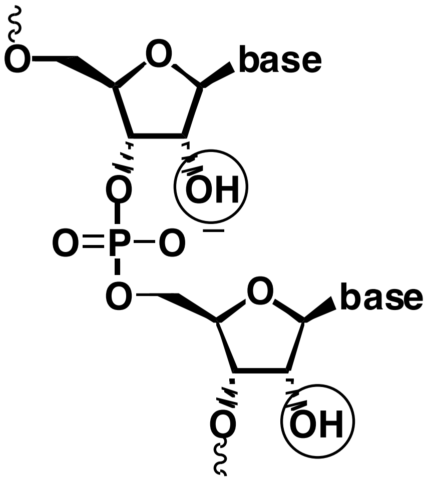
- ***DNA-based Asymmetric Catalysis***
  - *Concept*
  - *Synthesis of ligands*
  - *Application to a copper-catalyzed Diels-Alder reaction*
  - *Perspectives*

Deoxyribozymes (DNAzymes): DNA Catalysts for Bioorganic Chemistry

- Relatively few studies focus on nucleic acids as catalysts for bioorganic chemistry
  - The study of DNAzymes is only about a decade old, whereas that of RNAzymes goes back over 20 years
- Reasons for lack of development in this field
  - Compared with proteins, there are much less functional groups available

Proteins	**RNA/DNA**
20 *diverse* sidechains	4 *similar* monomers
H-bonding capability of the polyamide backbone	H-bonding, π-stacking and metal-coordination

  - DNA appears even less catalytically competent (lacks the 2'-hydroxyl group present in RNA)
  - Exists almost entirely in double-helical form (single-stranded conformations are *probably* required for catalysis)

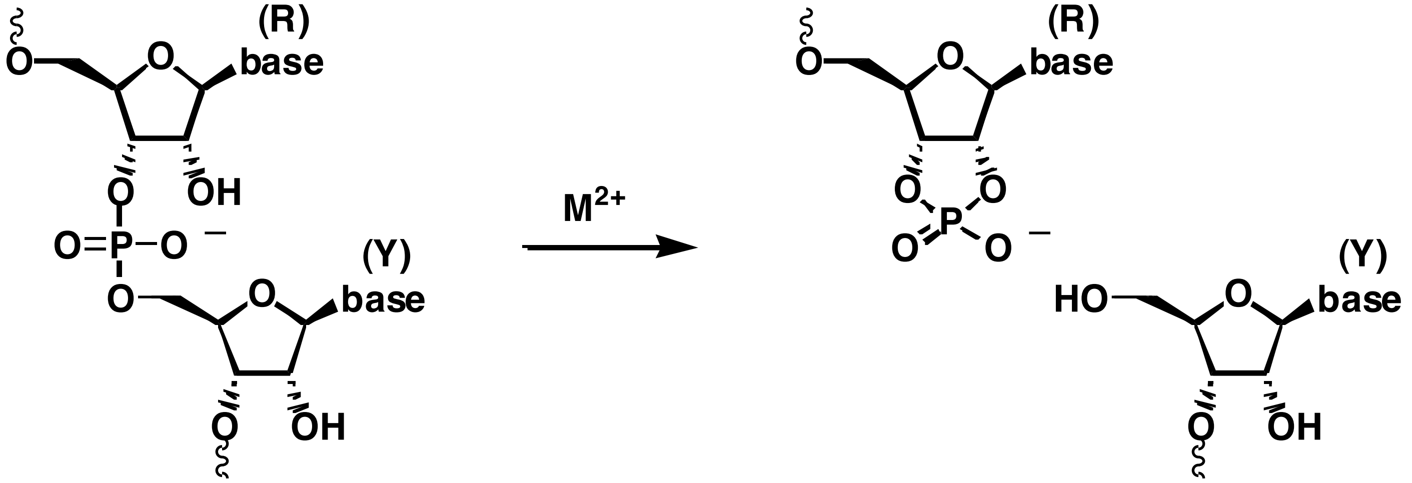


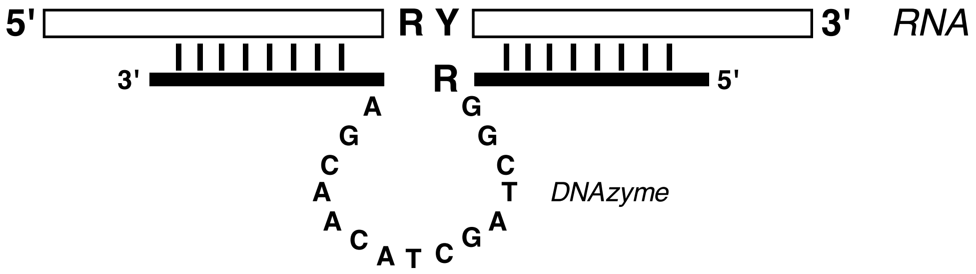
- No DNAzymes are known in nature (are they out there?)

Emerging Area: Silverman, S. K. *Org. Biomol. Chem.* **2004**, *2*, 2701

Deoxyribozymes (DNAzymes): DNA Catalysts for Bioorganic Chemistry

➢ First examples of catalytic DNA: deoxyribozymes that cleave RNA





- This reaction is the same as that promoted by most protein ribonucleases such as R Nase A
- An *in vitro* selection approach can be used to identify RNA-cleaving DNAzymes

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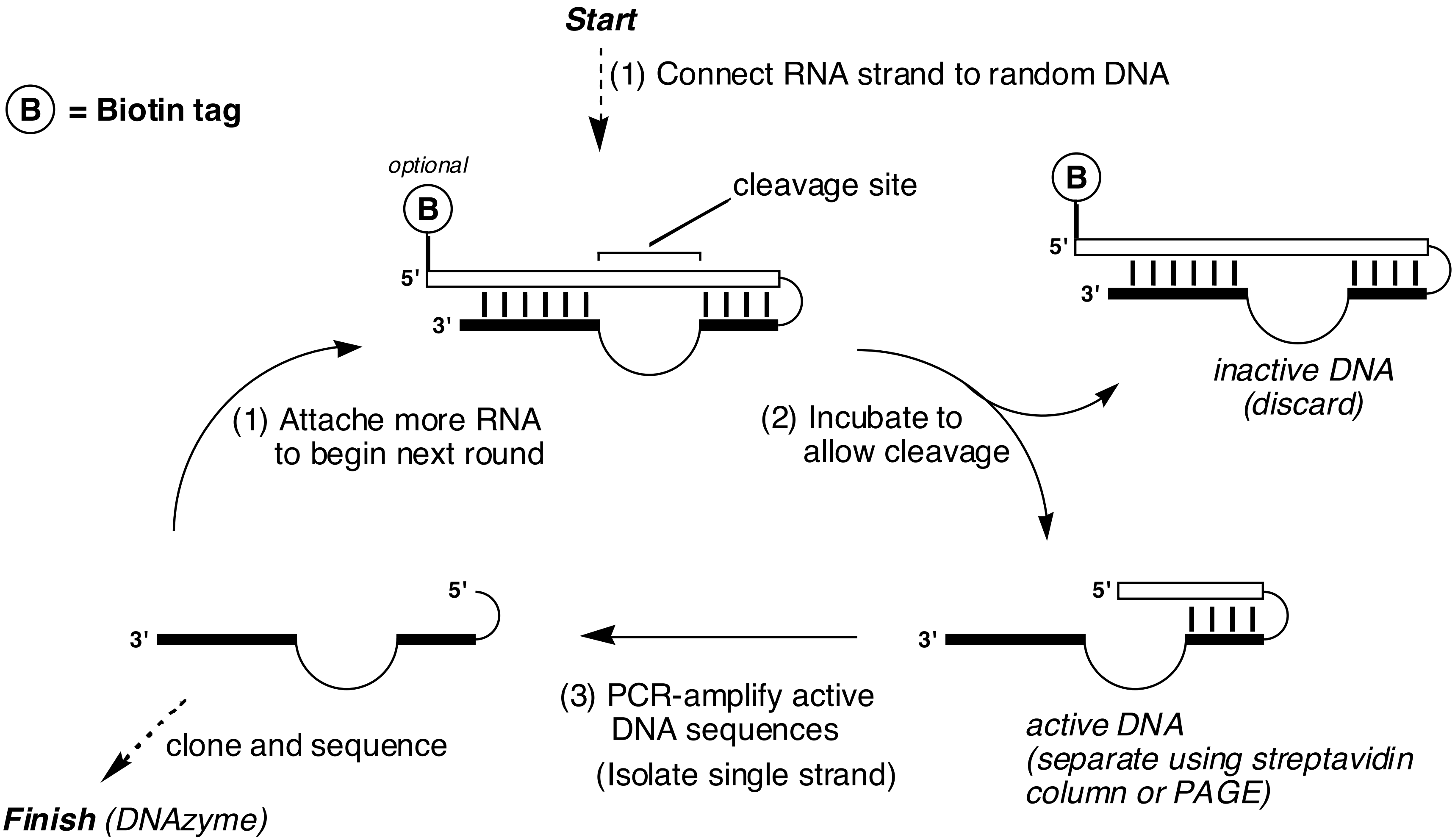
Deoxyribozymes (DNAzymes): DNA Catalysts for Bioorganic Chemistry

- *In vitro* selection approach to synthesize DNAzymes that cleave RNA



Start

(1) Connect RNA strand to random DNA

Ⓑ = Biotin tag

cleavage site

inactive DNA (discard)

(2) Incubate to allow cleavage

active DNA (separate using streptavidin column or PAGE)

(3) PCR-amplify active DNA sequences

(Isolate single strand)

(1) Attache more RNA to begin next round

clone and sequence

***Finish** (DNAzyme)*

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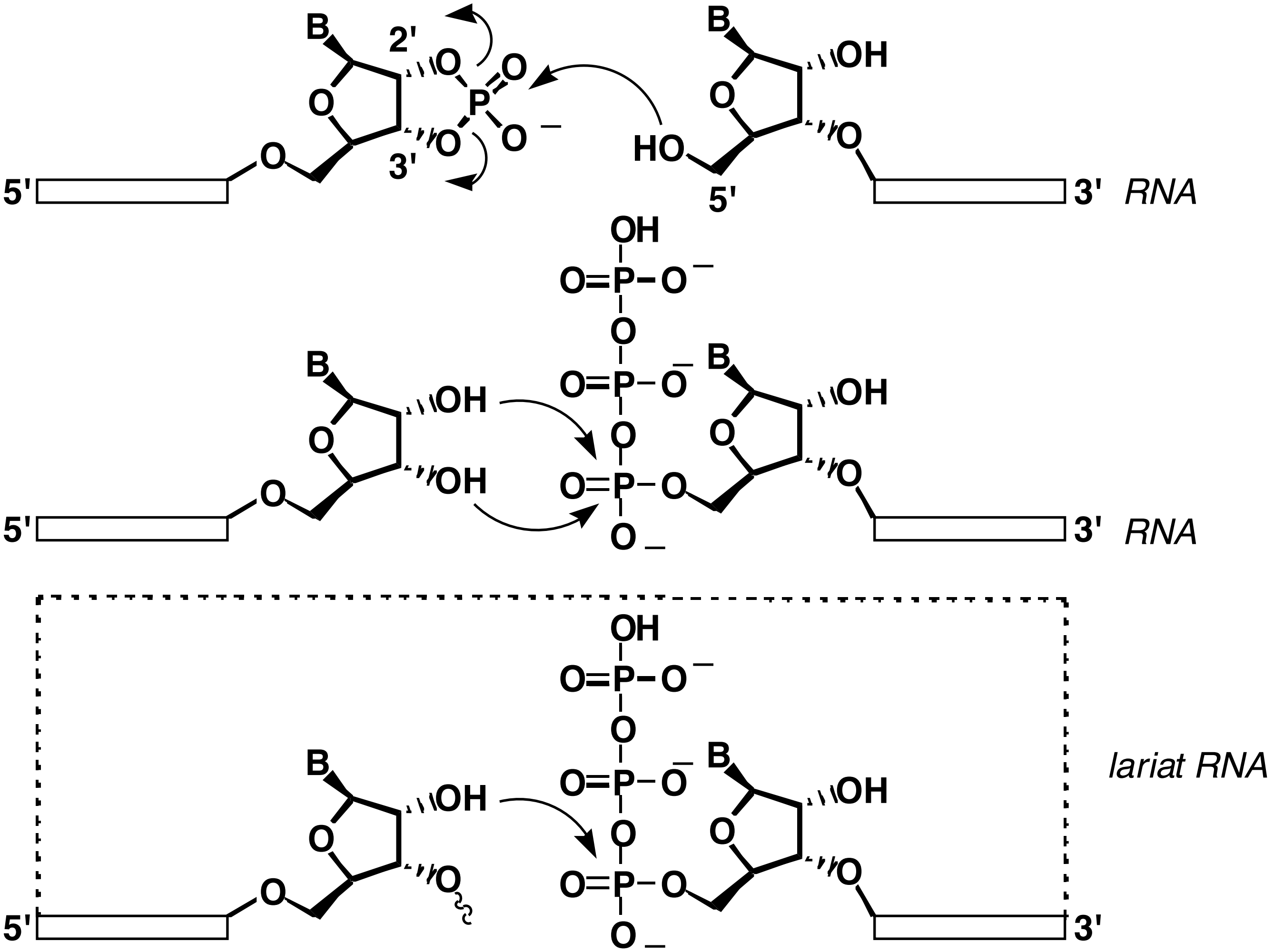
Deoxyribozymes (DNAzymes): DNA Catalysts for Bioorganic Chemistry

- Other examples of catalytic DNA: deoxyribozymes that ligate RNA



Emerging Area: Silverman, S. K. *Org. Biomol. Chem.* **2004**, *2*, 2701

Deoxyribozymes (DNAzymes): DNA Catalysts for Bioorganic Chemistry

- Deoxyribozyme catalytic parameters, mechanism and structures

  - Quantitative assessment of a DNAzyme's catalytic activity can be made by comparing its rate constant to that of an appropriate background reaction

$$\boxed{\text{rate enhancement} = k_{obs} / k_{bkgd}}$$

rate enhancements of 10^6 to 10^7 for RNA ligation reactions

rate enhancement as high as 10^{10} observed for other DNAzymes

  - Little is known about the structures and mechanisms of any DNAzymes

- Why study DNAzymes instead of RNAzymes?

  - If DNA and RNA have similar catalytic potential, practical concerns favor the use of DNA;

    1) DNA less expensive to make by solid-phase synthesis (*ca* 7 times less)
    2) DNA can generally be made in longer sequence lenghts and higher purity
    3) Relative chemical and biochemical stability (ubiquitous ribonucleases)

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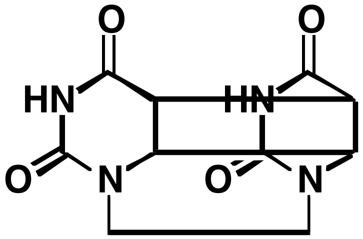
Deoxyribozymes (DNAzymes): DNA Catalysts for Bioorganic Chemistry

- Other reactions catalyzed by DNAzymes that covalently modify nucleic acids
  - Change in the phosphorylation status of an RNA or DNA strand

    DNA phosphorylation
    DNA adenylation (capping)

  - DNA deglycosylation
  - Porphyrin metalation
  - Thymine dimer photoreversion
  - DNA cleavage



- What do all these processes have in common?

  The use of *single-stranded* DNA for catalysis

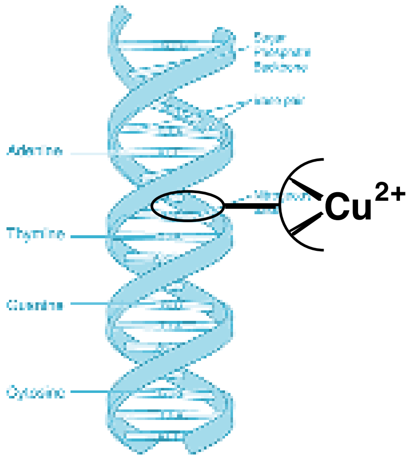
- Is it possible to use duplex-DNA to catalyze a specific reaction?

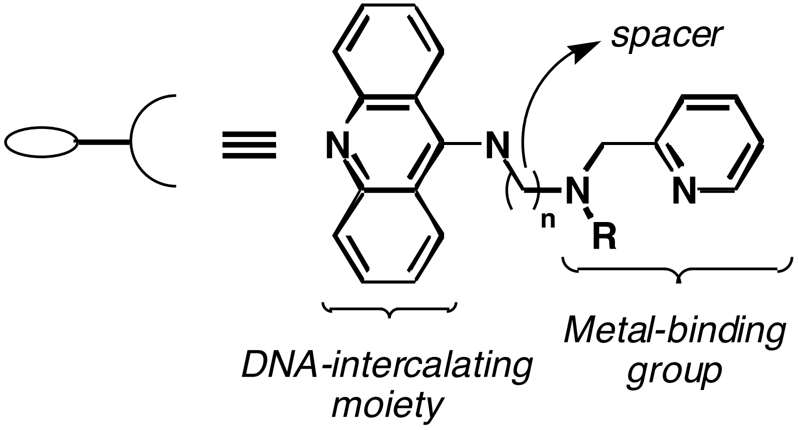
Emerging Area: Silverman, S. K. *Org. Biomol. Chem.* **2004**, *2*, 2701

DNA-Based Asymmetric Catalysis

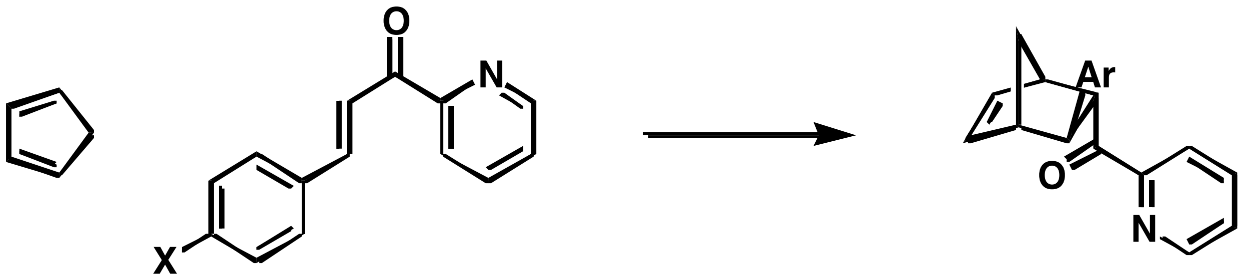
➢ Can the chirality of the DNA double helix be transfered directly to a metal-catalyzed reaction?

- Exploit the propensity of small aromatic molecules to interact with DNA in a noncovalent, yet kinetically stable way





- The reaction...



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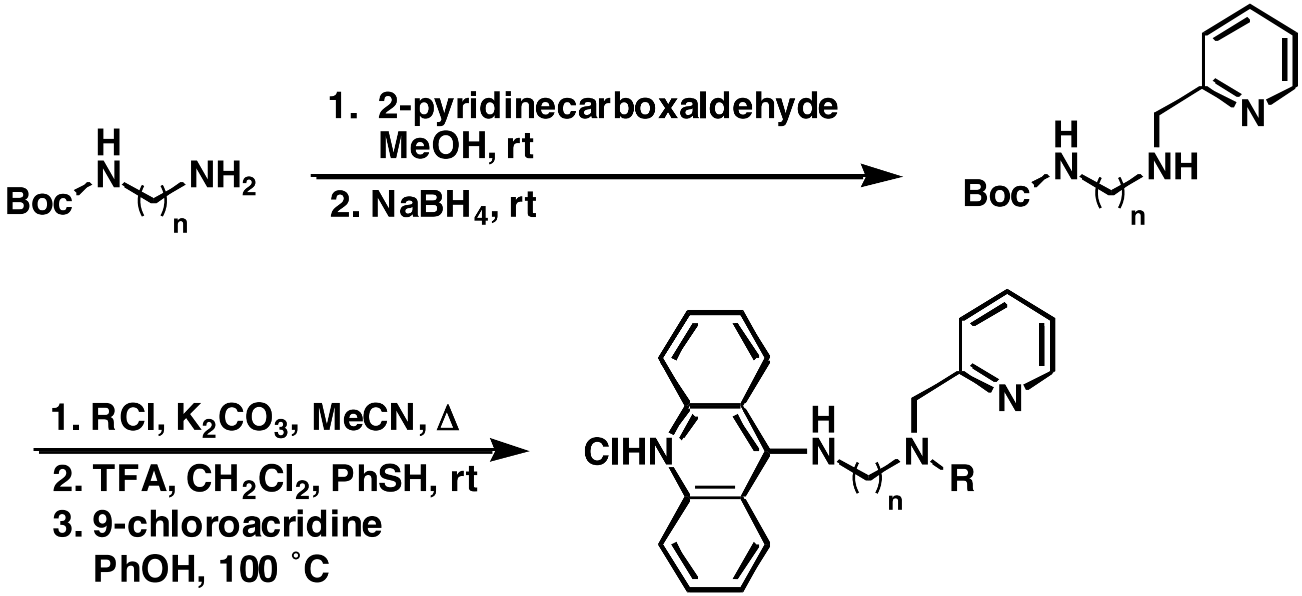
DNA-Based Asymmetric Catalysis

- Synthesis of the ligands



n = 3

R = Me, *t*-Bu, Benzyl, 1-Naphthylmethyl, 2-Naphthylmethyl
4-MeO$C_6H_4CH_2$, 3,5$(MeO)_2C_6H_3CH_2$

n = 2,4,5

R = 1-Naphthylmethyl, 3,5$(MeO)_2C_6H_3CH_2$

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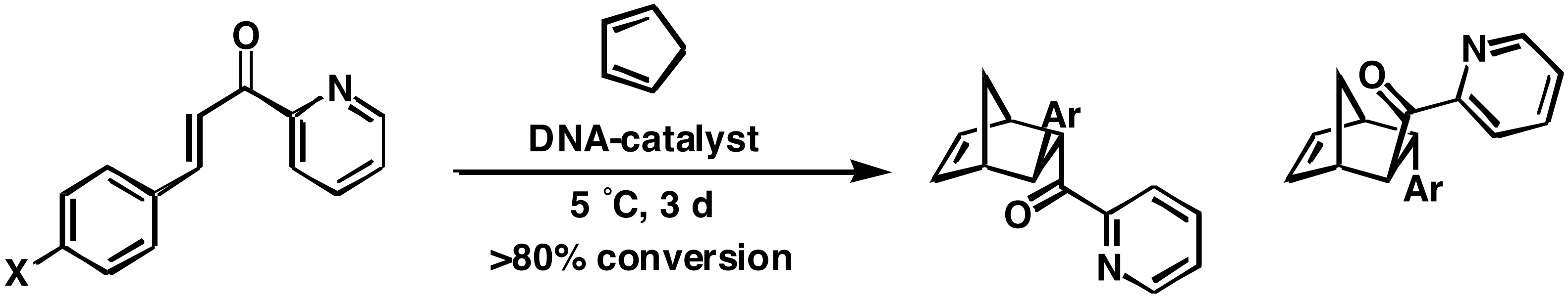
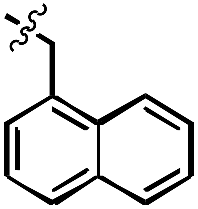
DNA-Based Asymmetric Catalysis



Entry	R	n	Dienophile (X)	Endo/Exo	Endo (%ee)	Exo (%ee)
1		3	H	98:2	49	18
2[a]		3	H	97:3	49	23
3[b]		3	H	98:2	47	23
4[c]		3	NO_2	96:4	37	16
5[c]		3	Me	98:2	48	24
6		4	H	98:2	33	19
7		5	H	97:3	<5	<5
8		2	H	96:4	-48	-37

[a] Catalyst (0.18 mM), dienophile (4 mM), cyclopentadiene (34 mM)

[b] Calf thymus DNA [c] *ca.* 50% conversion

- No significant ee when R = 2-Naphthylmethyl

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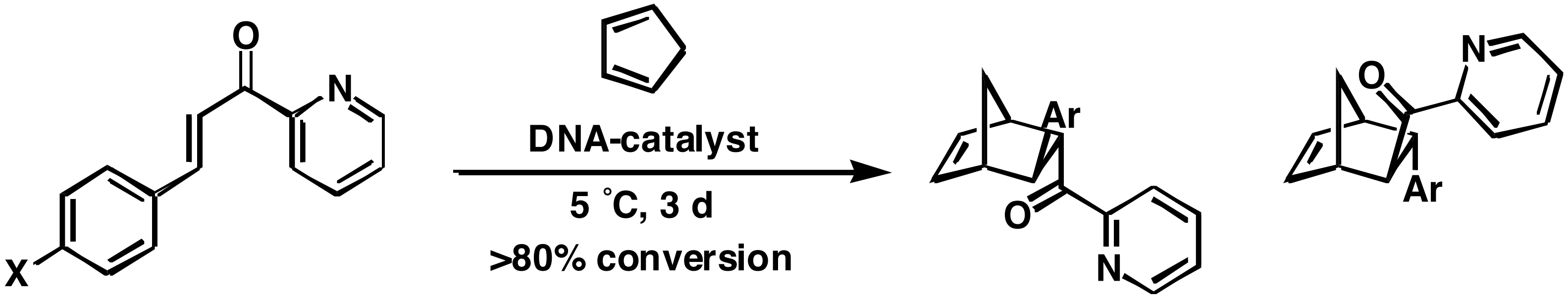
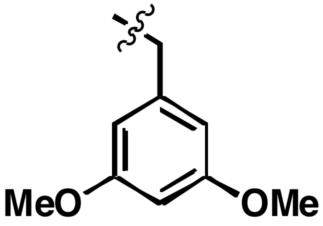
DNA-Based Asymmetric Catalysis



Entry	R	n	Dienophile (X)	Endo/Exo	Endo (%ee)	Exo (%ee)
1		3	H	98:2	-37	-7
2		2	H	92:8	-37	-78
3[a]		2	H	92:8	-34	-74
4[b]		2	H	92:8	-35	-82
5[c]		2	H	82:18	-34	-80
6[d]		2	NO_2	88:12	-47	-78
7[d]		2	OMe	91:9	-53	-90

[a] Catalyst (0.18 mM), dienophile (4 mM), cyclopentadiene (34 mM)

[b] Calf thymus DNA

[c] DNA = synthetic duplex d(GACT)$_2$-(AGTC)$_2$ (0.39 mM), cyclopentadiene (21 mM)

[d] *ca.* 50% conversion

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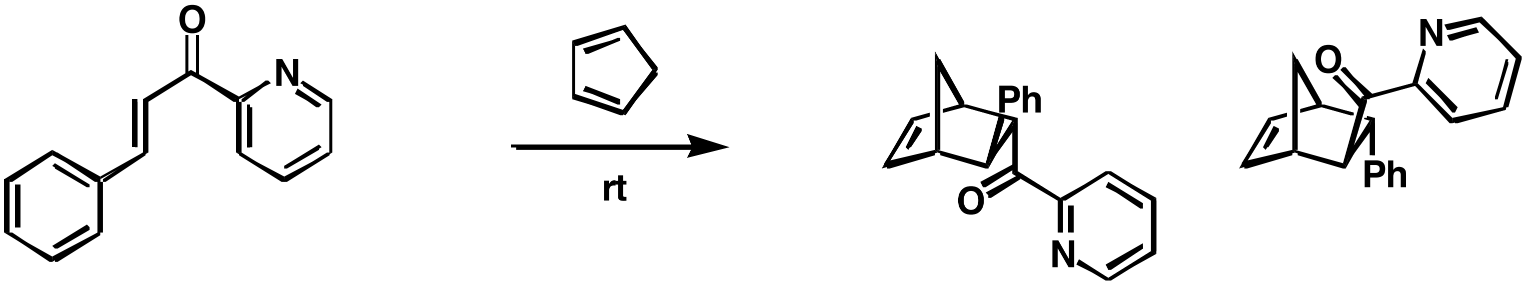
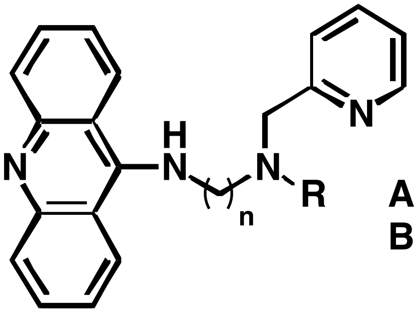
DNA-Based Asymmetric Catalysis



Entry	Catalyst	ligand	Conv. (%)	Endo/Exo	Endo (%ee)	Exo (%ee)
1	$Cu(NO_3)_2$/DNA	---	50-60	95:5	10	13
2	DNA	---	<5%[a]	n.d.	<5	n.d.
3	$Cu(NO_3)_2$/DNA/9-aminoacridine	---	100	94:6	7	18
4	$Cu(NO_3)_2$/ligand	A	50-70	95:5	---	---
5	$Cu(NO_3)_2$/ligand	B	50-70	93:7	---	---

[a] Reaction performed at 5 °C



A: n = 3, R = 2-Naphthylmethyl
B: n = 2, R = 3,5(OMe)$C_6H_4CH_2$

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Perspectives

- The chirality of duplex DNA *can* be transfered directly to a catalytic reaction
- *Both* enantiomers of the Diels-Alder adduct are accessible by a judicious choice of ligand
- Rapid structural variation and optimization of catalysts for new reactions
- Ease of purification (Cu-ligand-DNA complex remains in aqueous solution)

Futur work should focus on

- The possibilty to address specific DNA sequences by using a selective DNA binding moiety tethered to the catalyst
- Extending to other reactions that can be performed in buffered aqueous solutions (metal-catalyzed reactions, organocatalysis)