

# DNA-Polymerasen

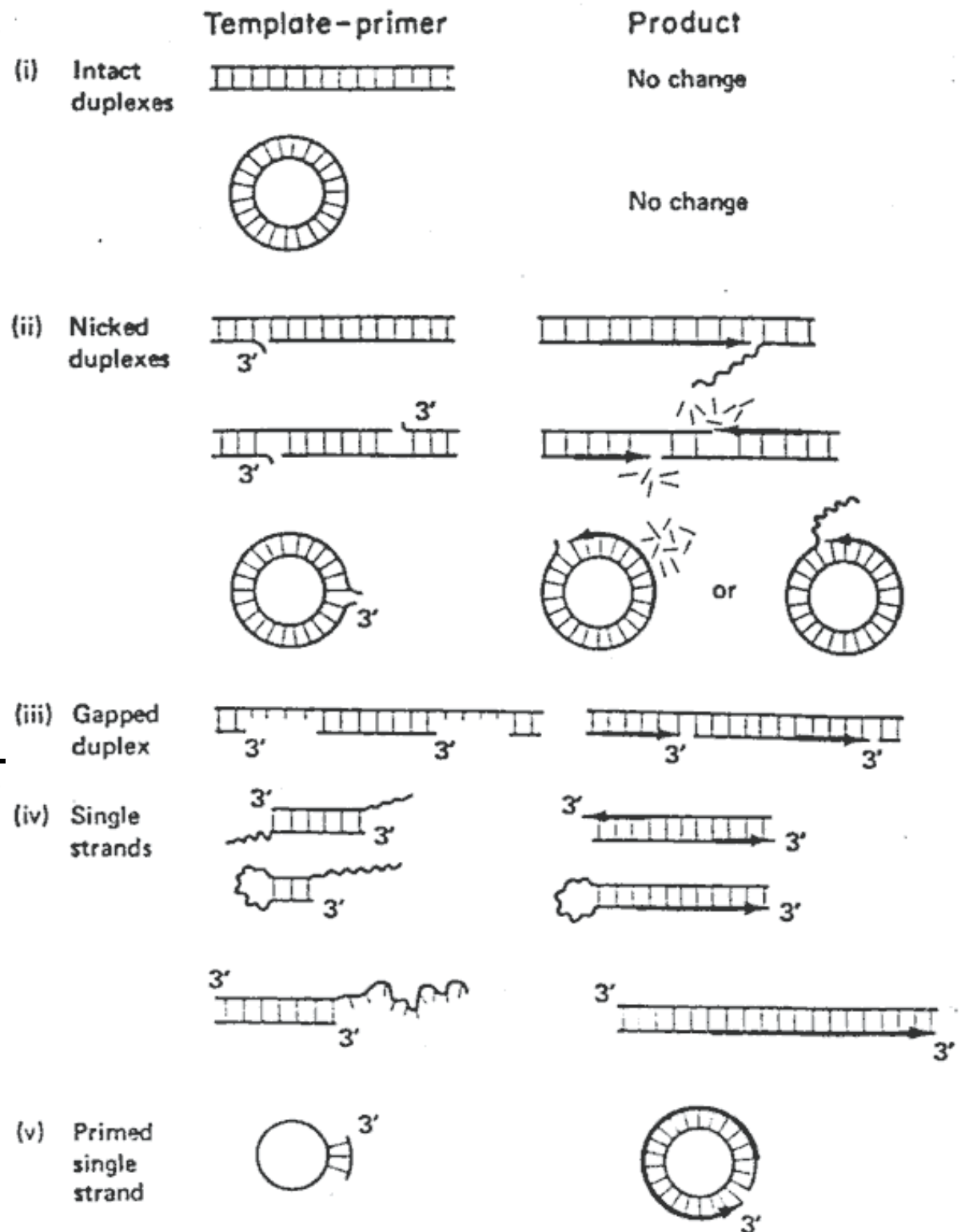
## Funktionen

Replikation, Transkription  
Reparatur von DNA

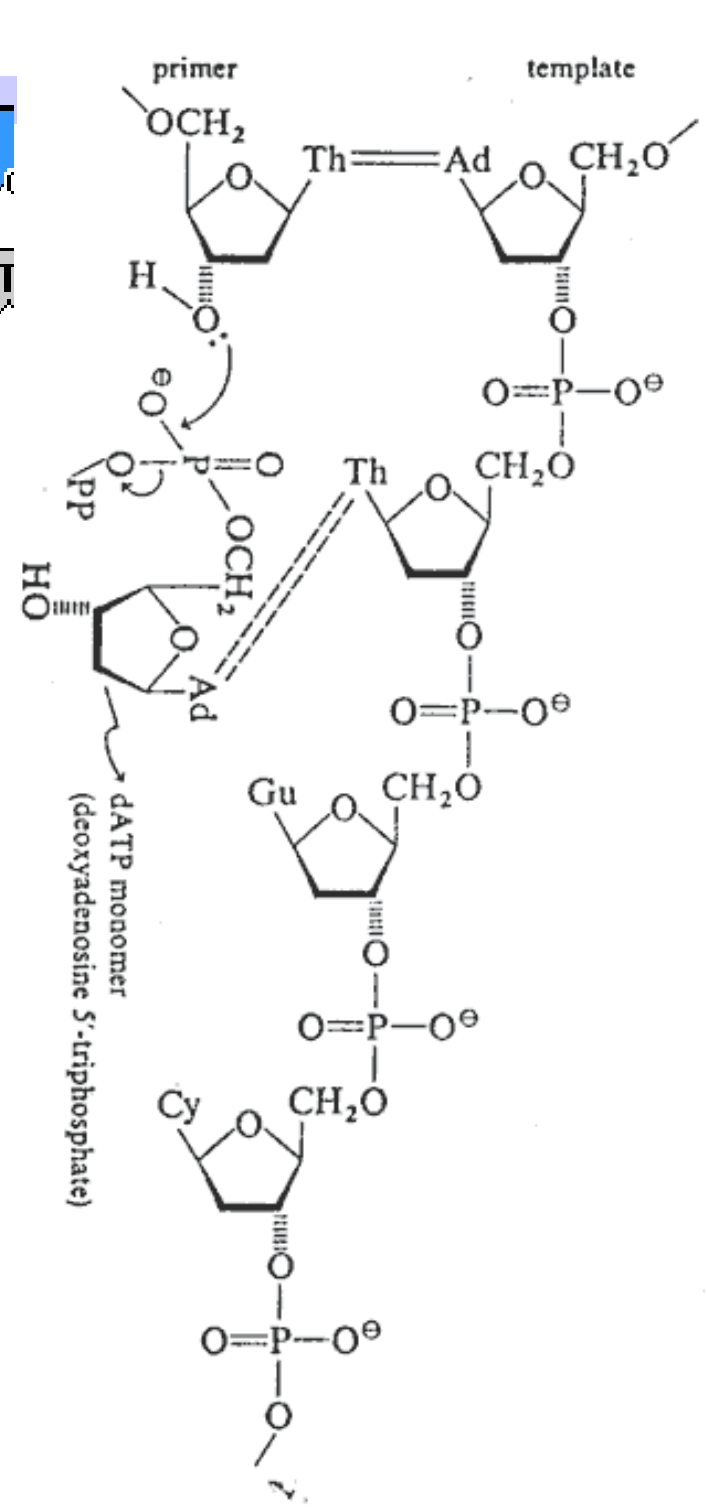
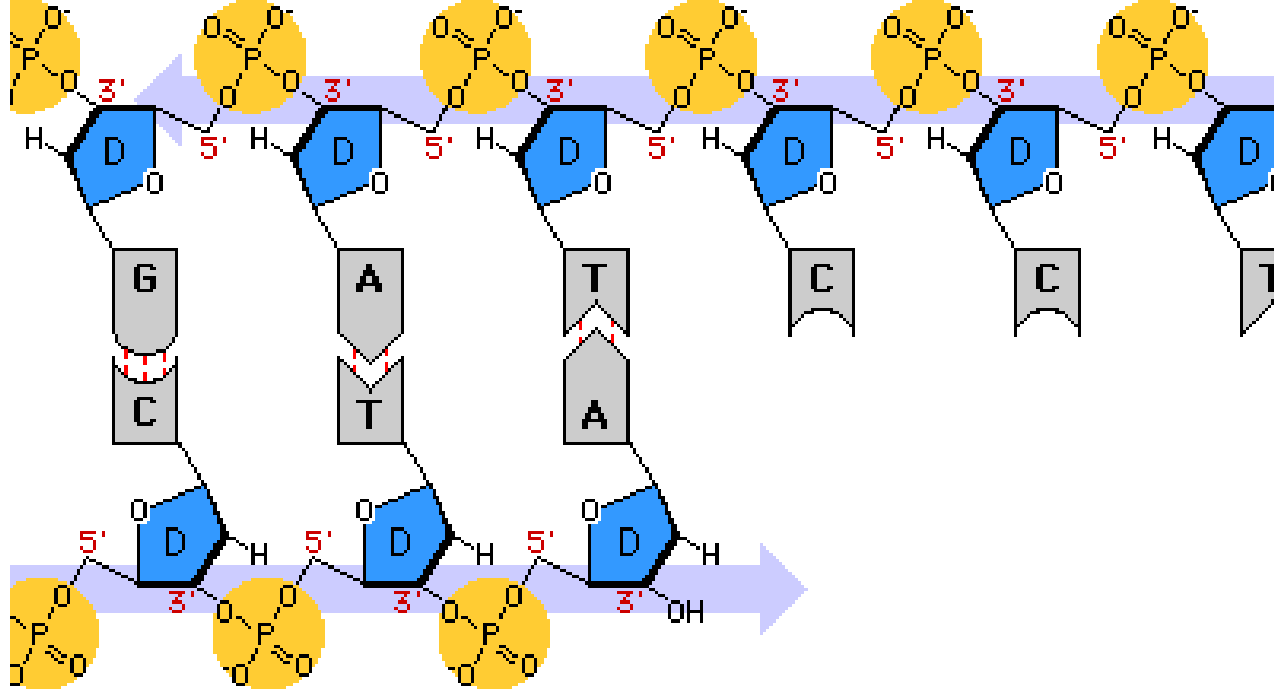
## Voraussetzungen

Startstrang (PRIMER)  
Matrize (TEMPLATE)  
Substrate (Desoxynucleosid-  
Triphosphate  
Magnesiumionen

Richtung 5' - 3'  
Pyrophosphorolyse







# DNA-Polymerase I

*Escherichia coli*

M: 103 kD, 928 Aminosäuren

400 Moleküle/Zelle

Aktivität: 670 Nucleotide / min

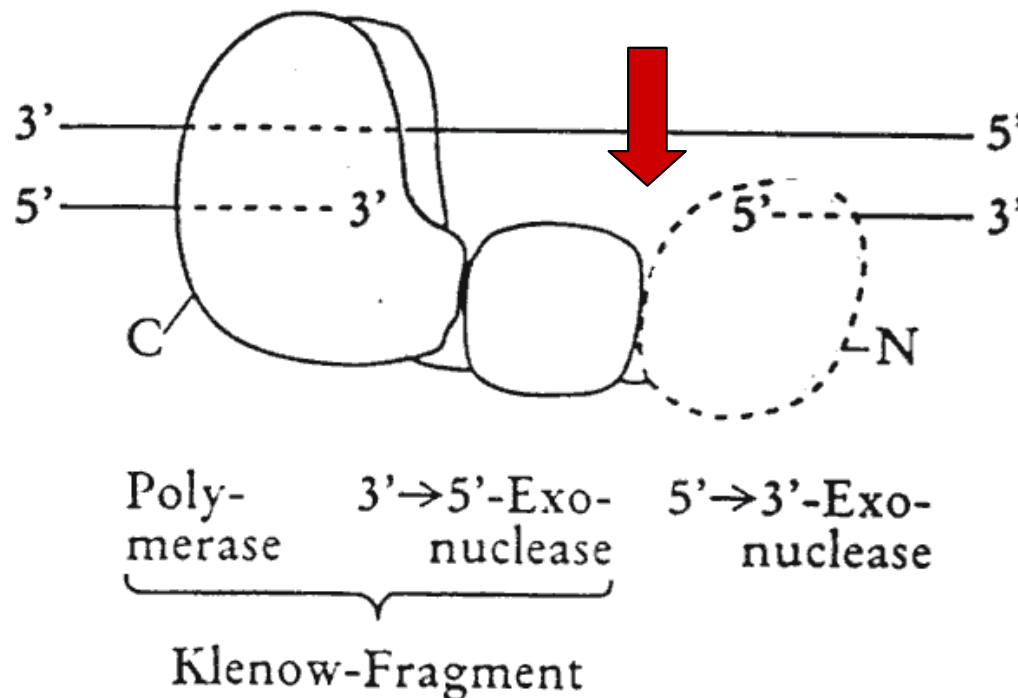
Funktion: Reparatur und Präzision der Basenpaarung

(Fehlerrate 1: 1.000.000)

(DNA-Polymerase III Replikationsenzym: 1000 Nucleotide / s)

Bindungsstellen für Matrize, Starter und Substrate

Zusätzlich Exonuklease-aktivität in 3'-5' und 5'-3' Richtung

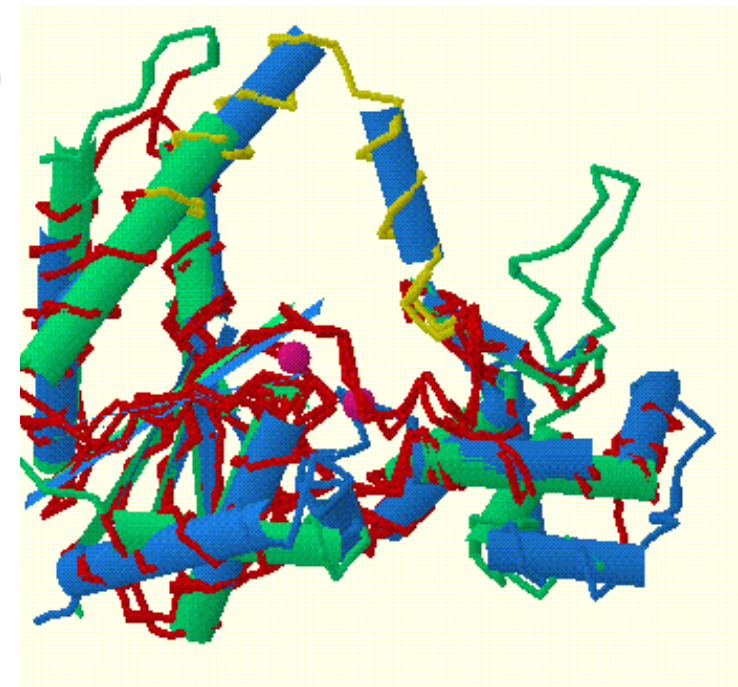
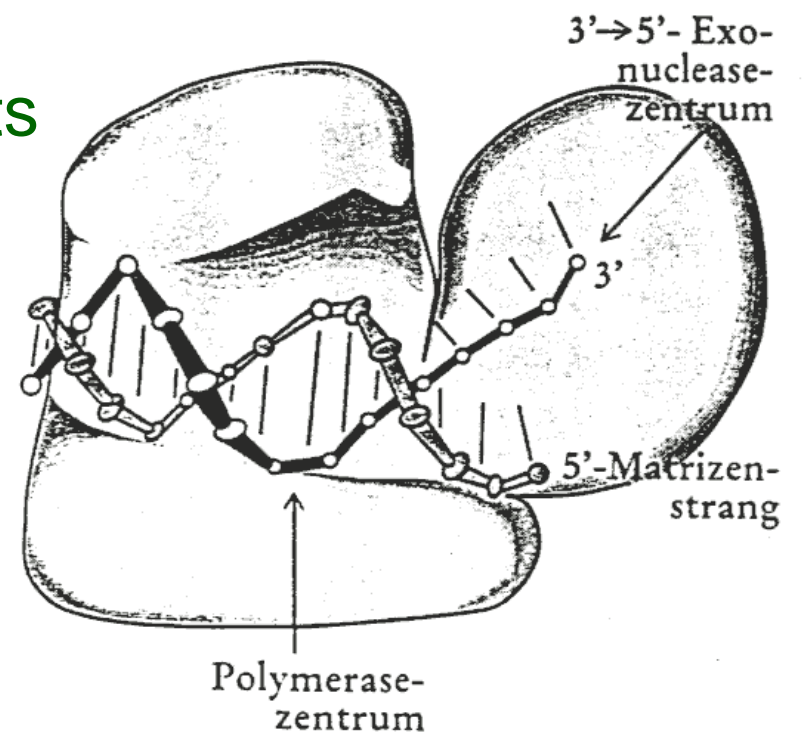
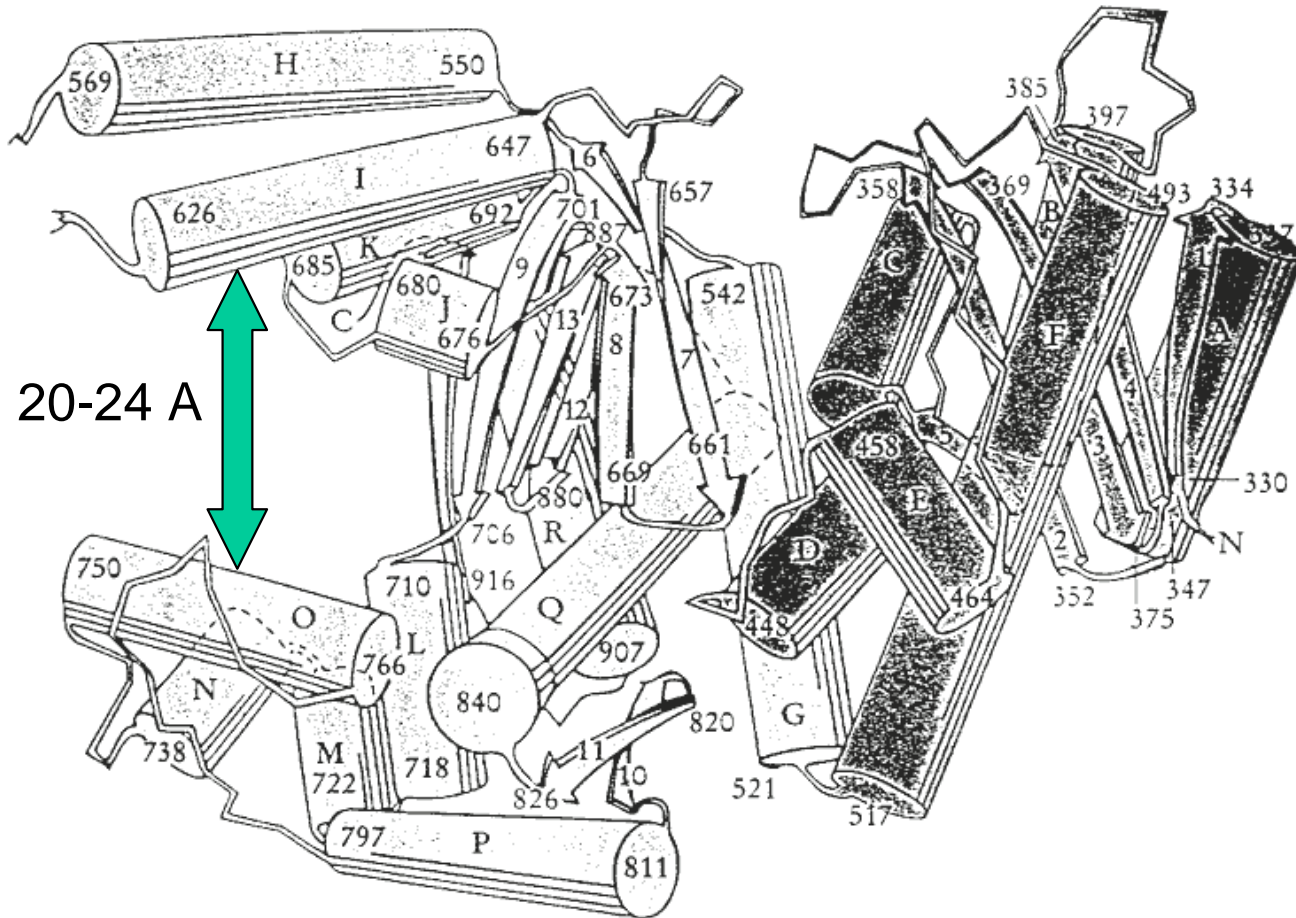


Spaltung mit Subtilisin

Abbau zu Einzelstrang  
(Startstrang)

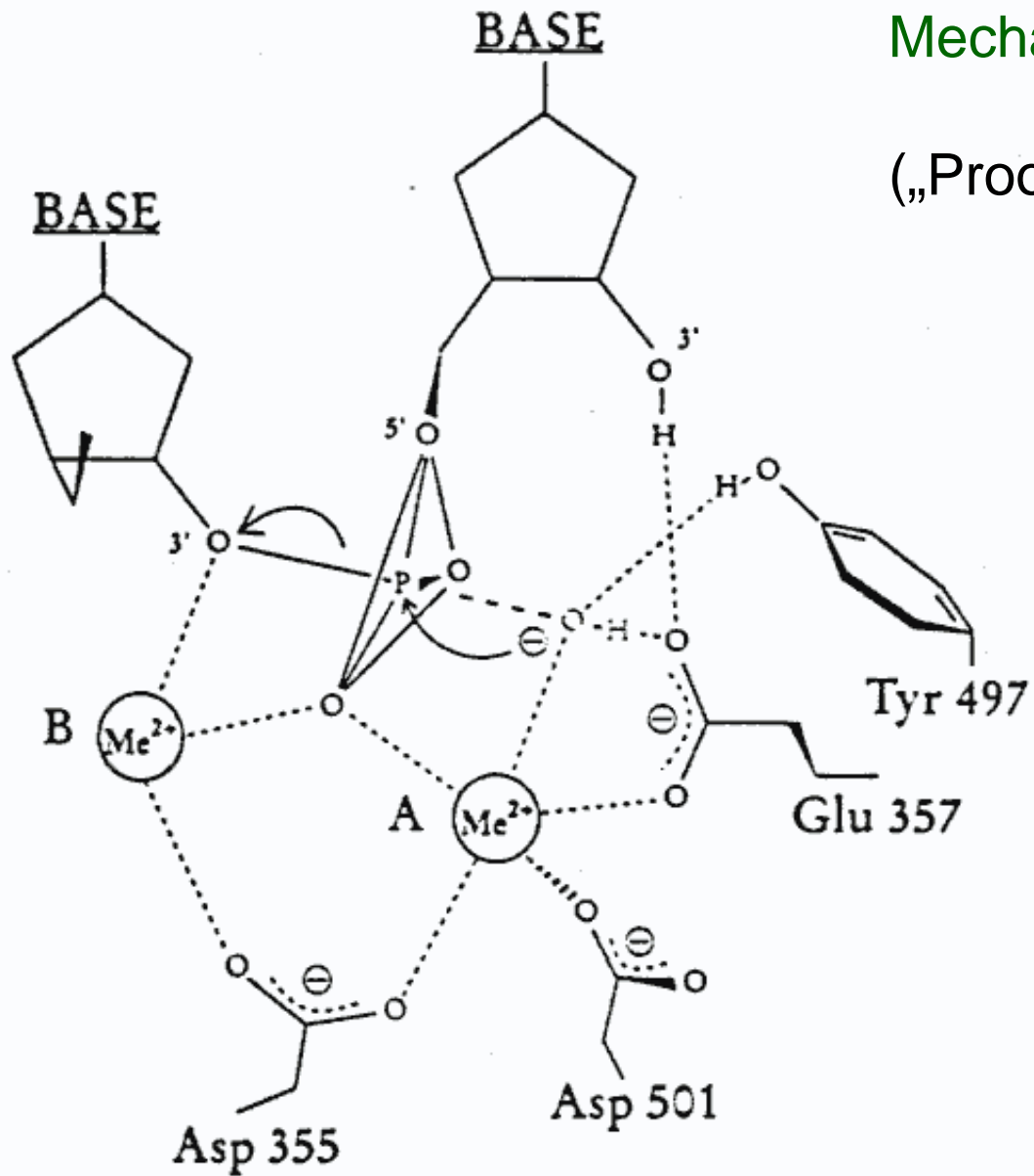
# Röntgenstruktur des Klenow-Fragments

605 Aminosäuren, PDB: 2KZM



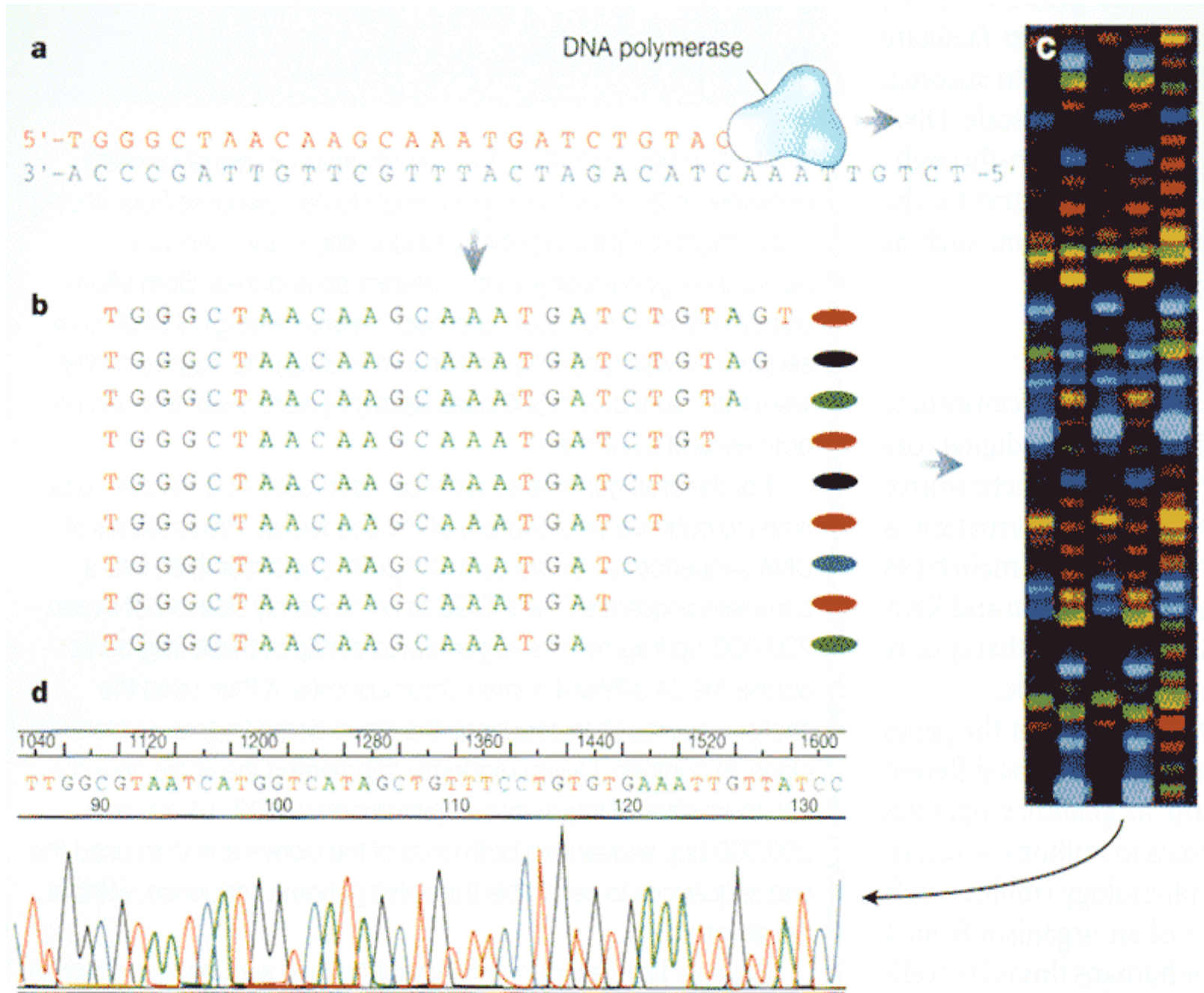
# Mechanismus der 3'-5' Exonuklease

(„Proofreading“)



# Einsatz der DNA-Polymerasen zur Sequenzbestimmung

## Taq-Polymerase (*Thermophilus aquaticus*)



# Chemische Oligonukleotidsynthesen

Aufklärung des genetischen Codes

Synthese von Startsequenzen

Modifikation von DNA und RNA

Linker, Adapter für Klonierungsexperimente

„Antisense“ Oligonukleotide, Hybridisierungsproben für mRNA und cDNA

Gensynthesen

Voraussetzungen:

Bildung des 3´-5´Phosphodiesters, Schutz aller anderen nukleophilen Zentren (Phosphat, Basen, Pentose)

Aktivierung zur Phosphodiesterbildung – Hohe Ausbeuten notwendig

Schutzgruppen: Möglichst quantitative Einführung und Abspaltung

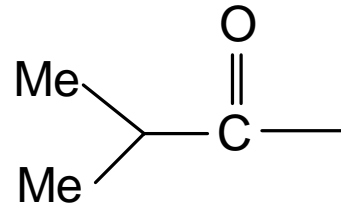
Stabilität unter Synthesebedingungen

Festphasensynthese an polymeren Trägern

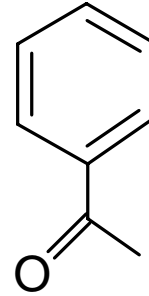


# Schutzgruppen

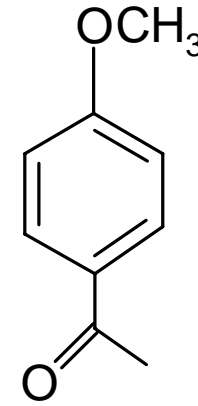
Aminogruppen der N-Basen:



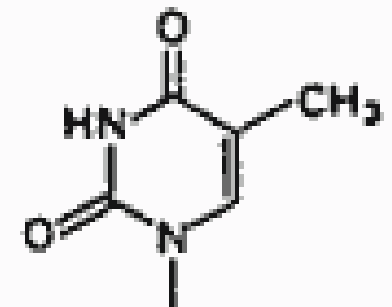
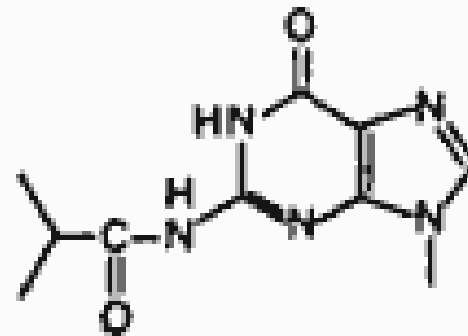
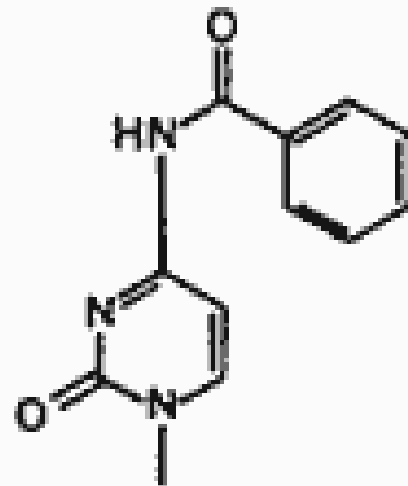
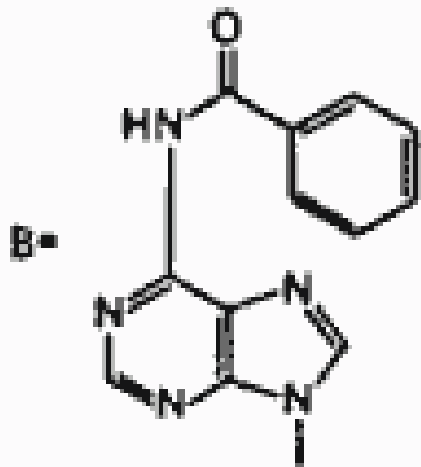
Isobutyryl



Benzoyl

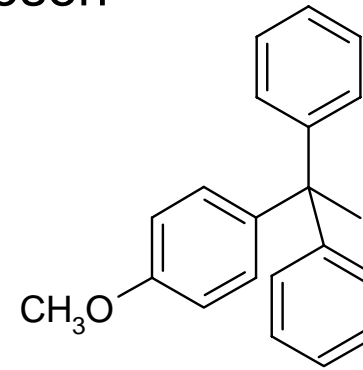
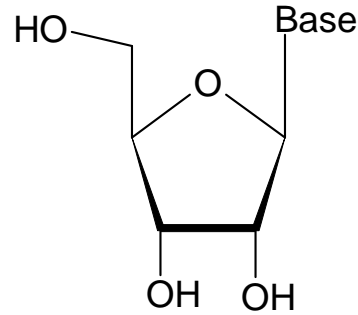


Methoxybenzoyl (Anisoyl)

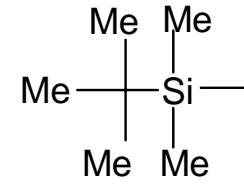
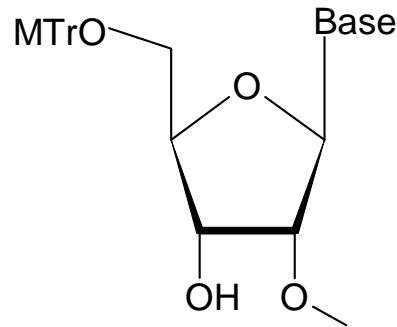


# Schutzgruppen

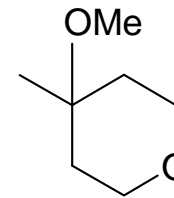
## OH-Gruppen der Pentosen



Triphenylmethyl-  
(Trityl)  
Methoxytrityl-

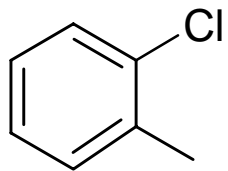


*tert*-Butyldimethylsilyl  
tBDMSi

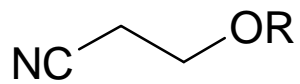
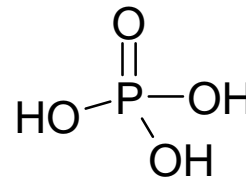


Methoxytetrahydropyranyl

## OH-Gruppen der Phosphate

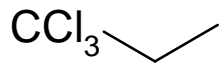
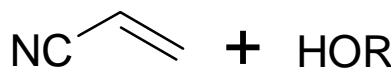


2-Chlorphenyl-



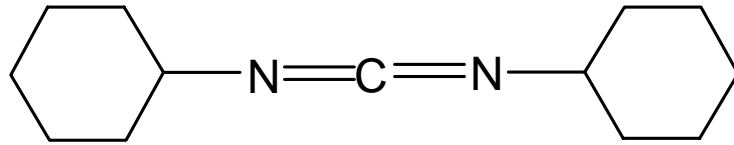
2-Cyanoethyl-

$F^-$

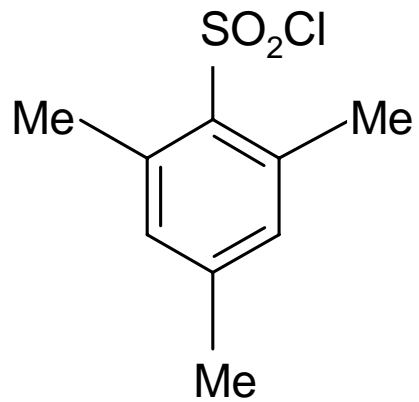


Trichlorethyl-

# Kopplung

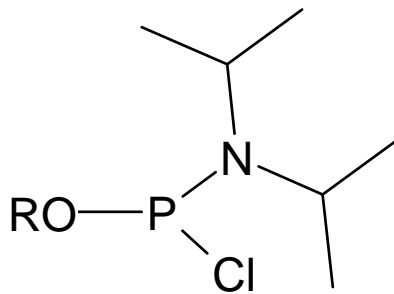


Dicyclohexylcarbodiimid (DCC)

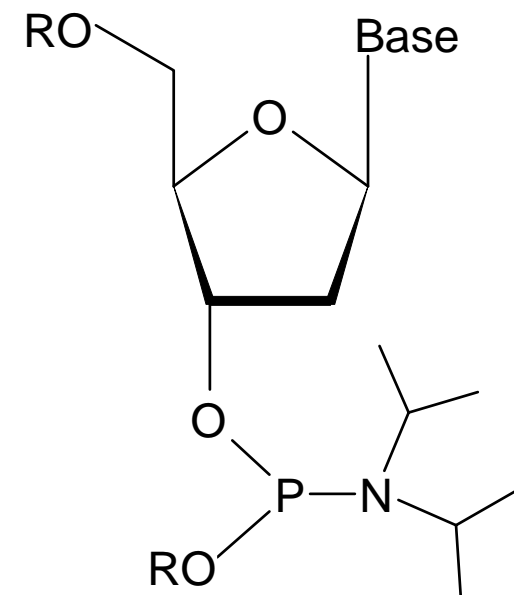


Mesitylsulfochlorid

## Aktivierung als Phosphoramidit

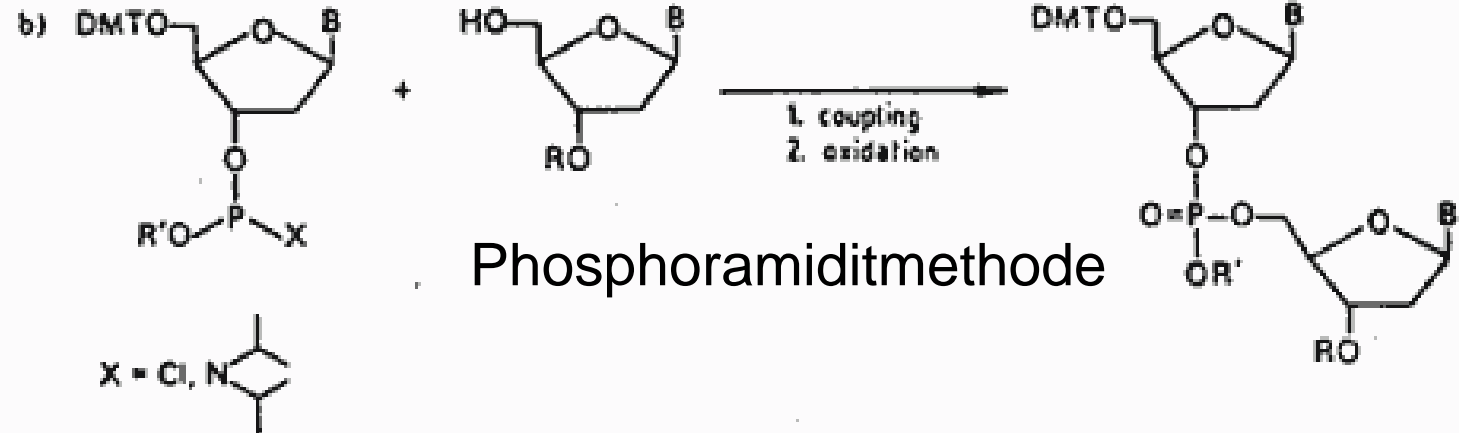
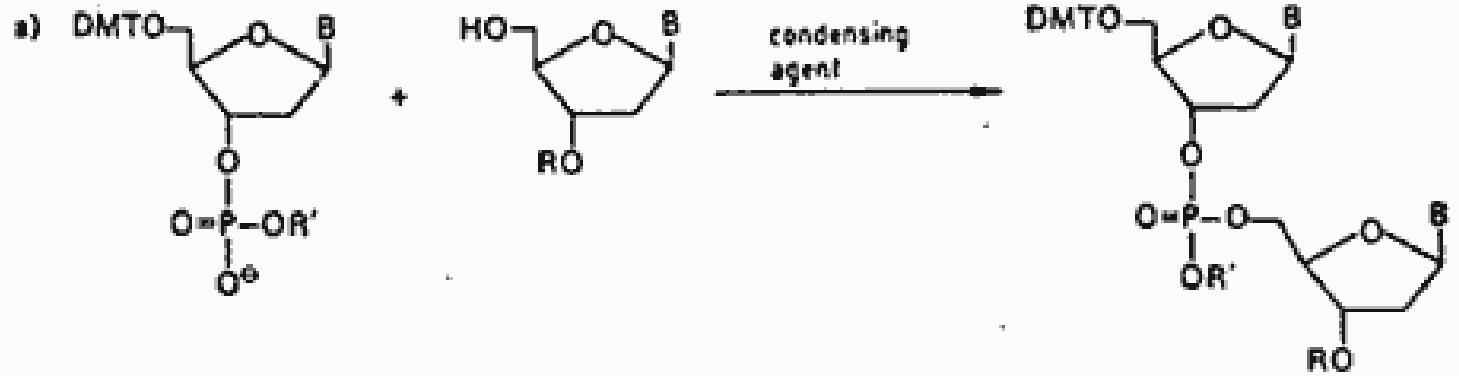


Chloro-N,N-Diisopropylphosphoramidit

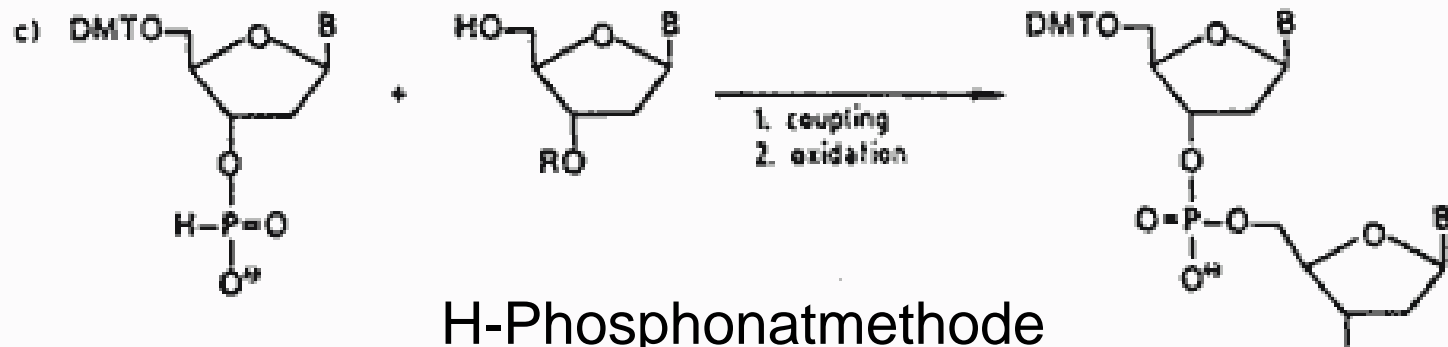


Umkehr der enzymatischen Reaktion:  
5'-OH als Nukleophil

## Phosphotriestermethode



## Phosphoramiditmethode



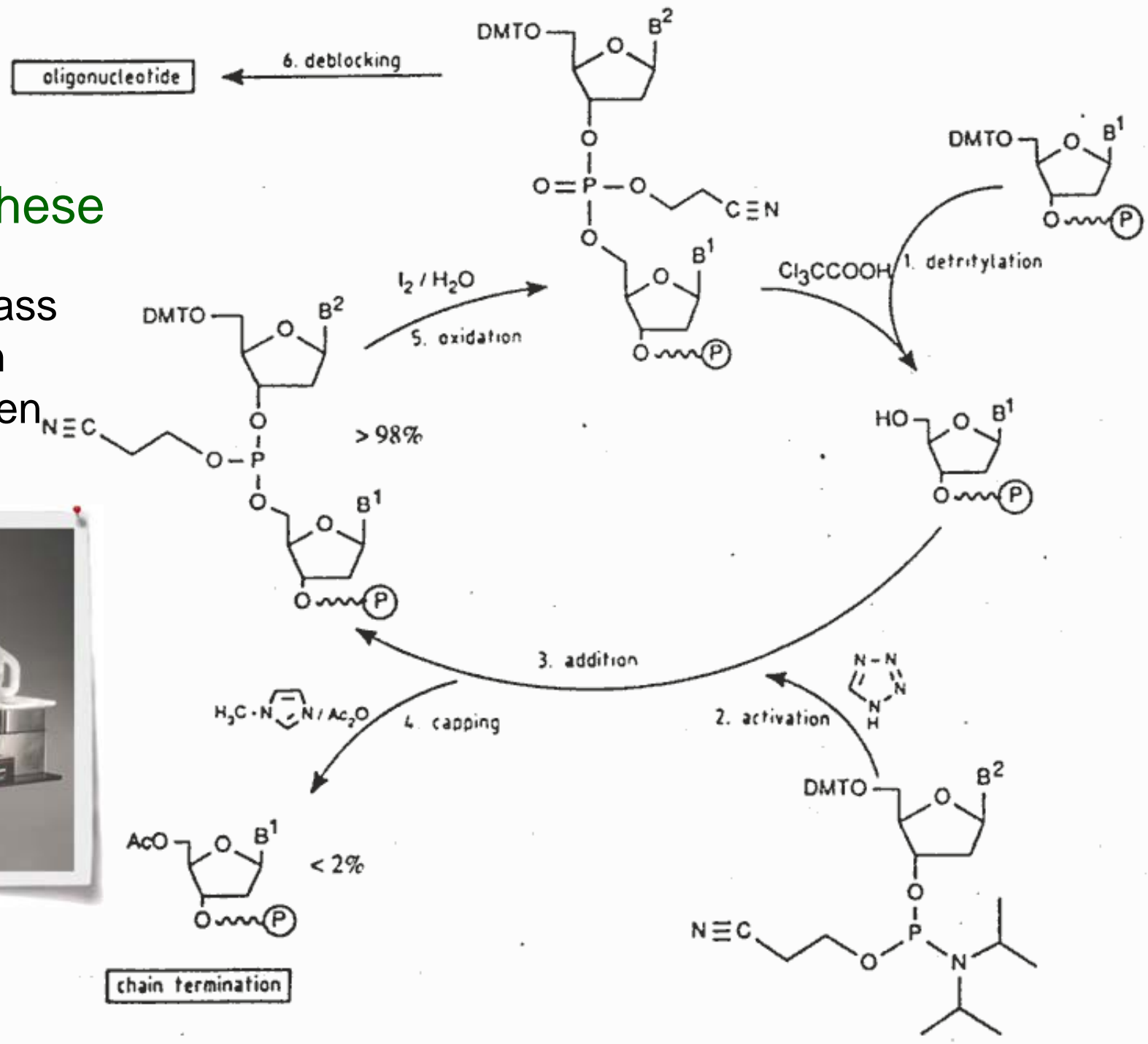
## H-Phosphonatmethode

# Festphasensynthese

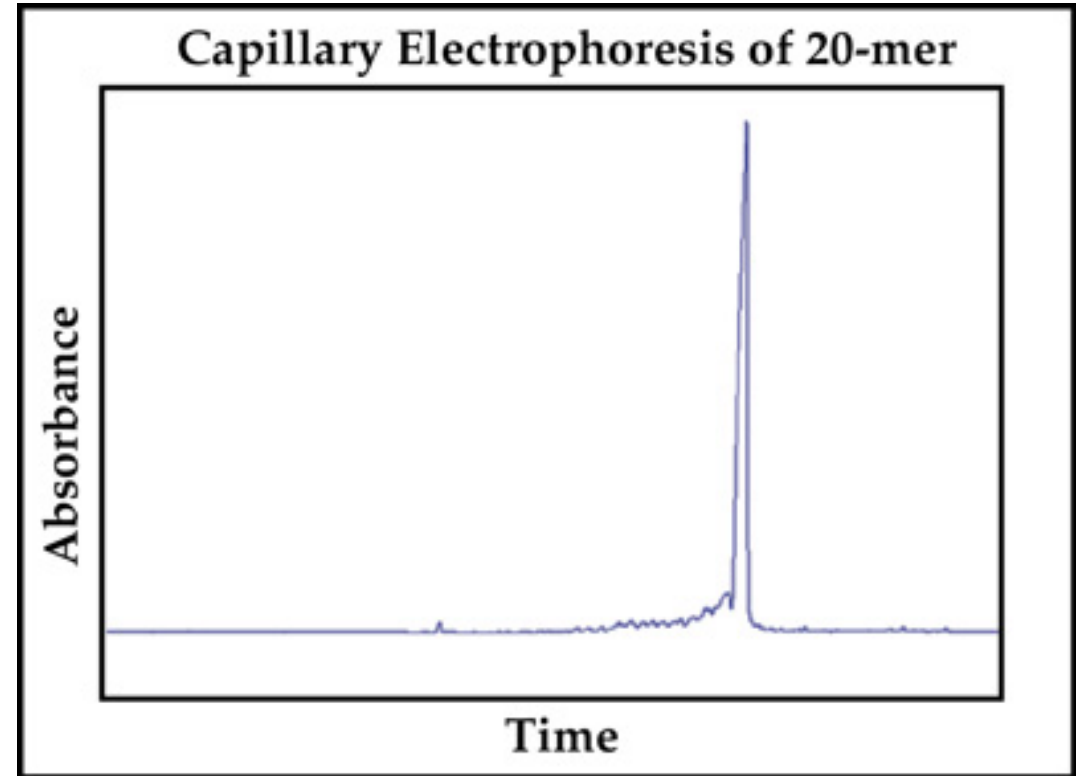
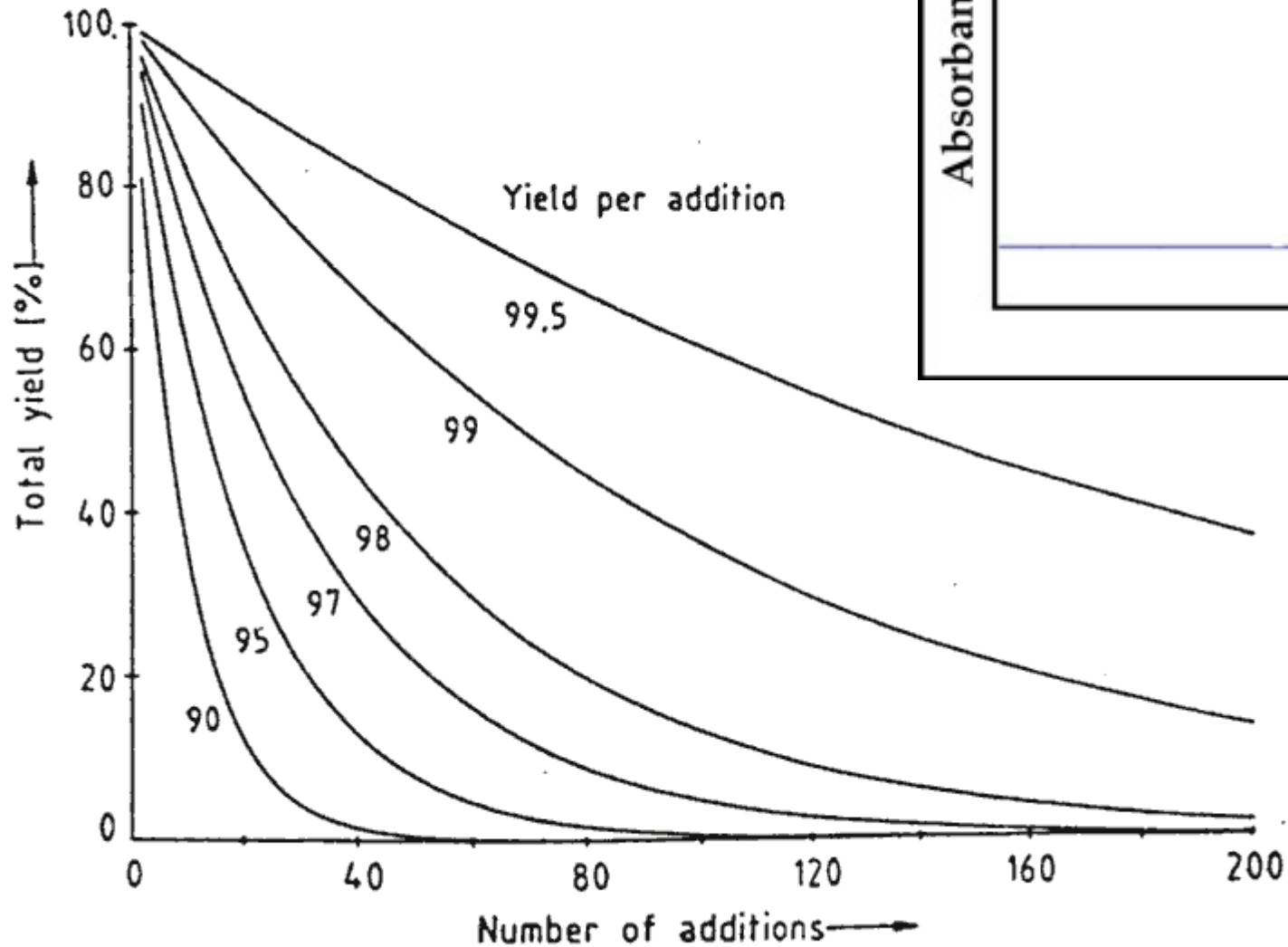
Controlled pore glass  
500 A: ~ 80 Basen  
1000 A: ~200 Basen



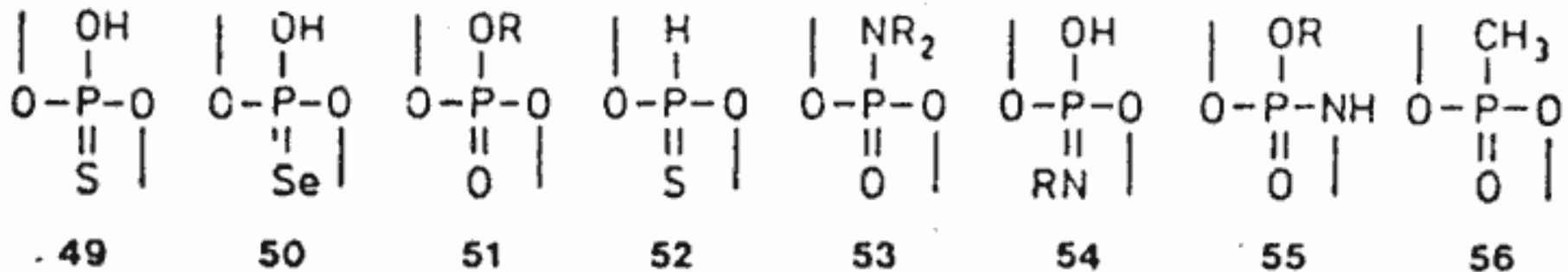
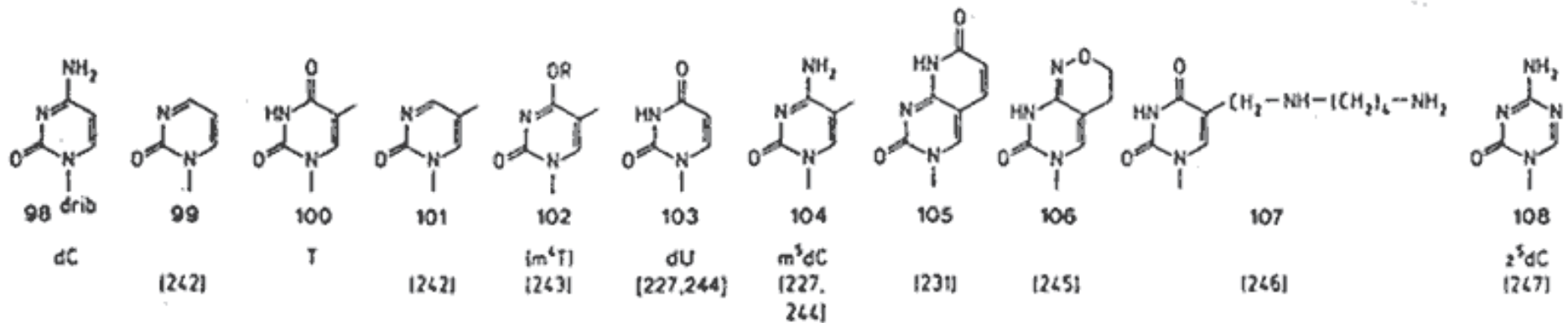
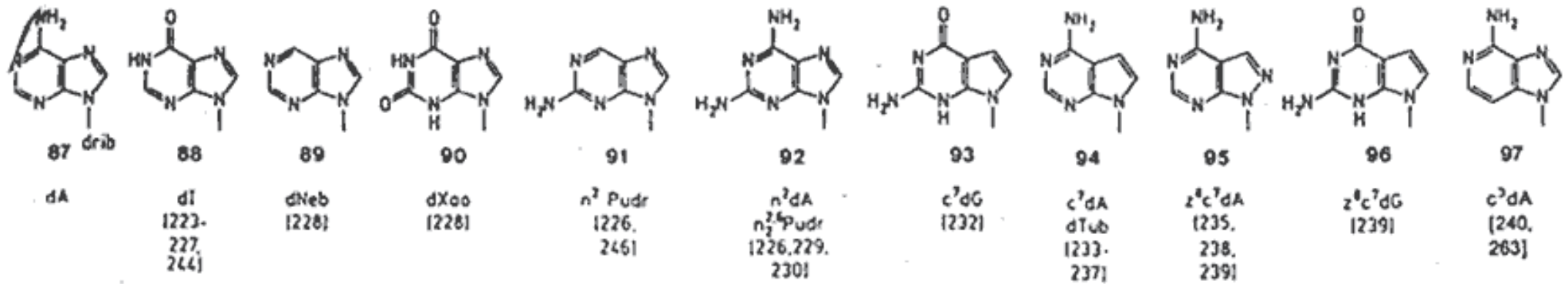
PolyPlex



# Gesamtausbeute und Reinheit bei vielstufigen Synthesen

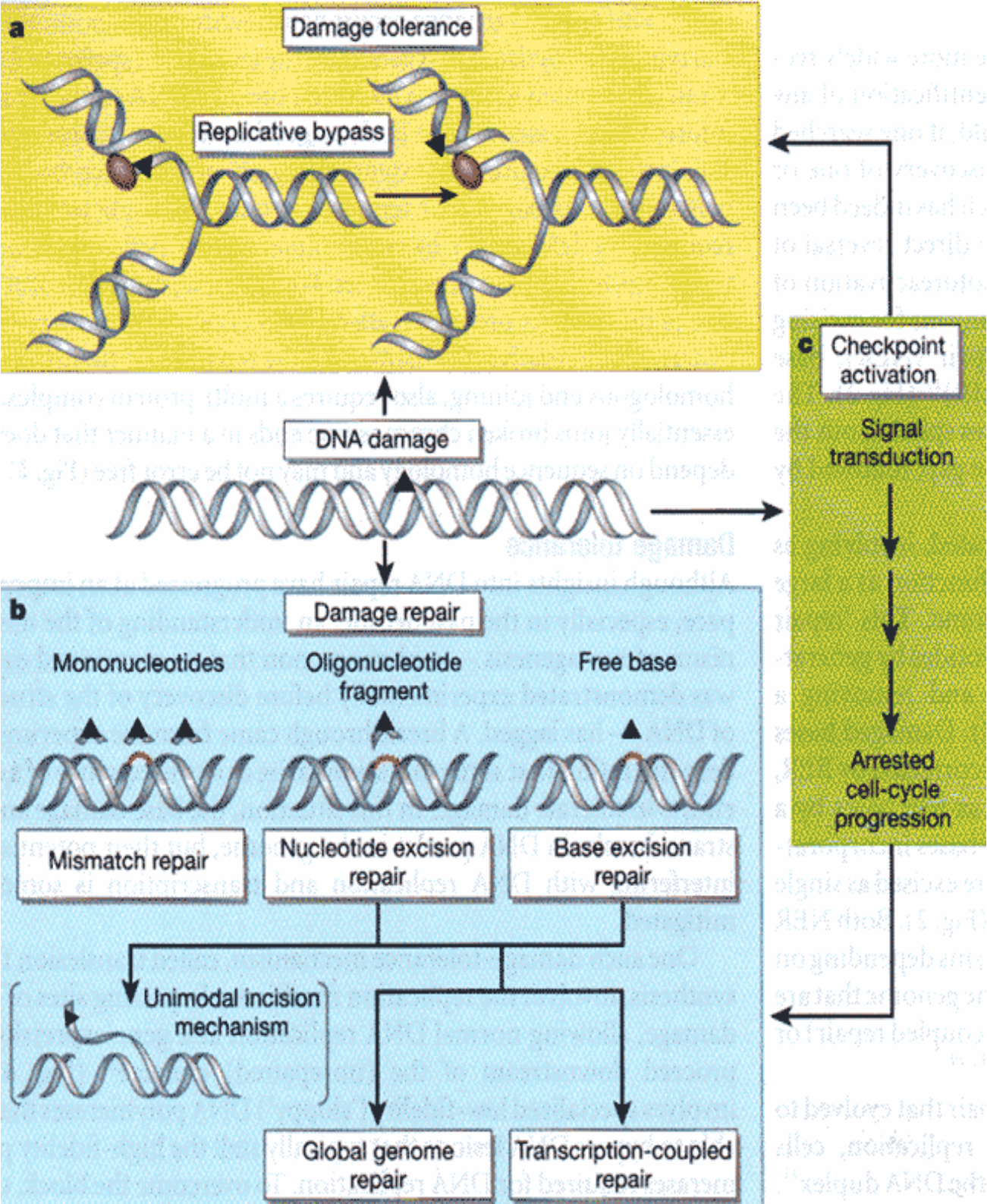


# Modifizierte Nukleotide



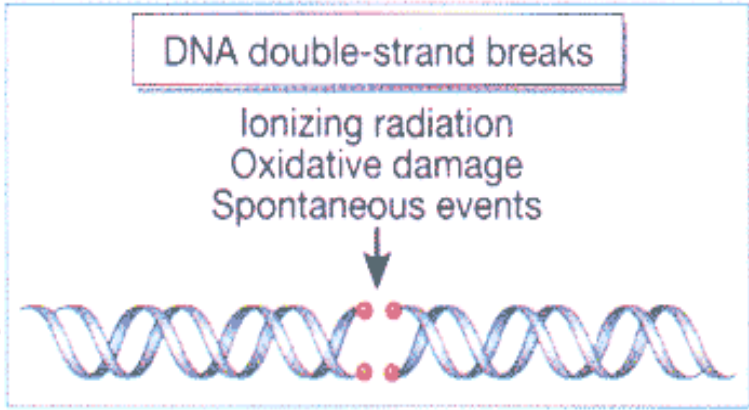
# DNA-Schäden

## Reparaturmechanismen

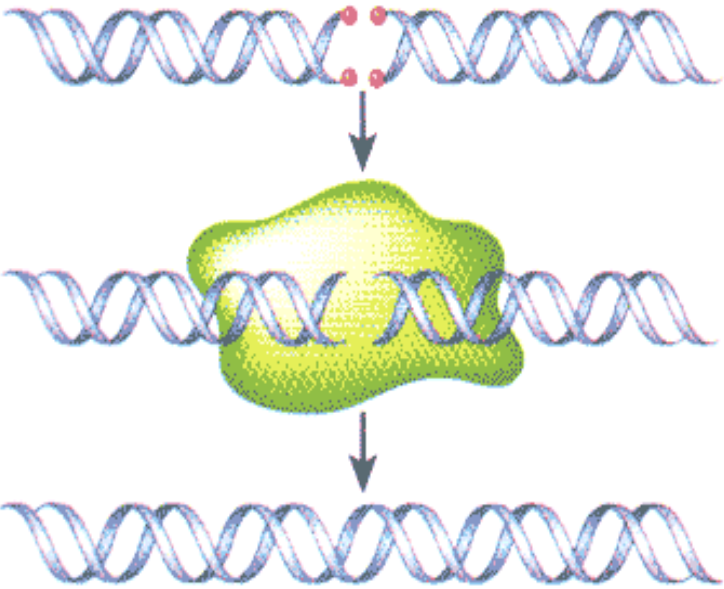




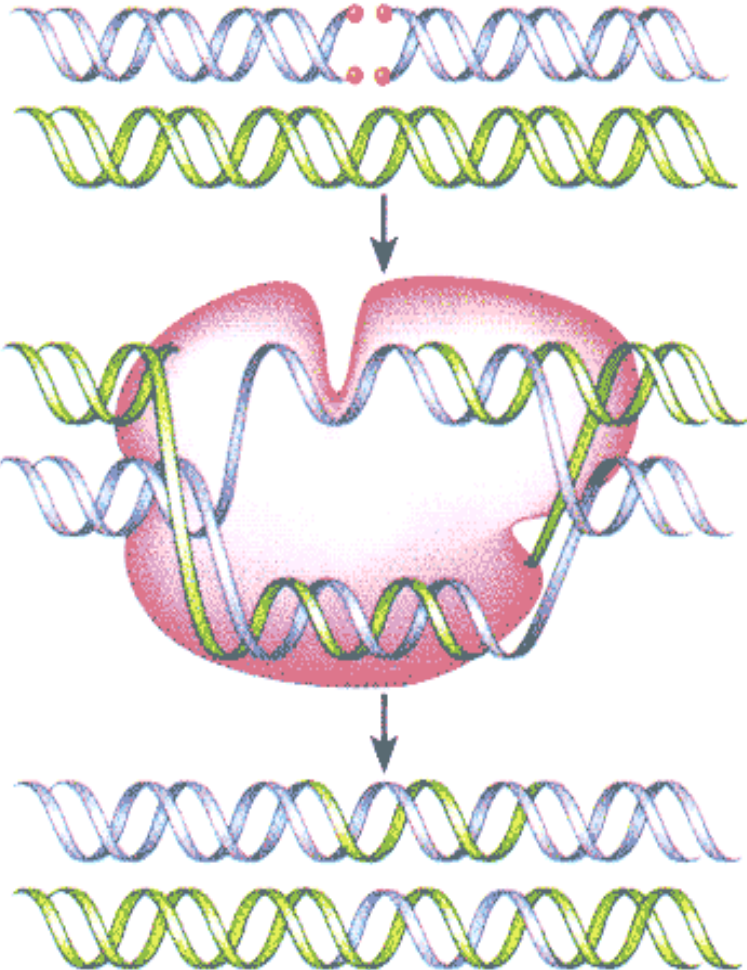
# DNA-Schäden



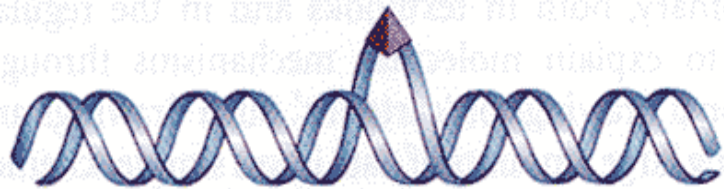
Double-strand break rejoining



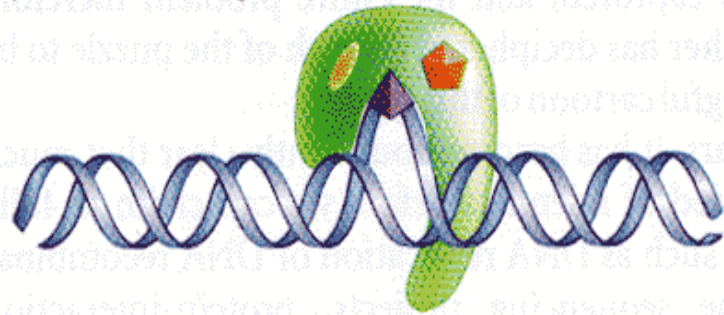
Homologous recombination



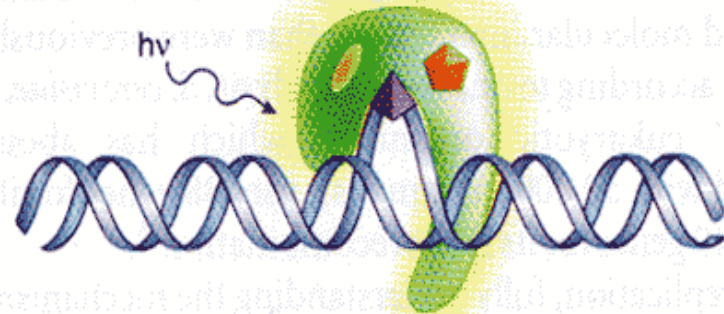
Pyrimidine dimer in UV-exposed DNA



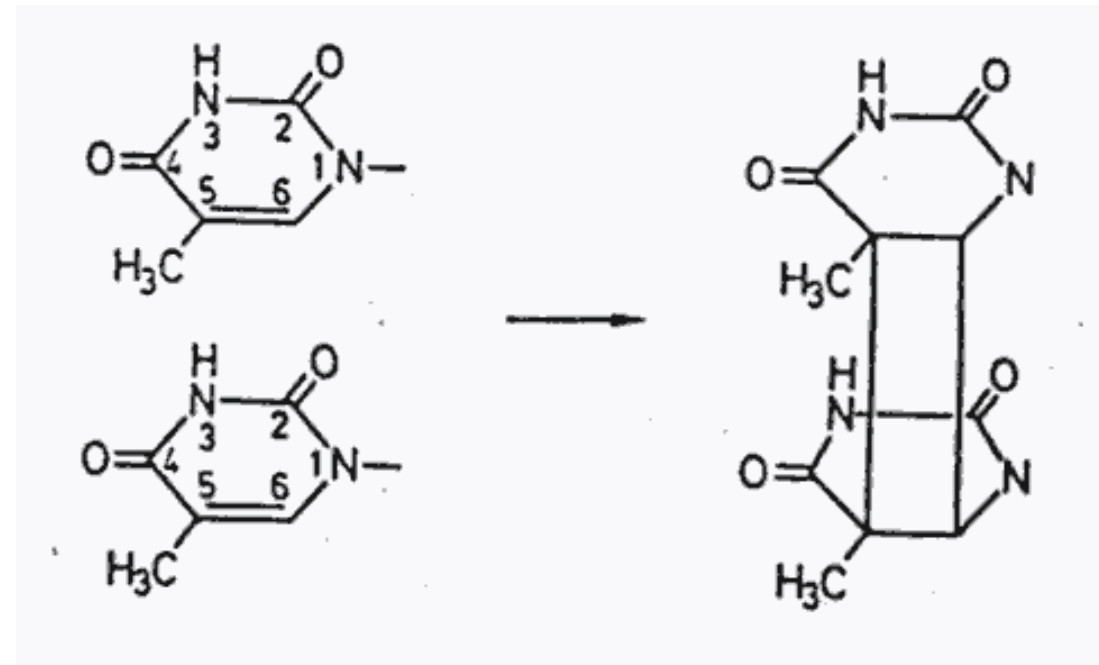
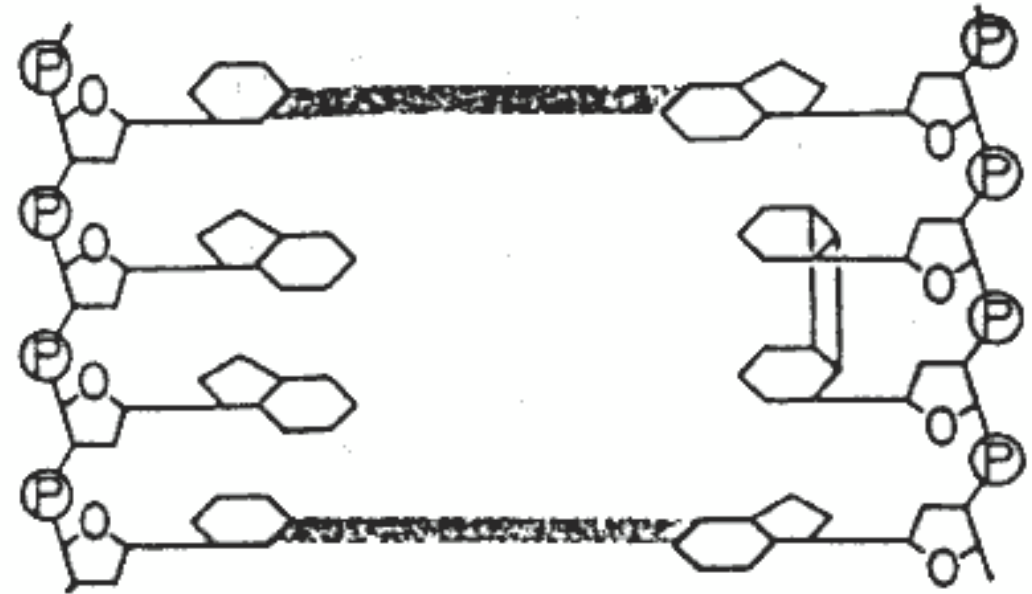
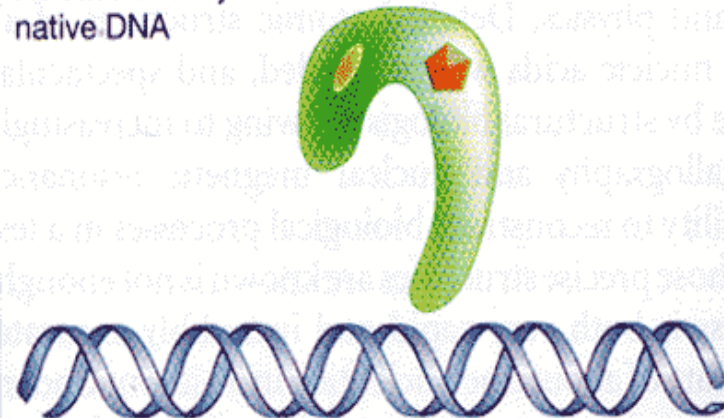
Complex of DNA with photoreactivating enzyme



Absorption of light (>300 nm)

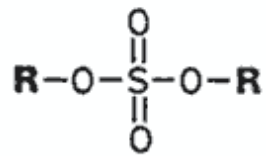


Release of enzyme to restore native DNA

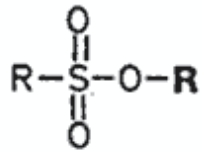


# Mutagene Substanzen

## Alkyl-Sulfate

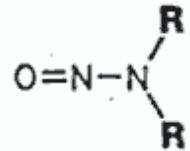


Dialkylsulfat  
Beispiel:  
Dimethylsulfat

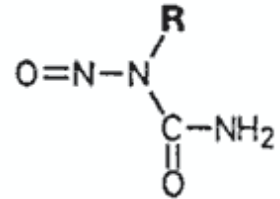


Alkyl-Alkan-Sulfonat  
Beispiele: Methylmethan-  
sulfonat, MMS; Ethyl-  
methansulfonat, EMS

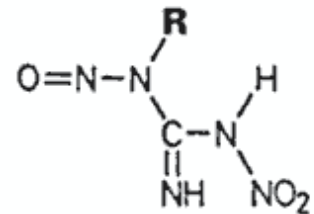
## N-Nitroso-Verbindungen



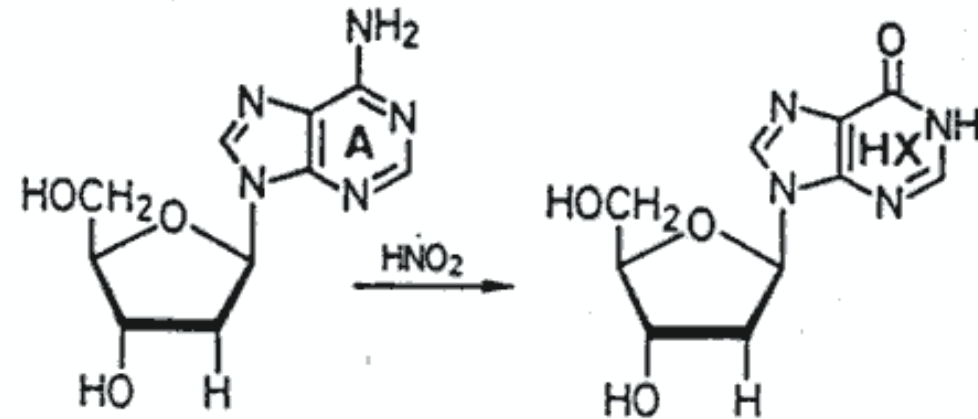
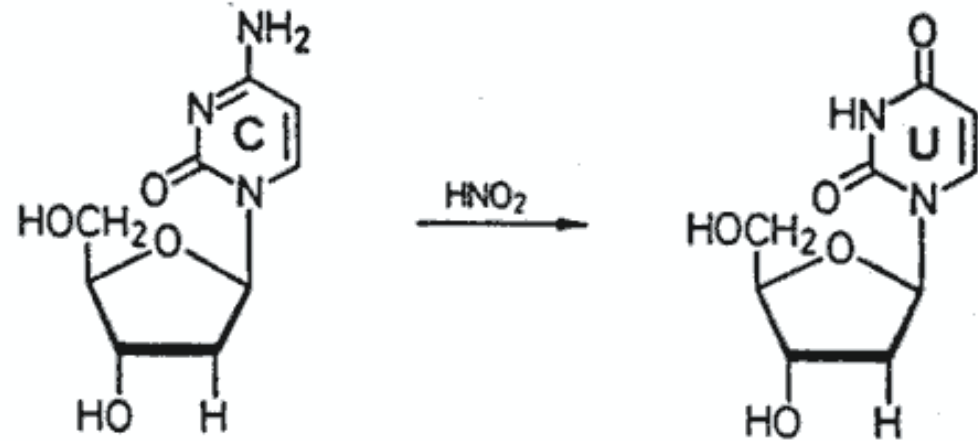
Dialkylnitrosamine  
Beispiel:  
Dimethylnitrosamin



N-Nitrosoharnstoff-  
Derivate  
Beispiel: Methyl-  
Nitrosoharnstoff, MNN



N-Alkyl-N'-Nitro-  
N-Nitrosoguanidin  
Beispiel: N-Methyl-N'-  
Nitro-N-Nitroso-  
guanidin (NNG)



## Beispiel

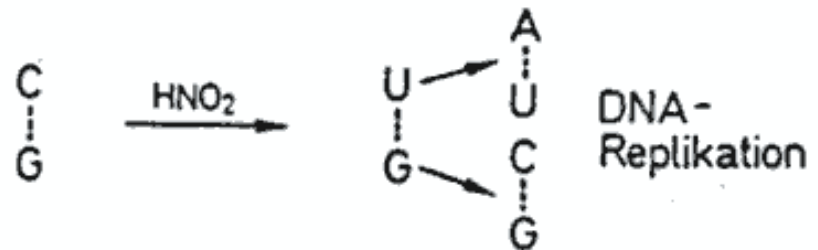
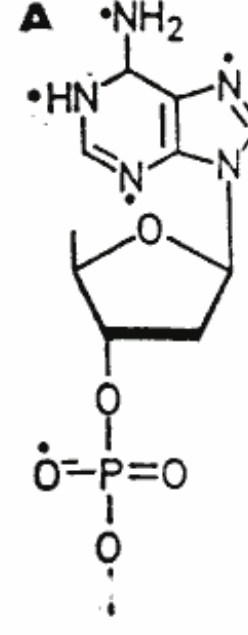
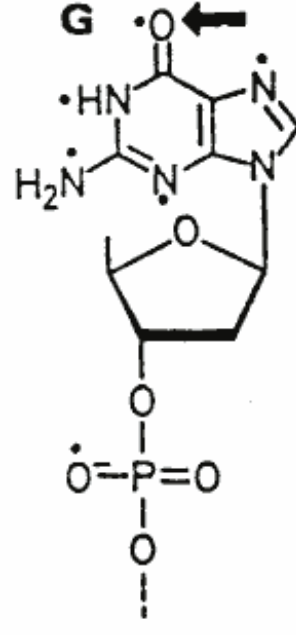
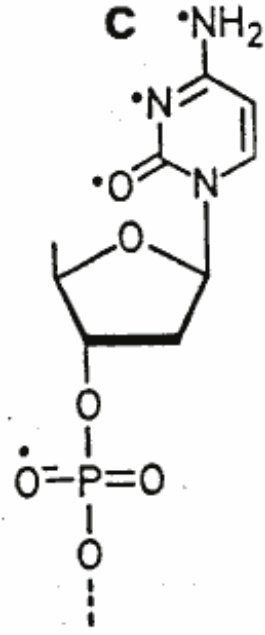
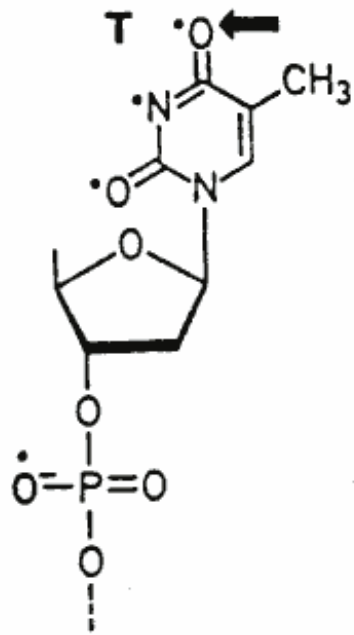
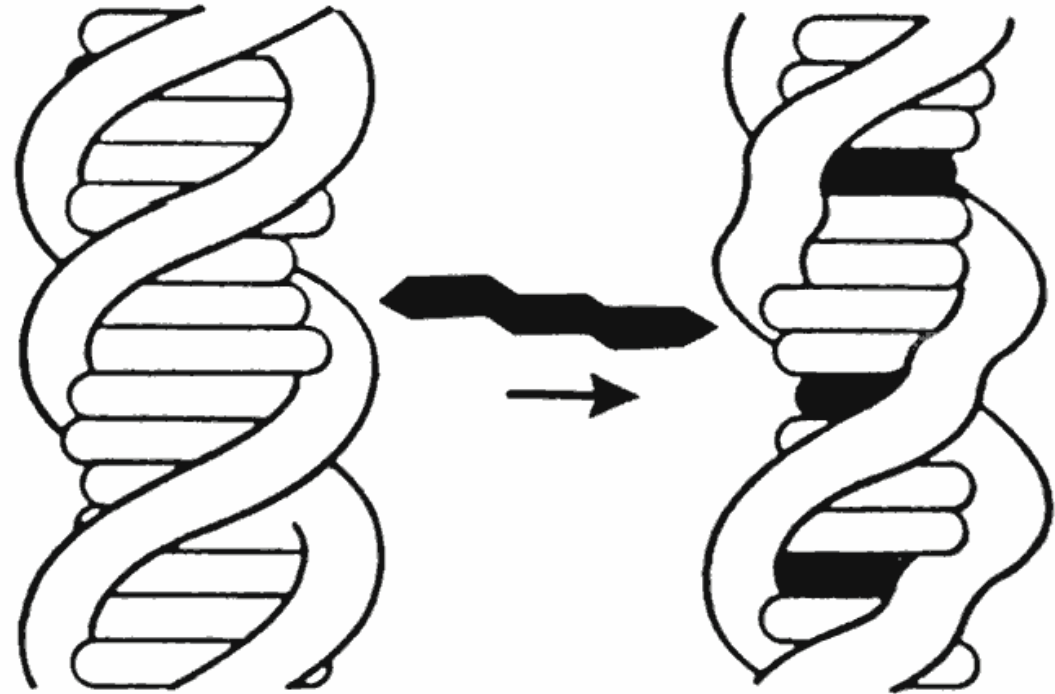


Abb. 8.16 Alkylierende Mutagene (R:  $-\text{CH}_3$   
oder  $-\text{CH}_2-\text{CH}_3$ ).

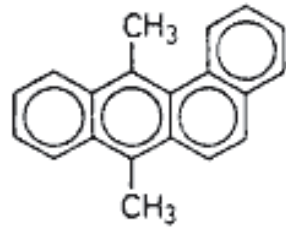


„Frame-shift“ Mutationen

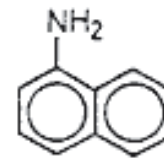


# Mutagene Substanzen

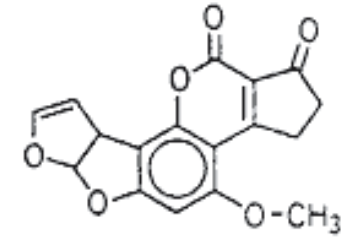
nicht aktive Formen



7,12-Dimethylbenz(a)anthracen (DMBA)

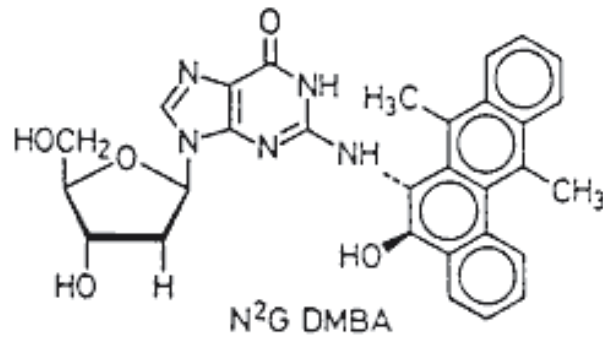


1-Naphtylamin (1-NA)

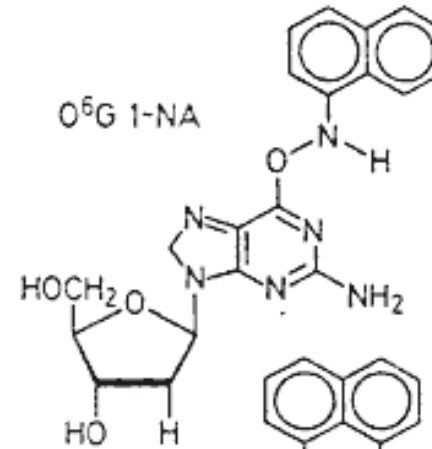


Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>)

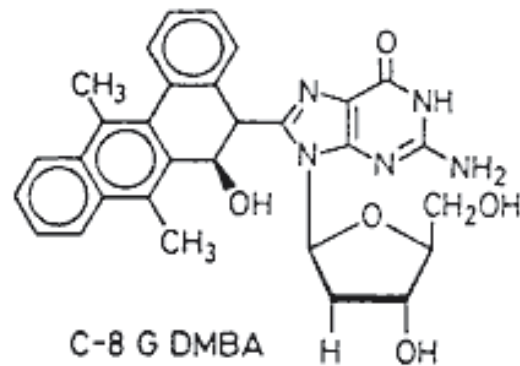
Reaktionsprodukte  
mit DNA-Basen



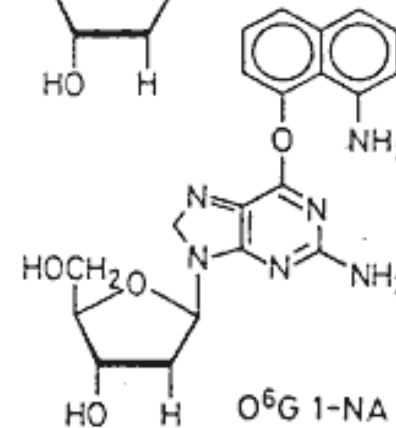
N<sup>2</sup>G DMBA



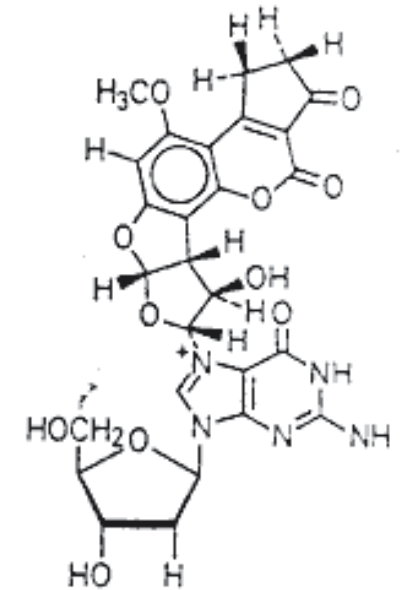
O<sup>6</sup>G 1-NA



C-8 G DMBA

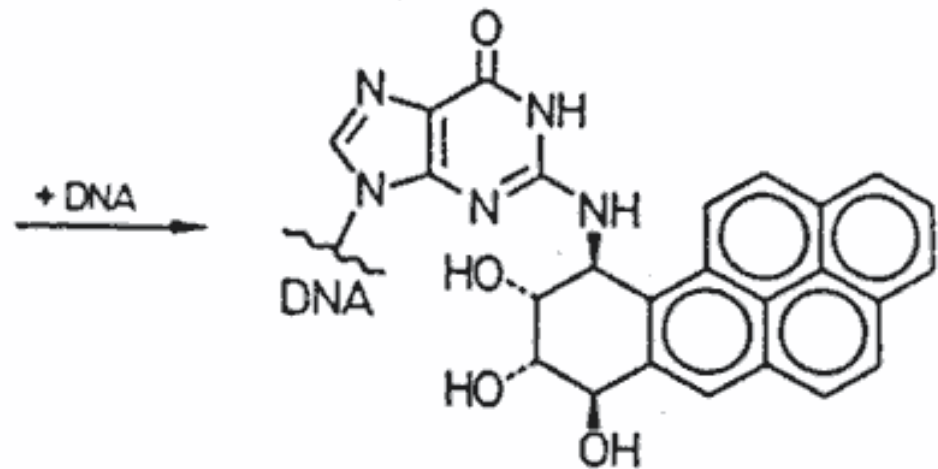
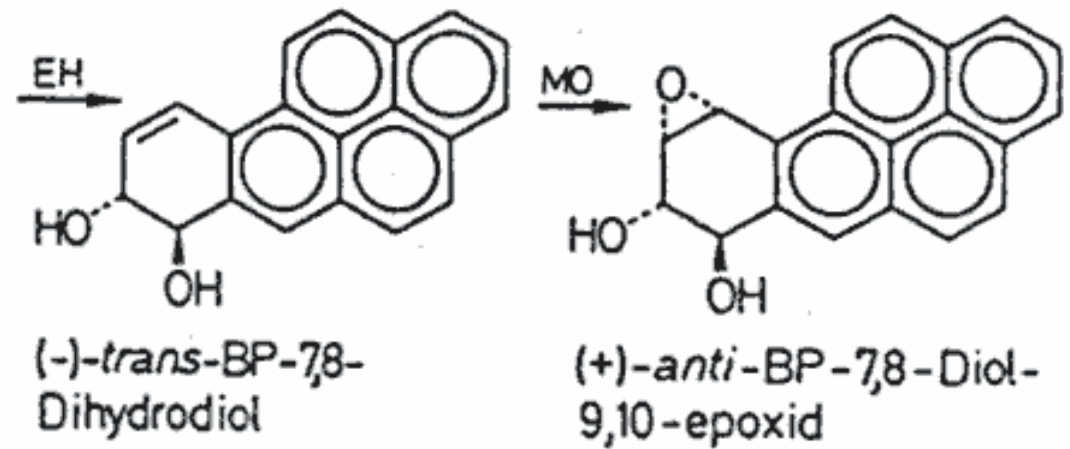
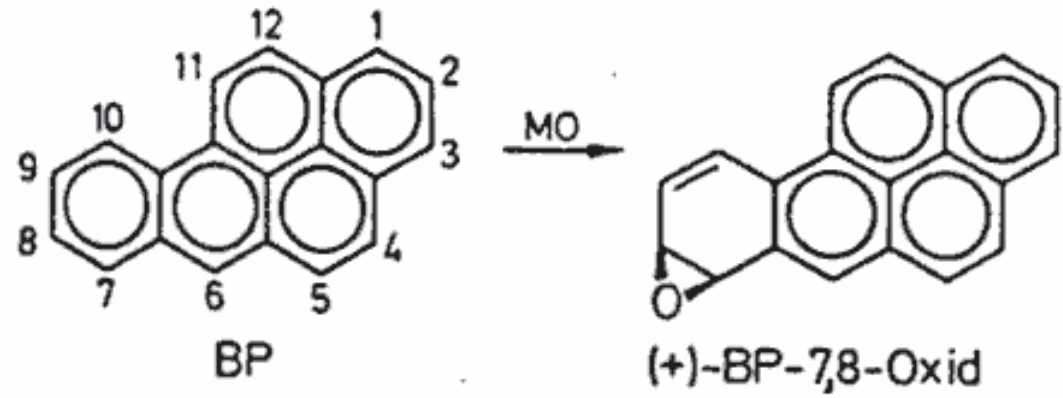


O<sup>6</sup>G 1-NA



N-7G AFB<sub>1</sub>

# Benzpyren



# Calicheamycin

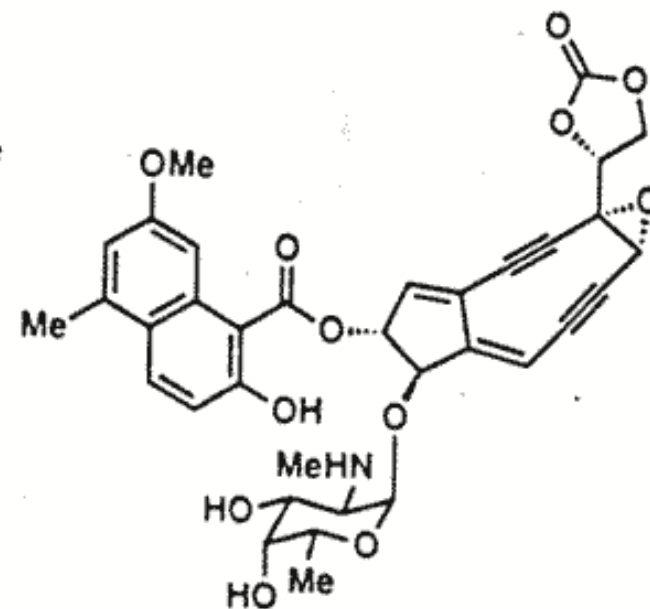
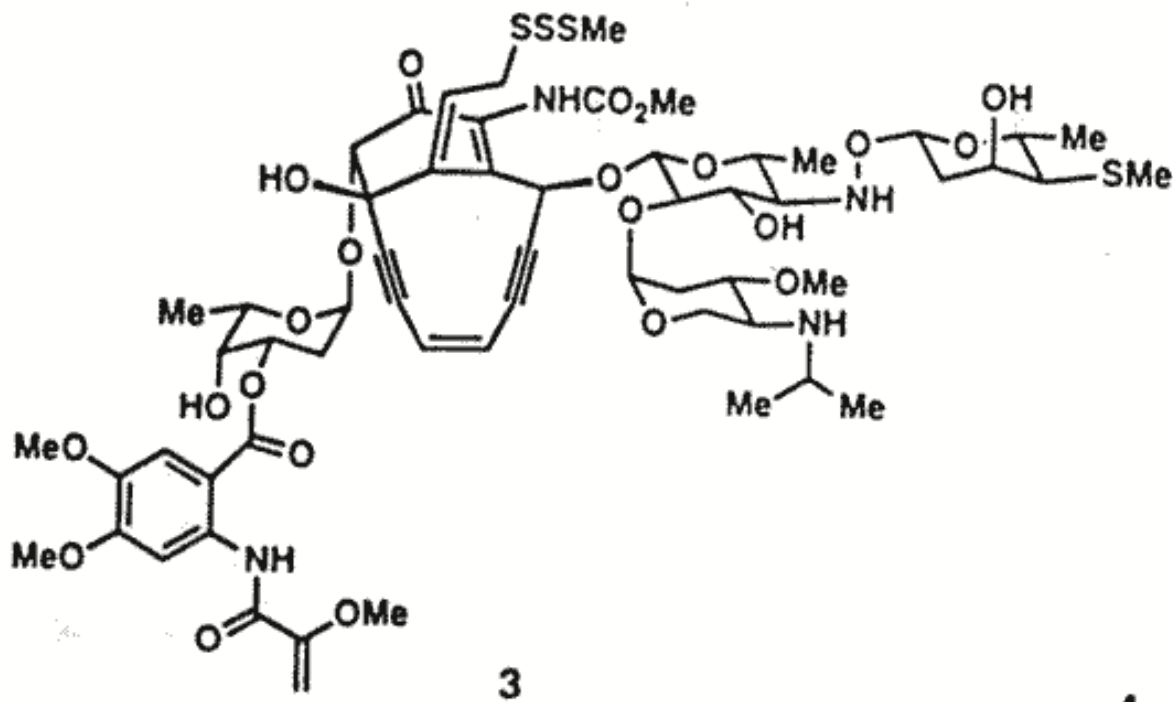
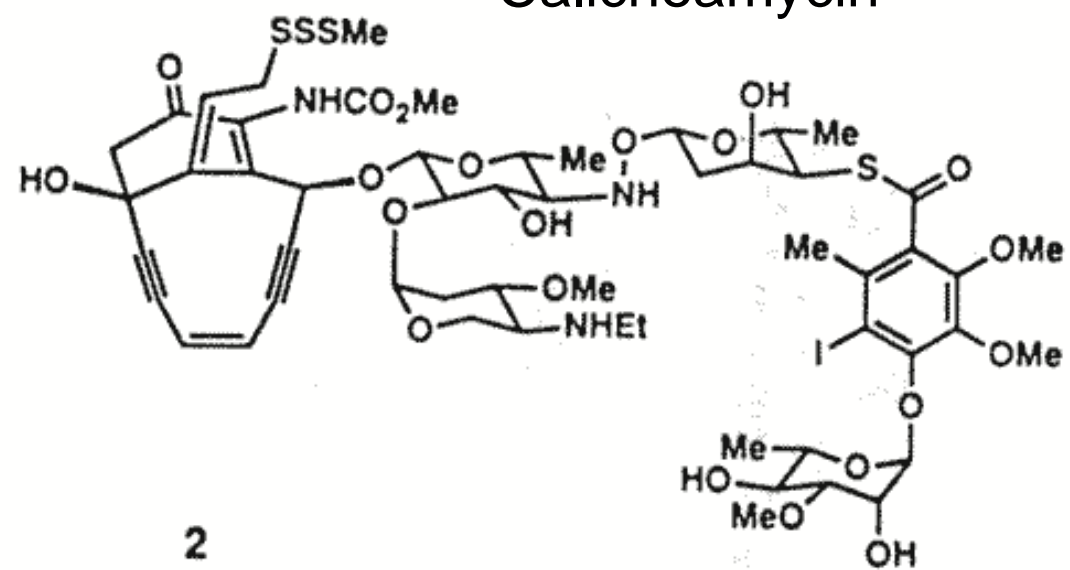
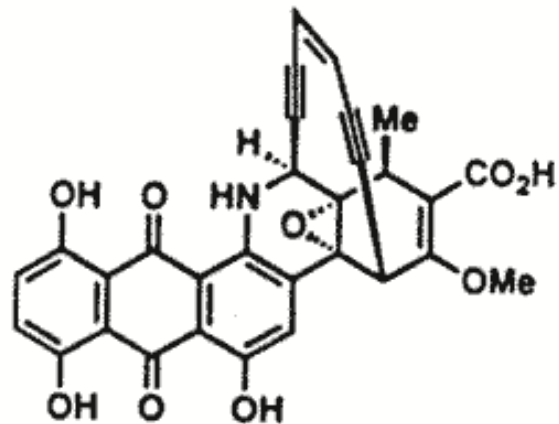
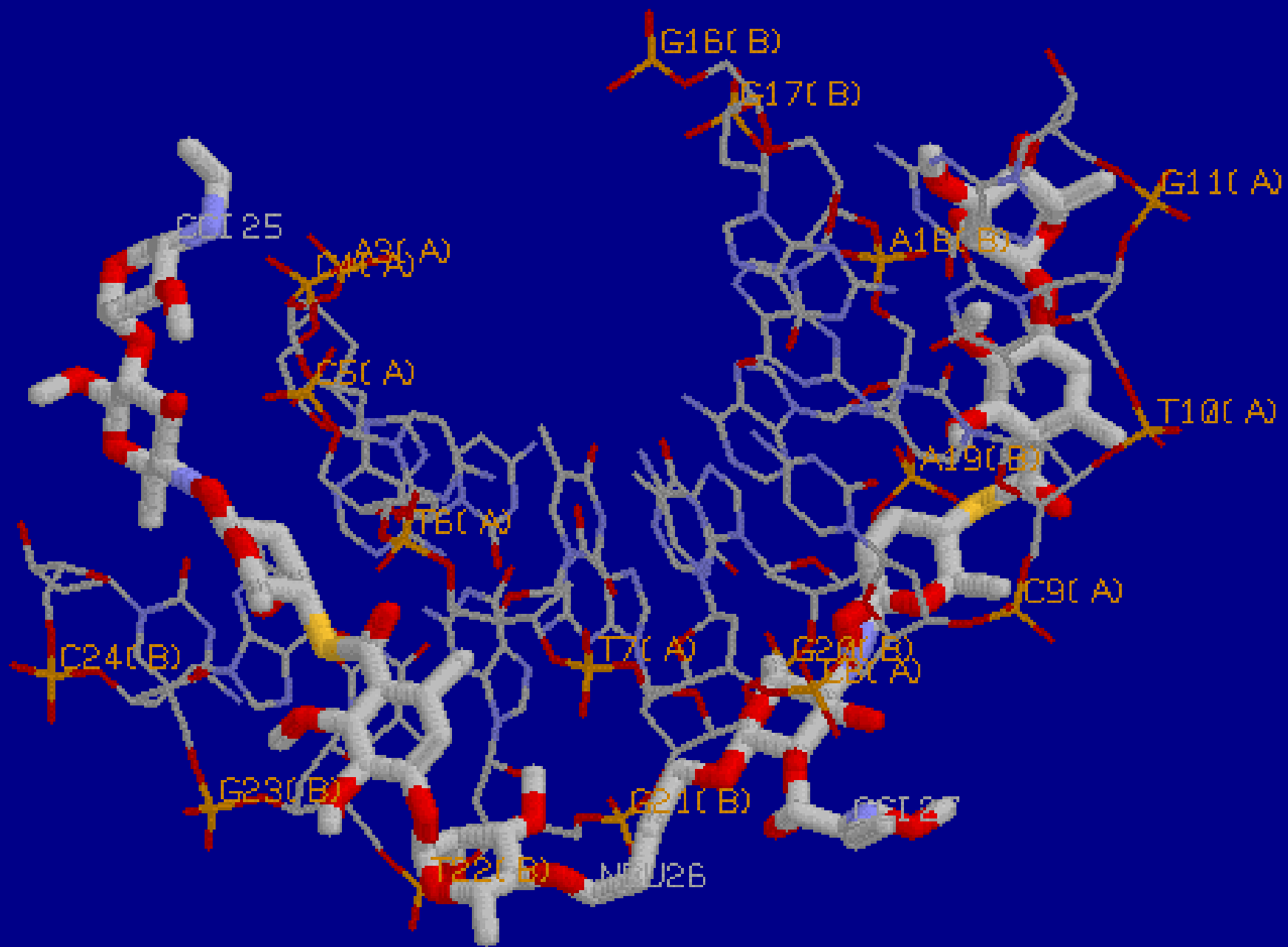


FIG. 1 Structures of naturally occurring enediyne anticancer antibiotics: **1**, dynemicin A; **2**, calicheamycin  $\gamma_1$ ; **3**, esperamicin A<sub>1</sub>; **4**, neocarzinostatin chromophore.





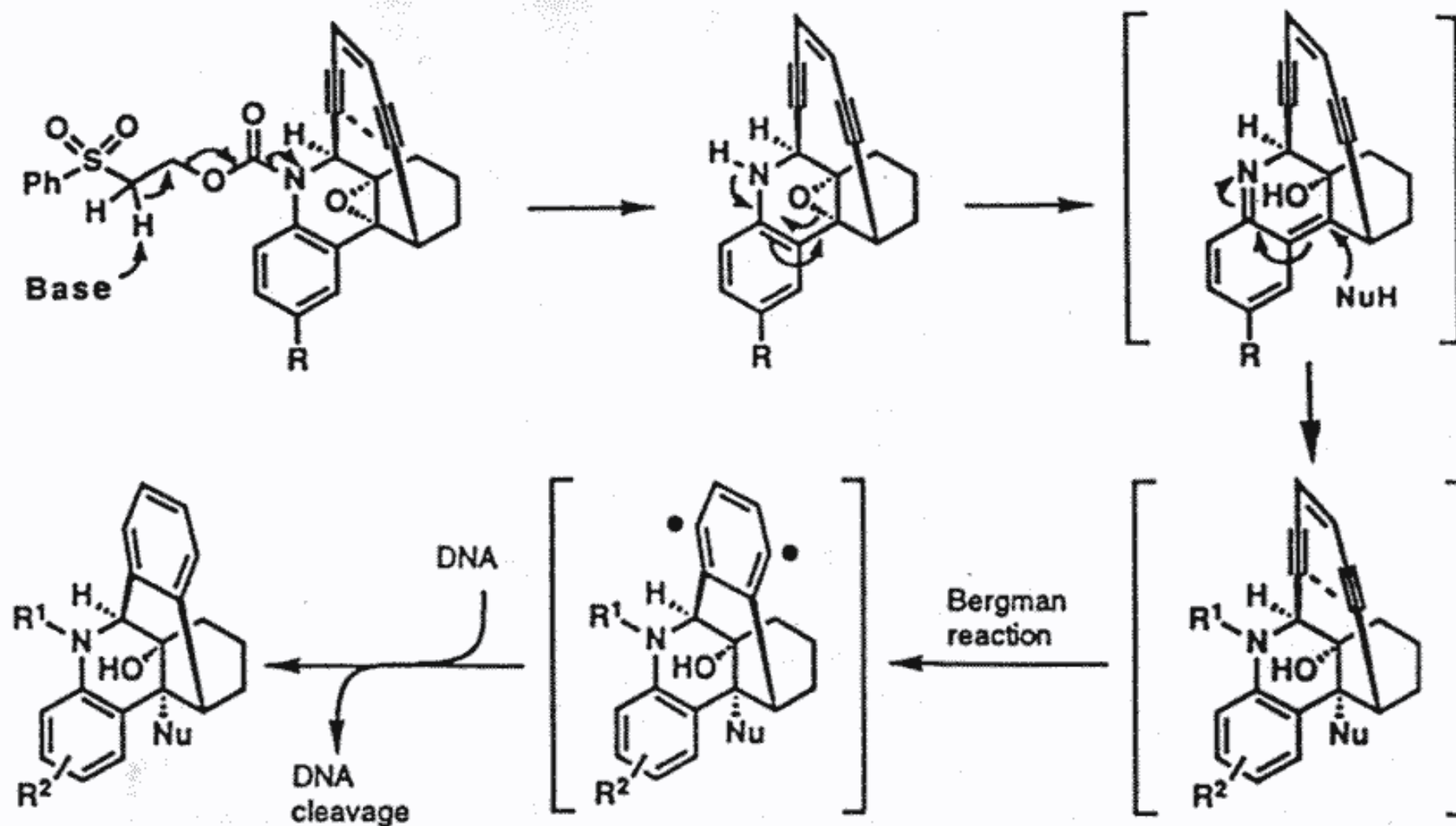
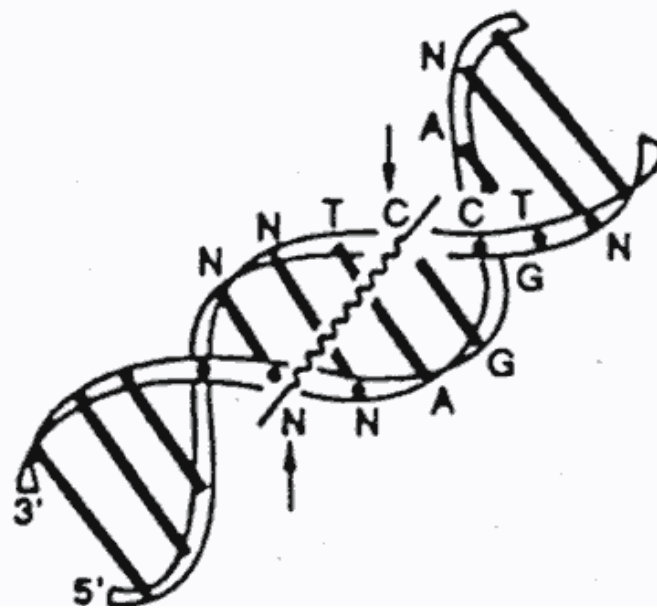


FIG. 3. Eneidyne design, synthesis and biological action.



CYTOTOXICITIES OF DESIGNED ENEDIYNE 7 AGAINST 19 TUMOUR CELL LINES (TOP) AND FOUR NORMAL CELL LINES (BOTTOM)

Cell type	Cell line	IC <sub>50</sub> (M)	Cell type	Cell line	IC <sub>50</sub> (M)
Melanoma	SK-Mel-28	$3.1 \times 10^{-6}$	Lung carcinoma	UCLA P-3	$9.8 \times 10^{-8}$
Melanoma	M-14	$1.6 \times 10^{-6}$	Pancreatic carcinoma	Capan-1	$3.1 \times 10^{-9}$
Melanoma	M-21	$1.6 \times 10^{-6}$	T-cell leukaemia	TCAF	$1.1 \times 10^{-9}$
Colon carcinoma	HT-29	$1.6 \times 10^{-6}$	Multidrug resistant T-cell leukaemia	TCAF-DAX	$1.7 \times 10^{-9}$
Ovarian carcinoma	Ovcar-3	$7.8 \times 10^{-7}$	Myeloma	RPMI-8226	$7.7 \times 10^{-9}$
Astrocytoma	U-87 UG	$7.8 \times 10^{-7}$	Mouse leukaemia	P-388	$4.6 \times 10^{-9}$
Glioblastoma	U-251 MG	$3.9 \times 10^{-7}$	Mouse leukaemia	L-1210	$1.3 \times 10^{-9}$
Breast carcinoma	MCF-7	$7.8 \times 10^{-7}$	Promyelocytic leukaemia	HL-60	$3.6 \times 10^{-11}$
Lung carcinoma	H-358	$2.0 \times 10^{-7}$	T-cell leukaemia	Molt-4	$2.0 \times 10^{-14}$
Lung carcinoma	H-522	$9.8 \times 10^{-8}$			
Bone marrow	HNBM	$5.0 \times 10^{-5}$	Normal human dermal fibroblast	NHDF	$5.0 \times 10^{-6}$
Human mammary epithelial cells	HMEC	$6.3 \times 10^{-6}$	Chinese hamster ovary	CHO	$3.1 \times 10^{-6}$

