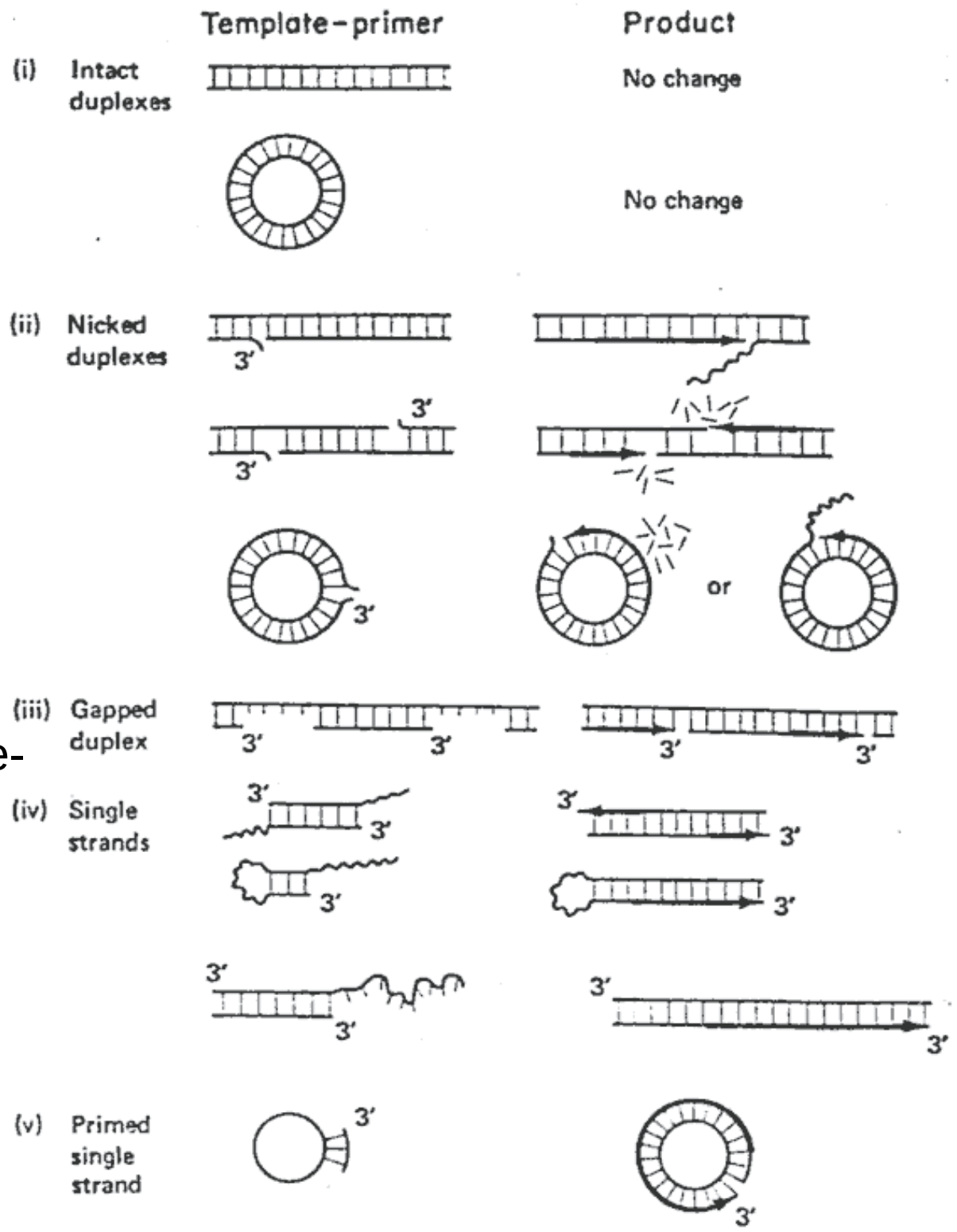
DNA-Polymerases

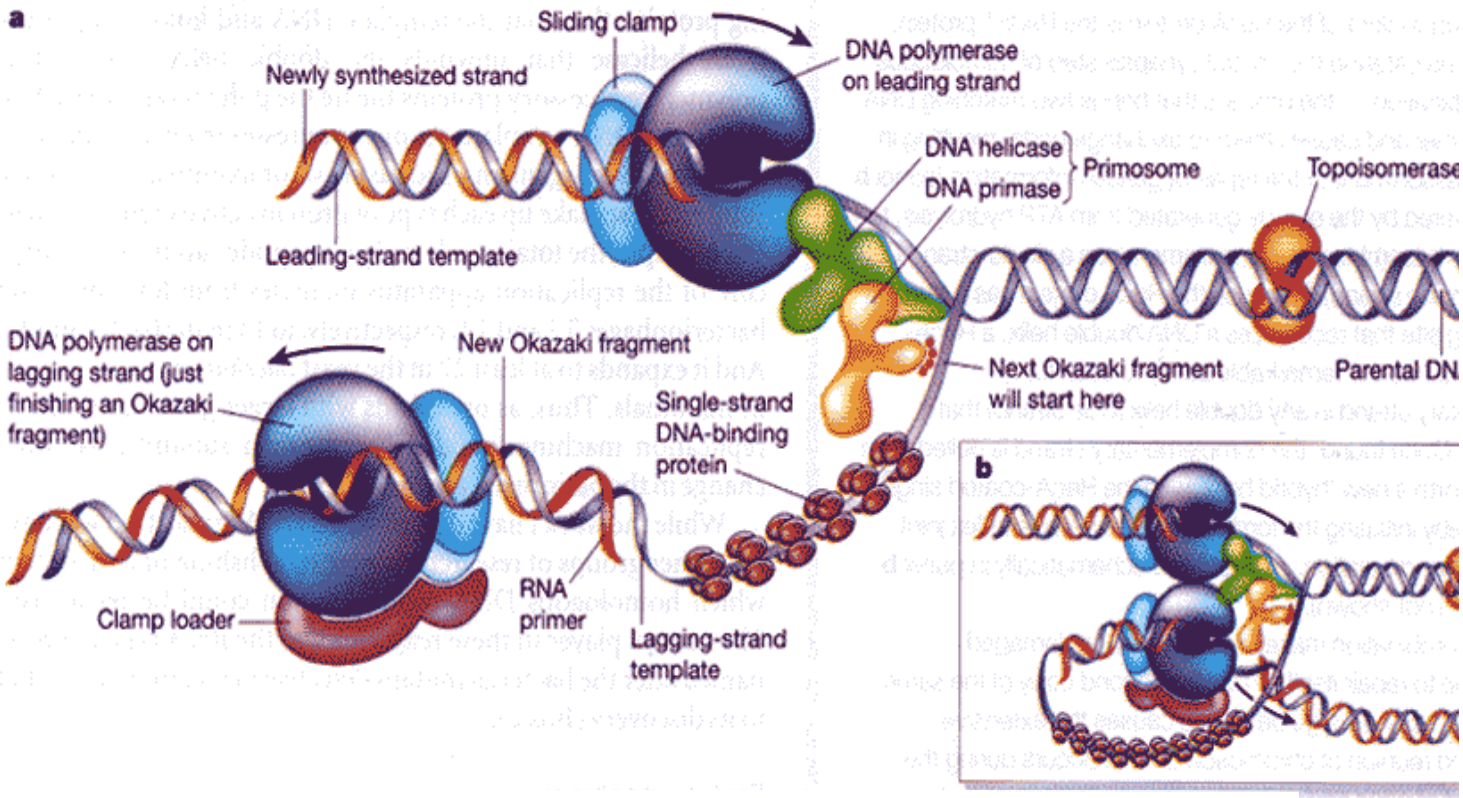
Involved in
Replication, transcription
DNA repair

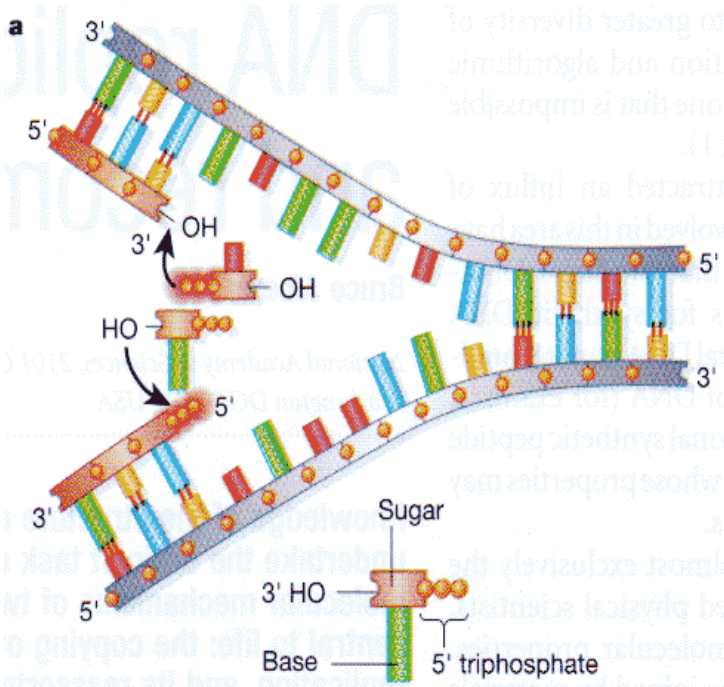
Requirements for activity

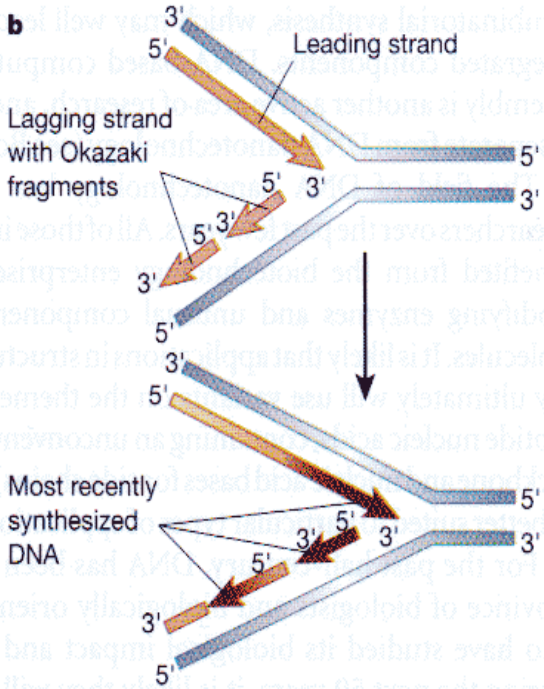
Primer
Template
Substrates (deoxynucleoside-triphosphates)
Magnesium ions

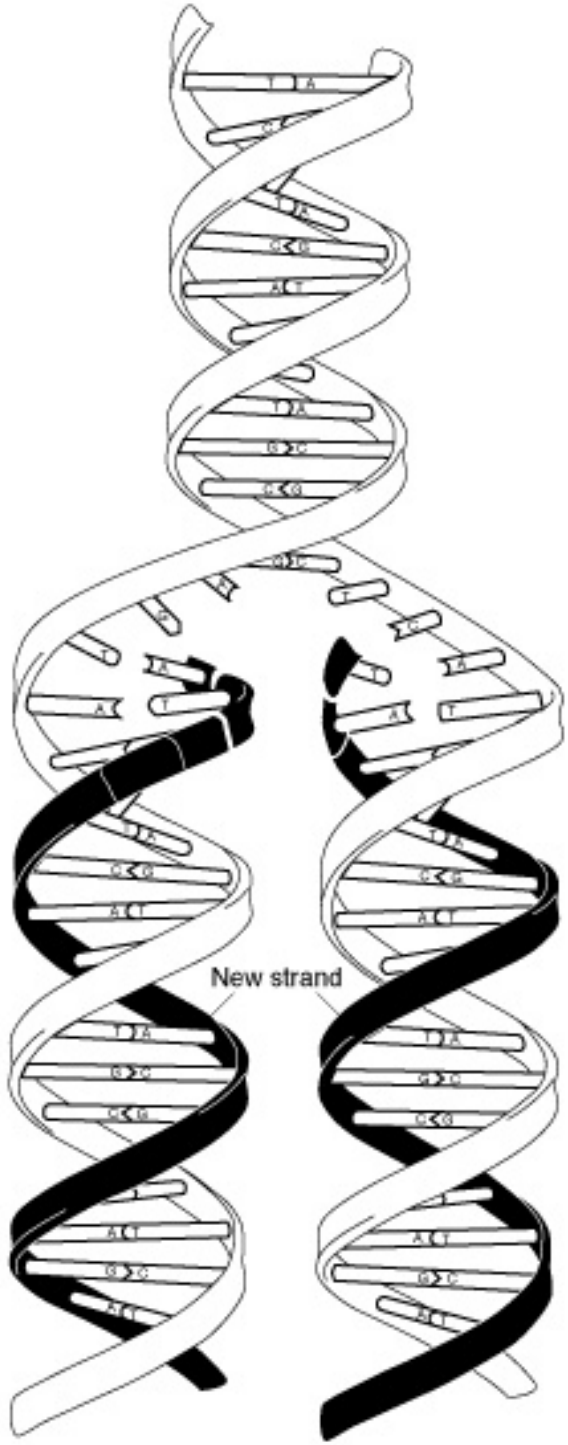
Direction 5´ - 3´
Pyrophosphorolysis

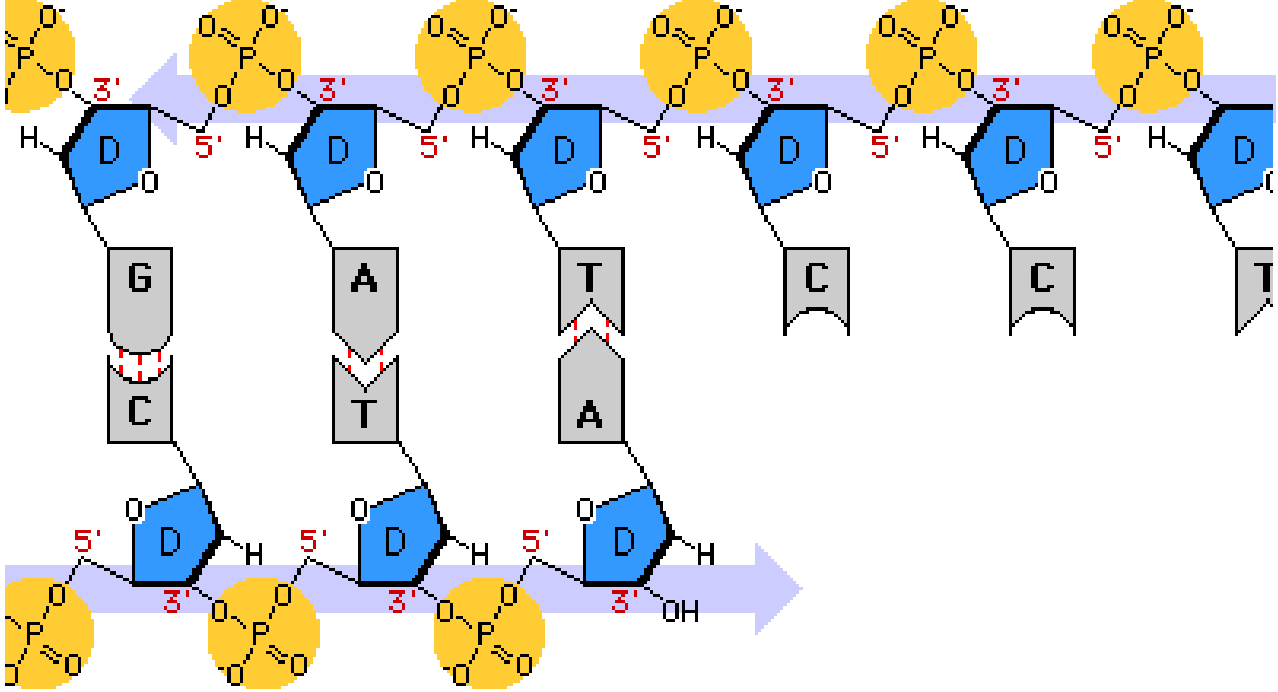


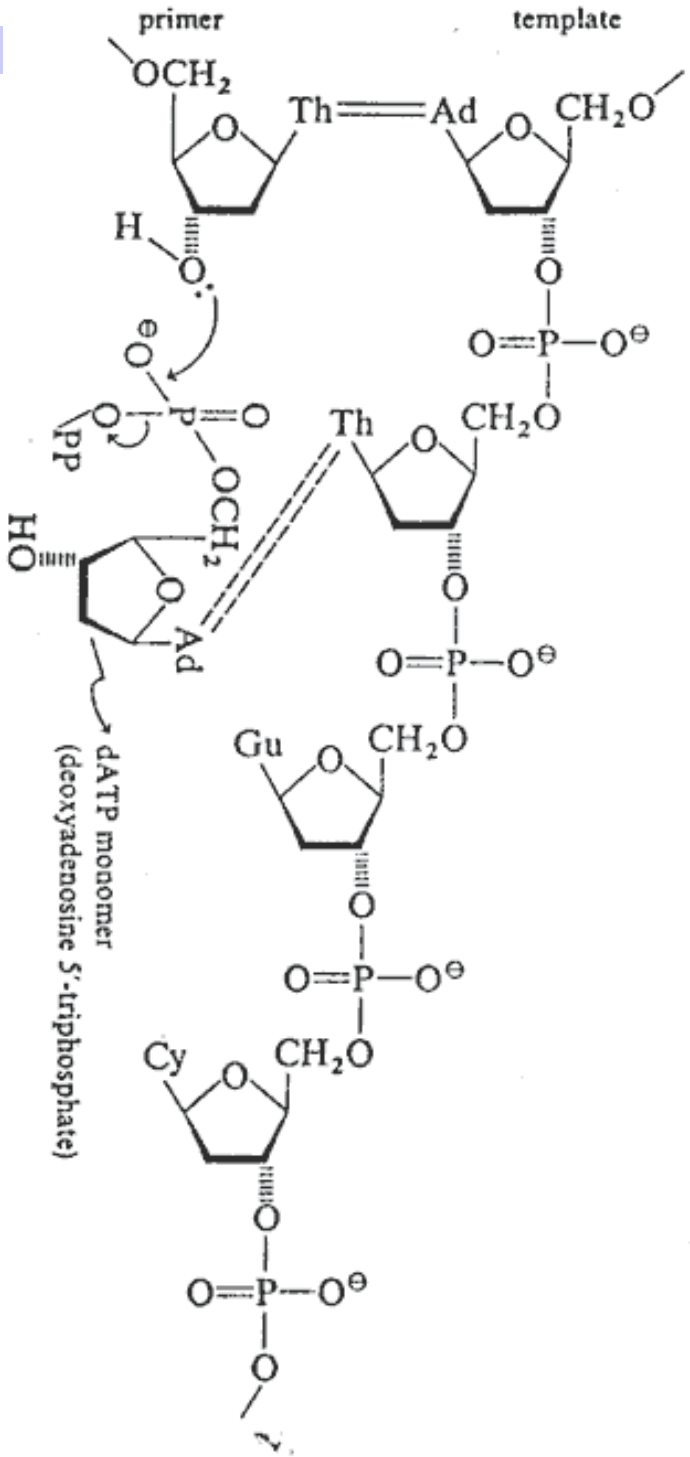

a
Sliding clamp
DNA polymerase on leading strand
Newly synthesized strand
DNA helicase
DNA primase
Primosome
Topoisomerase
Leading-strand template
DNA polymerase on lagging strand (just finishing an Okazaki fragment)
New Okazaki fragment
Single-strand DNA-binding protein
Next Okazaki fragment will start here
Parental DN
Clamp loader
RNA primer
Lagging-strand template
b


a
3'
5'
OH
3'
OH
HO
5'
5'
3'
3'
5'
Sugar
3' HO
Base
5' triphosphate


b
3'
5'
Leading strand
Lagging strand with Okazaki fragments
5'
3'
5'
3'
5'
3'
5'
3'
3'
5'
Most recently synthesized DNA
5'
3'
5'
3'
5'
3'
3'
5'


New strand


G
A
T
C
C
T
C
T
A
5'
3'
OH

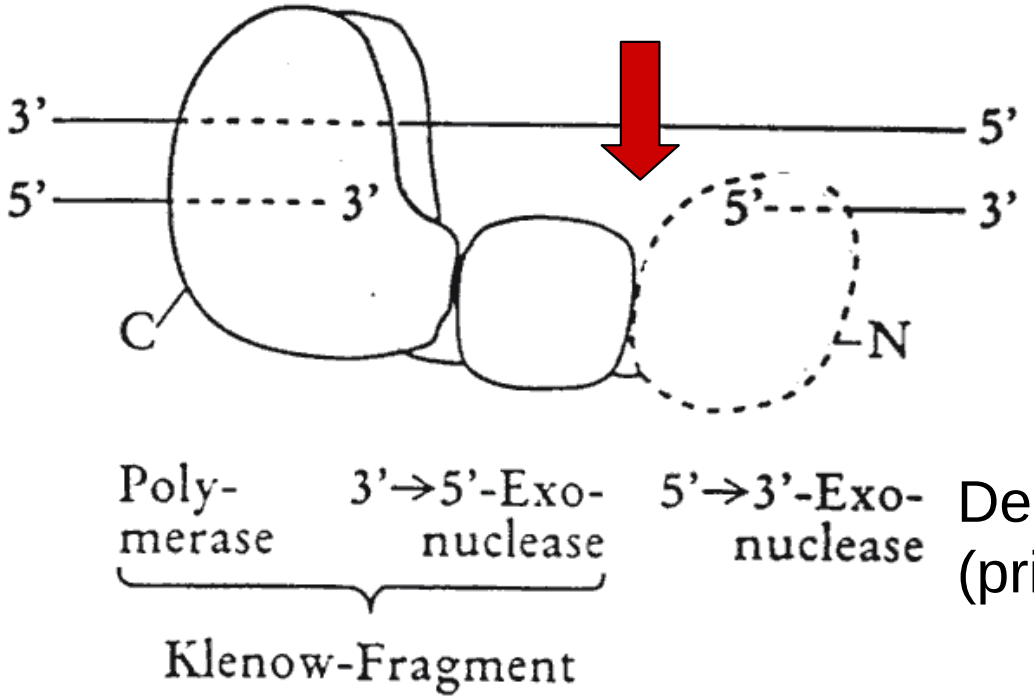

primer
template
dATP monomer
(deoxyadenosine 5'-triphosphate)

DNA-Polymerase I

Escherichia coli
M: 103 kD, 928 Amino acids
400 Molecules / cell
Activity: 670 nucleotides / min
Function: repair and precision of base pairing
(error rate 1: 1.000.000)

(DNA-Polymerase III Replication enzyme: 1000 Nucleotides / s)

Binding sites for template, primer and substrate
additional exonuclease-activity in 3´-5´and 5´-3´direction

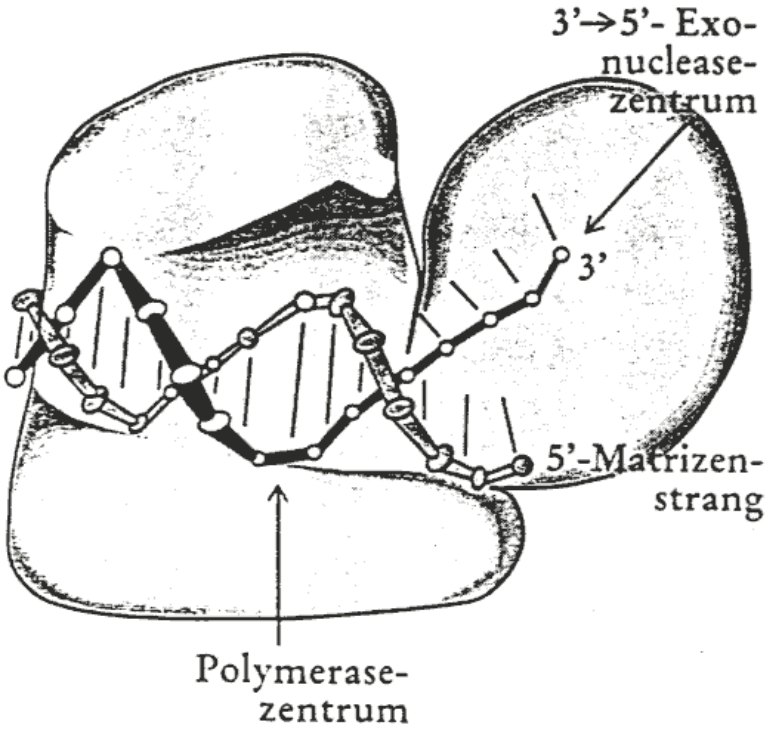


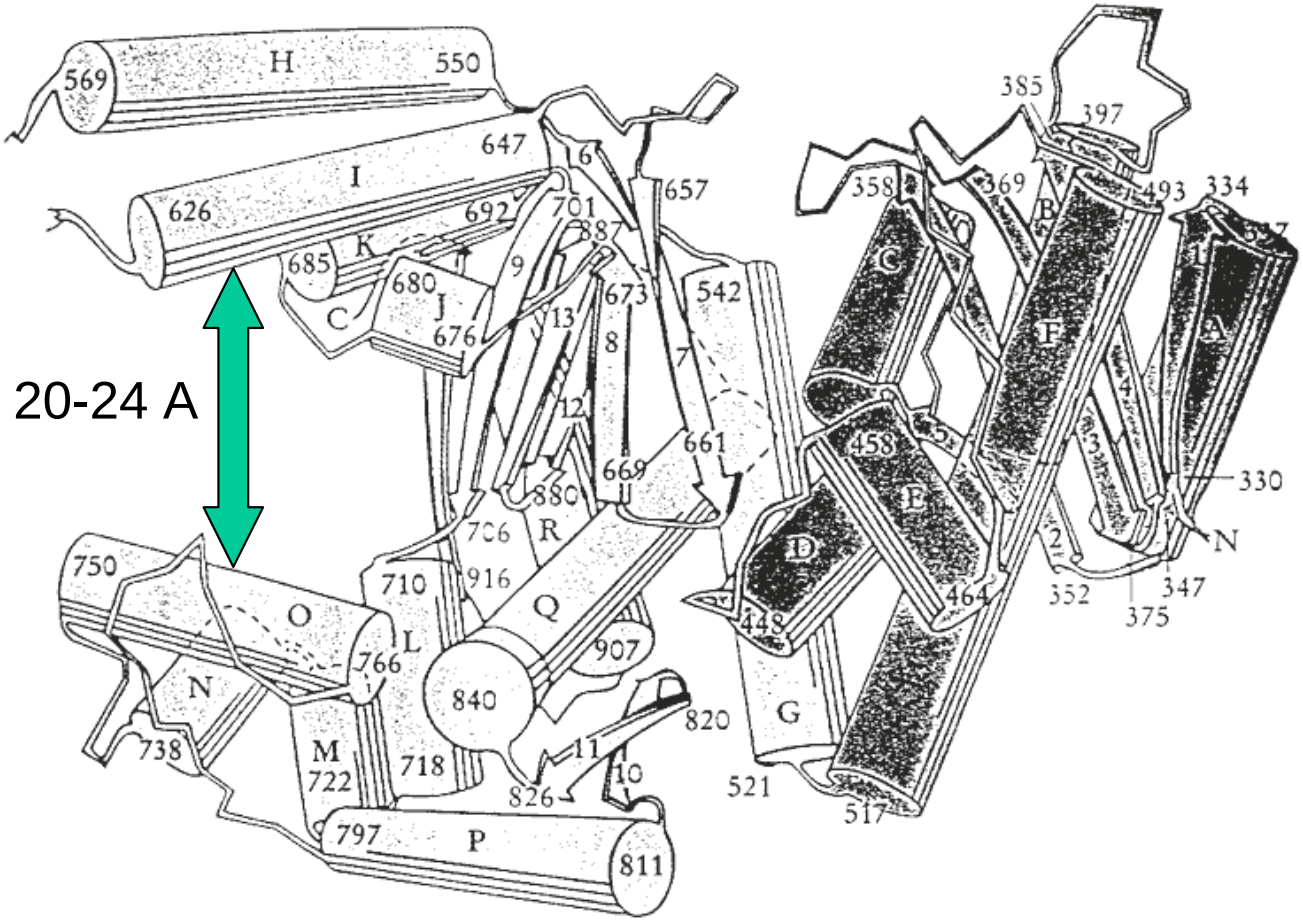
Cleavage by subtilisin

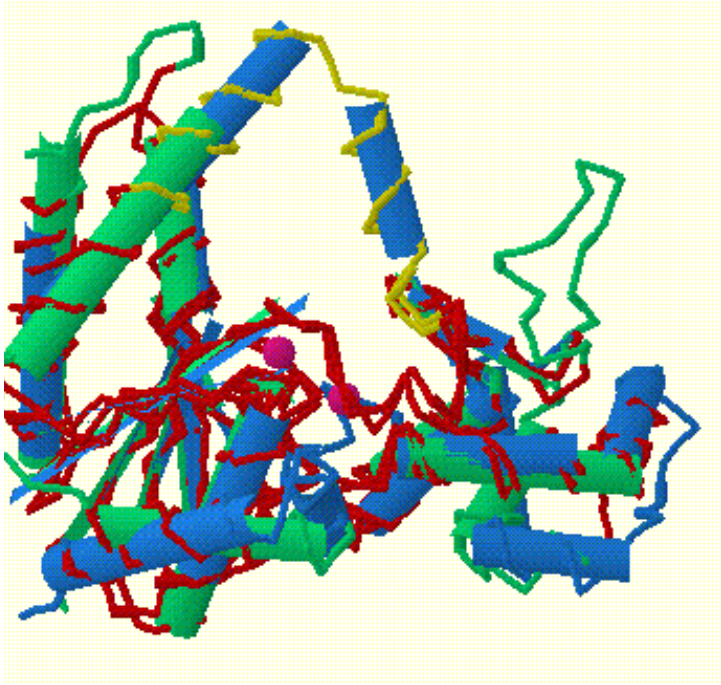
Degradation leads to a single strand (primer)

Crystal structure of Klenow-fragment

605 Amino acids, PDB: 2KZM

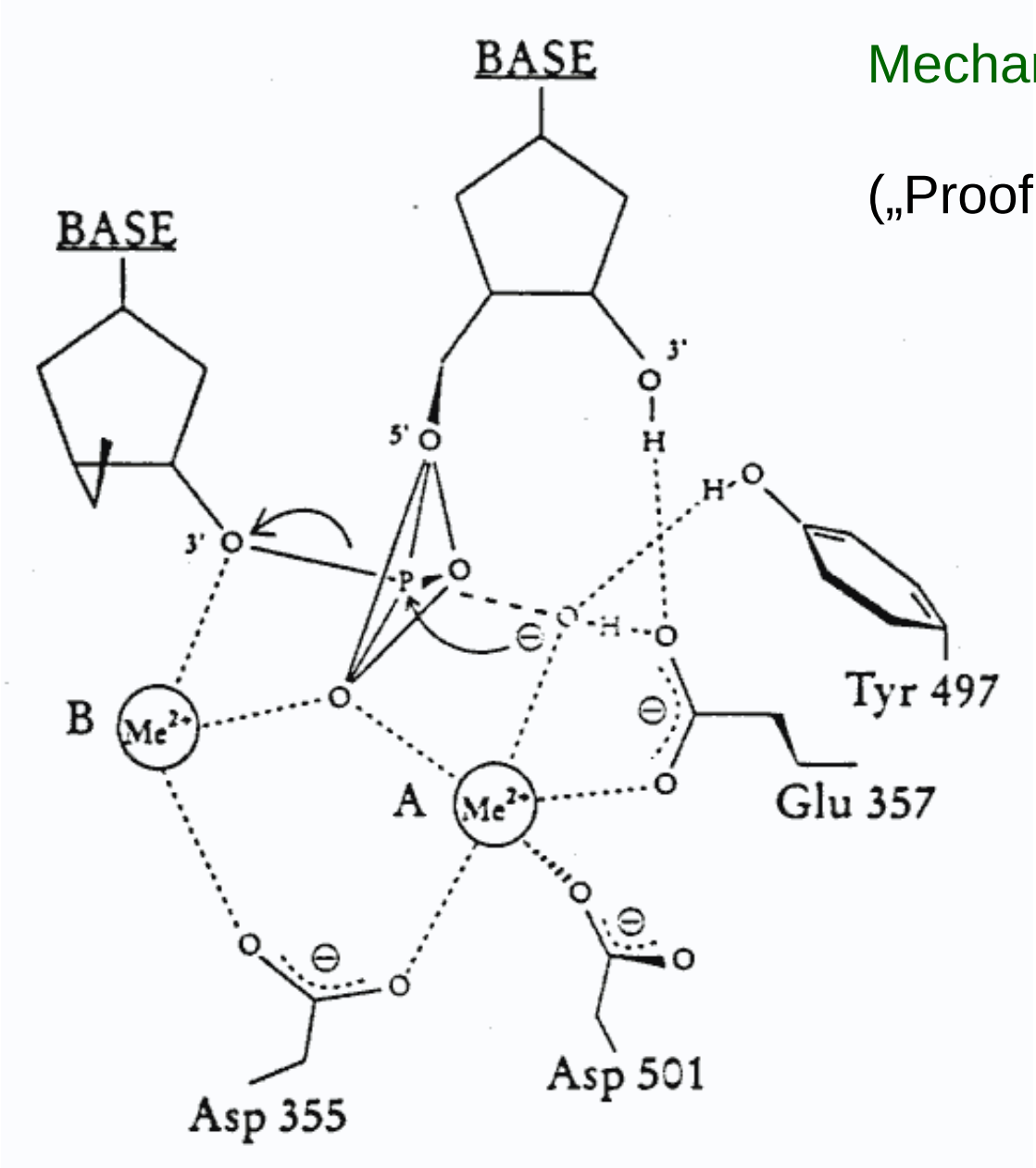






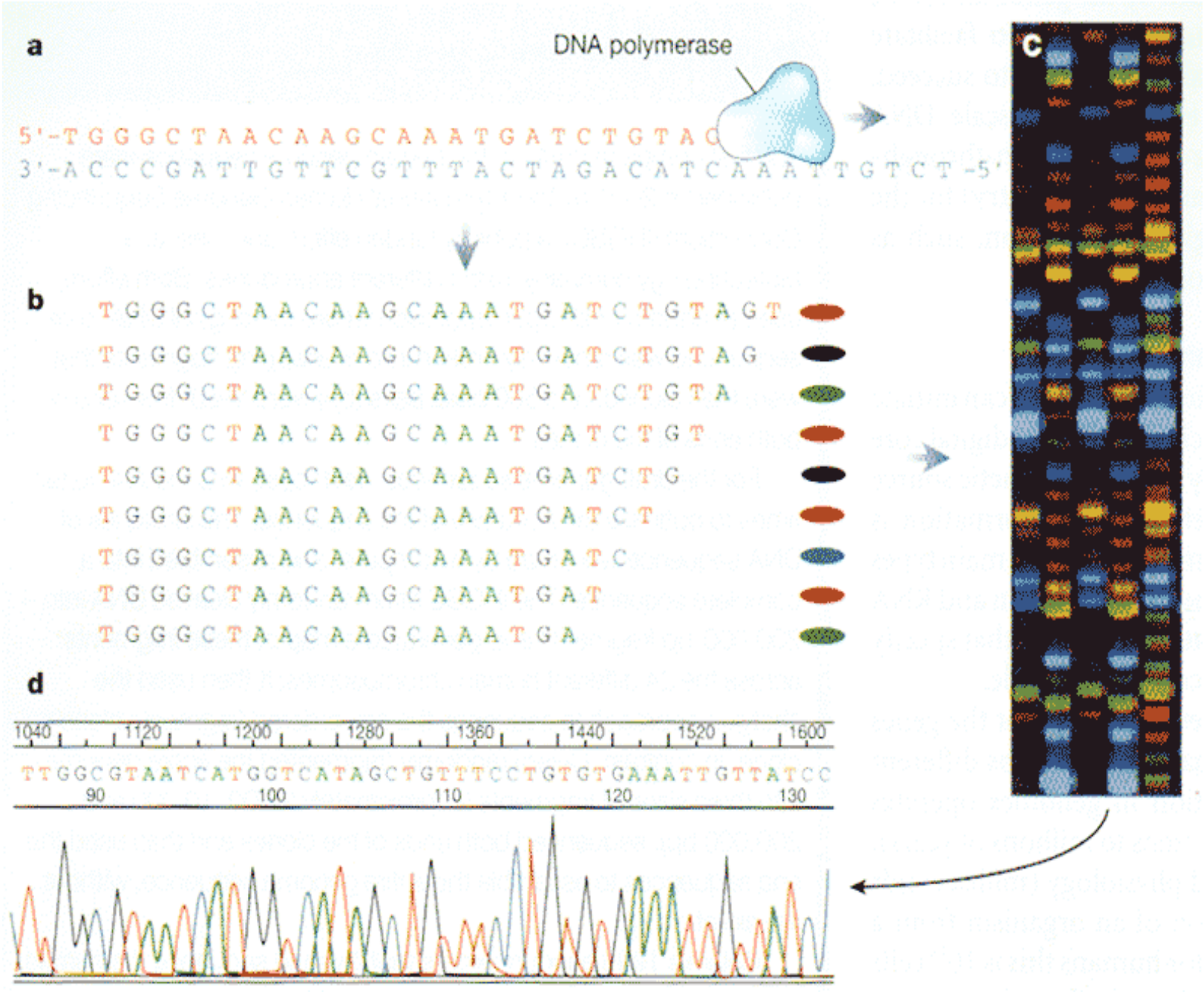
Mechanism of 3´-5´ exonuclease

(„Proofreading“)



Sequence determination using DNA-polymerases

Taq-Polymerase (*Thermophilus aquaticus*)



Chemical oligonucleotide synthesis

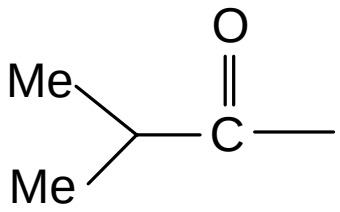
Elucidation of the genetic code
Synthesis of primers
Modification of DNA and RNA
Linker, Adapter for cloning experiments
„Antisense“ oligonucleotide, hybridisation probes for mRNA and cDNA
Gene synthesis

Challenges:
Formation of 3´-5´phosphodiester, protecting groups for other nucleophilic sites (phosphate, base, pentose)
Activation needed for phosphodiester formation –in high yields

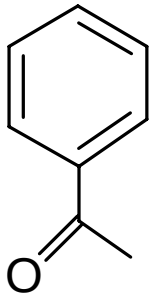
Protecting groups:
Ideally quantitative introduction and cleavage
Stable at various reaction conditions
Solid phase synthesis on polymers

Protecting groups

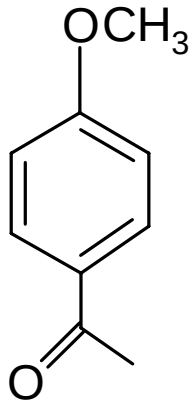
Amino groups of N-bases:



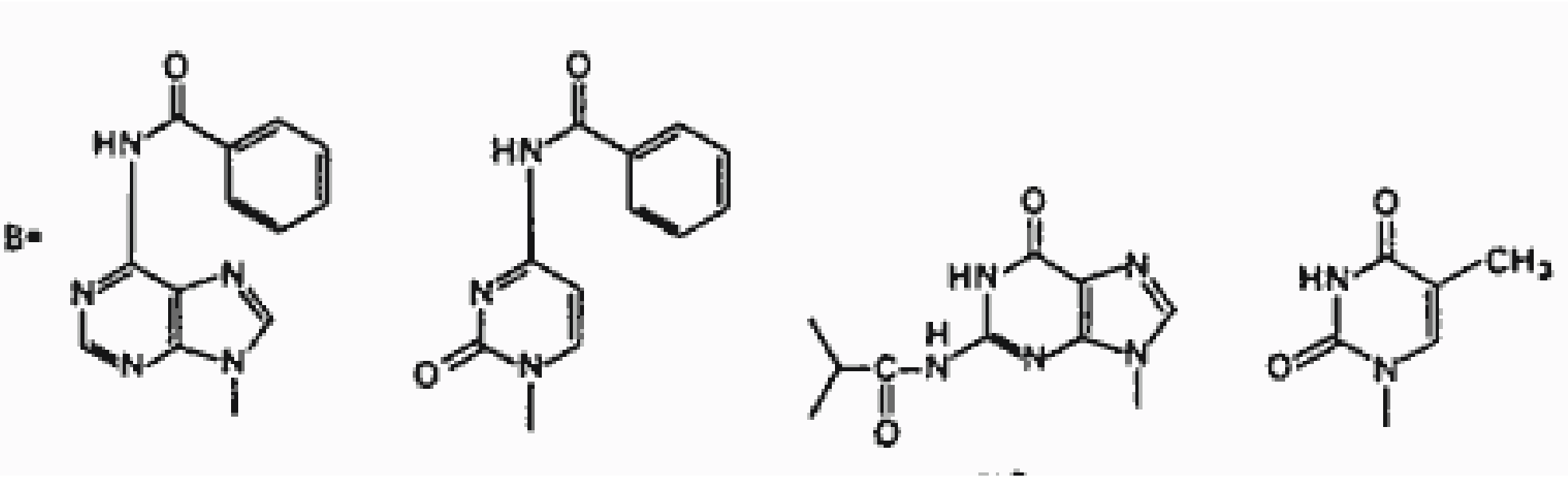
Isobutyroyl



Benzoyl

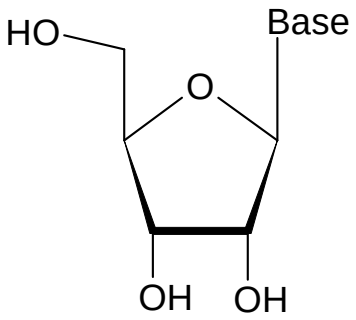


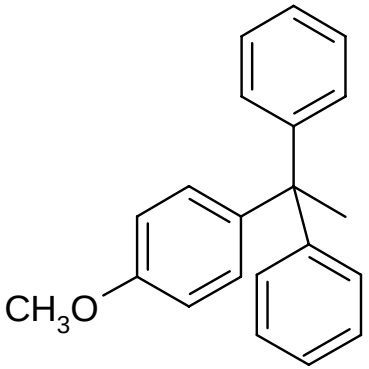
Methoxybenzoyl (Anisoyl)



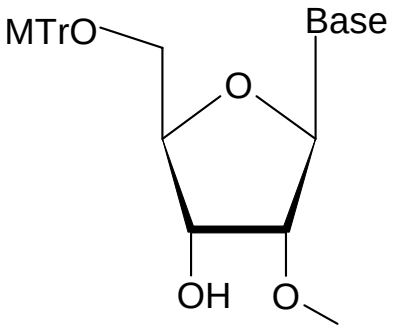
Protecting groups

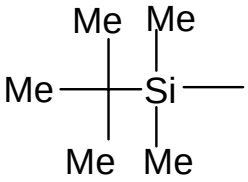
OH-groups of pentoses



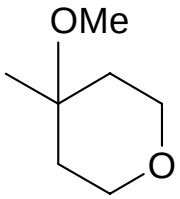


Triphenylmethyl-
(Trityl)
Methoxytrityl-



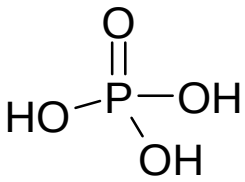


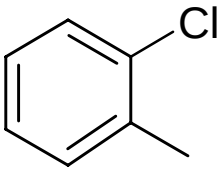
tert-Butyldimethylsilyl
*t*BDMSi



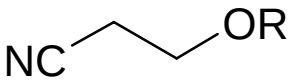
Methoxytetrahydropyranyl

OH-goups of phosphate

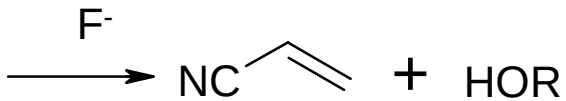


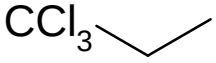


2-Chlorophenyl-



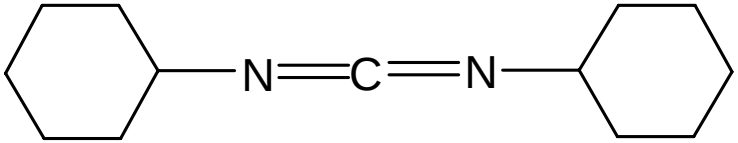
2-Cyanoethyl-



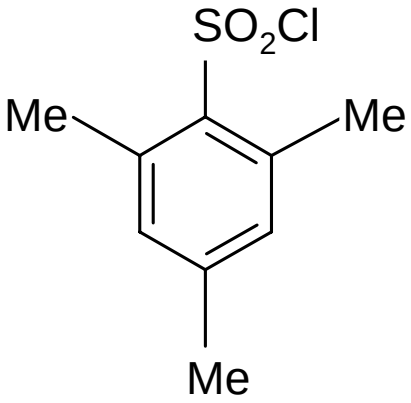


Trichloroethyl-

Coupling

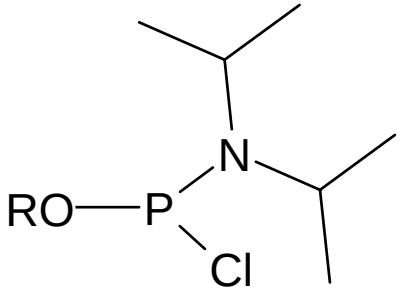


Dicyclohexylcarbodiimide (DCC)

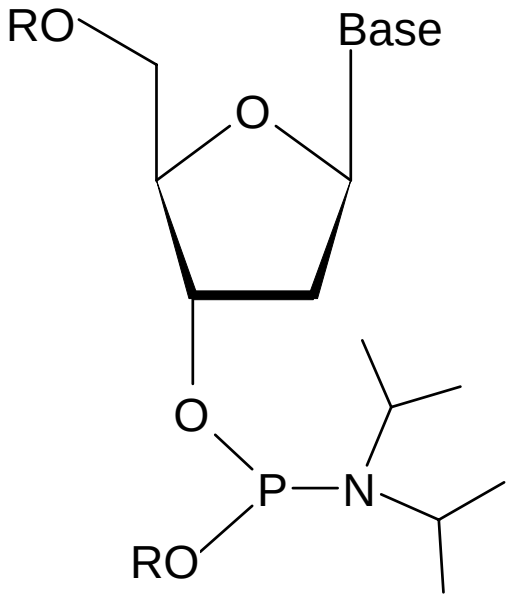


Mesitylene sulfochloride

Activation as phosphoramidite

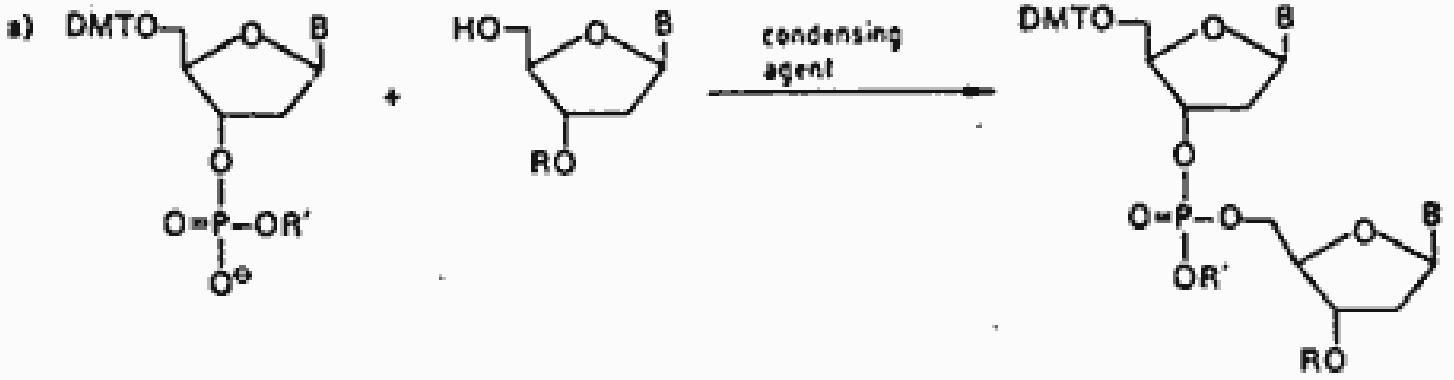


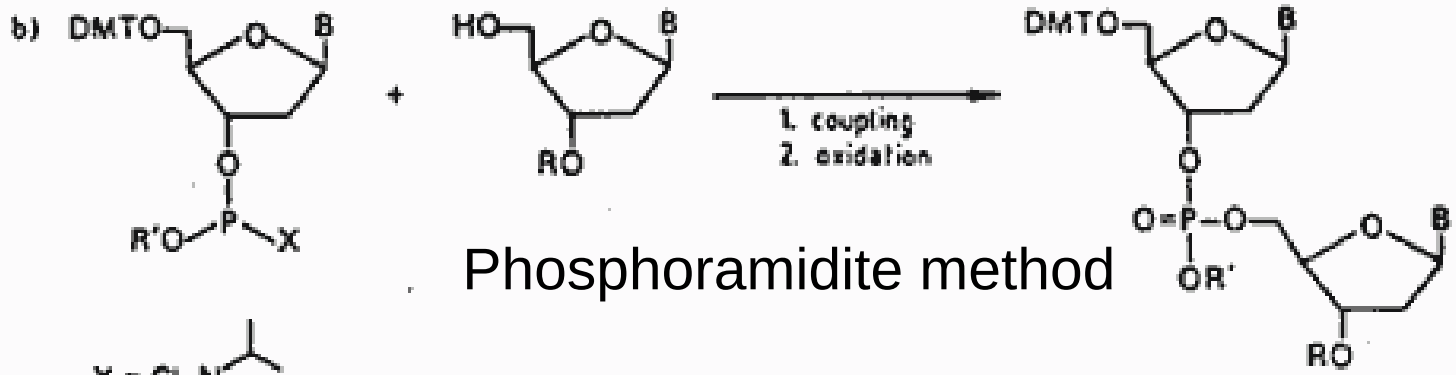
Chloro-N,N-diisopropylphosphoramidite



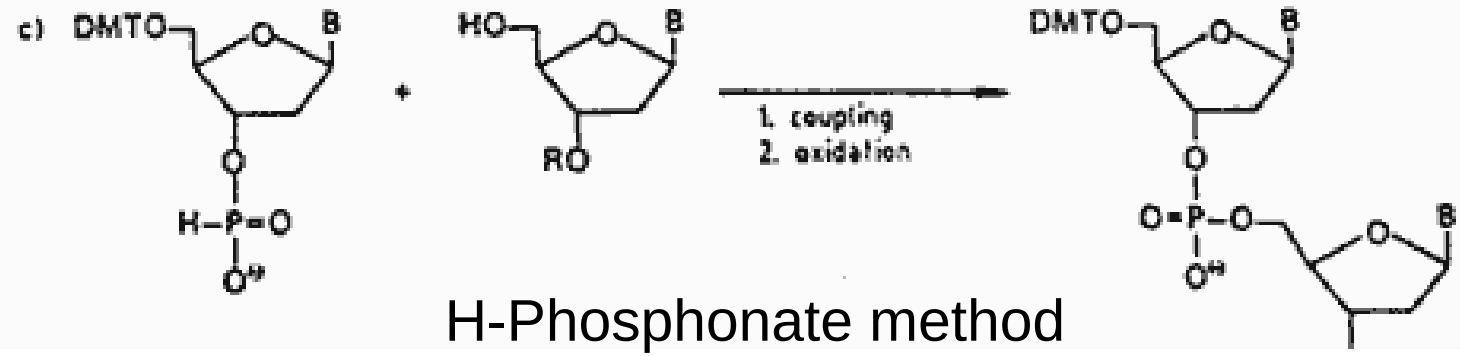
Reversal of the enzymatic reaction: 5´-OH as nucleophile

Phospho triester method





Phosphoramidite method

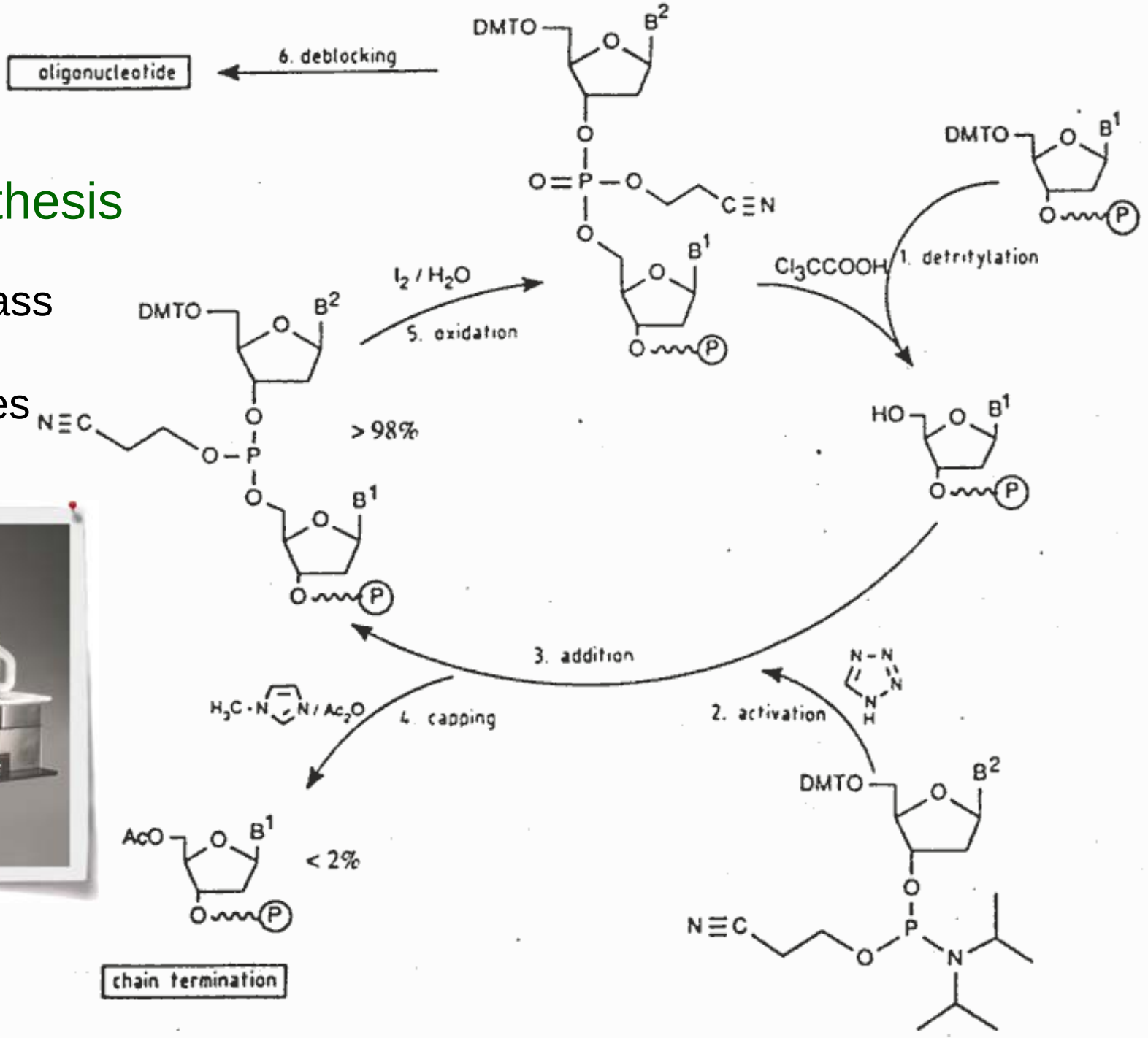


H-Phosphonate method

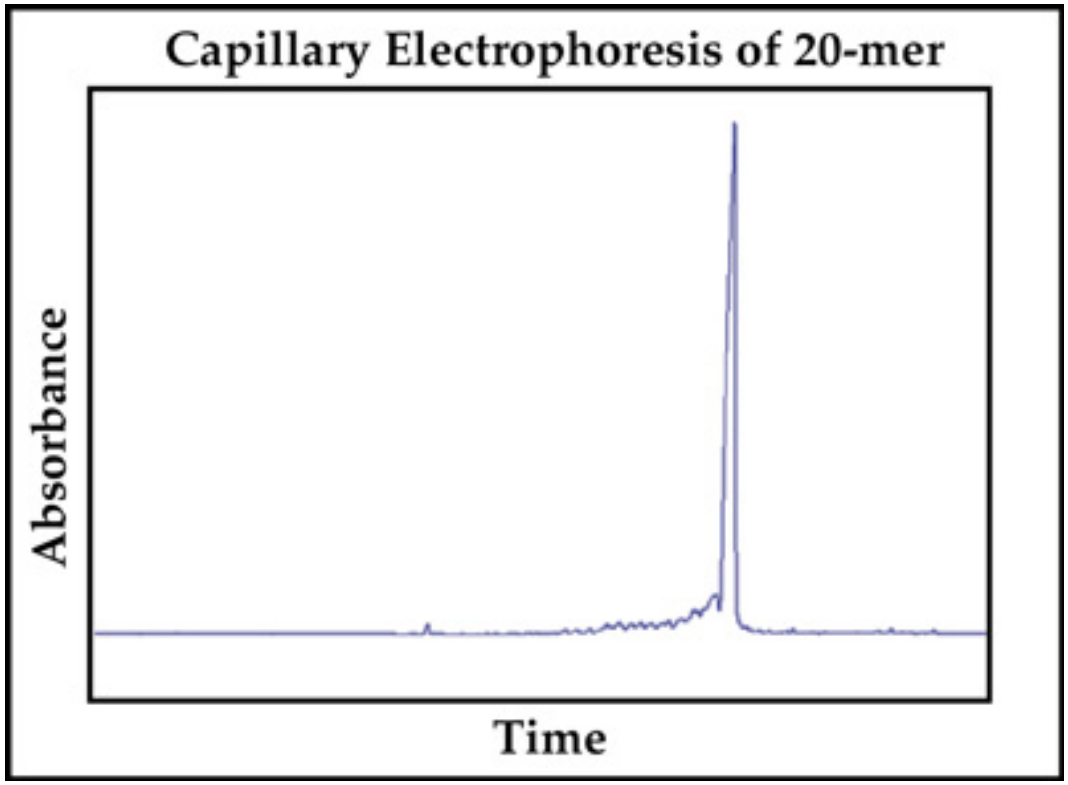
Solid phase synthesis

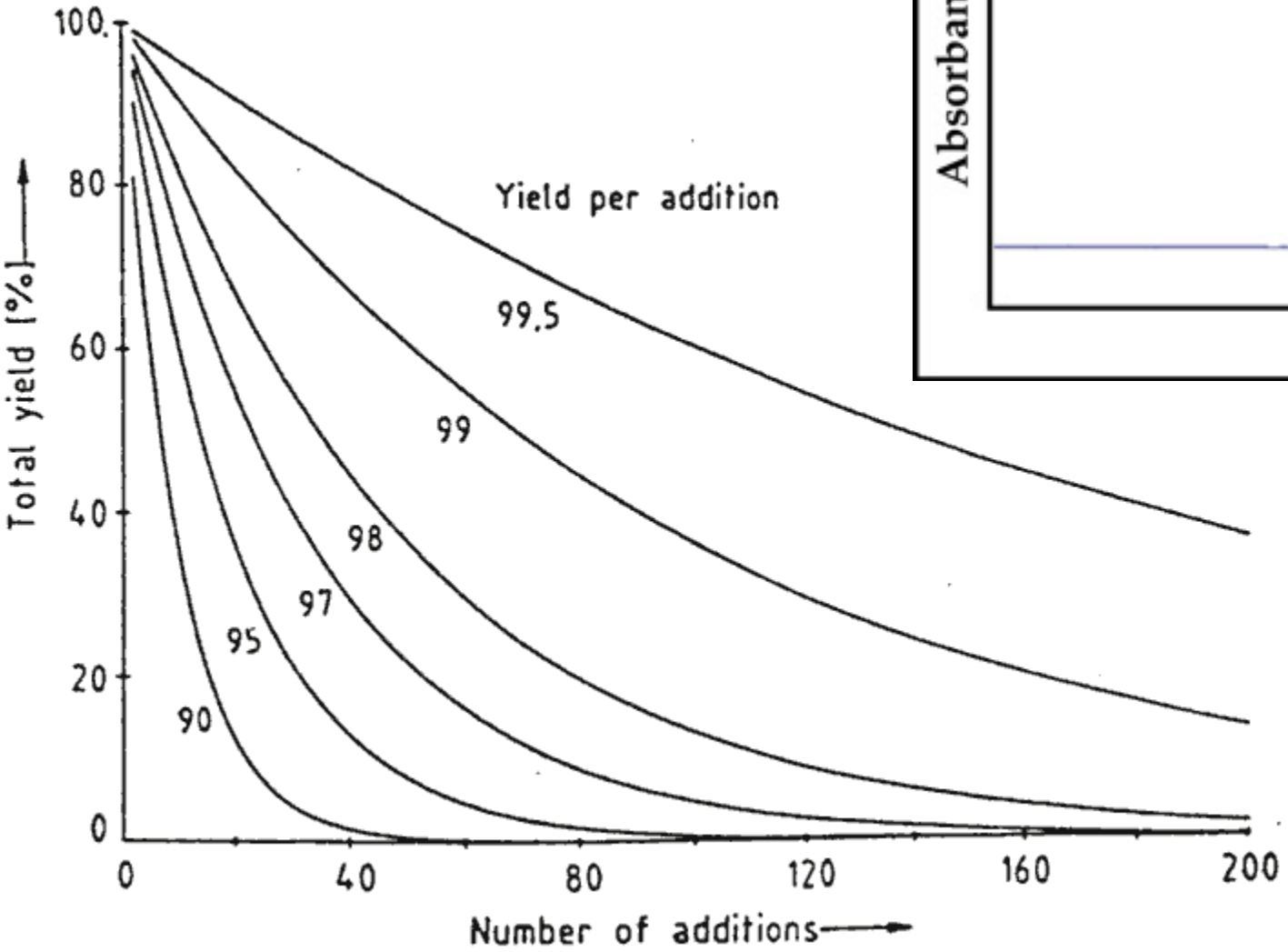
Controlled pore glass
500 A: ~ 80 bases
1000 A: ~200 bases

PolyPlex

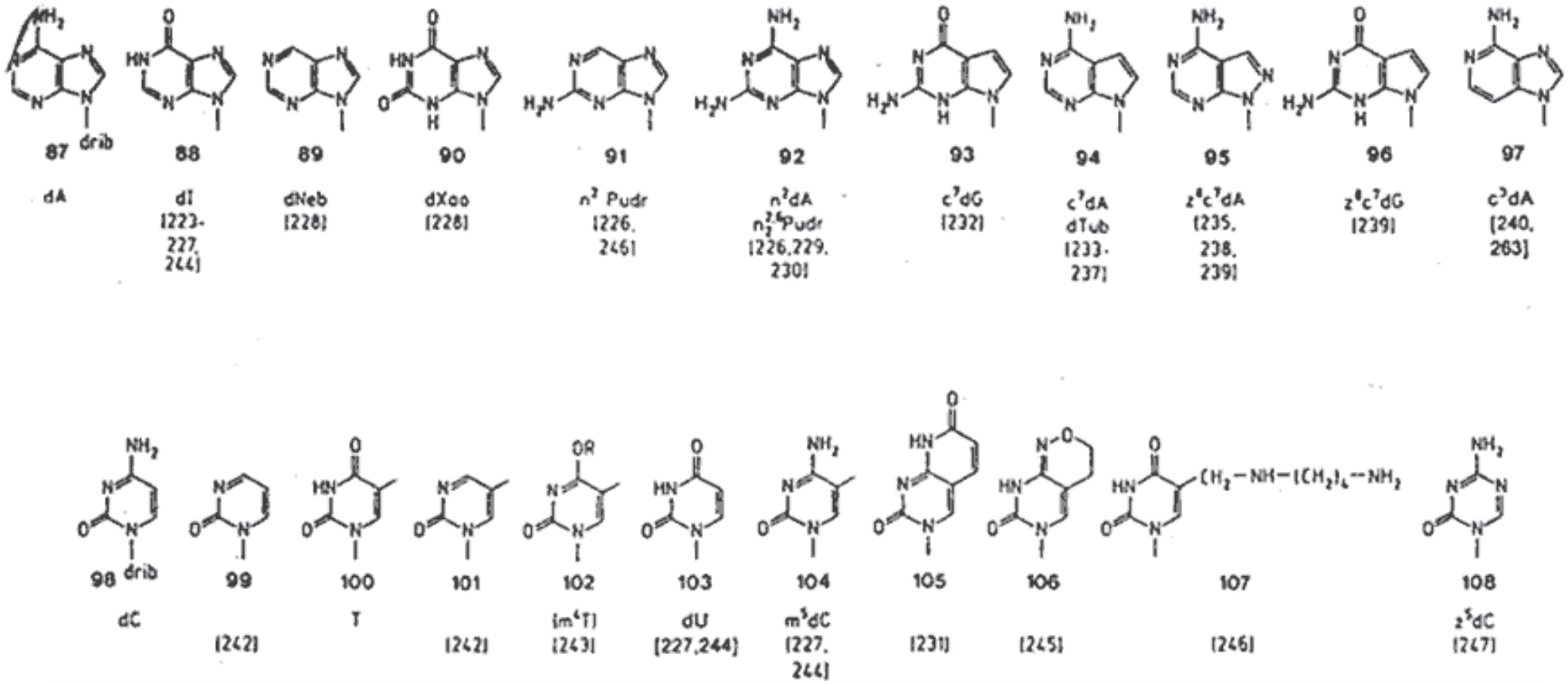


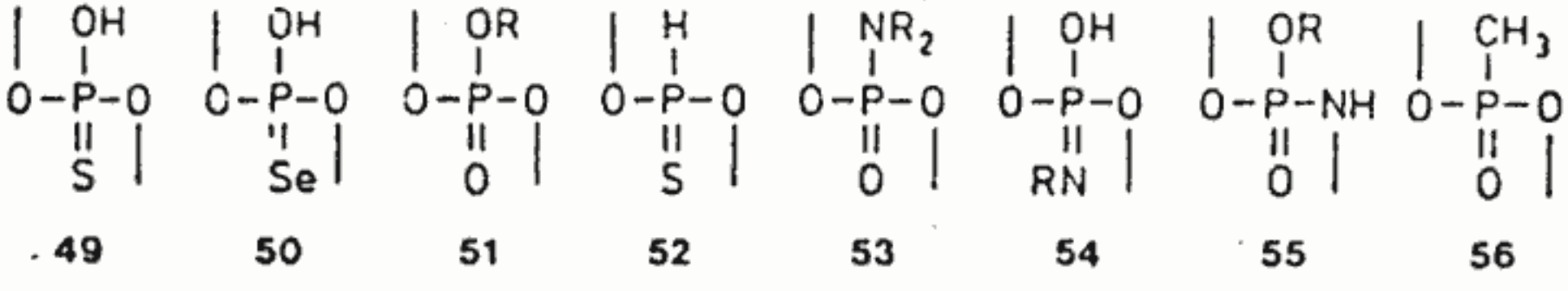
Total yield and purity of products in multistep syntheses





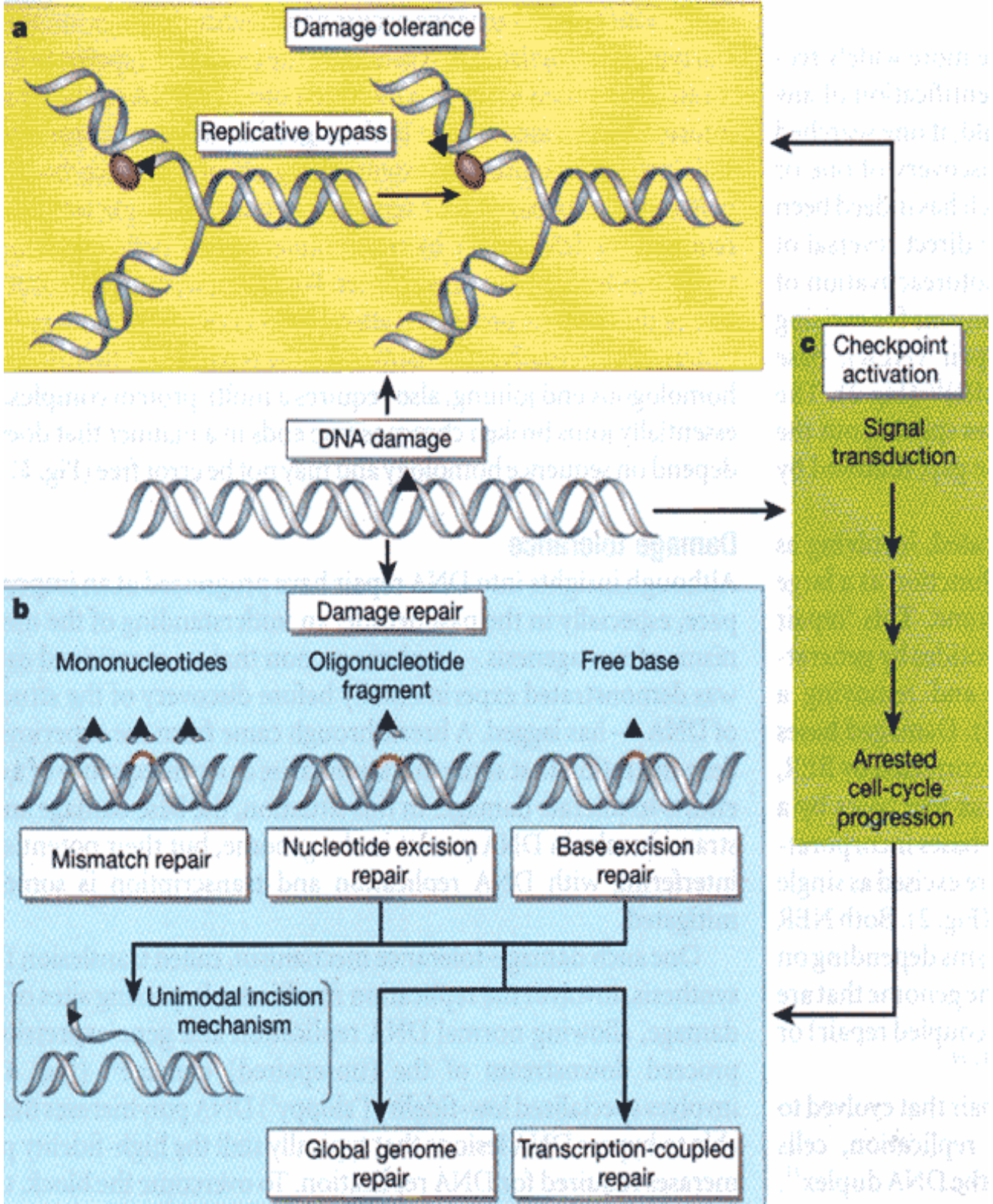
Modified nucleotides



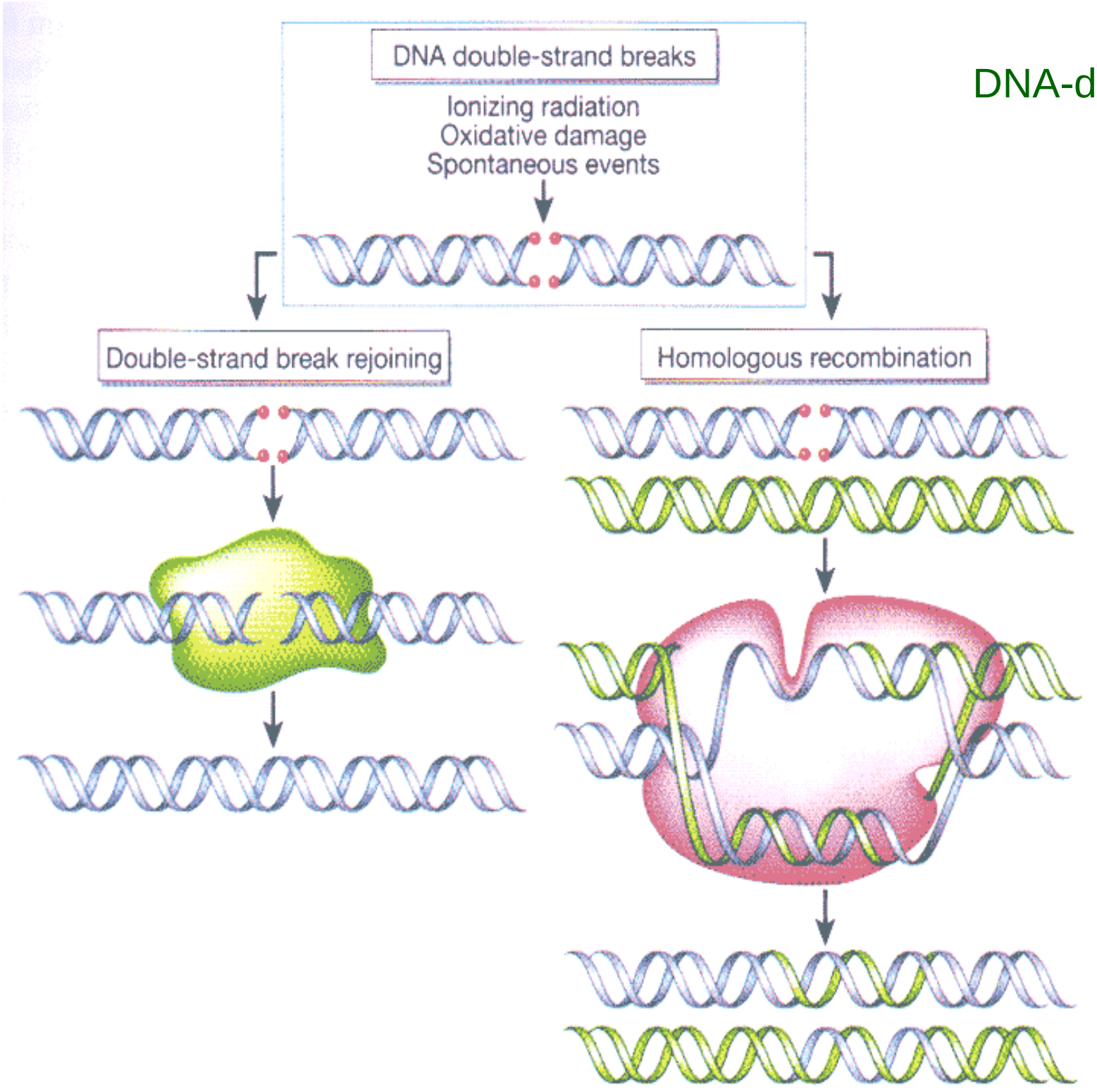


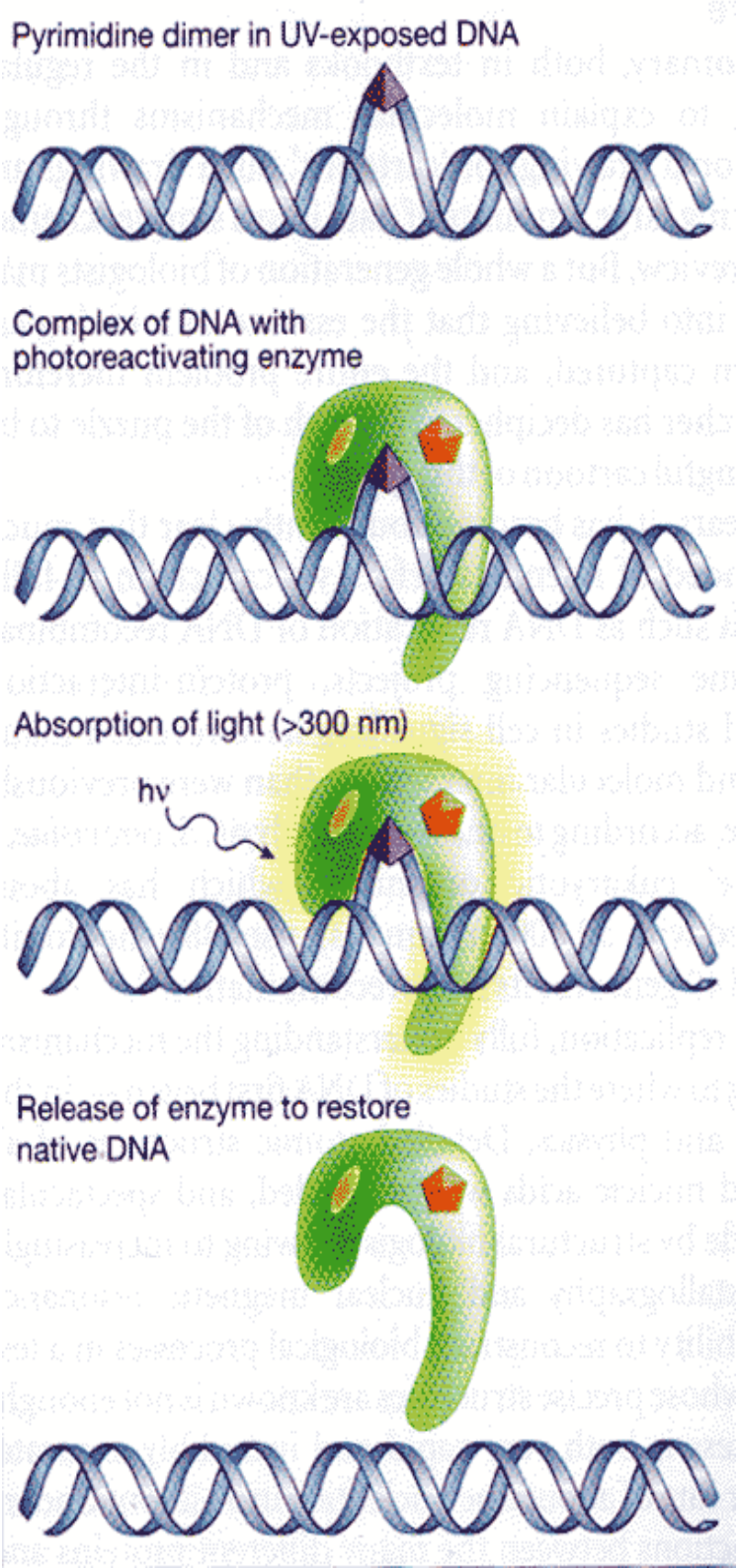
DNA-damage

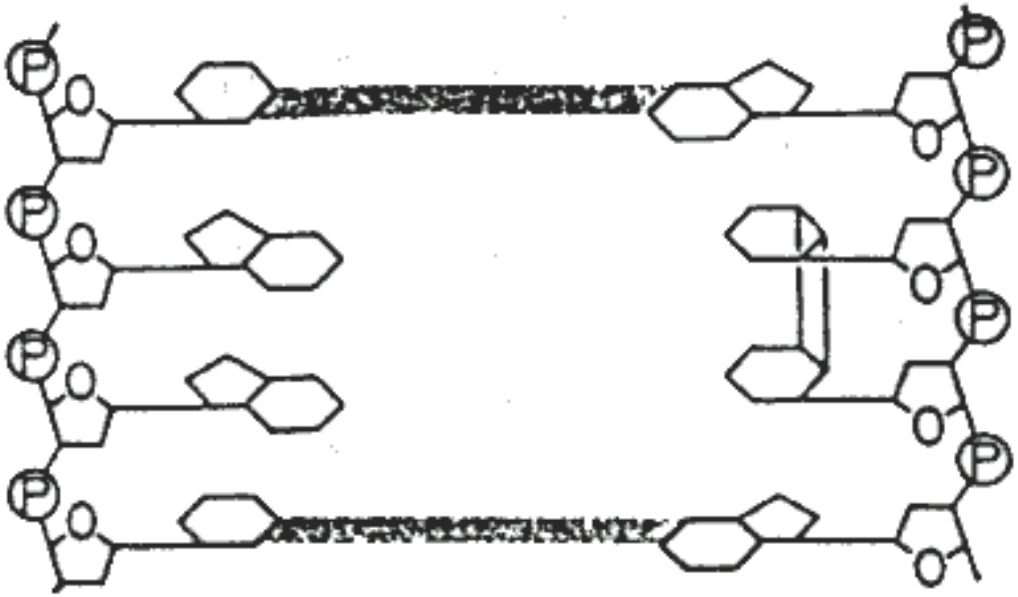
Repair mechanisms

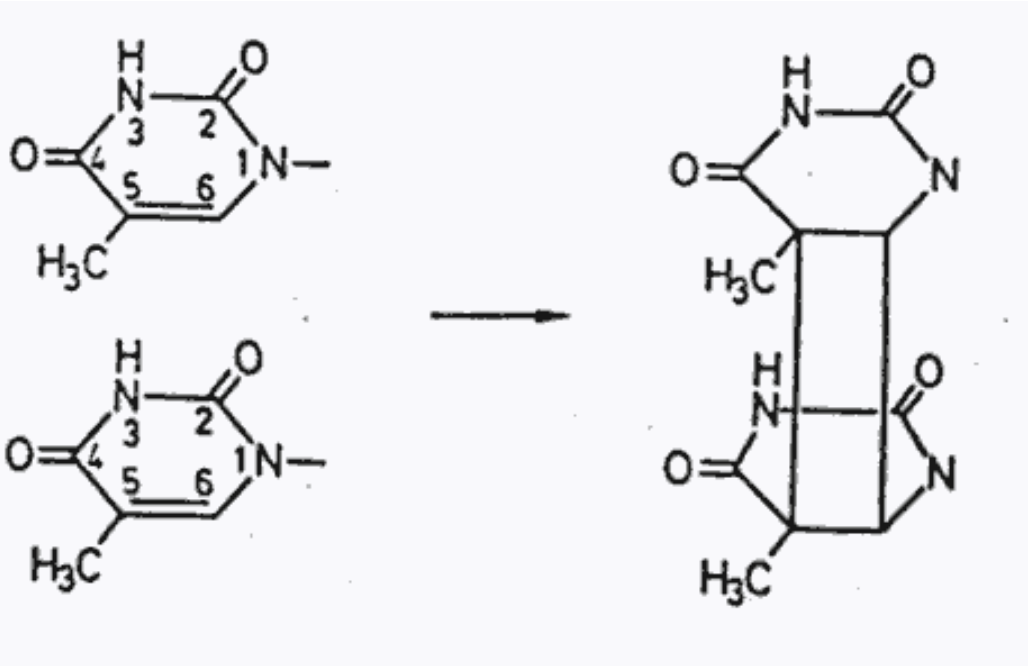


DNA-damage


DNA double-strand breaks
Ionizing radiation
Oxidative damage
Spontaneous events
Double-strand break rejoining
Homologous recombination

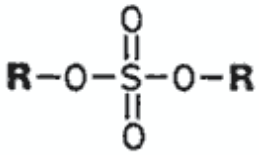

Pyrimidine dimer in UV-exposed DNA
Complex of DNA with photoreactivating enzyme
Absorption of light (>300 nm)
hν
Release of enzyme to restore native DNA



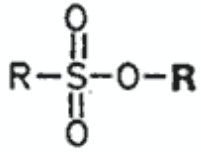

H
N
O
3
2
4
1
N—
5
6
H3C

Mutagenic compounds

Alkyl-Sulfate

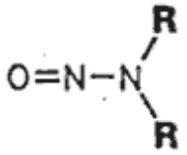


Dialkylsulfat
Beispiel:
Dimethylsulfat

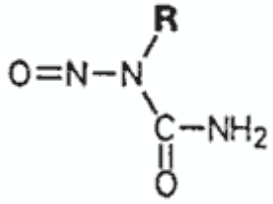


Alkyl-Alkan-Sulfonat
Beispiele: Methylmethan-sulfonat, MMS; Ethyl-methansulfonat, EMS

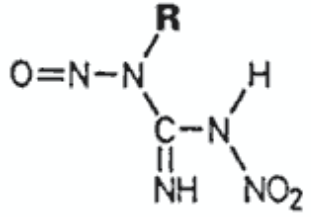
***N*-Nitroso-Verbindungen**



Dialkylnitrosamine
Beispiel:
Dimethylnitrosamin

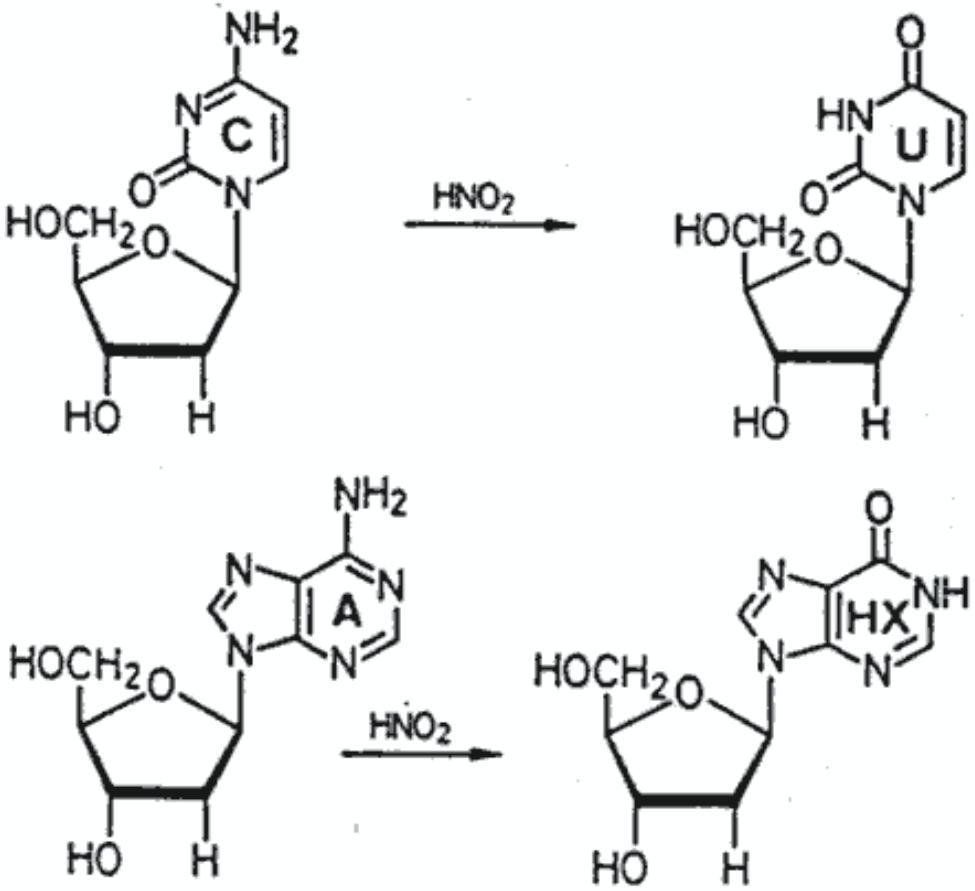


N-Nitrosoharnstoff-Derivate
Beispiel: Methyl-Nitrosoharnstoff, MNN

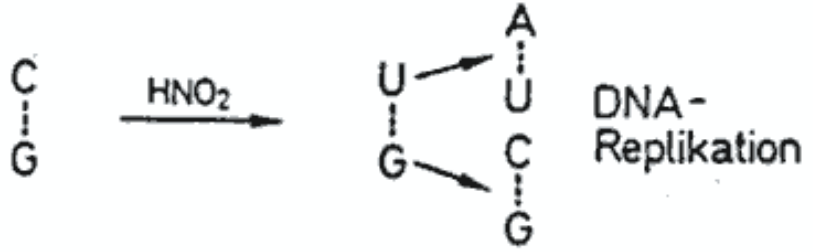


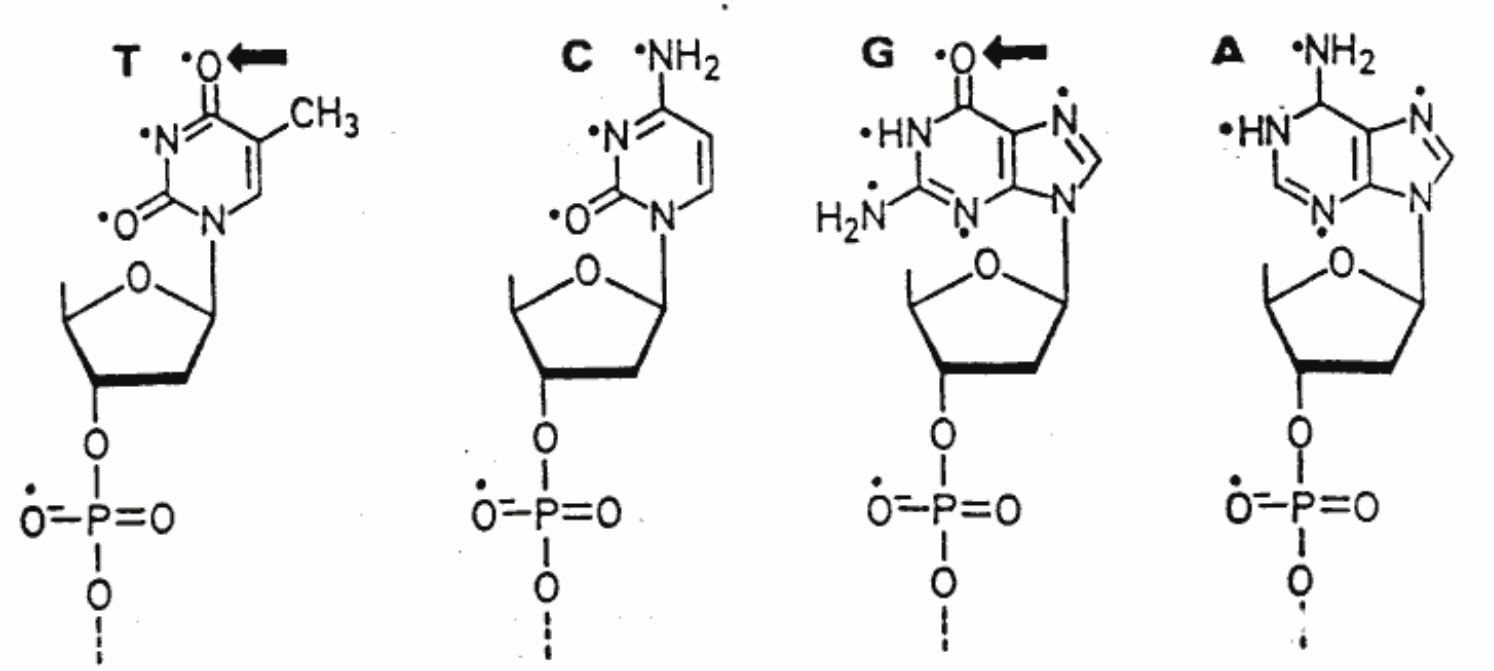
N-Alkyl-*N'*-Nitro-*N*-Nitrosoguanidin
Beispiel: *N*-Methyl-*N'*-Nitro-*N*-Nitroso-guanidin (NNG)

Abb. 8.16 Alkylierende Mutagene (R: $-CH_3$ oder $-CH_2-CH_3$).

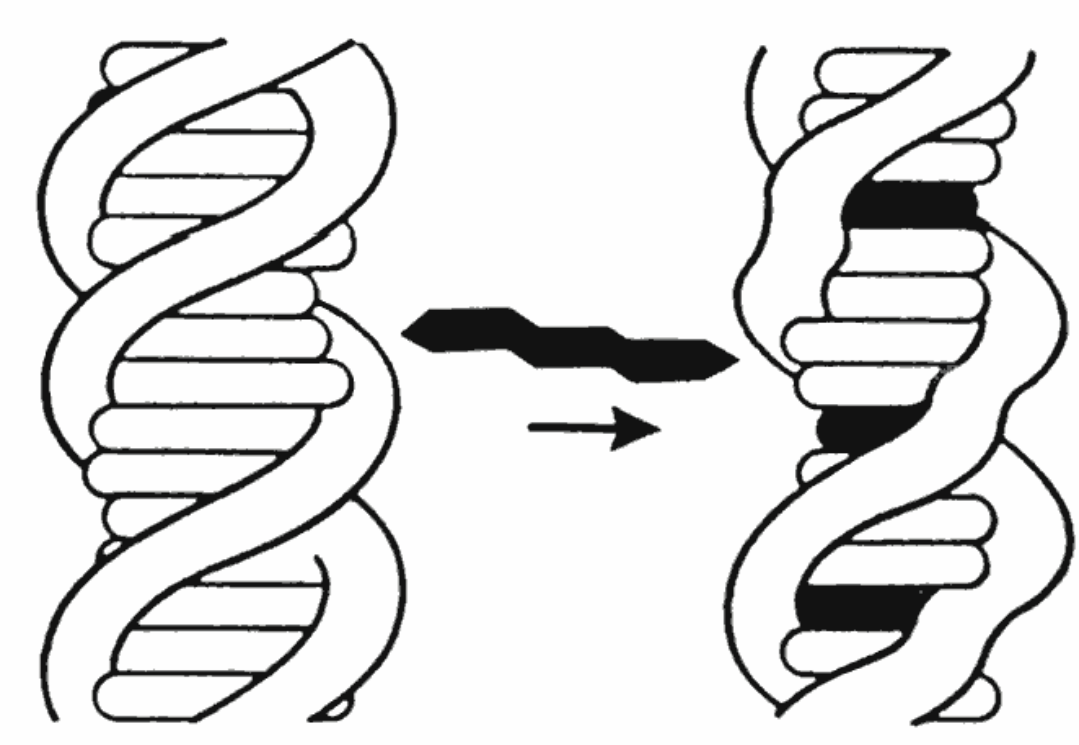


Beispiel



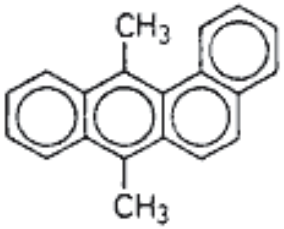


„Frame-shift“ mutation

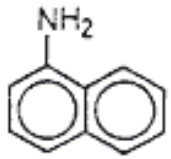


Mutagenic compounds

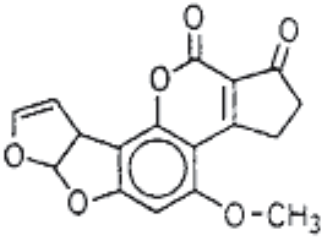
nicht aktive Formen



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

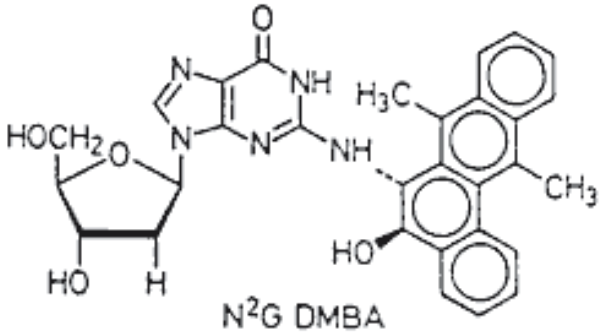


1-Naphtylamin (1-NA)

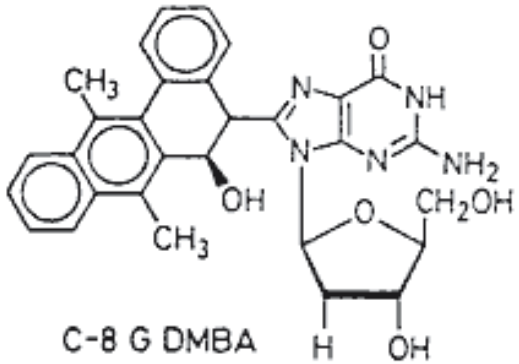


Aflatoxin B_1 (AFB_1)

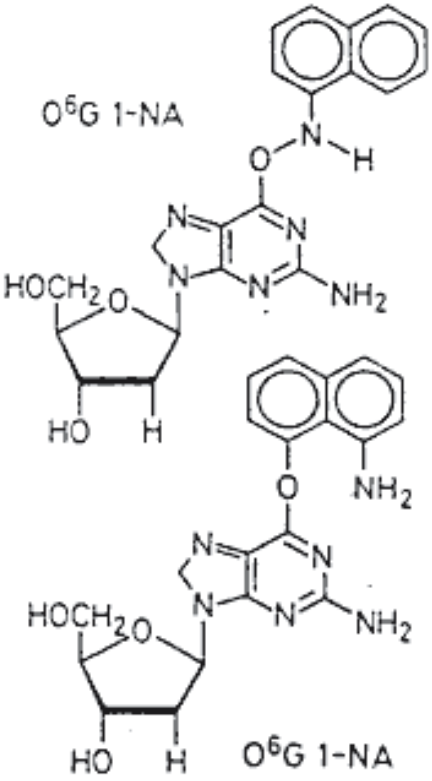
Reaktionsprodukte mit DNA-Basen



N^2G DMBA

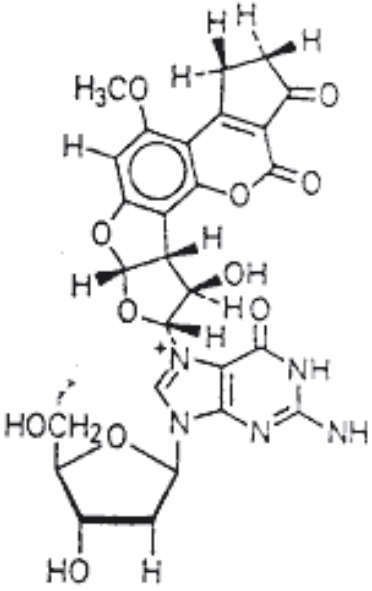


C-8 G DMBA



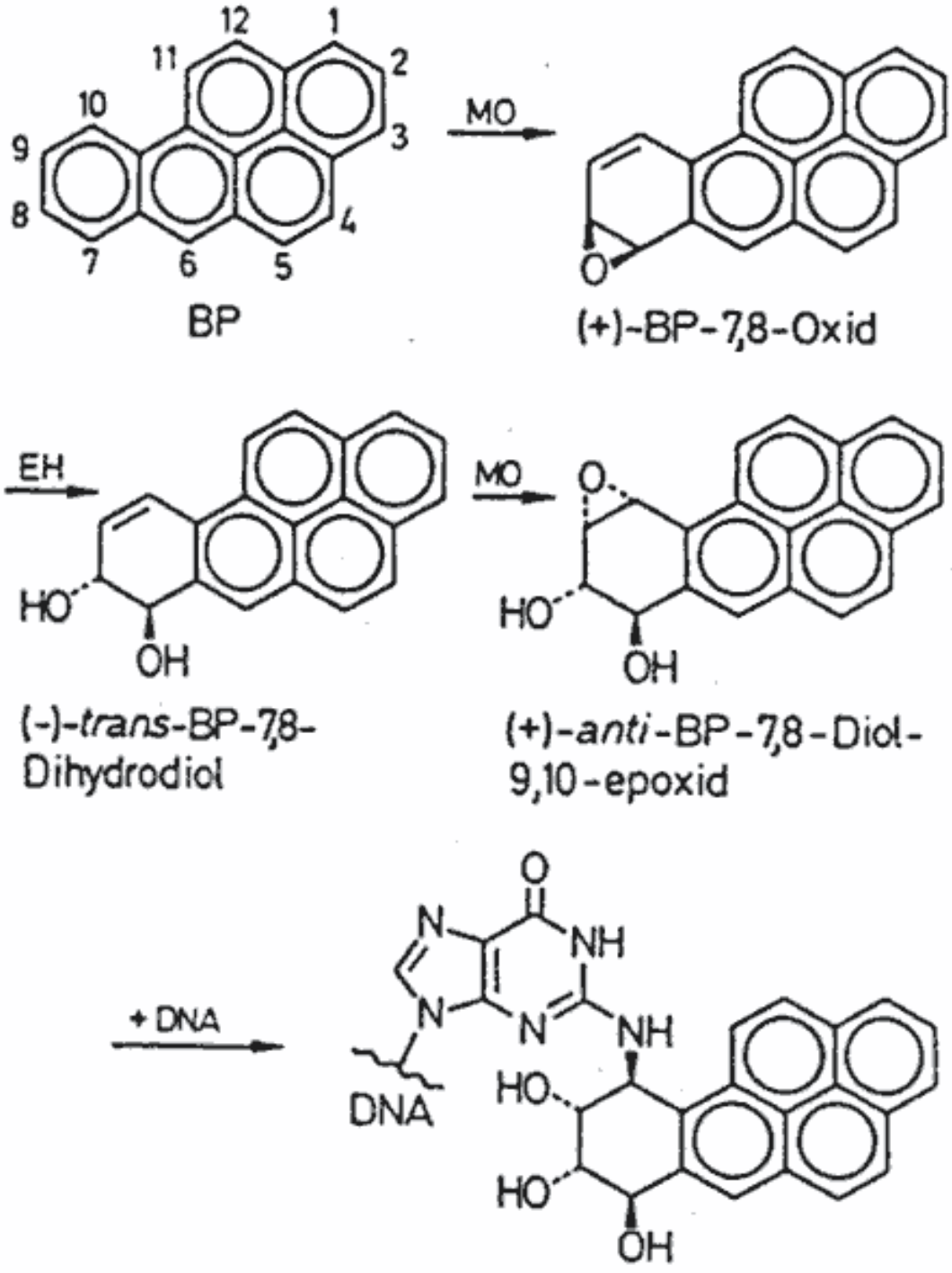
O^6G 1-NA

O^6G 1-NA



N-7 G AFB_1

Benzpyrene


12
1
11
2
10
3
9
4
8
7
6
5
BP
MO
O
(+)-BP-7,8-Oxid
EH
HO
OH
(-)-trans-BP-7,8-
Dihydrodiol
MO
O
HO
OH
(+)-anti-BP-7,8-Diol-
9,10-epoxid
+ DNA
DNA
O
N
NH
N
N
NH
HO
HO
OH

Calicheamicin

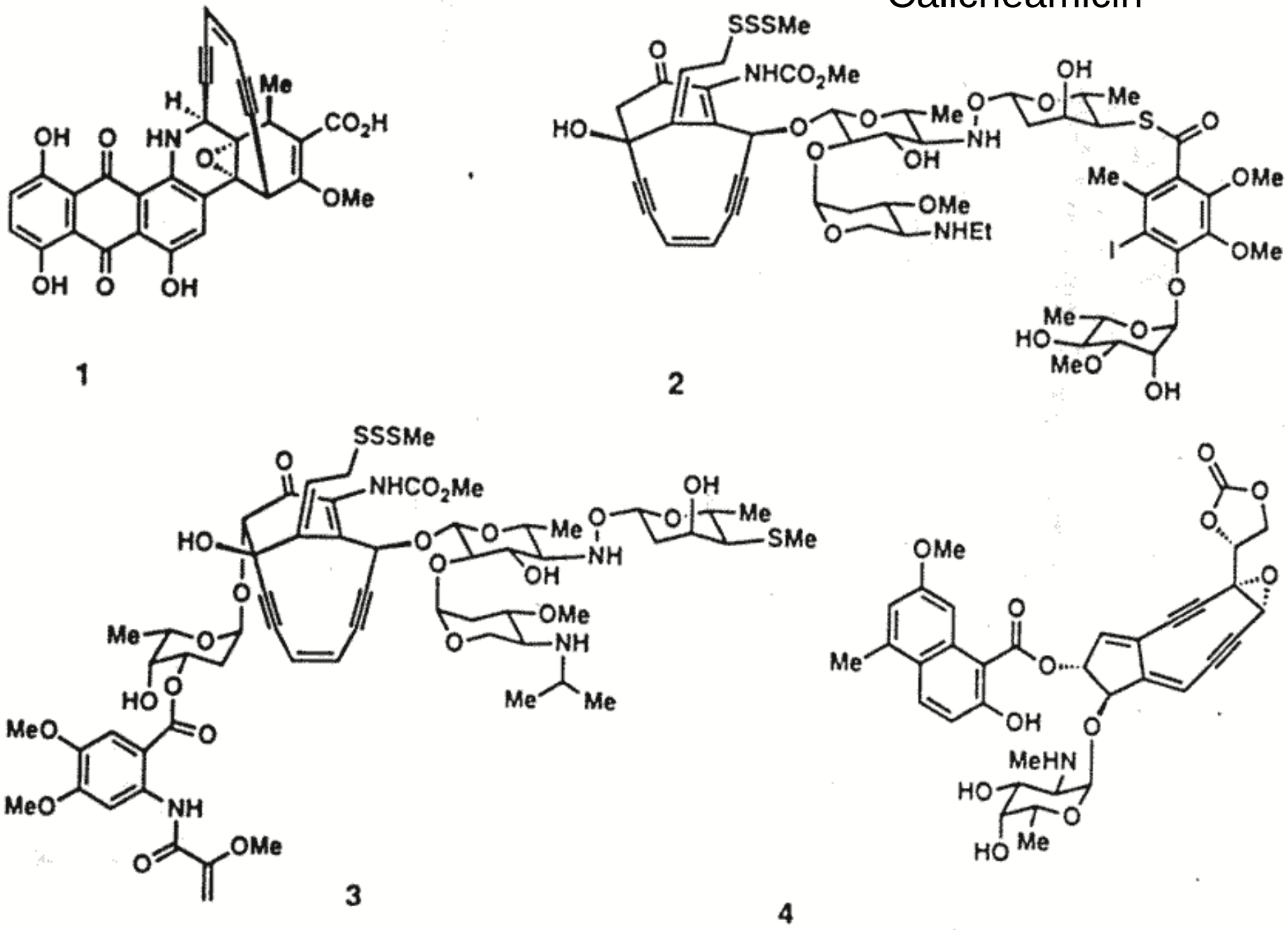
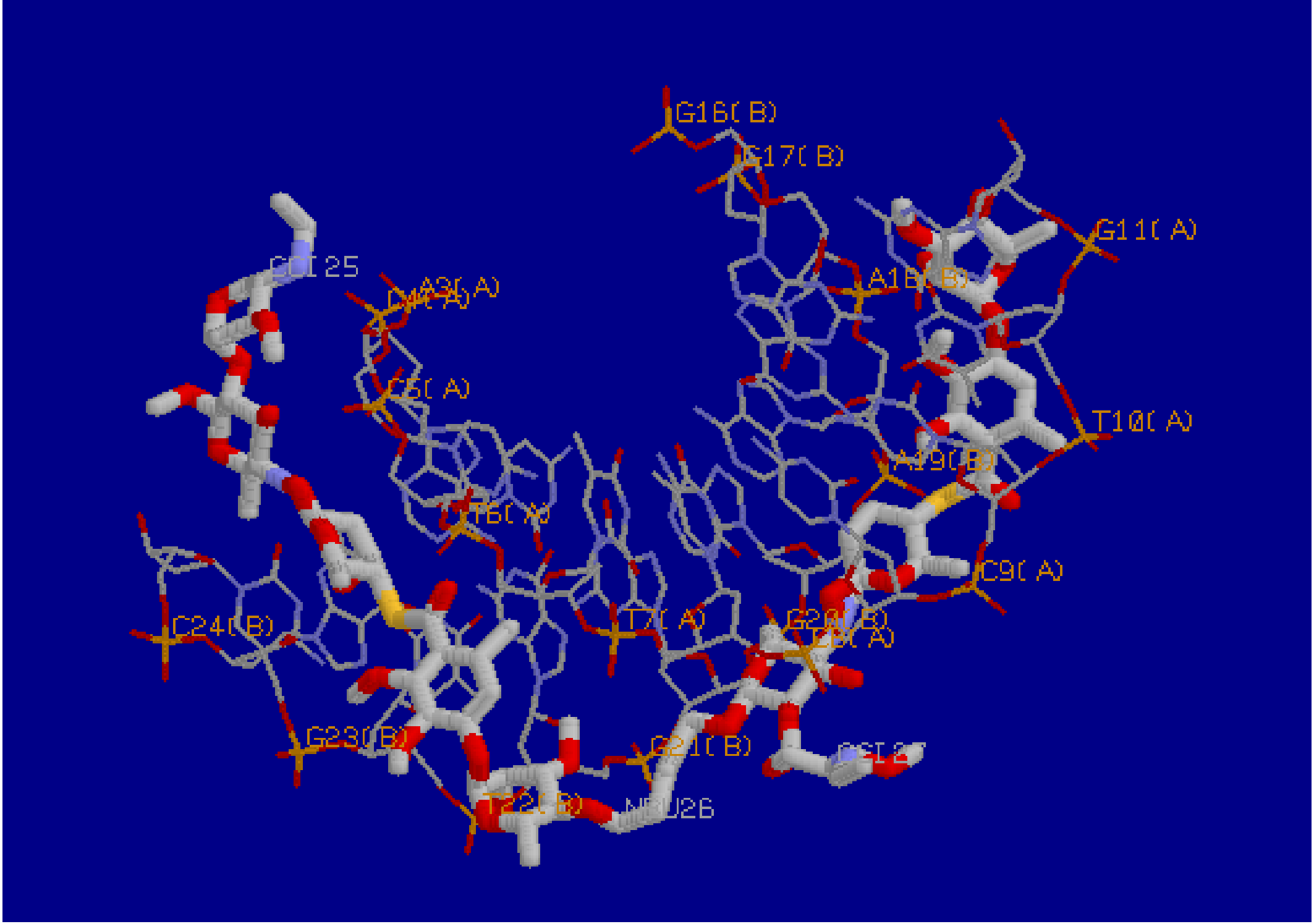


FIG. 1 Structures of naturally occurring enediyne anticancer antibiotics: **1**, dynemicin A; **2**, calicheamicin γ_1; **3**, esperamicin A_1; **4**, neocarzinostatin chromophore.


G16(B)
G17(B)
G11(A)
A18(B)
T10(A)
A19(B)
C9(A)
T7(A)
C8(A)
C5(A)
T6(A)
C24(B)
G23(B)
T22(B)

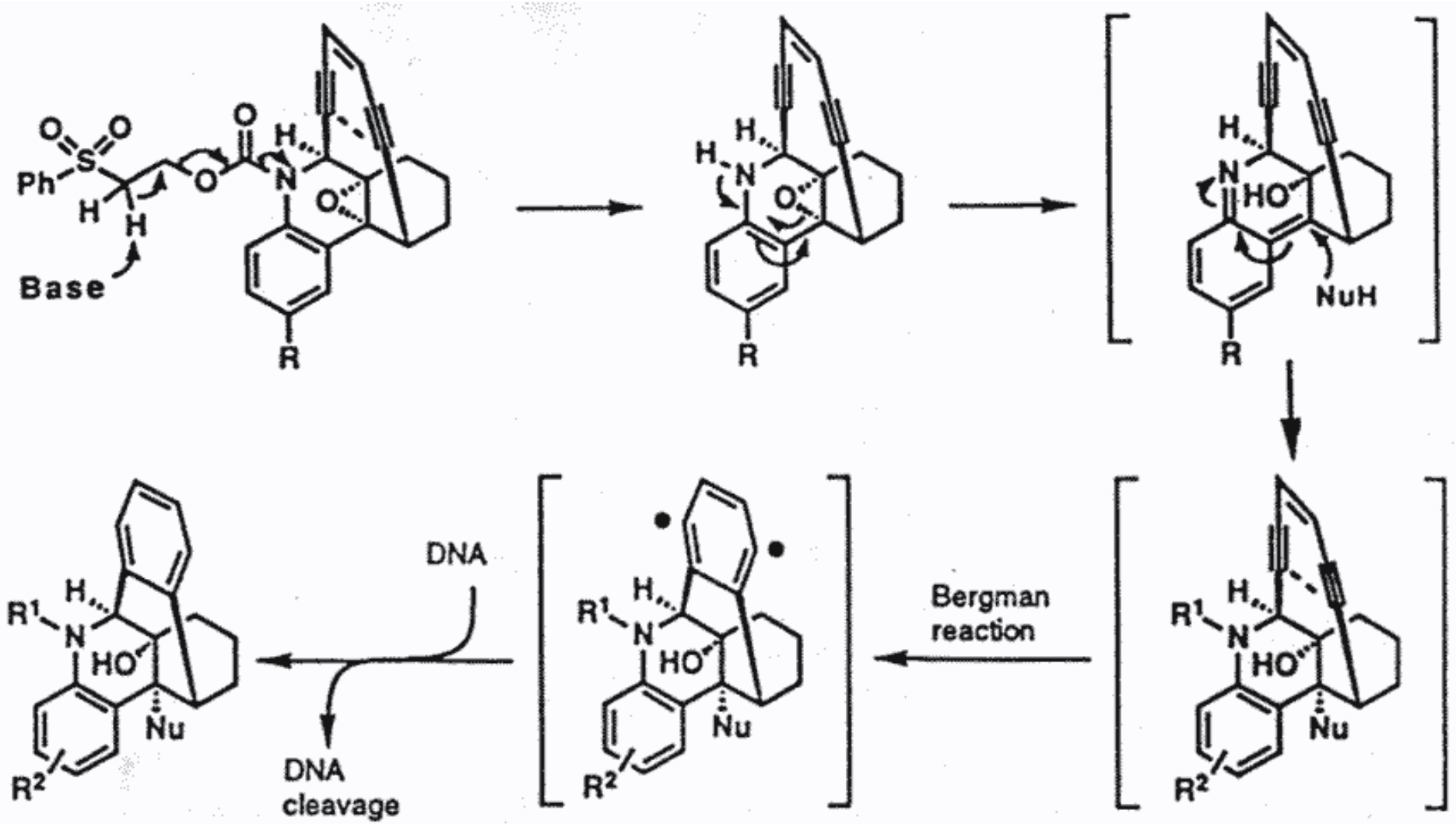
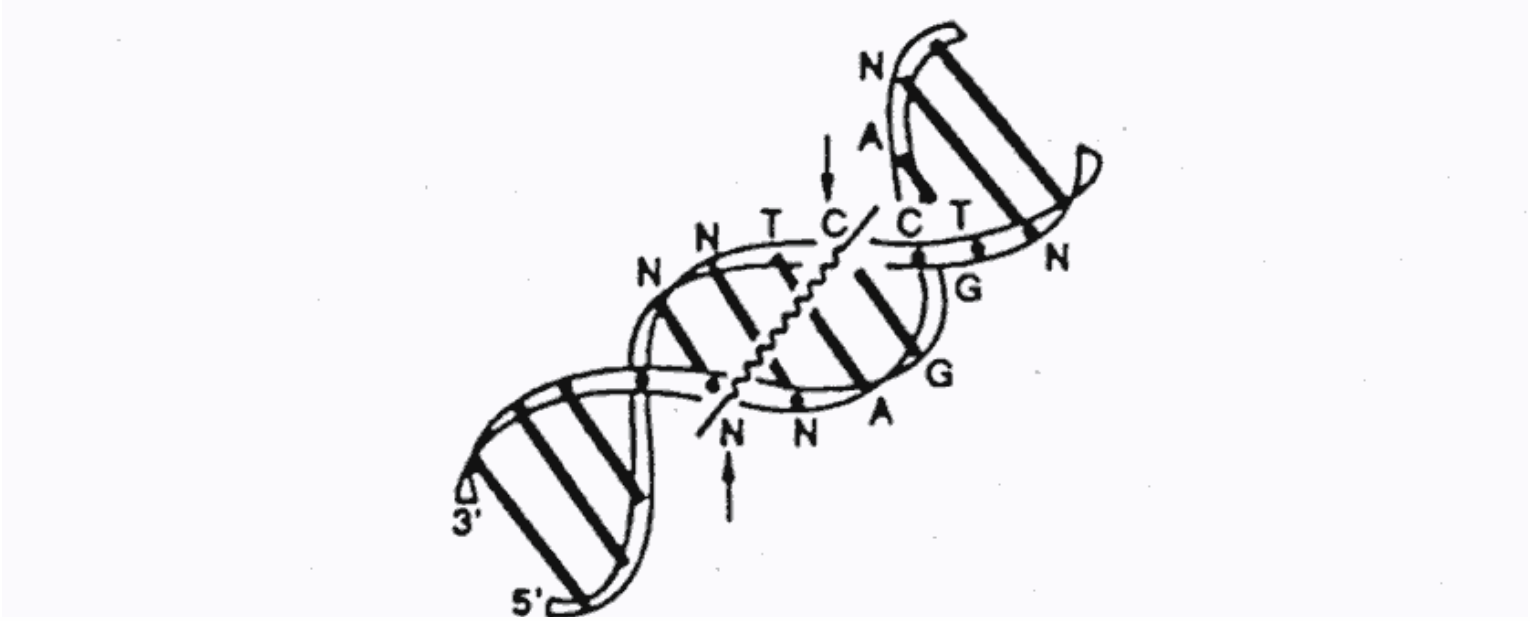
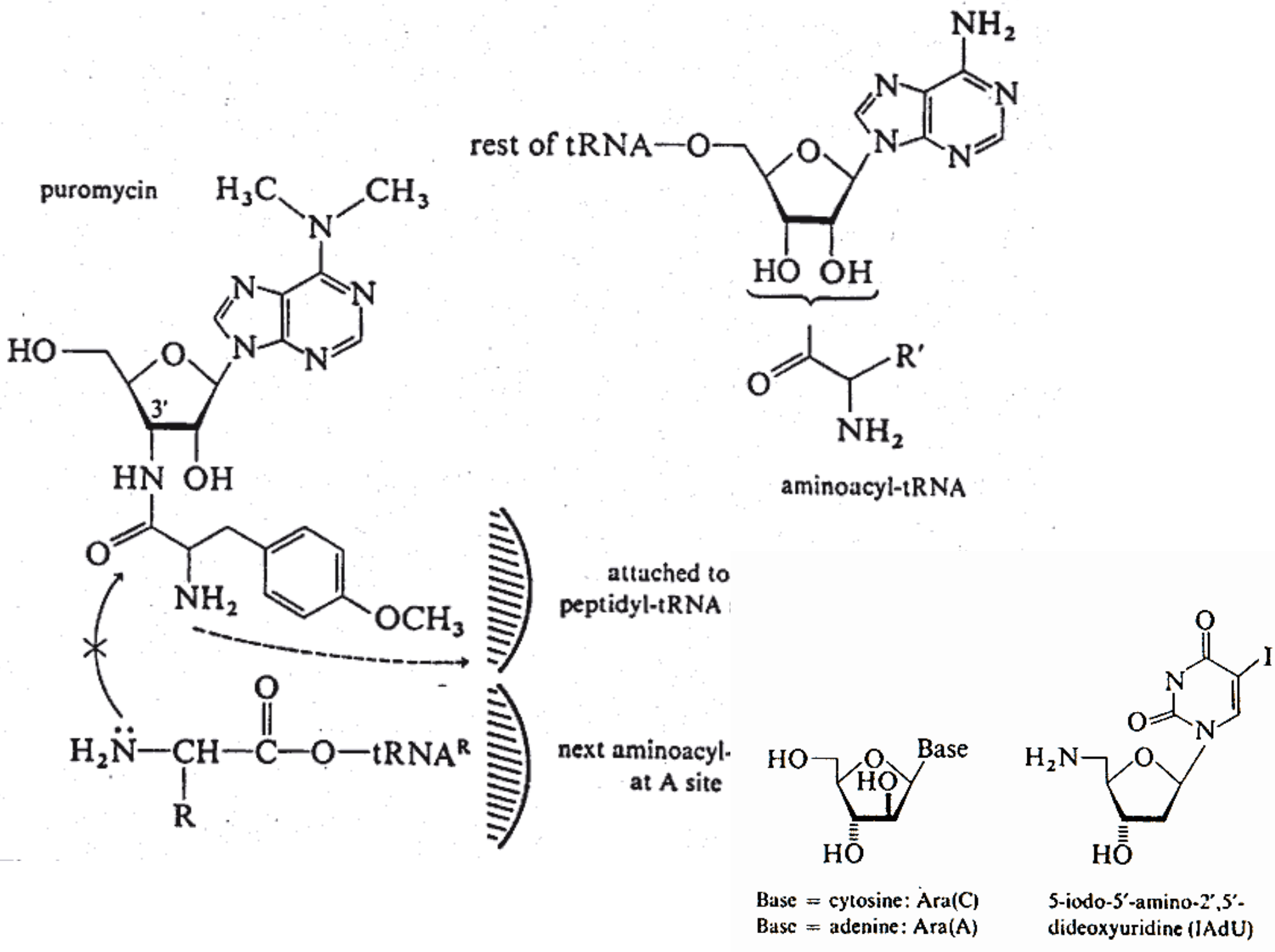


FIG. 3. Enediyne design, synthesis and biological action.



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

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


puromycin
rest of tRNA—O
aminoacyl-tRNA
attached to peptidyl-tRNA
next aminoacyl- at A site
Base = cytosine: Ara(C)
Base = adenine: Ara(A)
5-iodo-5′-amino-2′,5′-dideoxyuridine (IAdU)