Lecture 4

Organic Pharm. Chemistry for Pharmacy Students

By

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Examples of Carrier-Linked Bipartate Prodrugs

Prodrug approaches for enhancing administration, permeability or absorption

Bipartate prodrugs

Prodrugs with increased aqueous solubility

Poor aqueous solubility is considered as a serious problem limiting the therapeutic use of numerous drugs

One frequently employed means of improving the aqueous solubility of a drug is by the use of

1. Phosphate derivatives
2. Esters derivatives
3. Hemisuccinate derivatives

1. Phosphate

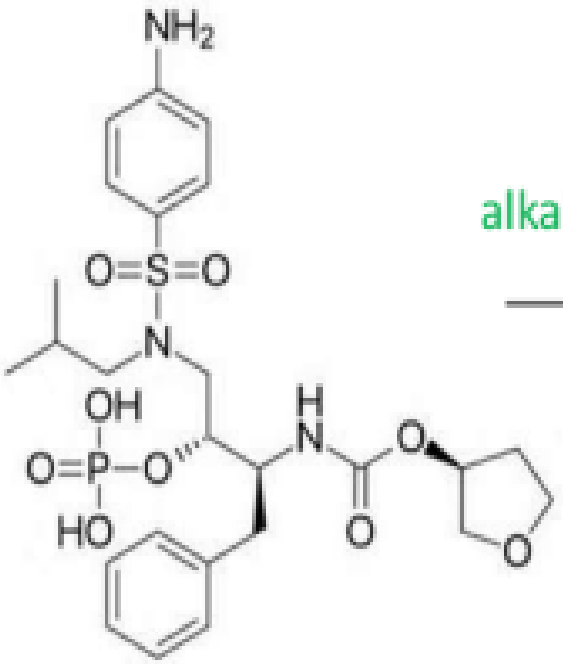
Due to the ionic nature of the phosphate group

Phosphate derivatives display high chemical stability, often even higher than the parent compound.

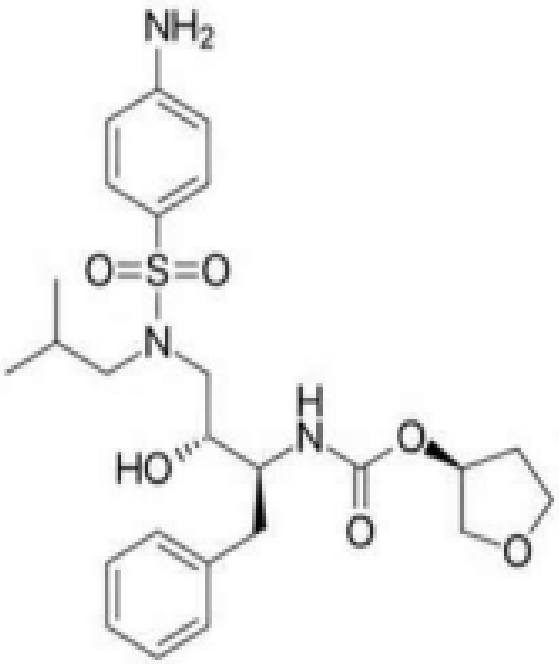
Under physiological conditions phosphate prodrugs undergo rapid biotransformation by endogenous phosphatases, such as alkaline phosphatase, of the intestine, plasma, and the liver

Fosamprenavir; phosphate ester of amprenavir
a HIV protease inhibitor.
Antiviral, HIV infections.

Bioconverted by alkaline phosphatases to amprenavir,



alkaline phosphatases

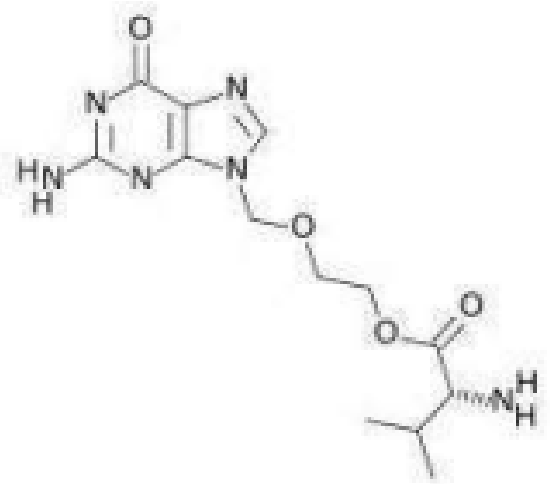


Fosamprenavir

amprenavir

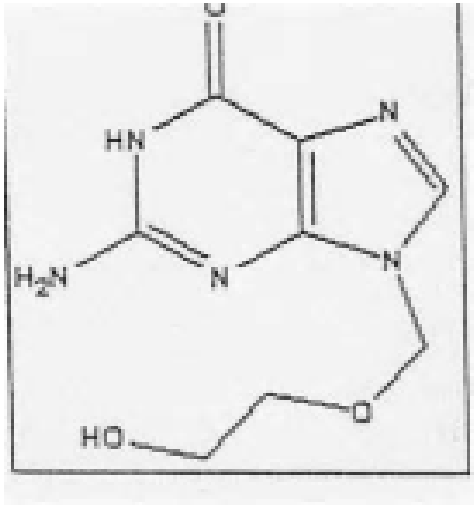
2-Another approach to increase absorption is esterification with amino acids.

valacyclovir which is valine esters of the antiviral drugs acyclovir
are substrates for PEPT1 (a specific transport system for amino acid)



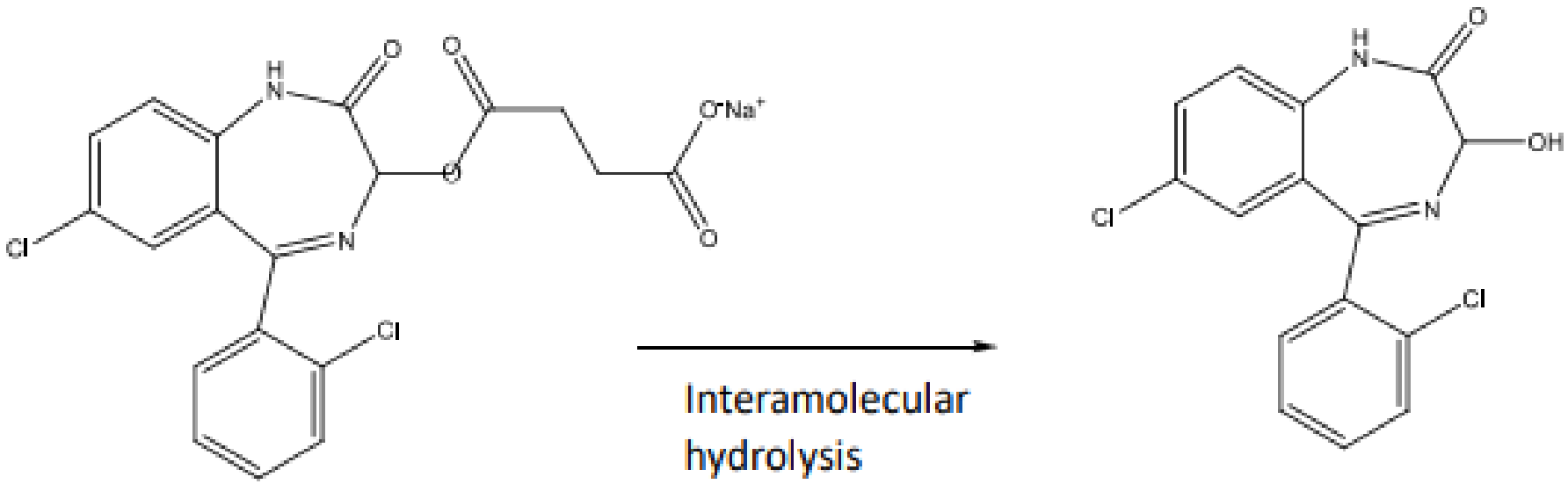
Valaciclovir

esterase



3-A hemisuccinate group can be conveniently used to increase water solubility, as it contains a free carboxylic group, which is suitable for the formation of dissociated salts.

hemi succinate esters is cinazepam, a novel benzodiazepine anxiolytic drug suitable for intravenous injections



Prodrugs with increased lipid solubility

1- Oral preparation

In order to improve lipophilicity, and thus passive transport through biological membranes, compounds containing polar or ionizable groups can be converted into ester prodrugs

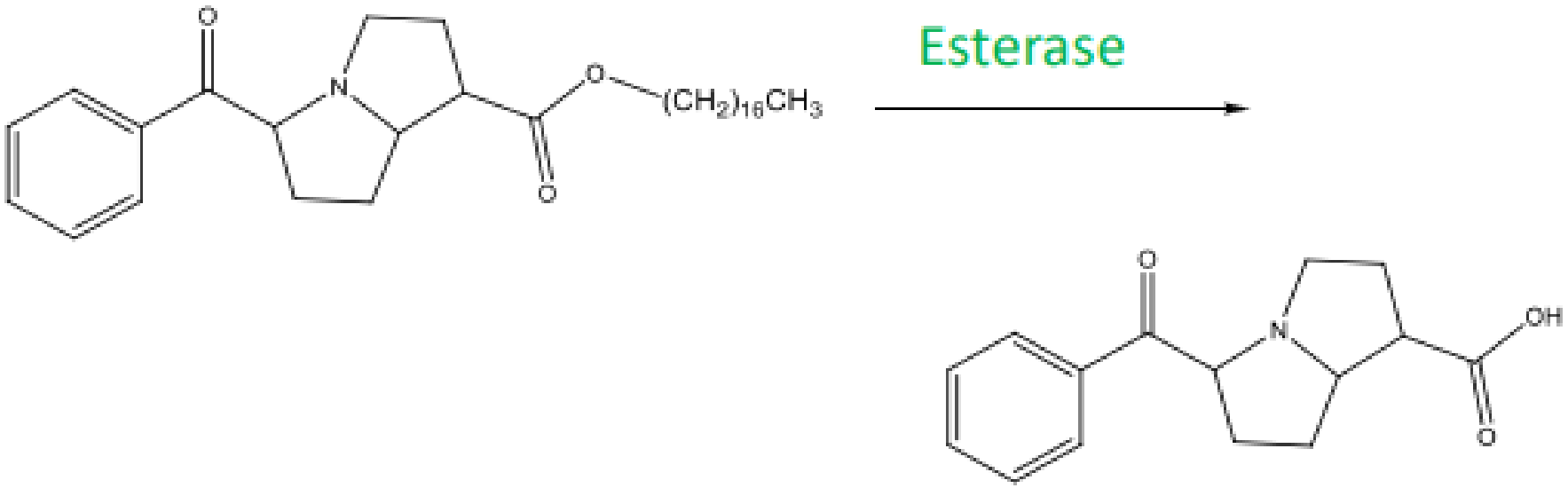
Enalapril In the liver bioconverted by esterases to enalaprilic acid, an angiotensin-converting enzyme inhibitor. Used in the treatment of hypertension, ischemic heart disease

Enalapril

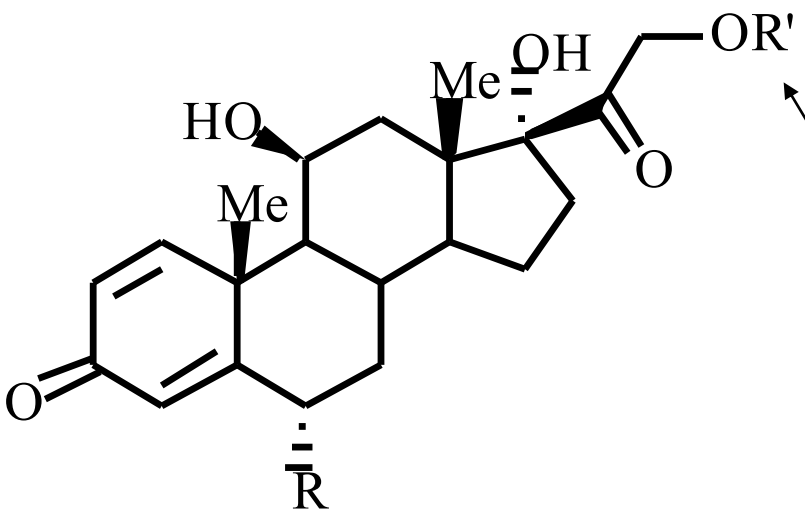
enalaprilic acid

2-Prodrugs with increased lipophilicity are also designed for topical administration

Esters of ketolac (a non-steroidal anti-inflammatory drug with potent analgesic activity) and fatty acids (stearic acid) allow the drug to accumulate in the skin with concomitant low skin permeation, leading to increased therapeutic efficiency and reduced side effects of the parent drug



1. Prodrug for **Increased Water Solubility**



$R' = C(=O)CH_2CH_2CO_2Na$

Prodrug forms

$R' = PO_3Na_2$

for aqueous injection or opthalmic use

prednisolone (R = R' = H)
methylprednisolone (R = CH_3, R' = H)

poor water solubility

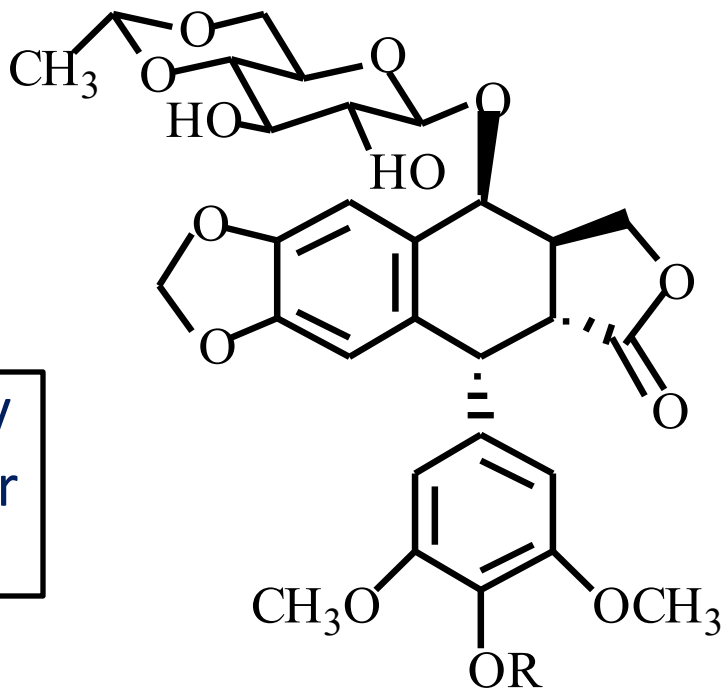
8.11

corticosteroid

(Choice of water solubilizing group: The ester must be stable enough for a shelf life of > 2 years but must be hydrolyzed in vivo with a half-life < 10 minutes).

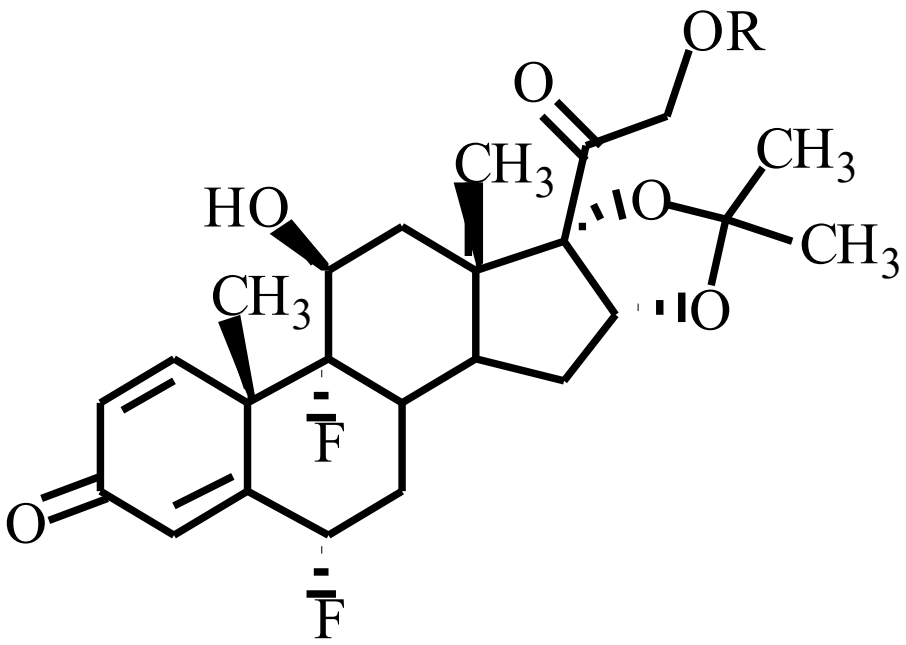
To avoid formulation of **etoposide** with detergent, PEG, and EtOH (used to increase water solubility), it has been converted to the phosphate prodrug.

Chemotherapy used for cancer treatment



etoposide (R = H)
etoposide phosphate (R = PO_3H_2)
8.12

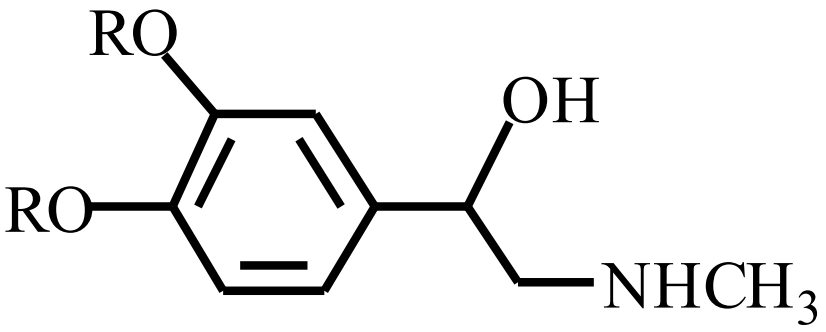
2. Prodrug for **Improved Absorption** Through Skin



fluocinolone acetonide (R = H)
fluocinonide (R = $COCH_3$)
8.14

corticosteroids - inflammation, allergic, pruritic skin conditions

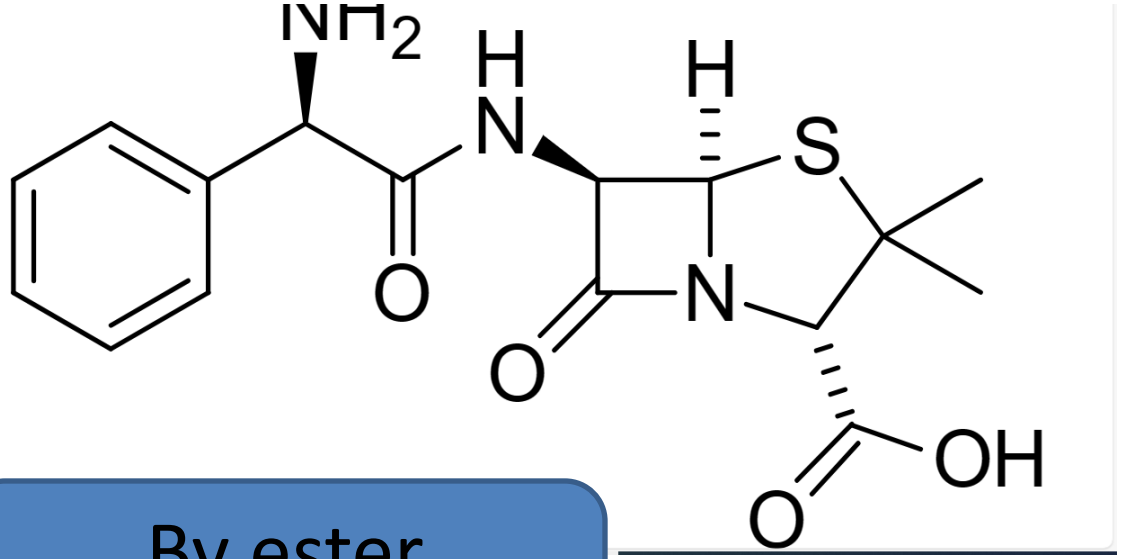
Better absorption into cornea for the treatment of glaucoma



dipivefrin (R = Me_3CCO)
epinephrine (R = H)
8.15

The cornea has significant esterase activity

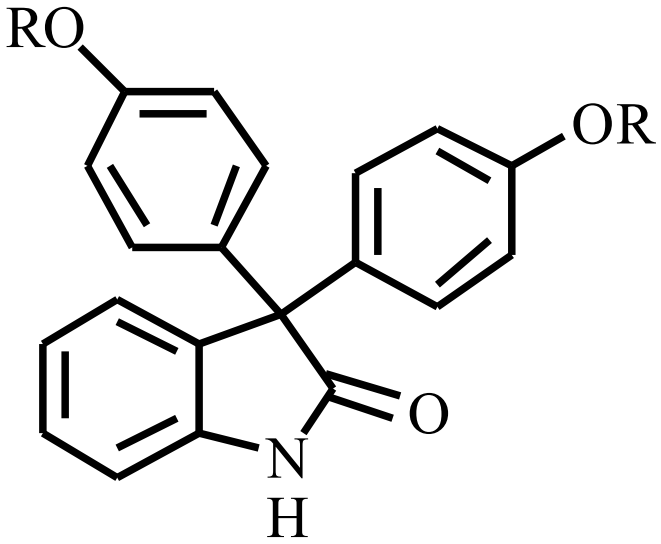
- How **to increase absorption** of ampicillin ?
- To increase lipid solubility for better absorption



By ester formation

3.Prodrug for **Site Specificity**

Bowel sterilant



oxyphenisatin (R = H) (administer rectally)
8.16

ما الفرق

prodrug R = Ac (administer orally)
hydrolyzed in intestines

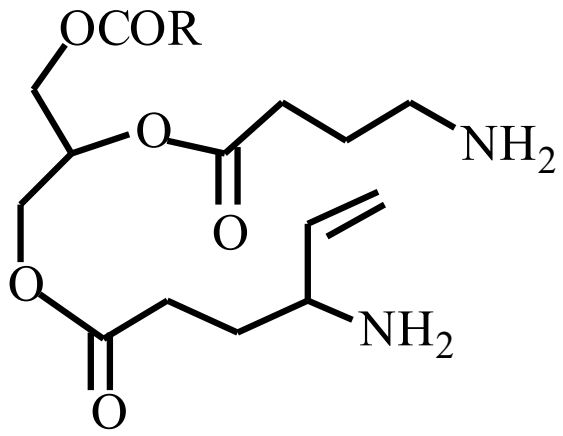
3. Prodrug for Site Specificity (cont.…)

The blood-brain barrier prevents hydrophilic molecules from entering the brain, unless actively transported. The anticonvulsant drug **vigabatrin** crosses poorly.

A glyceryl lipid (**8.17**, R = **linolenoyl**) containing one GABA ester and one vigabatrin ester was 300 times more potent in vivo than vigabatrin.

GABA : gama amino butyric acid
NH2-CH2-CH2-CH2-COOH

فين ؟



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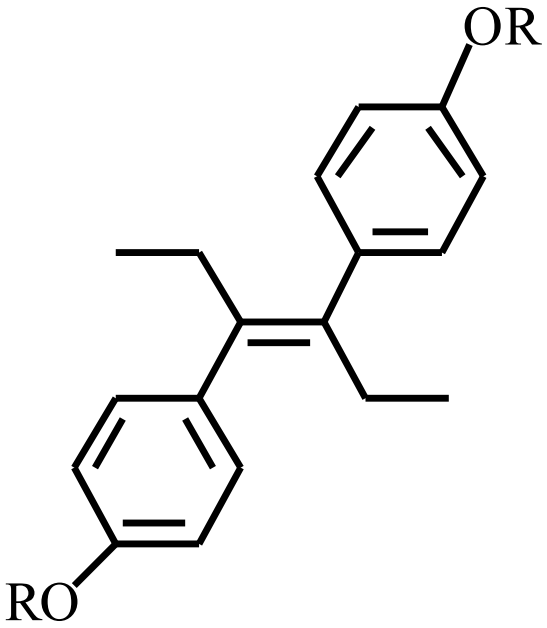
Site Specificity Using Enzymes at the Site of Action



diethylstilbestrol diphosphate (R = $PO_3^{=}$)

diethylstilbestrol (R = H)

8.18

Phosphatase should release the drug selectively in tumor cells.

(This approach has not been successful because the prodrugs are too polar, enzyme selectivity is not sufficient, or tumor cell perfusion rate is poor.)

Enzyme-Prodrug Therapies (Selective Therapy)

For selective activation of prodrugs in tumor cells

Two steps:

1. incorporate a prodrug-activating enzyme into a target tumor cell

2. administer a nontoxic prodrug which is a substrate for the exogenous enzyme incorporated

Criteria for Success with Enzyme-Prodrug Therapies

1. The prodrug-**activating enzyme** is either nonhuman or a human protein expressed poorly
2. The prodrug-activating enzyme must have high catalytic activity
3. The prodrug must be a good substrate for the incorporated enzyme and not for other endogenous enzymes
4. The prodrug must be able to cross tumor cell membranes

5. The prodrug should have low cytotoxicity and the drug high cytotoxicity

6. The activated drug should be highly diffusable to kill neighboring nonexpressing cells (**bystander killing effect**)*(تقرير)

7. The half-life of the active drug is long enough for bystander killing effect but short enough to avoid leaking out of tumor cells

- ***Bystander Effect**
- Bystander effects refer to effects seen in cells, tissues, or organisms which receive some type of a signal from an irradiated cell, tissue, or organism.

* Bystander and Distant Bystander Effects

- A bystander effect occurs when nontransduced or genetically unmodified cells are killed during death of transduced tumor cells.
- Bystander effect is one of the important features of a useful therapeutic gene for cancer gene therapy. The classical example is the bystander effect generated by the herpes simplex virus thymidine kinase (TK) gene. In the presence of the prodrug ganciclovir, TK expression kills not only the TK-transfected cells but also the nearby untransfected cells .

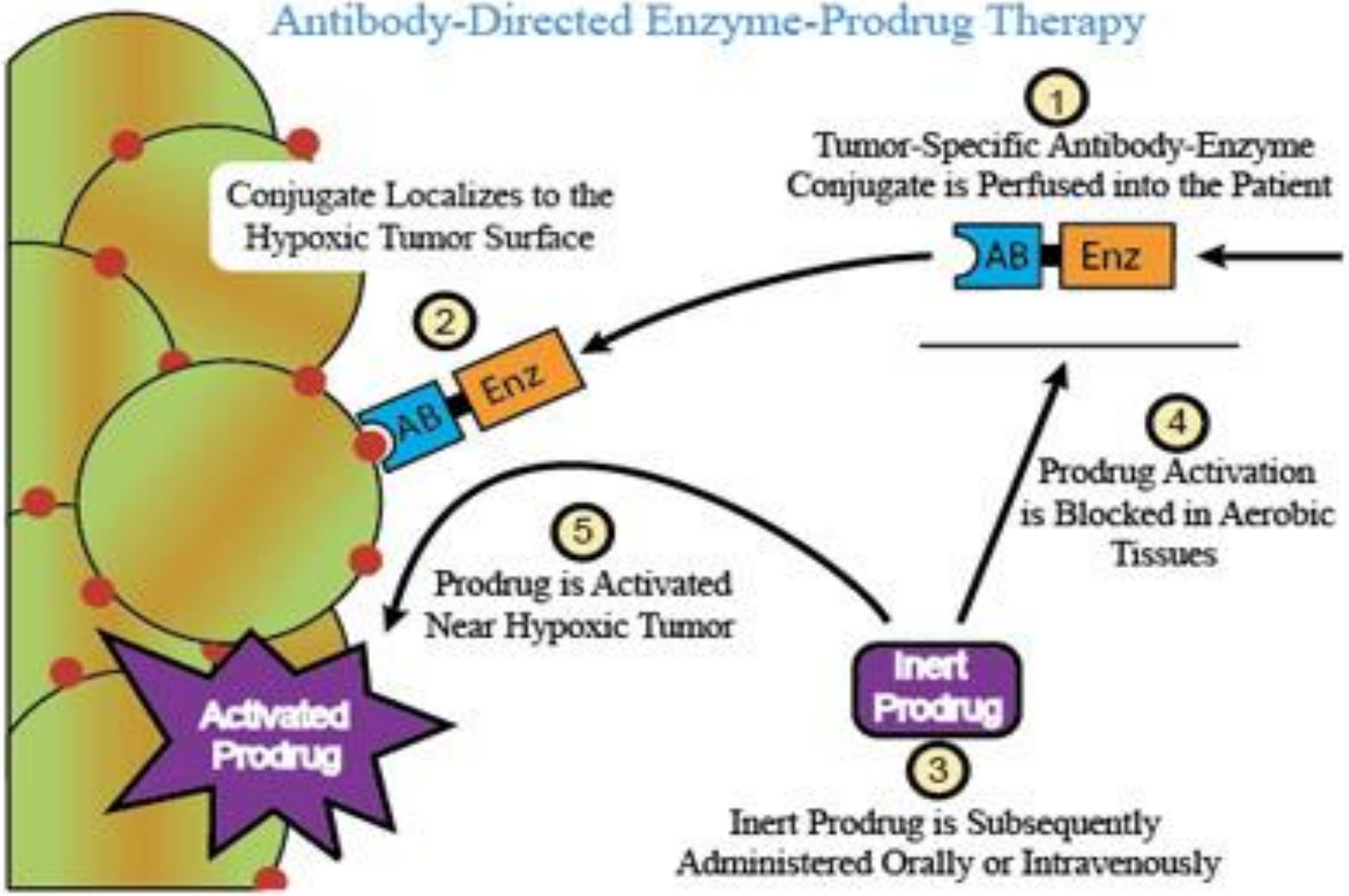
Antibody-Directed Enzyme Prodrug Therapy (ADEPT)

An approach **for site-specific delivery of cancer drugs.**

Phase One:

An antibody-enzyme conjugate is administered which binds to the surface of the tumor cells. The antibody used has been targeted for the particular tumor cell. The enzyme chosen for the conjugate is one that will be used to cleave the carrier group off of the prodrug administered in the next phase.

- **Phase Two**:
- After the antibody-enzyme has accumulated on the tumor cell and the excess conjugate is cleared from the blood and normal tissues, the prodrug is administered. The enzyme conjugated with the antibody at the tumor cell surface catalyzes the conversion of the prodrug to the drug when it reaches the tumor cell.


Antibody-Directed Enzyme-Prodrug Therapy
1
Tumor-Specific Antibody-Enzyme Conjugate is Perfused into the Patient
AB
Enz
Conjugate Localizes to the Hypoxic Tumor Surface
2
AB
Enz
4
Prodrug Activation is Blocked in Aerobic Tissues
5
Prodrug is Activated Near Hypoxic Tumor
Activated Prodrug
Inert Prodrug
3
Inert Prodrug is Subsequently Administered Orally or Intravenously

ADEPT

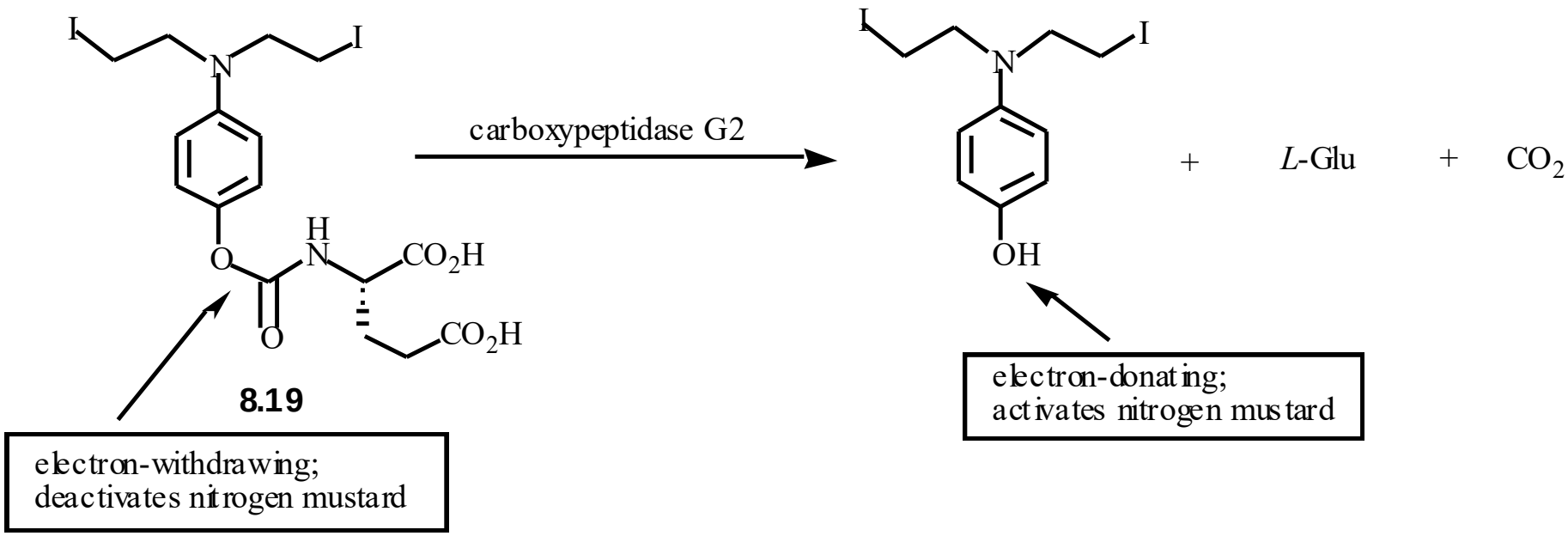
Advantages:

1. Increased selectivity for targeted cell
2. Each enzyme molecule converts many prodrug molecules
3. The released drug is at the site of action
4. Demonstrated to be effective at the clinical level
5. Concentrates the drug at the site of action

Disadvantages:

1. Immunogenicity and rejection of antibody-enzyme conjugate
2. Complexity of the two-phase system and i.v. administration
3. Potential for leakback of the active drug

An **example** is carboxypeptidase G2 or alkaline phosphatase linked to an antibody to activate a nitrogen mustard prodrug.



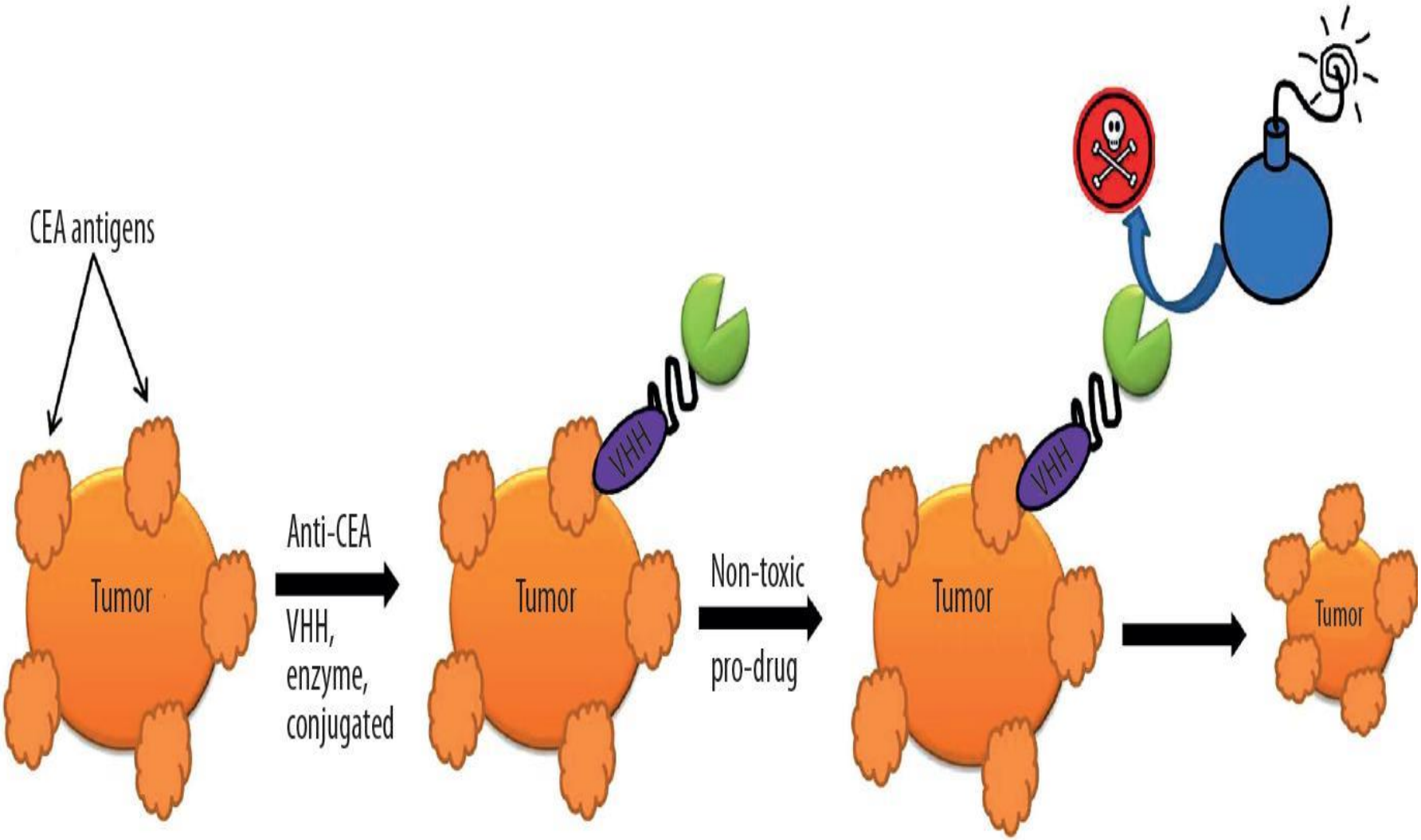
Humanization of antibodies minimizes immunogenicity. Note the prodrug-activating enzyme is a bacterial enzyme.

Antibody-Directed Abzyme Prodrug Therapy (ADAPT)

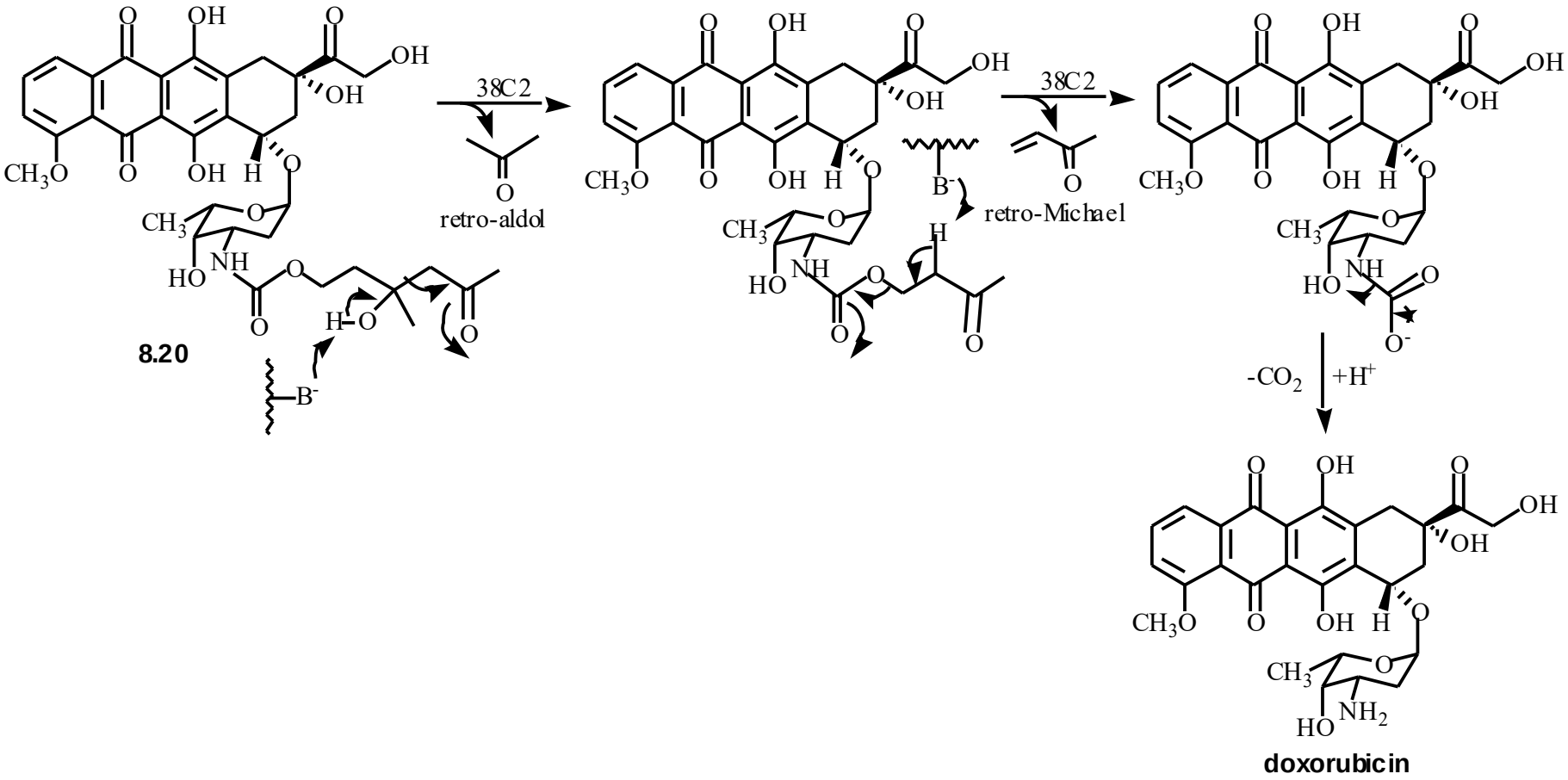
Instead of using a prodrug-activating enzyme, a humanized prodrug-activating catalytic antibody (**abzyme**) can be used.

Ideally, the abzyme catalyzes a reaction not known to occur in humans, so the only site where the prodrug could be activated is at the tumor cell where the abzyme is bound.

Antibody 38C2 catalyzes sequential retro-aldol and retro-Michael reactions not catalyzed by any known human enzyme found to be long-lived in vivo, to activate prodrugs selectively, and to kill colon and prostate cancer cells.


CEA antigens
Tumor
Anti-CEA
VHH,
enzyme,
conjugated
VHH
Tumor
Non-toxic
pro-drug
VHH
Tumor
Tumor

Abzyme 38C2 Activation of a Doxorubicin Prodrug

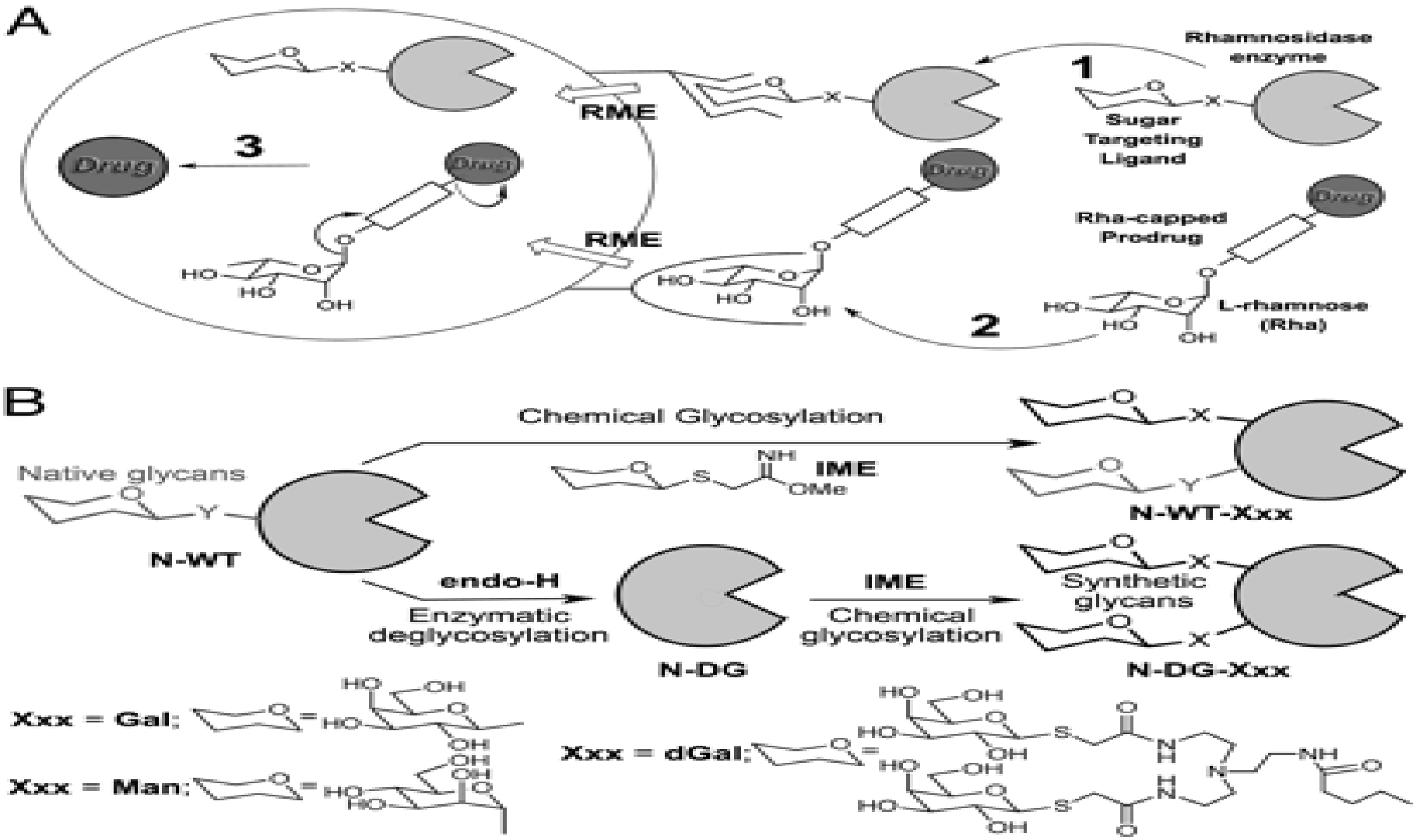


Lectin-directed enzyme-activated prodrug therapy

The LDEAPT strategy.
(*A)* Concept. LDEAPT is a bipartite delivery system.
(Step 1) Site-selective delivery of a glycosylated rhamnosidase (Rha-cleaving) enzyme by sugar-mediated RME.
(Step 2) Delivery of a Rha-capped prodrug that can be cleaved only by the delivered glycosylated rhamnosidase. When these two steps are combined, activation of the prodrug results in site-selective release of the parent drug (Step 3). (*B)* Glycosylated enzyme construction

- . Pure wild-type α-L-rhamnosidase N-WT with native “Y-linked” glycosylation was
- **(*i*) chemically glycosylated with sugar**-IME reagents to yield a N-WT-Xxx (Xxx = Gal, Man, or dGal) series with mixed synthetic (“X-linked”) and native (Y-linked) glycosylation or **(*ii*) enzymatically** DG with endo-H, yielding N-DG. N-DG was then chemically reglycosylated with sugar-IME reagents to yield only synthetic (X-linked) N-DG-Xxx (15).
- An IME reagent bearing two terminal Gal units (dGal) was also synthesized from D-galactose.

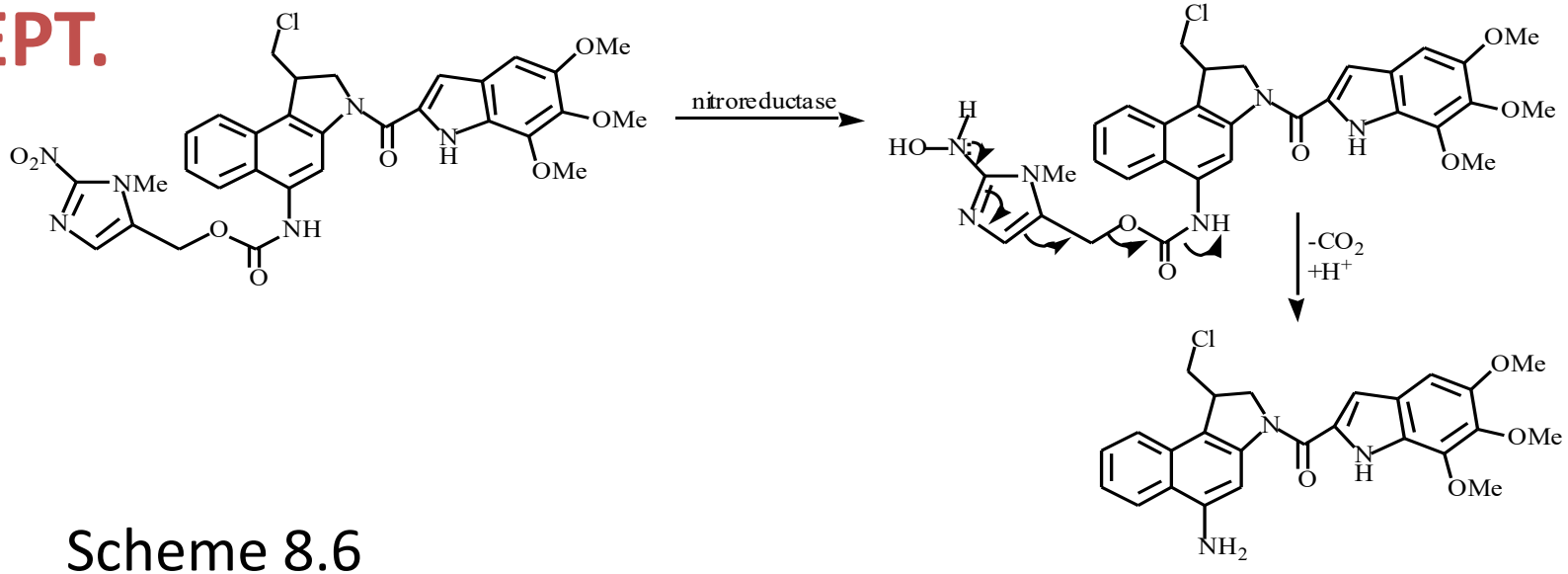
Lectin-directed enzyme-activated prodrug therapy



Gene-Directed Enzyme Prodrug Therapy (GDEPT)

Also known as suicide gene therapy

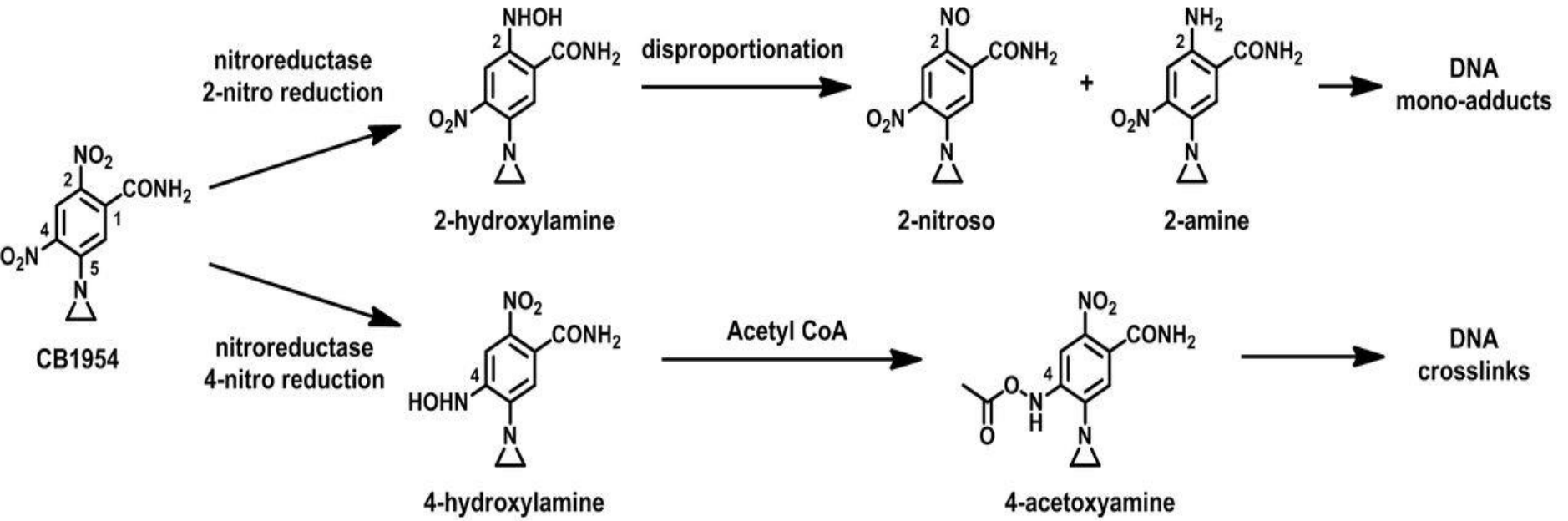
A gene encoding (التشفير) the prodrug-activating enzyme is expressed in target cancer cells under the control of tumor-selective promoters or by viral transfection. These cells activate the prodrug as in ADEPT.

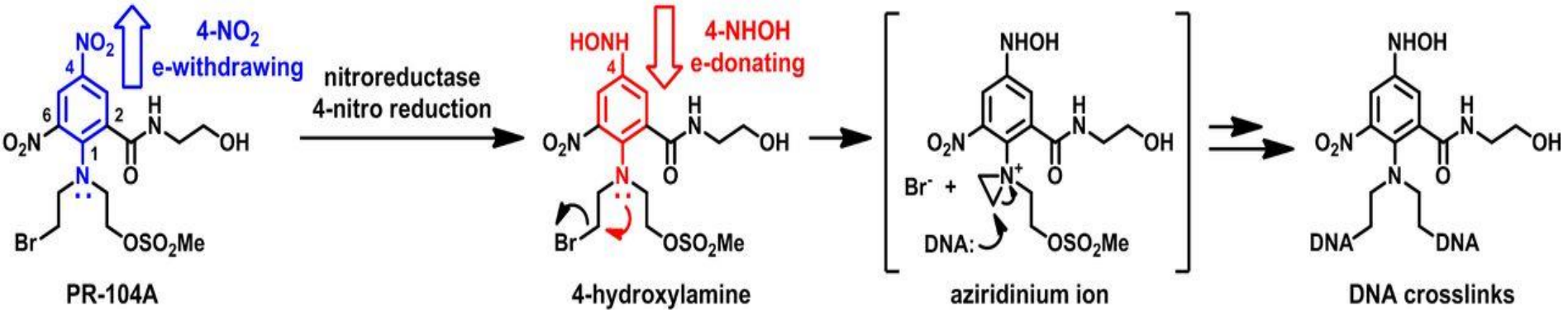


amino- *seco*-CBI-TMI
8.21

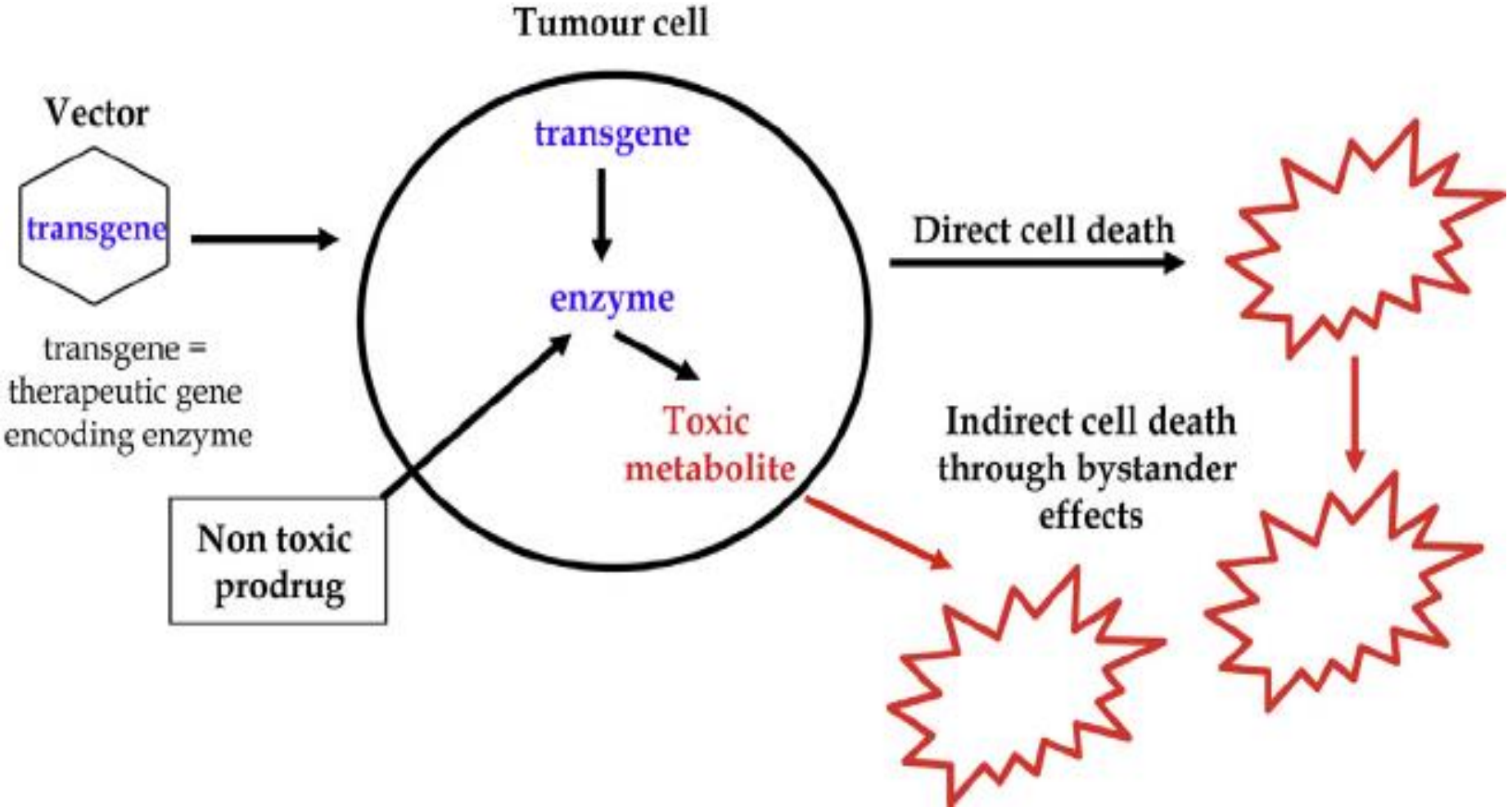
Scheme 8.6

Nitroreductase gene-directed enzyme prodrug therapy

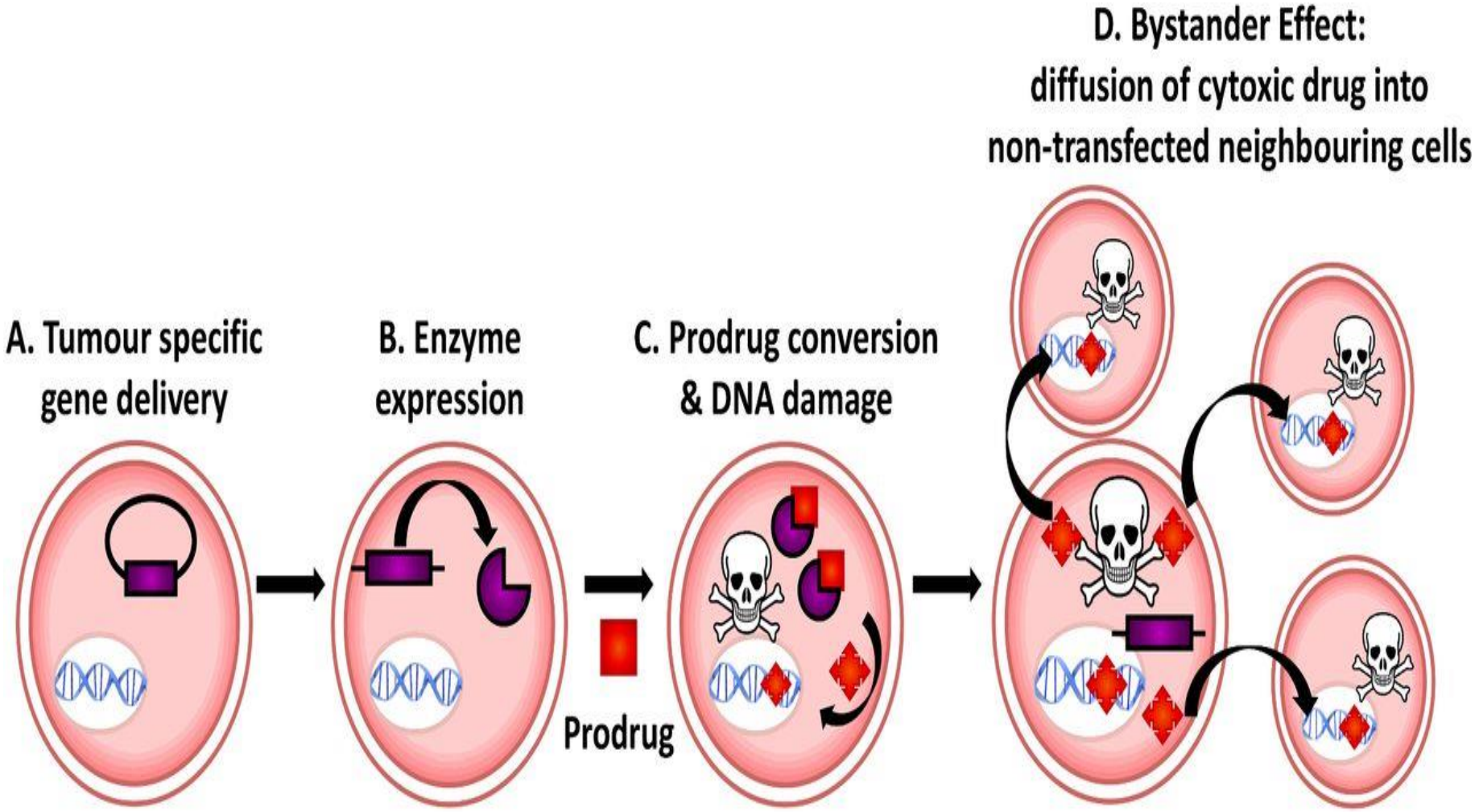




suicide gene therapy



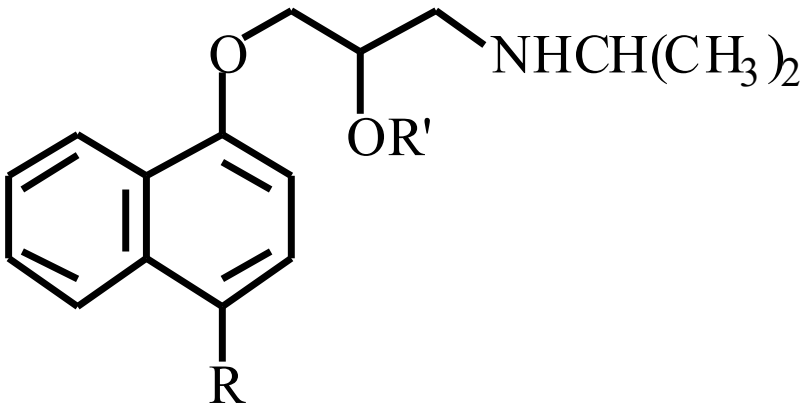
suicide gene therapy



4.Prodrug **for Stability**

protection from first-pass effect

Oral administration has lower bioavailability than i.v. injection.



propanolol (R = R' = H)
8.22

antihypertension

prodrug R' = $\overset{\overset{O}{\|}}{C}CH_2CH_2COOH$

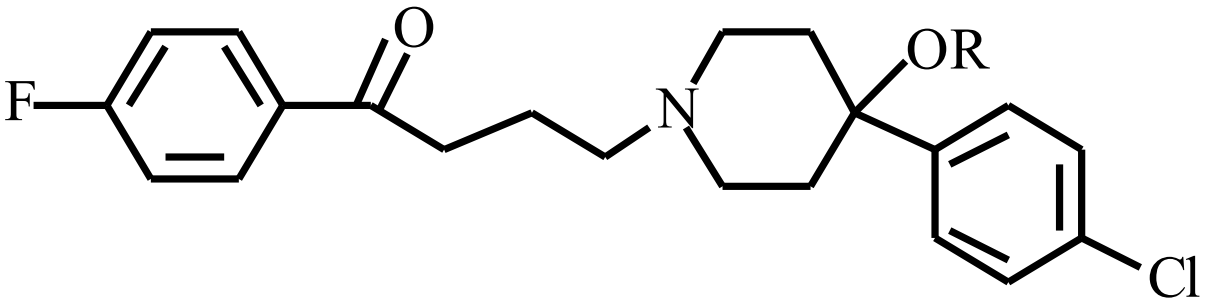
plasma levels 8 times that with propanolol

5.Prodrugs for **Slow and Prolonged Release**

1. To reduce the number and frequency of doses
2. To eliminate night time administration
3. To minimize patient noncompliance
4. To eliminate peaks and valleys of fast release

   (relieve strain on cells)
5. To reduce toxic levels
6. To reduce GI side effects

(Long-chain fatty acid esters hydrolyze slowly.
Intramuscular injection is used also).



haloperidol (R = H)
haloperidol decanoate (R = $CO(CH_2)_8CH_3$)
8.24

Sedative/tranquilizer/antipsychotic

prodrug **R' =** $C(CH_2)_8CH_3$ slow release

inject i.m.

Antipsychotic activity for about 1 month

6.Prodrugs **to Minimize Toxicity**

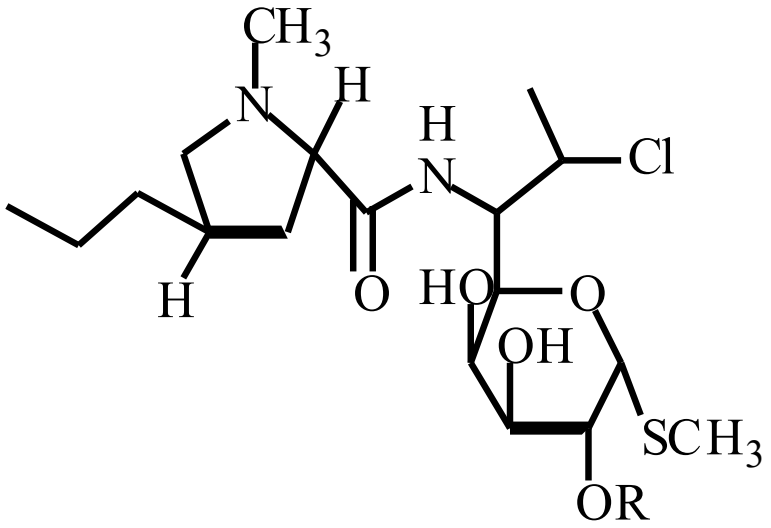
Many of the prodrugs just discussed also have lowered toxicity.

For example, **epinephrine** (for glaucoma) has ocular and systemic side effects not found in **dipivaloylepinephrine**.

7.Prodrug to **Increase Patient Acceptance**

The antibacterial drug **clindamycin** (**8.28**) is bitter and not well tolerated by children.

Clindamycin pulmitate is not bitter.



clindomycin (R = H)
clindomycin phosphate (R = PO_3H_2)
clindomycin palmitate (R = $O(CH_2)_{14}CH_3$)
8.28

Either not soluble in saliva or does not bind to the bitter taste receptor or both.

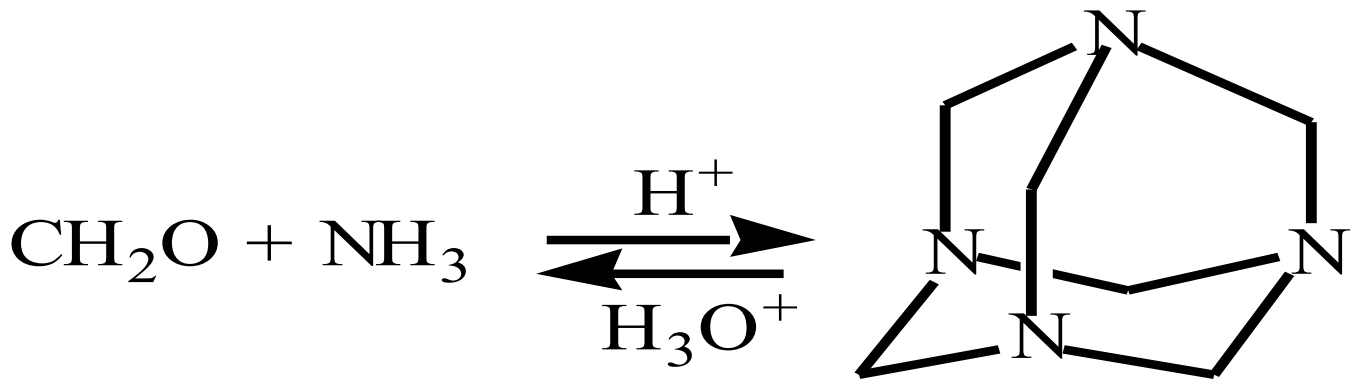
8.Prodrug **to Eliminate Formulation Problems**

Formaldehyde is a gas with a pungent odor that is used as a disinfectant. Too toxic for direct use.

Methamine :It is a stable solid that decomposes in aqueous acid.

The pH of urine in the bladder is about 4.8, so methenamine is used as a urinary tract antiseptic.

It has to be enteric coated to prevent hydrolysis in the stomach.



methenamine
8.30