生理活性分子工学特論

Special Lecture on
Bioactive Molecular Engineering

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Mechanisms of Drug Resistance
薬物耐性

1. Altered drug uptake - exclusion of drug from site of action by blocking uptake of drug - altered membrane with more + or – charges 薬物の取り込みの減少

2. Overproduction of the target enzyme - gene induced 酵素の過剰発現

3. Altered target enzyme (mutation of amino acid residues at the active site) - drug binds poorly to altered form of the enzyme 酵素の変異

4. Production of a drug-destroying enzyme - a new enzyme is formed that destroys the drug 薬物分解酵素の生産

Mechanisms of Drug Resistance (cont'd)

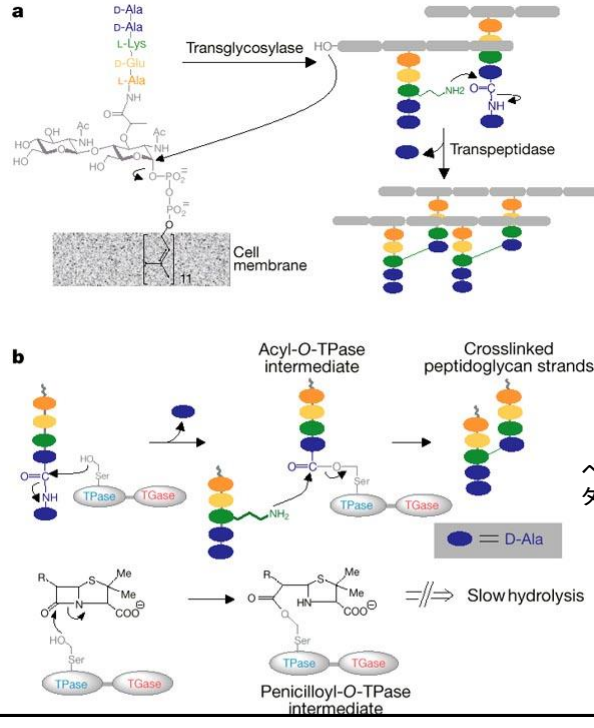
5. Deletion of a prodrug-activating enzyme - the enzyme needed to activate a prodrug is missing プロドラッグ活性化酵素の消失

6. Overproduction of the substrate for the target enzyme - blocks inhibitor binding 標的酵素の基質の過剰生産

7. New metabolic pathway for formation of the product of the target enzyme - bypass effect of inhibiting the enzyme 別な酵素の反応による物質生産

8. Efflux pump - protein that transports molecules out of the cell

薬物を細胞から排出するポンプの作動

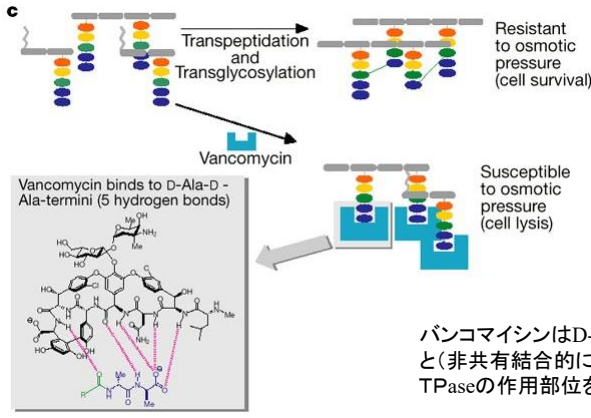


"正常な"細胞壁形成。
紺色部分はD-Ala-D-ala

ペニシリンはTPase(トランスペプチダーゼ)に結合して阻害

C. Walsh,
Nature, **406**, 775 (2000)

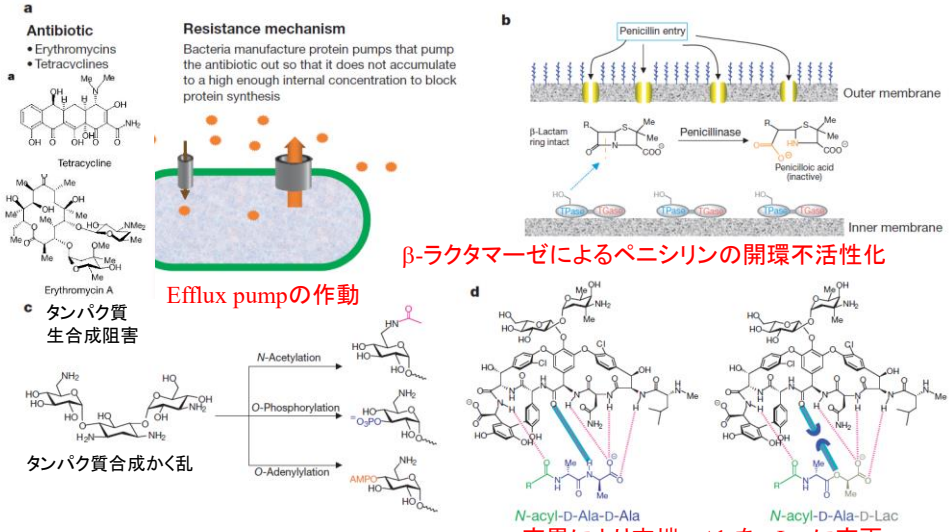
Vancomycin – another inhibitor for bacterial cell wall formation



バンコマイシンはD-Ala-D-ala
と(非共有結合的に)結びついて
TPaseの作用部位をふさぐ

C. Walsh,
Nature, **406**, 775 (2000)

Examples for antibiotic resistance 抗生物質への薬物耐性の例



β-ラクタマーゼによるペニシリンの開環不活性化

Efflux pumpの作動

糖構造をもつ抗生物質のOH基修飾
(cf. phase II transformation in metabolism)

変異により末端D-AlaをD-Lacに変更
(アミド結合→エステル結合)

C. Walsh,
Nature, **406**, 775 (2000)

Antidote for Drug Resistance
薬物耐性への対抗策

1. Inhibition of a drug-destroying enzyme　薬物分解酵素の阻害（cf. β-lactamase inhibitor）

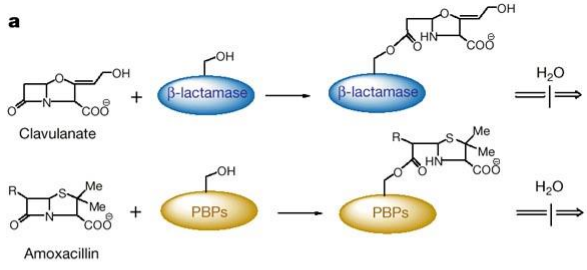
2. Sequential blocking - inhibition of two or more consecutive steps in a metabolic pathway - 連続する酵素反応の阻害

3. Inhibition of enzymes in different metabolic pathways-　他の代謝ルートを担当する酵素の阻害

4. Efflux pump inhibitors 薬物排出ポンプタンパク質の阻害

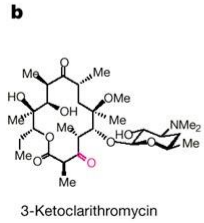
5. Use of multiple drugs for same target　一つの標的酵素に2種類の阻害剤

Examples…

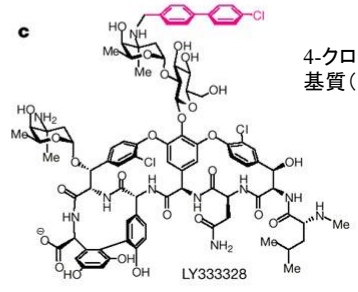


こちらはβ-ラクタマーゼを阻害

こっちはトランスペプチダーゼを阻害



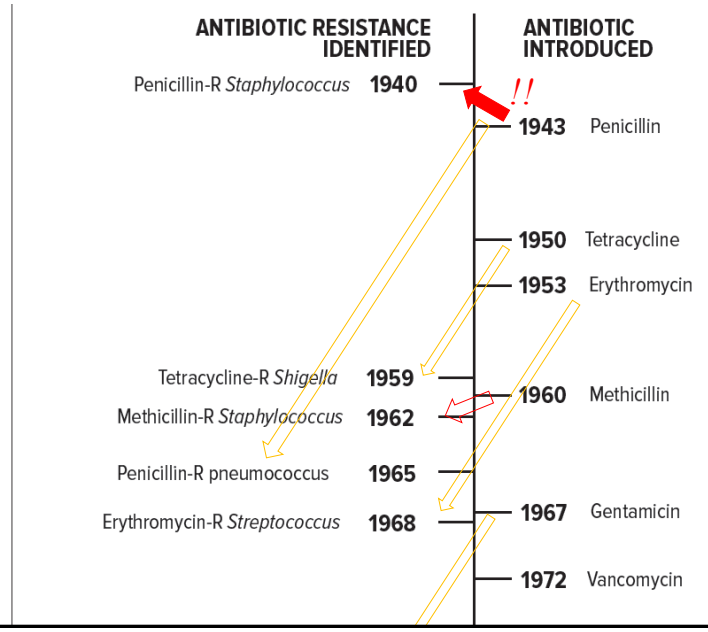
ケト基を導入することでコンホメーションが変化して“捕まりにくく”なる

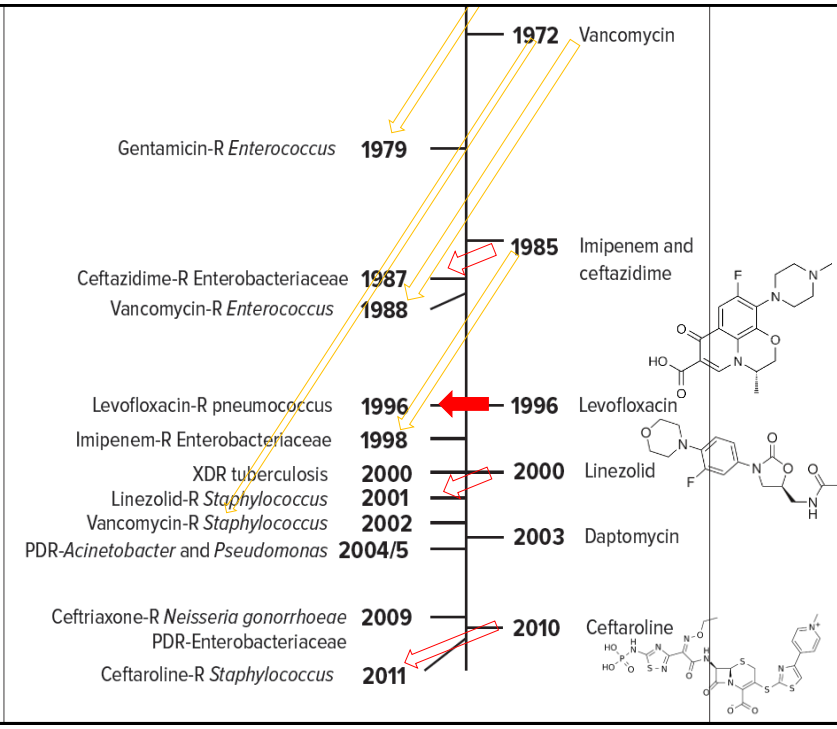


4-クロロビフェニル基の導入により基質（デプシペプチド）と強固に結合

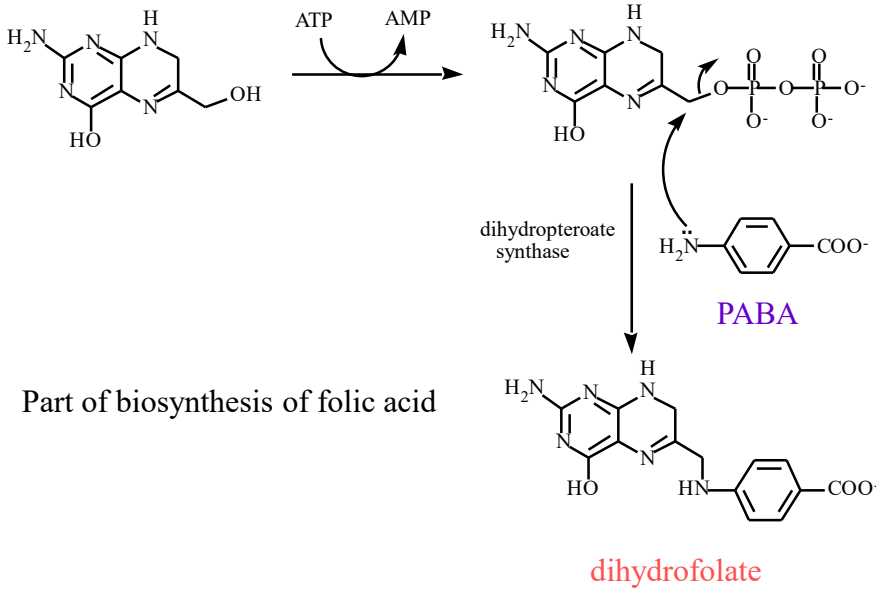
C. Walsh,
Nature, **406**, 775 (2000)

Developing drug resistance of antibiotics 耐性菌出現までの年数



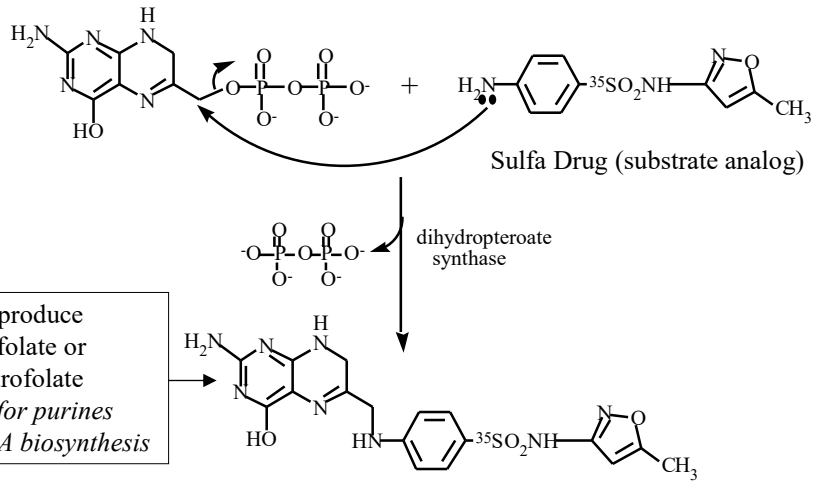


Resistance for Sulfa-drug



Part of biosynthesis of folic acid

Example for another type of antibiotic (sulfa drug)



Cannot produce dihydrofolate or tetrahydrofolate *needed for purines and DNA biosynthesis*

Drug Resistance

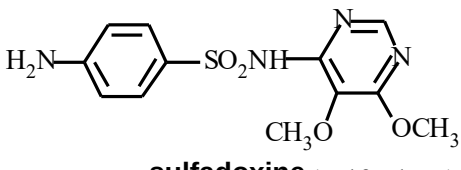
- overproduction of PABA 過剰生産
- formation of a dihydropteroate synthase that binds PABA normally, but binds sulfonamides thousands of times less tightly

基質には結合するが薬物に結合しない酵素に変異

- altered permeability of sulfonamides 薬物の膜透過性の変化

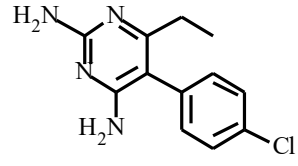
Drug Synergism 薬物相乗作用

- used in treatment of malaria



sulfadoxine (sulfa drug)

inhibits synthesis of dihydrofolate



pyrimethamine

inhibits dihydrofolate reductase

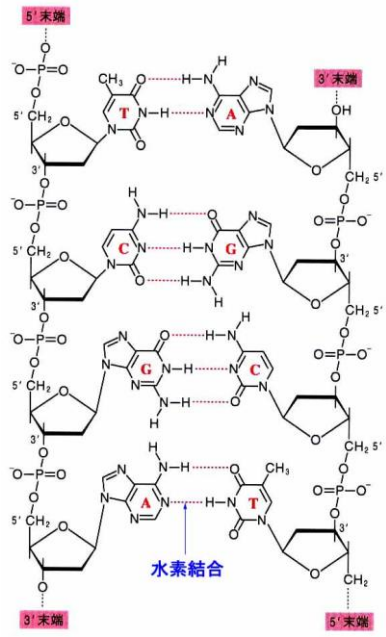
DNA の構造

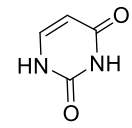
thymine

cytosine

guanine

adenine

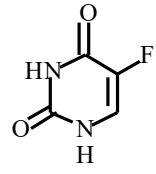




cf. uracil in RNA

5-Fluorouracil

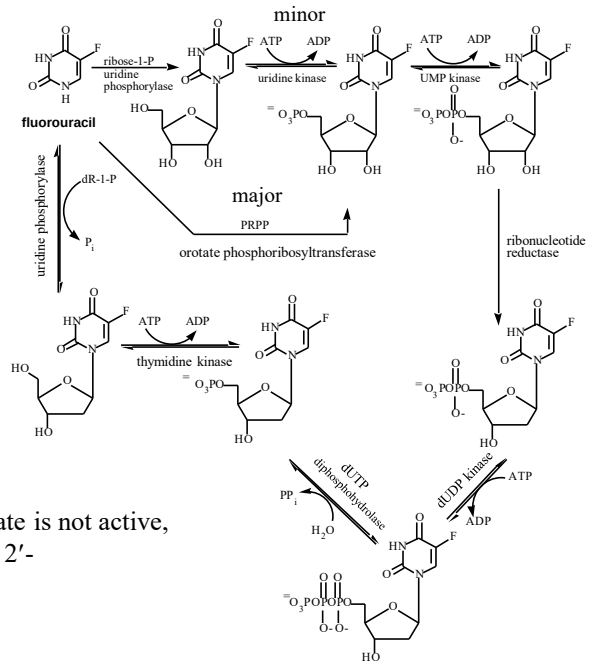
- Antitumor Agent -



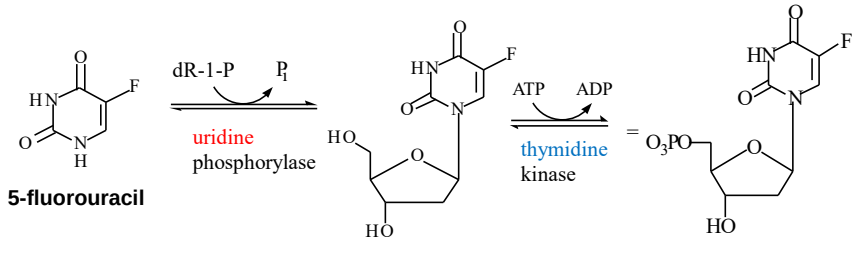
5-fluorouracil
antimetabolite of uracil

Cancer - abnormal and uncontrolled cell division

Antimetabolite design - structures related to pyrimidines and purines interfere with biosynthetic pathways of metabolites by enzyme inhibition or by incorporation into proteins or DNA. (抗がん剤分子設計アプローチの一つとして核酸アナログが有効)



5-Fluoro-2′-deoxyuridylate is not active, but is metabolized to the 2′-deoxynucleotide.

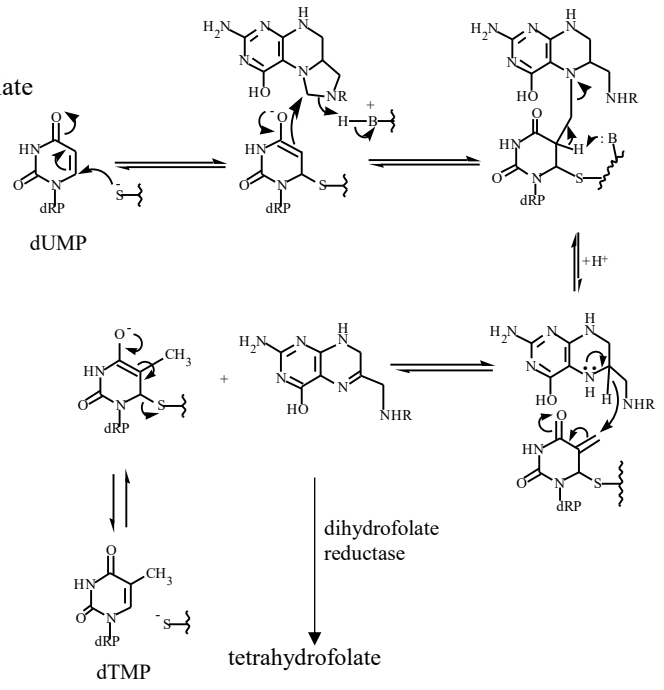


5-fluorouracil

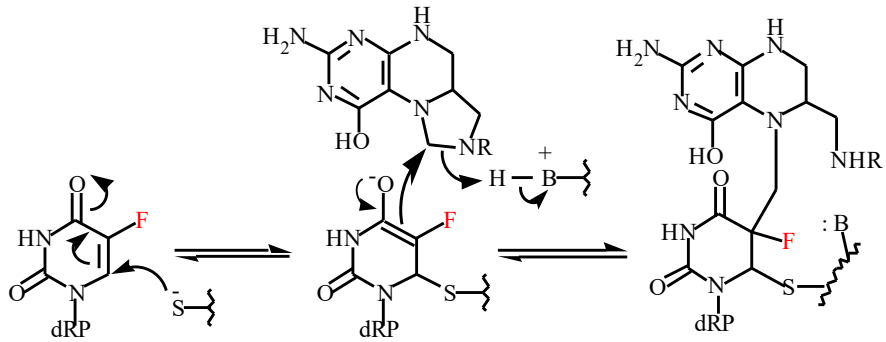
dUMP-analog

5-Fluoro-2′-deoxyuridylate is not active, but is metabolized to the 2′-deoxynucleotide.

Mechanism of Thymidylate Synthase



Mechanism of Inactivation of Thymidylate Synthase by 5-Fluoro-2′-deoxyuridylate



DNA interactive drugs

DNA – もう1つのレセプター

正常細胞のDNAとガン細胞のDNAで，化学的な違いはわずか

→ DNAをターゲットとする薬は一般に毒性が高く，ガンなど生命を脅かすような病気にのみ用いられる。

がん細胞

異常かつ迅速な細胞の増殖

常に DNAやその前駆体を要求

選択毒性

- がん細胞による外来分子の積極的取り込みを利用
- 修復機構の遅さをねらう
- p53など正常細胞で DNA修復に関わるタンパクを活性化することで，正常細胞でのDNA修復を促進，細胞周期の制御，アポトーシス誘導

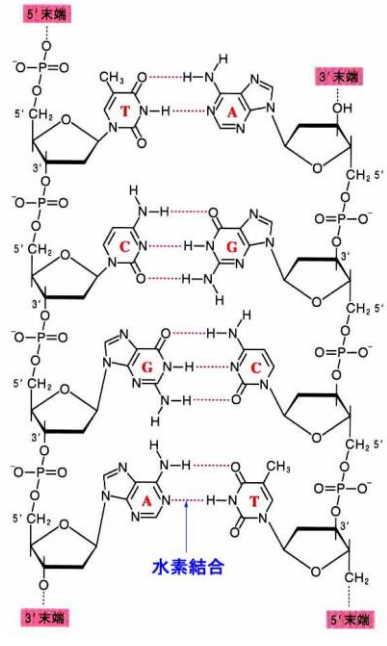
DNA の構造

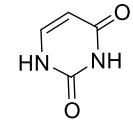
thymine

cytosine

guanine

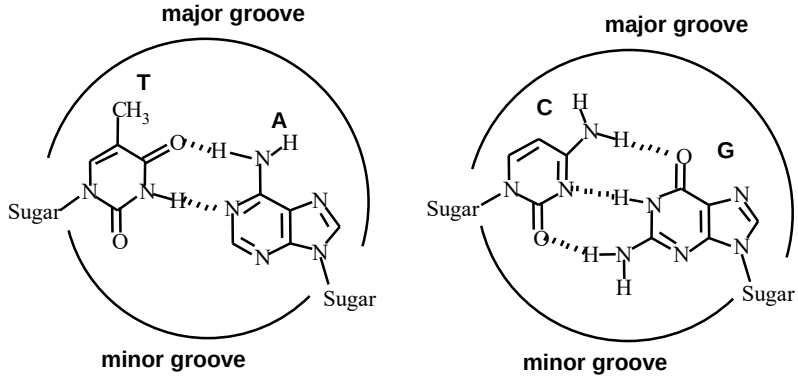
adenine



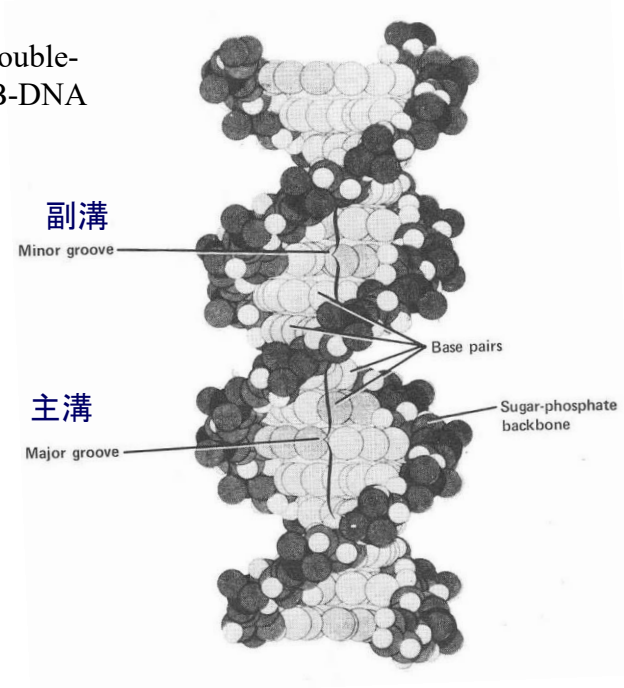


cf. uracil in RNA

核酸塩基間の水素結合（Watson-Crick Base Pair）。
糖部分の位置はちょうど反対側というわけではない。



Duplex (double-stranded) B-DNA



Classes of DNA-Interactive Drugs

1） 可逆的結合性分子 Reversible binders

– 相互作用も可逆的

2） アルキル化剤 Alkylators

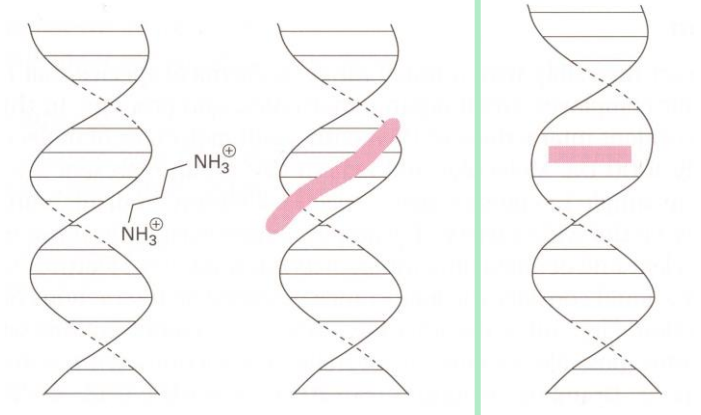
– DNA塩基と共有結合形成

3） 鎖切断剤 Strand breakers

– ラジカルを発生し，ポリヌクレオチド鎖を切断

1） 可逆的結合性分子 Reversible binders

DNAと可逆的結合分子との相互作用

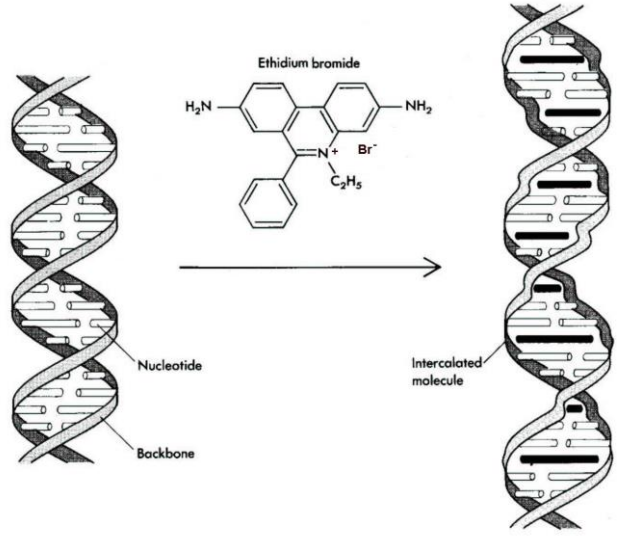


External electrostatic　Groove binder　Intercalator

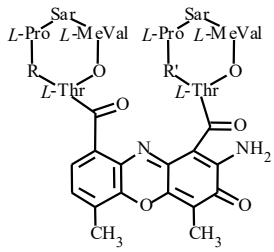
DNAインターカレーター

- 平板分子であり，通常は芳香族化合物
- 連続した塩基対間のスペースに挿入。非共有結合性
- 糖—リン酸鎖はひずむ
- 水素結合は壊さない
- 通常のらせんを壊し，DNAの“巻き戻し”を起こす
- 結果として，DNA topoisomeraseや DNA polymeraseを阻害して，合成や修復をできなくする

インターカレーションの例

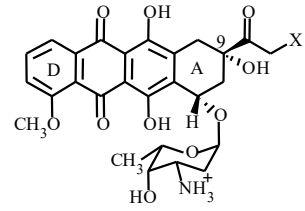


DNA Intercalators



dactinomycin (R = R' = *D*-Val)

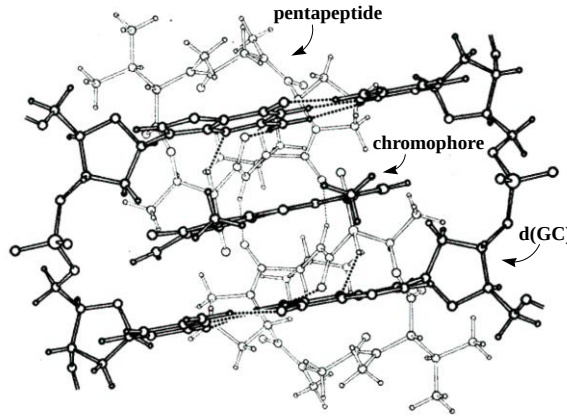
Actinomycins



doxorubicin (X = OH)
daunorubicin (X = H)

Anthracyclines

Crystal Stucture of an Actinomycin Analog Bound to a DNA



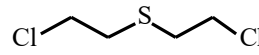
dactinomycin – antitumor抗腫瘍藥
from *Streptomyces*

2） アルキル化剤 Alkylators

Nitrogen mustards

Lead discovery

第一次大戦時におけるマスタードガス（下記）による死者の解剖で，白血球減少，骨髄異常，リンパ細胞の異常，腸内の潰瘍の所見 →全て分裂の速い細胞



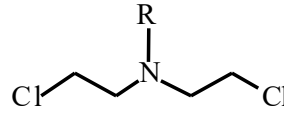
腫瘍細胞に対しても同様の作用があるものと推察。

⇒ 試されたが毒性強すぎ。

Lead Modification

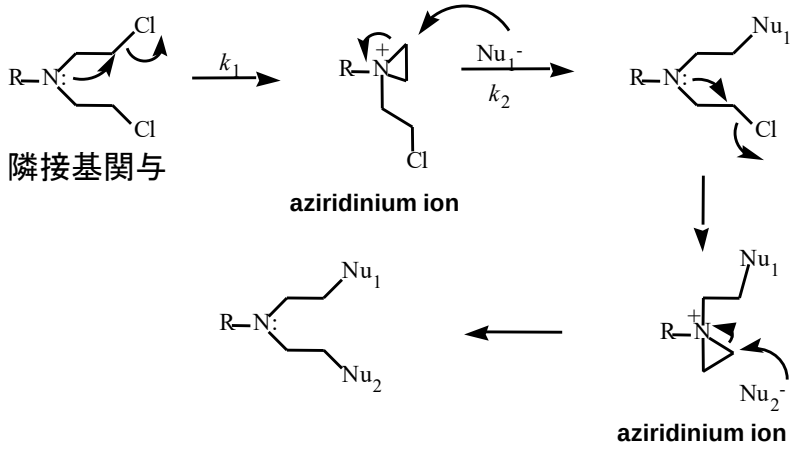
sulfur mustard → nitrogen mustard

現代的化学療法薬の始まり



mechlorethamine (R = CH_3)

(for advanced Hodgkin’s diseaseホジキンリンパ腫)



隣接基関与

aziridinium ion

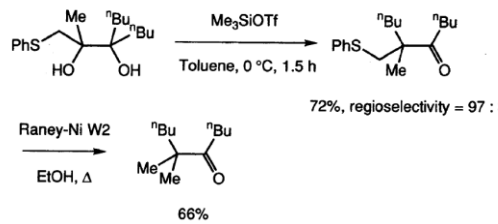
aziridinium ion

二官能性アルキル化剤→DNAの架橋（分子内・分子間）

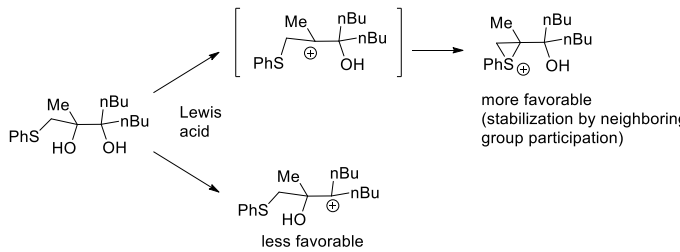
なぜNの方がSよりも反応性低い？

Sの隣接基関与の合成反応への利用

位置選択的pinacol転位



K. Kudo, K. Saigo, Y. Hashimoto, K. Saito, and M. Hasegawa, *Chem. Lett.*, 1449 (1992).



Chemistry of Alkylating Agents

A) Alkyl–X $\overset{S_N1}{\rightleftharpoons}$ Alkyl$^+$ X$^-$ $\xrightarrow{Nu^-}$ Alkyl–Nu

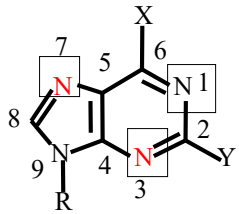
B) Alkyl–X $\xrightarrow[S_N2]{Nu^-}$ Alkyl–Nu + X$^-$

Reactivity of Nu^- in general:

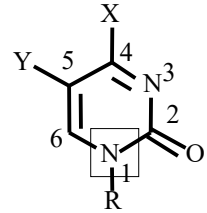
$RS^- > RNH_2 > ROPO_3^{=} > RCOO^-$

Where is the nucleophile in DNA?

*N*7 of G > *N*3 of A > *N*7 of A > other parts

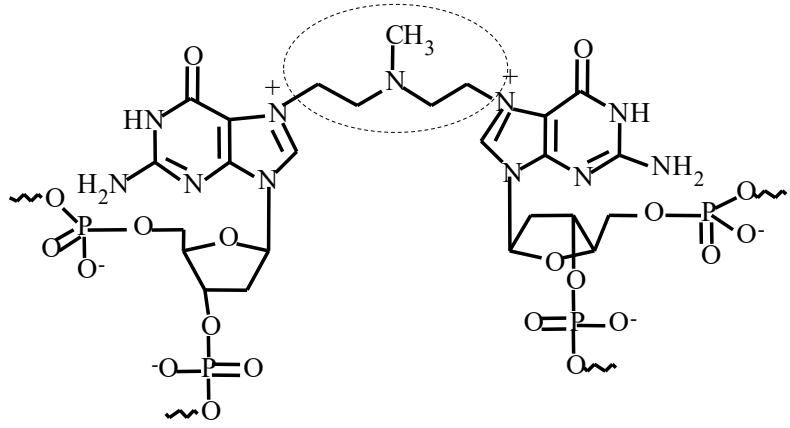


Purines
A/G

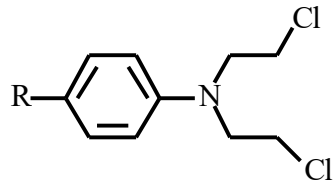


Pyrimidines
T/C

分子間（鎖間）架橋の状態



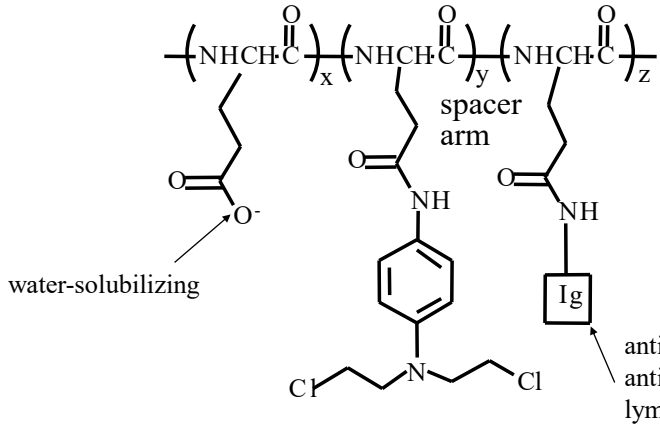
体内での分解速度の抑制



More stable
Slows rate of aziridinium formation

R = COOH　too stable, but soluble
R = $(CH_2)_3COOH$　chlorambucil

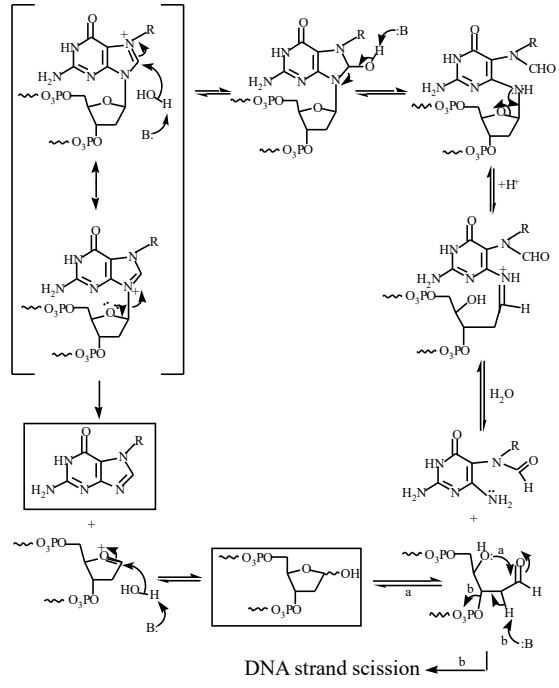
Nitrogen Mustardへの部位特性付与



All 5 mice tested were alive and tumor free after 60 days (all controls died).
Also, therapeutic index greatly enhanced (40 fold).

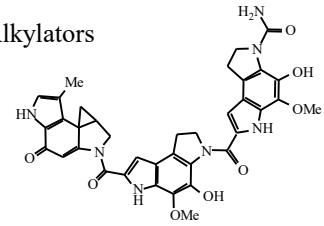
guanine *N*-7アルキル化は
DNAの切断にもつながる。

(化学的不安定化)

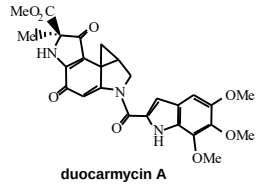


Cyclopropane-Containing Alkylators

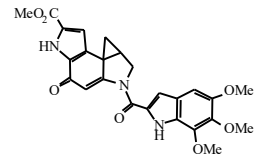
From *Streptomyces*



CC-1065

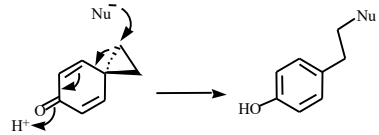


duocarmycin A

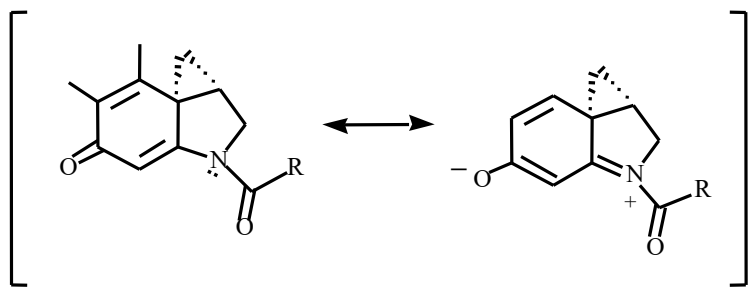


duocarmycin SA

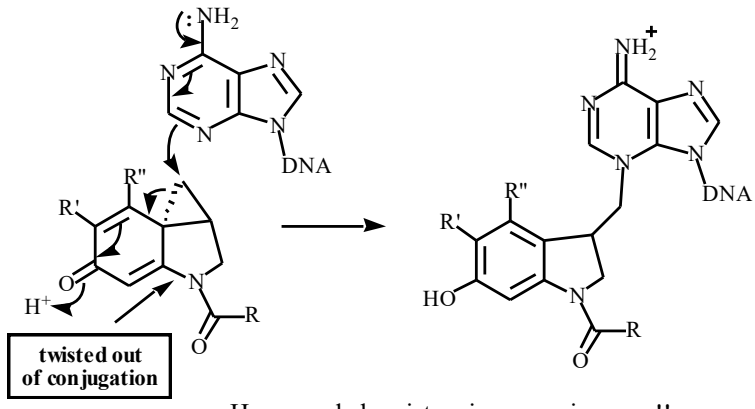
共通の反応駆動力（シクロプロパンひずみ解消＋芳香族化）



窒素原子は共役系に含まれていてシクロプロパン部の求電子反応性を低下させている

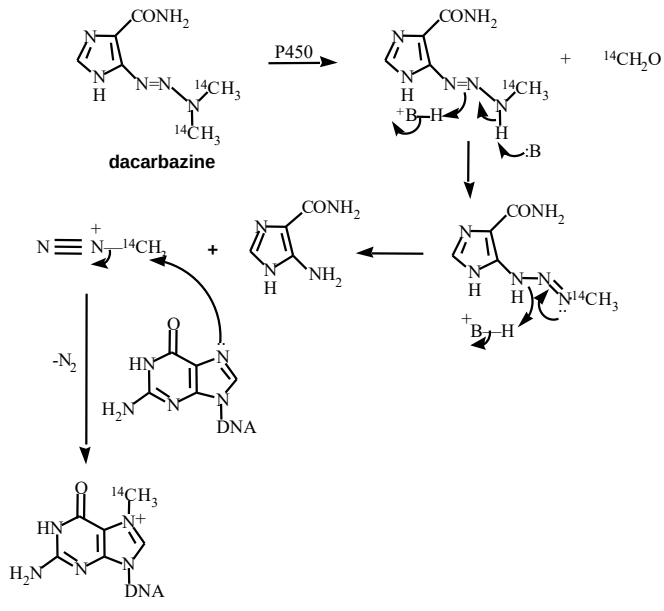


A-T領域に結合→Nが共役系から外れる→シクロプロパンの反応性が上昇
→adenine *N*-3が反応



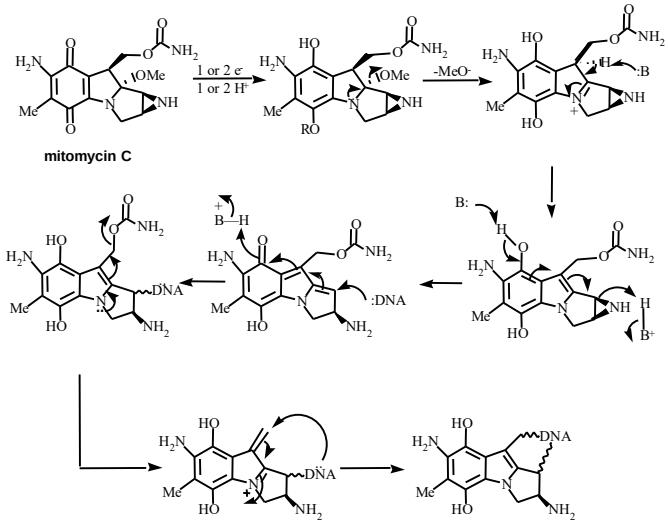
How good chemists microorganism are !!

アルキル化剤のprodrug



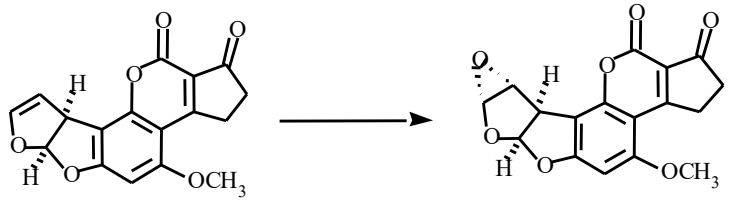
Mitomycin C（抗ガン剤）

還元的代謝でアルキル化剤へと変化する

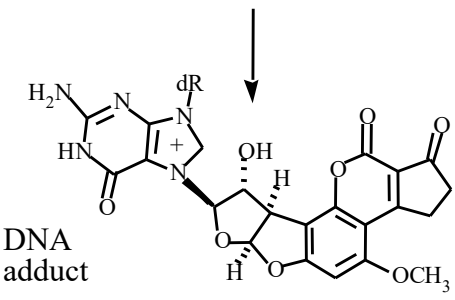


mitomycin C

Toxic Product of Alkene Oxygenation

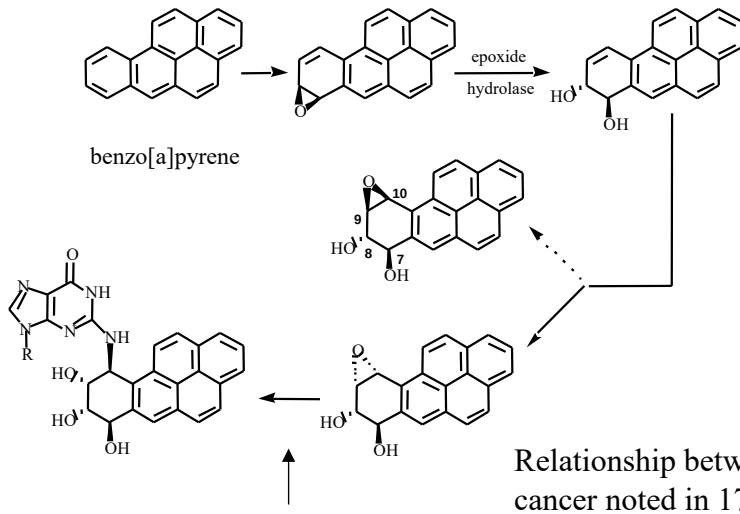


Aflatoxin B_1（カビ毒）



DNA adduct

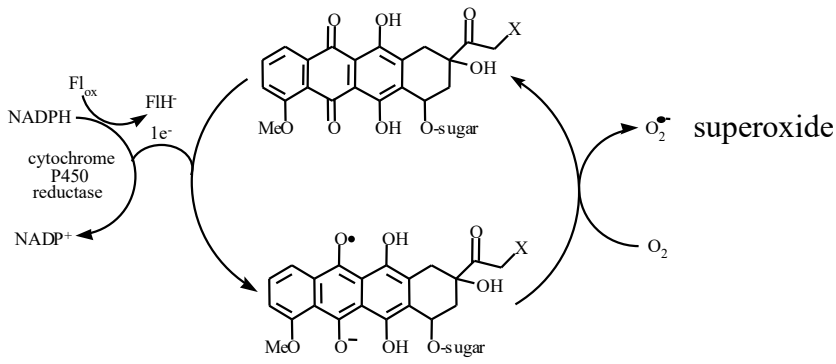
Toxicity from Arene Oxides



Relationship between soot and cancer noted in 1775 - chimney sweeps frequently developed skin cancer

3） 鎖切断剤 Strand breakers

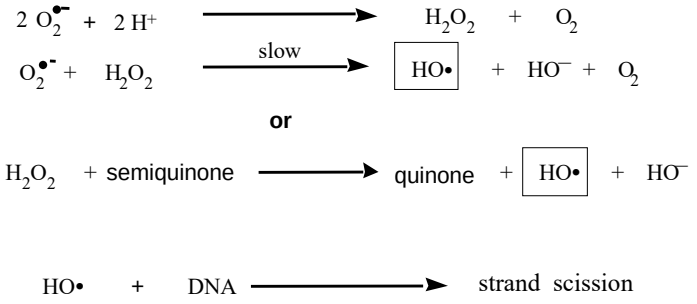
Anthracycline Radical Formation



$O_2^{-\bullet}$ and anthracycline semiquinone can generate HO•

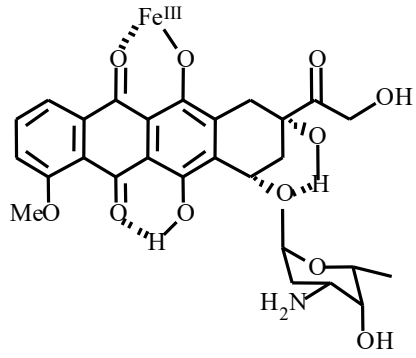
HO• Cleaves DNA

Generation of HO• from $O_2^{-\bullet}$ and from semiquinone



Other Possible Mechanism of DNA Damage by Anthracyclines

Ferric complex



This could react with $O_2^{-\bullet}$ to give O_2 + Fe(II) Fenton reaction of Fe(II) with H_2O_2 gives HO•

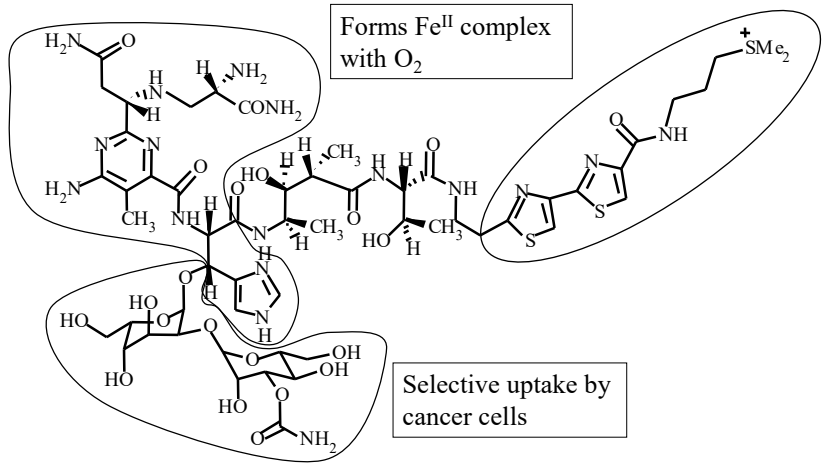
Bleomycin

From a *Streptomyces*

Principal domains in bleomycin

Intercalates into DNA

Forms Fe^{II} complex with O_2

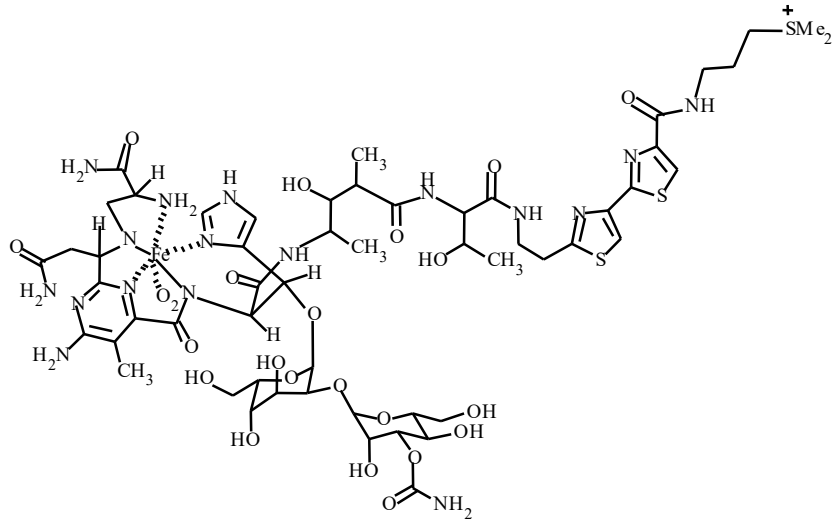


Selective uptake by cancer cells

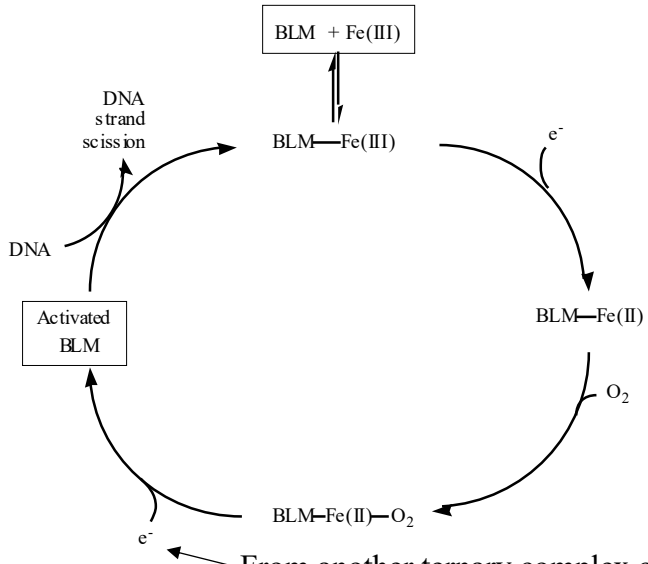
bleomycin A_2

Ternary Complex of Bleomycin, Fe (II), and O_2

Active Form



Activation of Bleomycin



Possible Mechanisms for Activation of Bleomycin

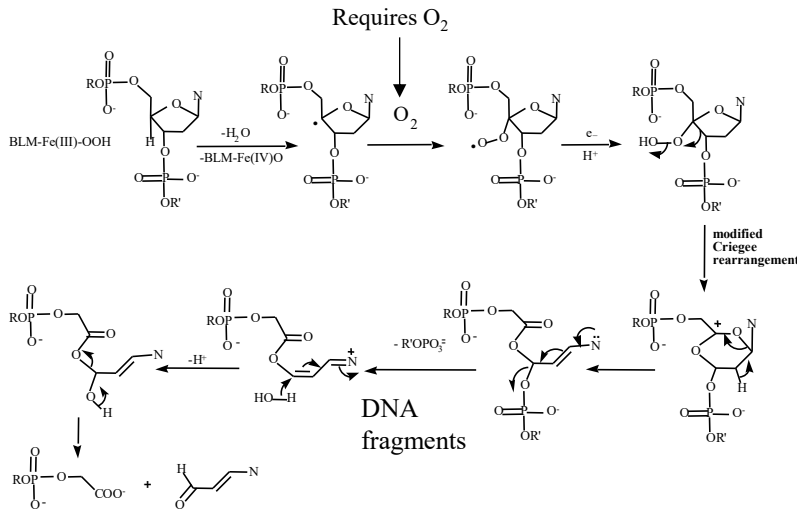
1. BLM-Fe(II)-O_2 ⟶ BLM-Fe(II)-OOH $\xrightarrow[2H^+]{e^-}$ BLM-Fe(V)=O

2. BLM-Fe(II)-O_2 ⟶ BLM-Fe(II)-O–OH ⟶ BLM-Fe(IV)=O + HO•

3. BLM-Fe(II)-O_2 ⟶ BLM-Fe(II)-O–OH $\xrightarrow{DNA}$ concerted reaction with DNA

All three mechanisms involve generation of free radicals that can abstract H• from DNA, leading to DNA strand scission.

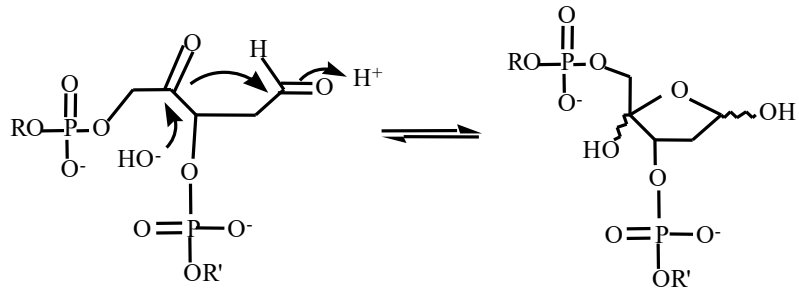
Proposed Mechanisms for the Reaction of Activated BLM with DNA



3′-phosphoglycolate nucleic base propenals (2 major products *isolated*)

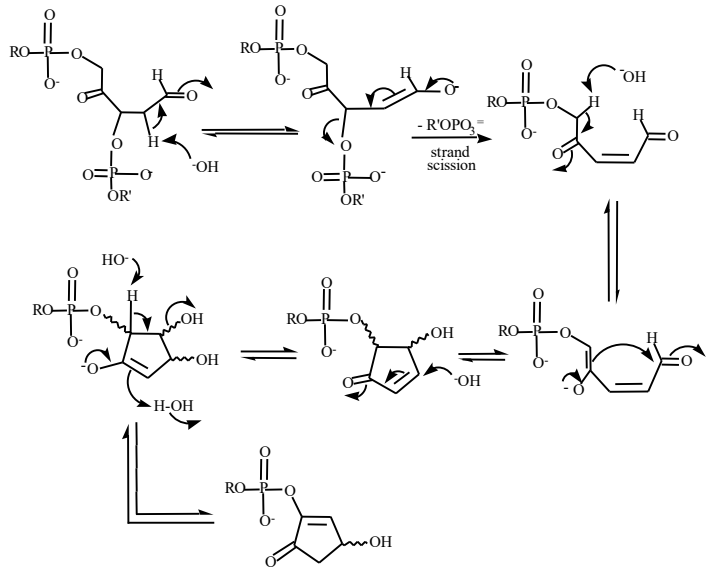
Alkali-Labile Lesion アルカリ不安定化損傷

Additional single-strand cleavage of DNA occurs in the presence of alkali.



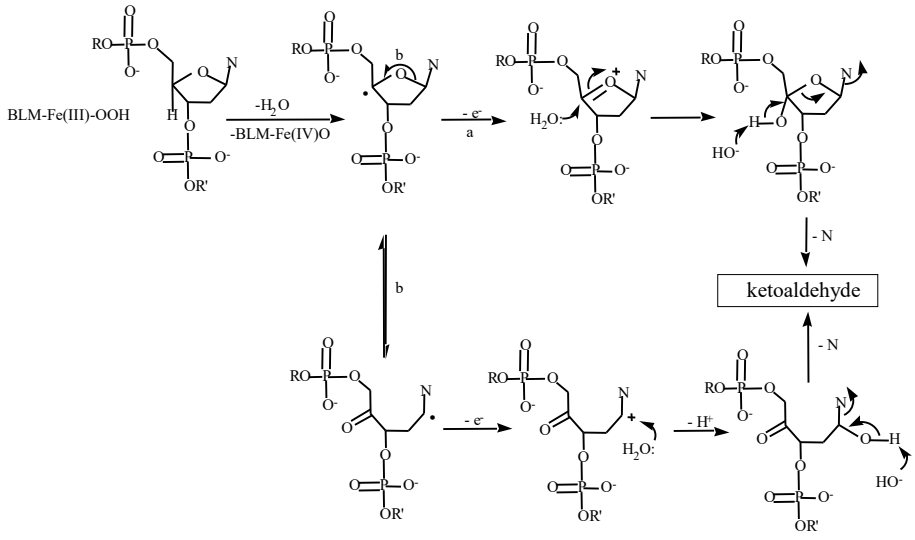
Alkali-labile lesion

Mechanism for DNA Cleavage by Alkali

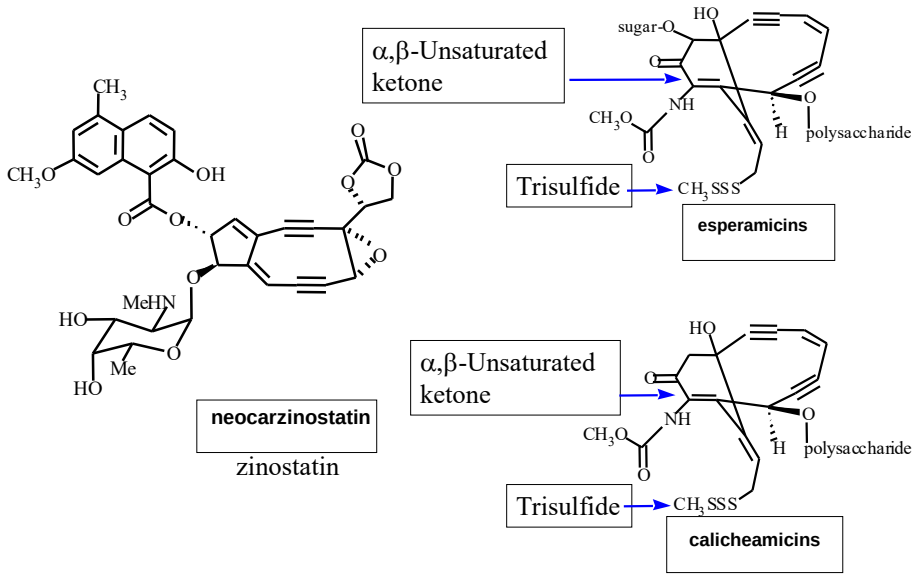


Isolated

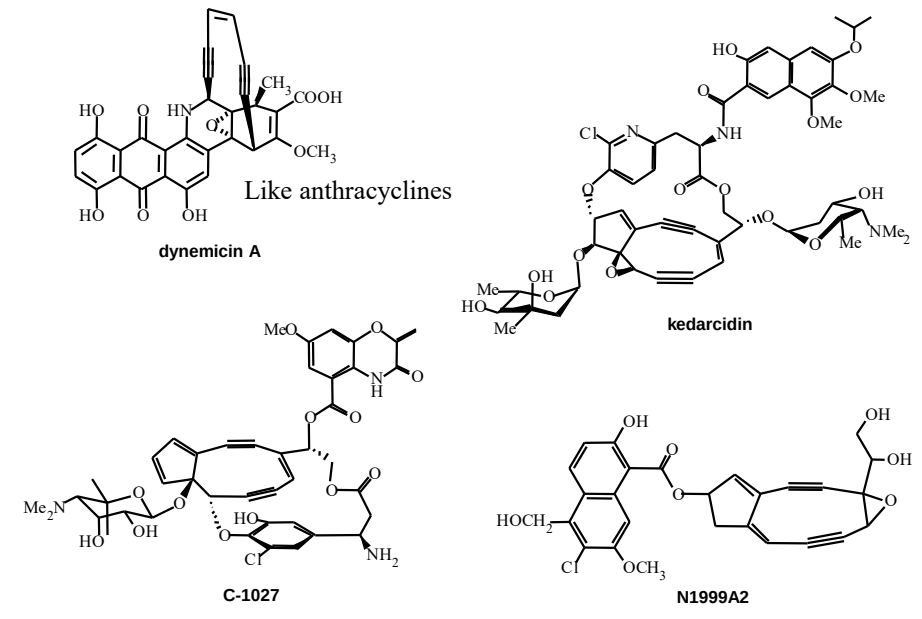
Mechanism to Account for Water Incorporation into Both C-4′ and C-1′ Positions of the Alkali-Labile Product



Enediyne Antitumor Antibiotics



Enediyne Antitumor Antibiotics (cont'd)

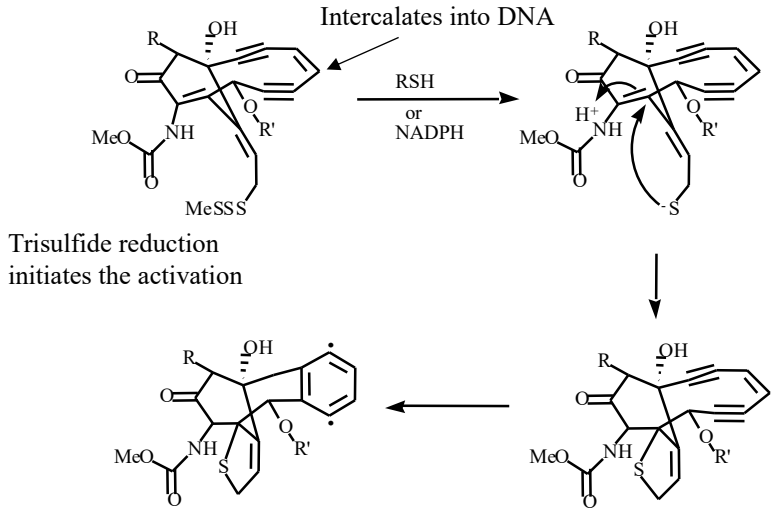


Common modes of action

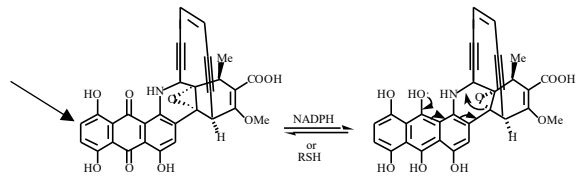
intercalation into minor groove

- reaction (activation) with either a thiol or NADPH - generates radical
- radical cleavage of DNA

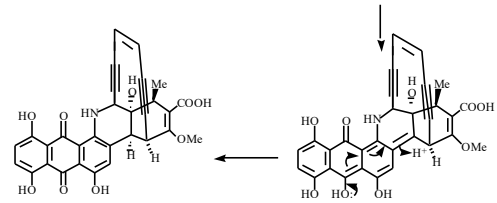
Mechanism for Esperamicins/Calicheamicins

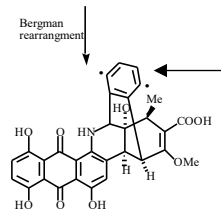


Intercalates into DNA



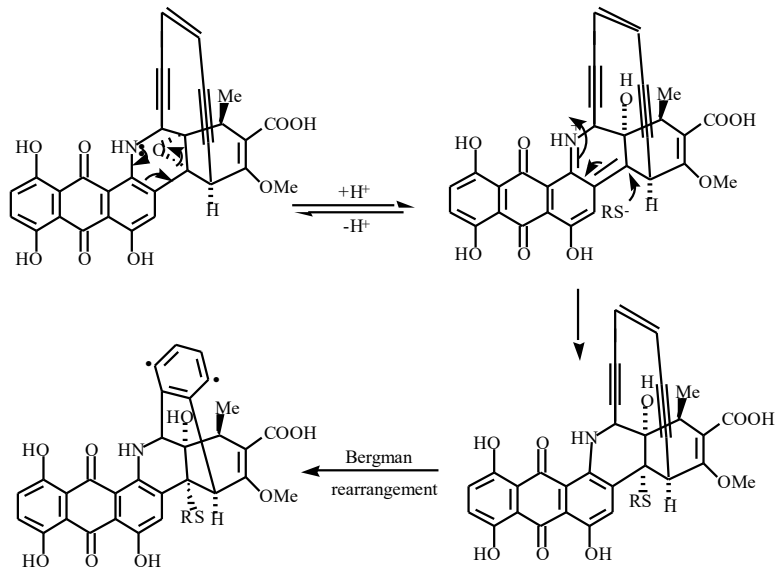
Dynemicin A Reductive Mechanism



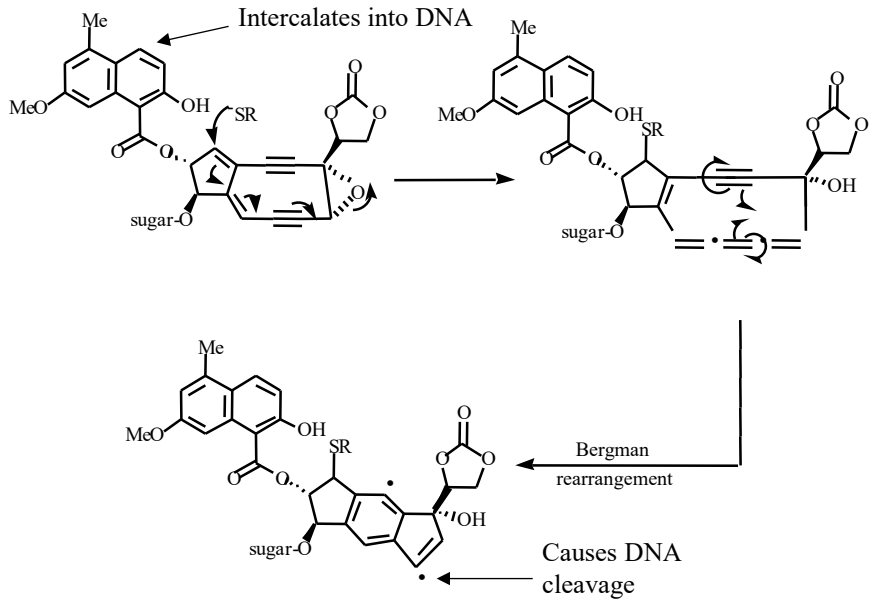


Causes DNA cleavage

Dynemicin A Nucleophilic Mechanism



Zinostatin Mechanism



Two mechanisms for DNA cleavage by any of the *biradicals* generated *in the presence of O_2* under reducing conditions

NCS: neocarzinostatin

X : DNA main chain