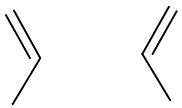
Strategies of bioconjugation

Homobifunctional

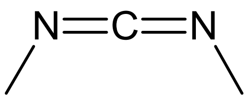
Identical functions

1. $-NH_2$ H_2N-
2. -SH HS-
3. -COOH HOOC-
4. -CHO OHC-
5. $-NH-NH_2$ H_2N-NH-
6. 

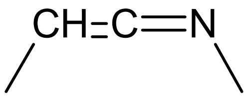
„Zero-length"

No insertion

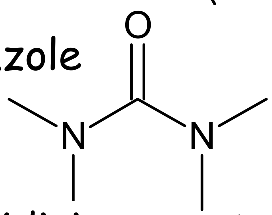
1. Carbodiimide



2. Woodward's reagent k



3. Diimidazole



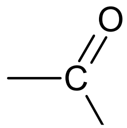
4. Oxidising agents

-SH + HS-

5. Enzymes

Heterobifunctional

Different functions

1. $-NH_2$ HS-
2. 

HS-
3. Photoreactive

$-NH_2$

-SH

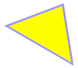
-COOH

-CHO

4. Photoaffinity labeling

3. Heterobifunctional reagents

Approach: selectivity vs. specificity

differences between  and  → two steps

3.1. amino- and thiol-groups

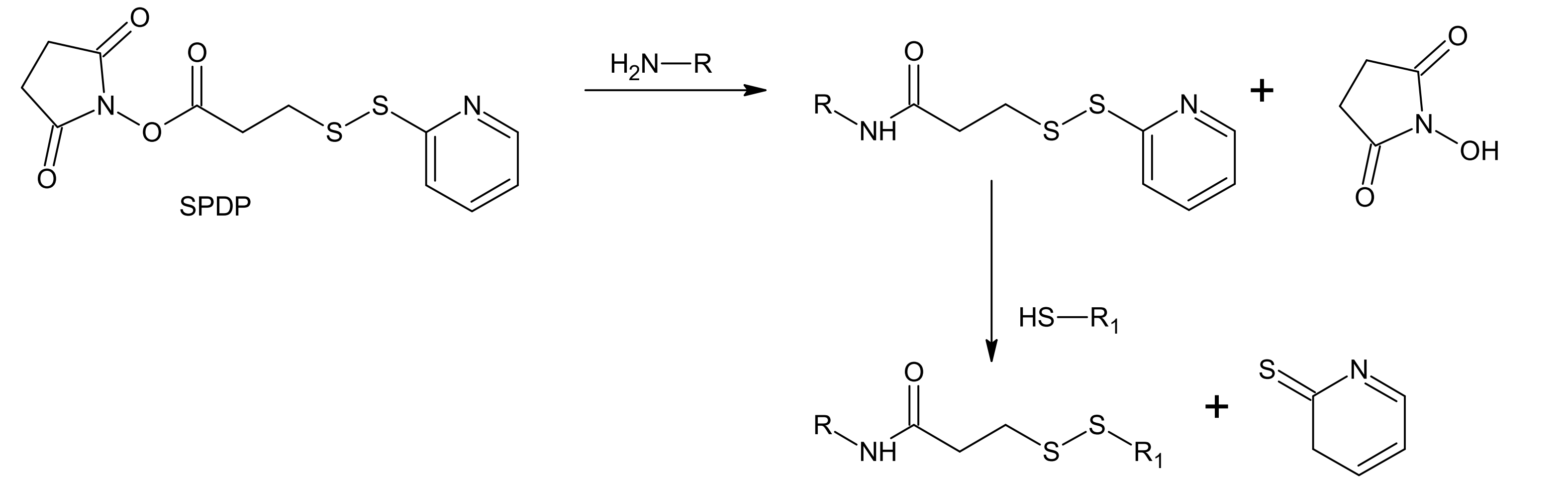
3.2. carboxyl- and thiol/amino-groups

3.3. carbonyl- and thiol-groups

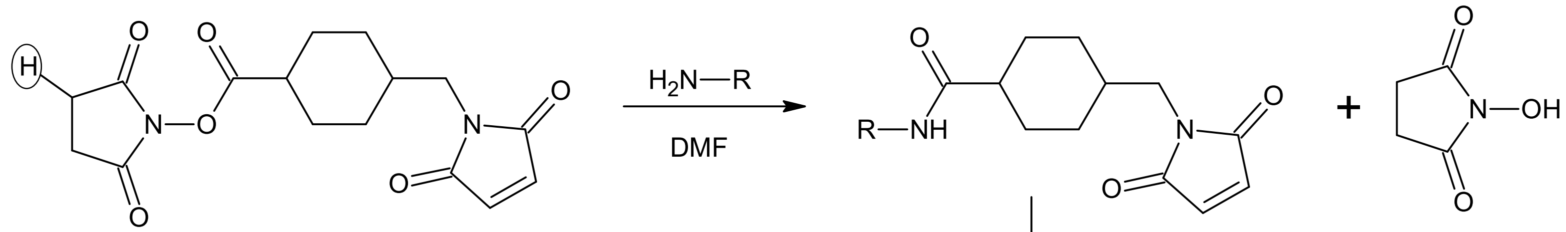
3.4. light sensitive (photoreactive) reagents

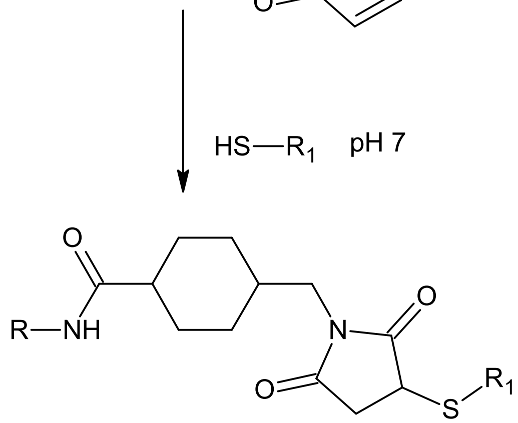
3.1. Connecting amino- and thiol-functions

a



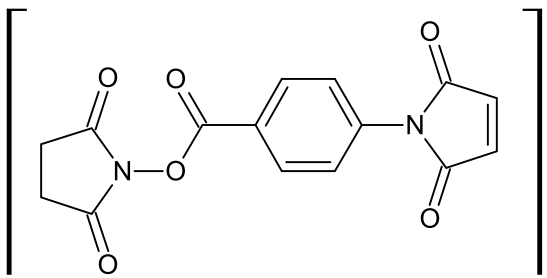
b



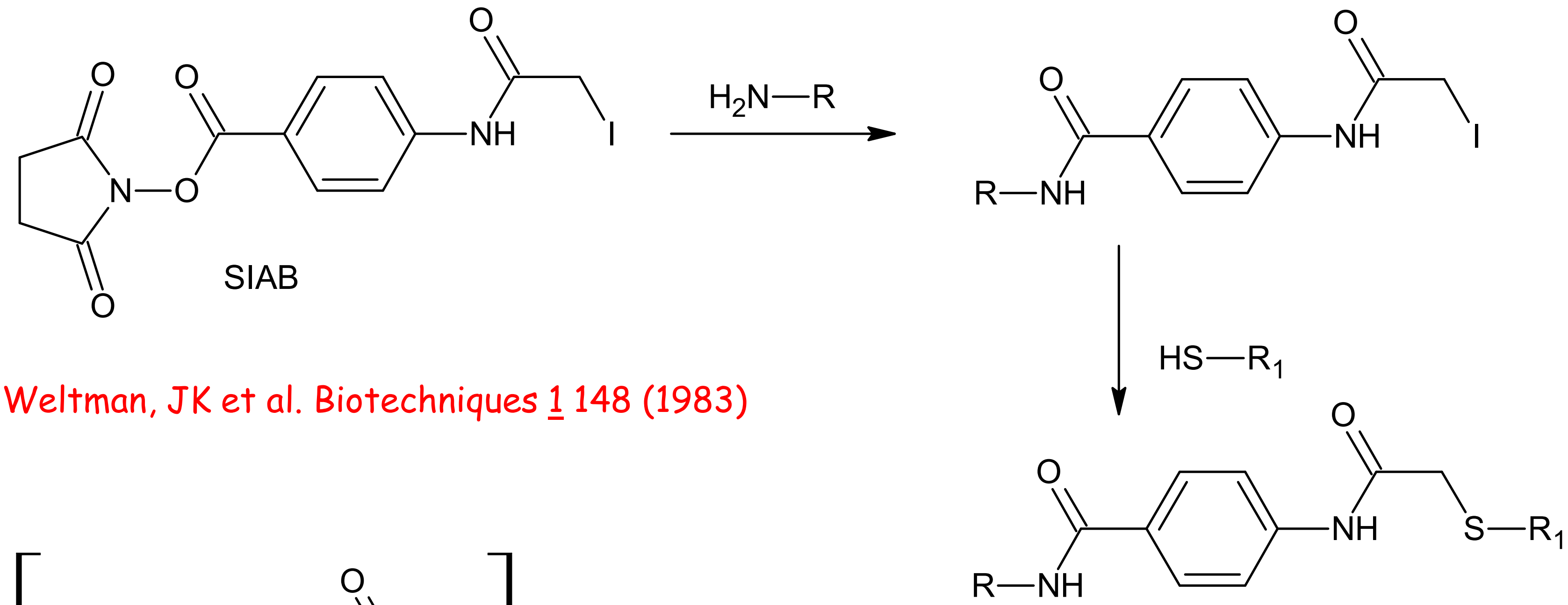


H = H, SMCC; - $SO_3^-Na^+$

Yoshitake, S. et al. 101 395 (1979)



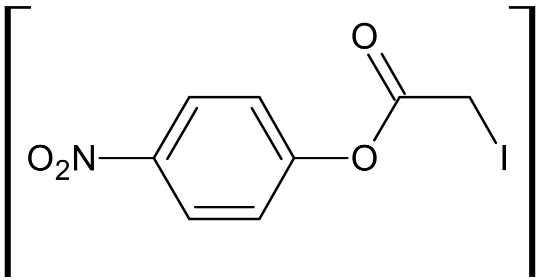
m-Maleimidobenzol-N-hydroxysuccinimide ester (MBS), 1981 c



SIAB

Weltman, JK et al. Biotechniques 1 148 (1983)

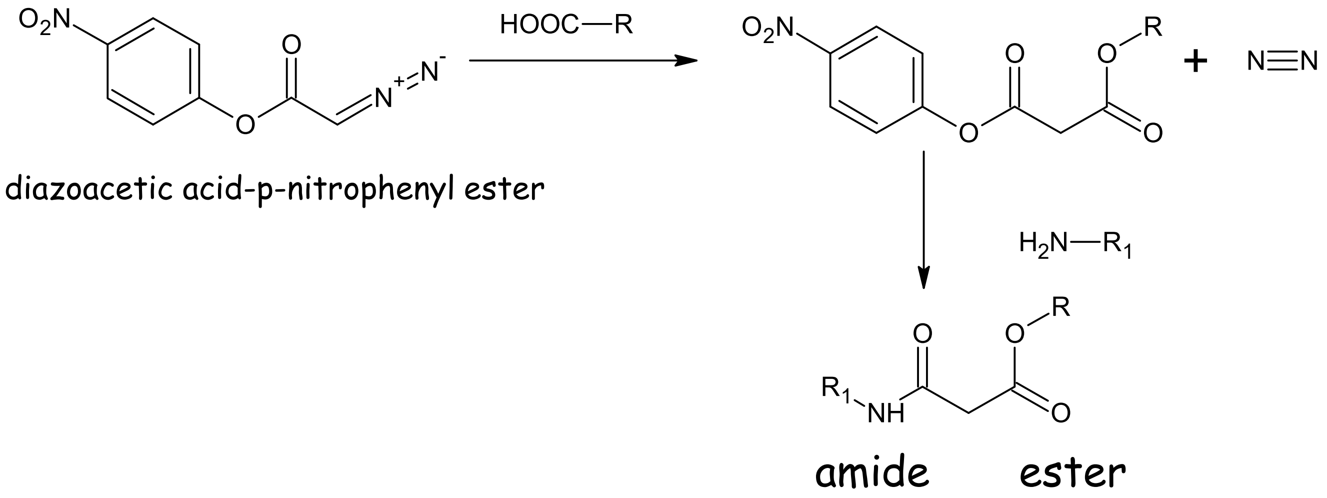
amide thioether



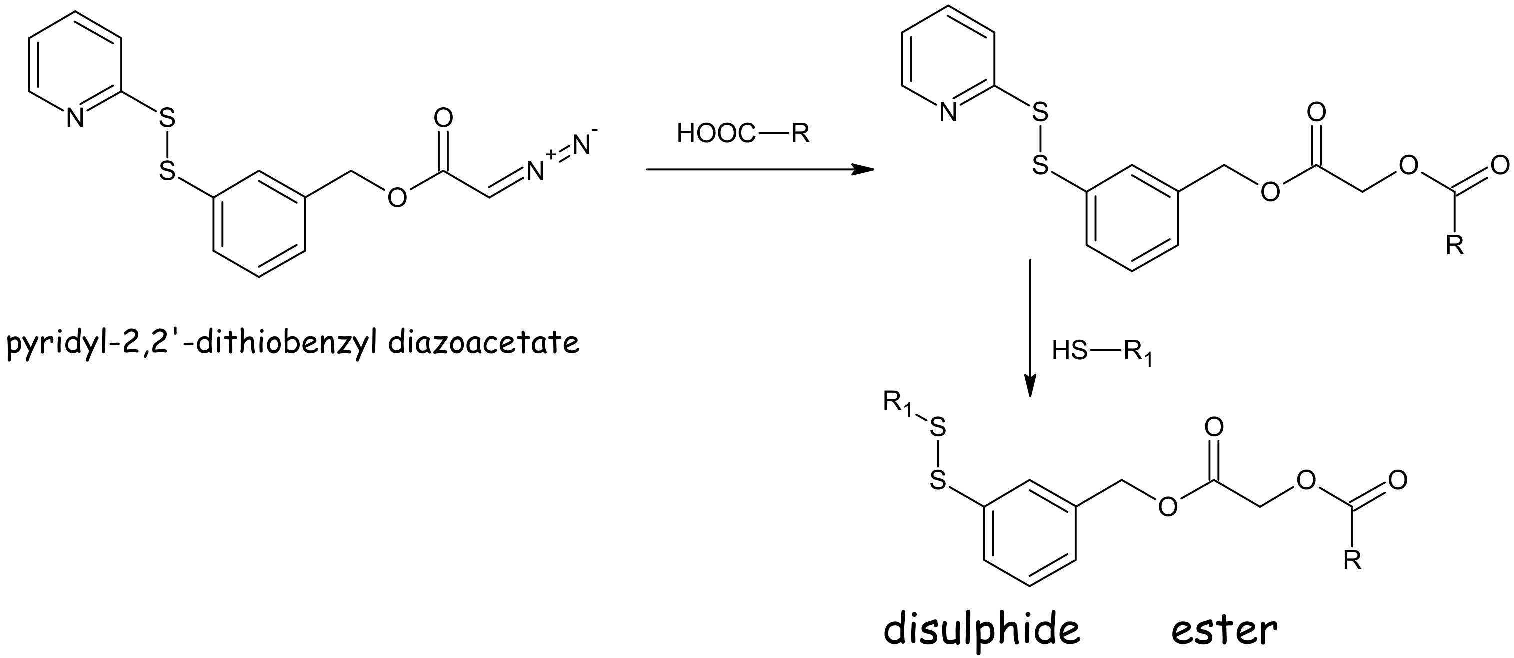
Iodoacetic acid-p-nitrophenyl ester
1975

3.2. Connecting carboxyl and amino/thiol-functions

a

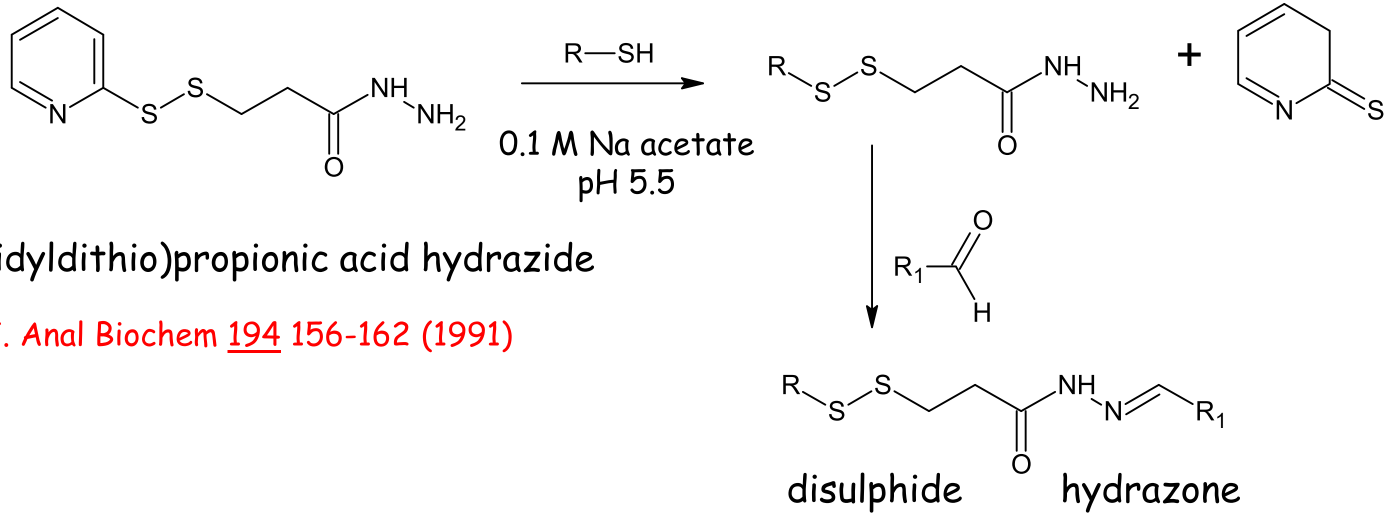


b



3.3. Connecting carbonyl and thiol-functions

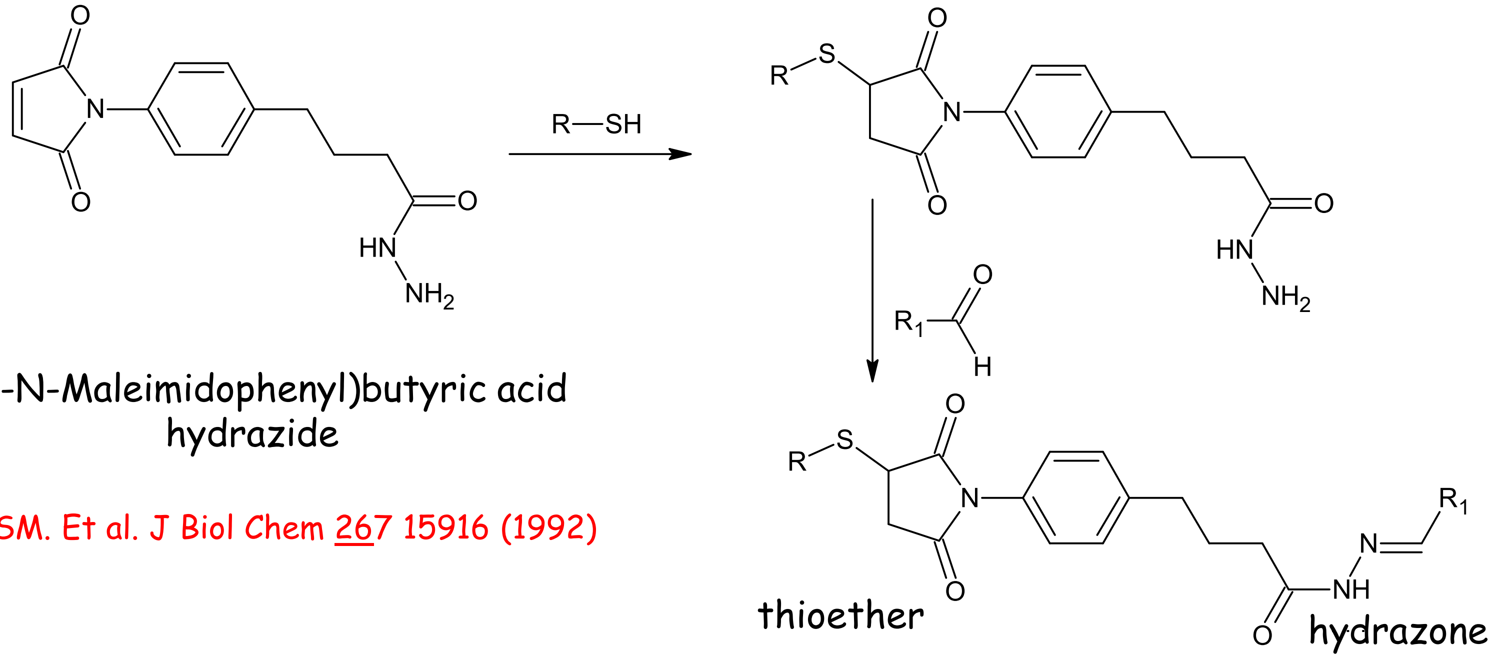
a



3-(2-pyridyldithio)propionic acid hydrazide

Zara JJ. Anal Biochem 194 156-162 (1991)

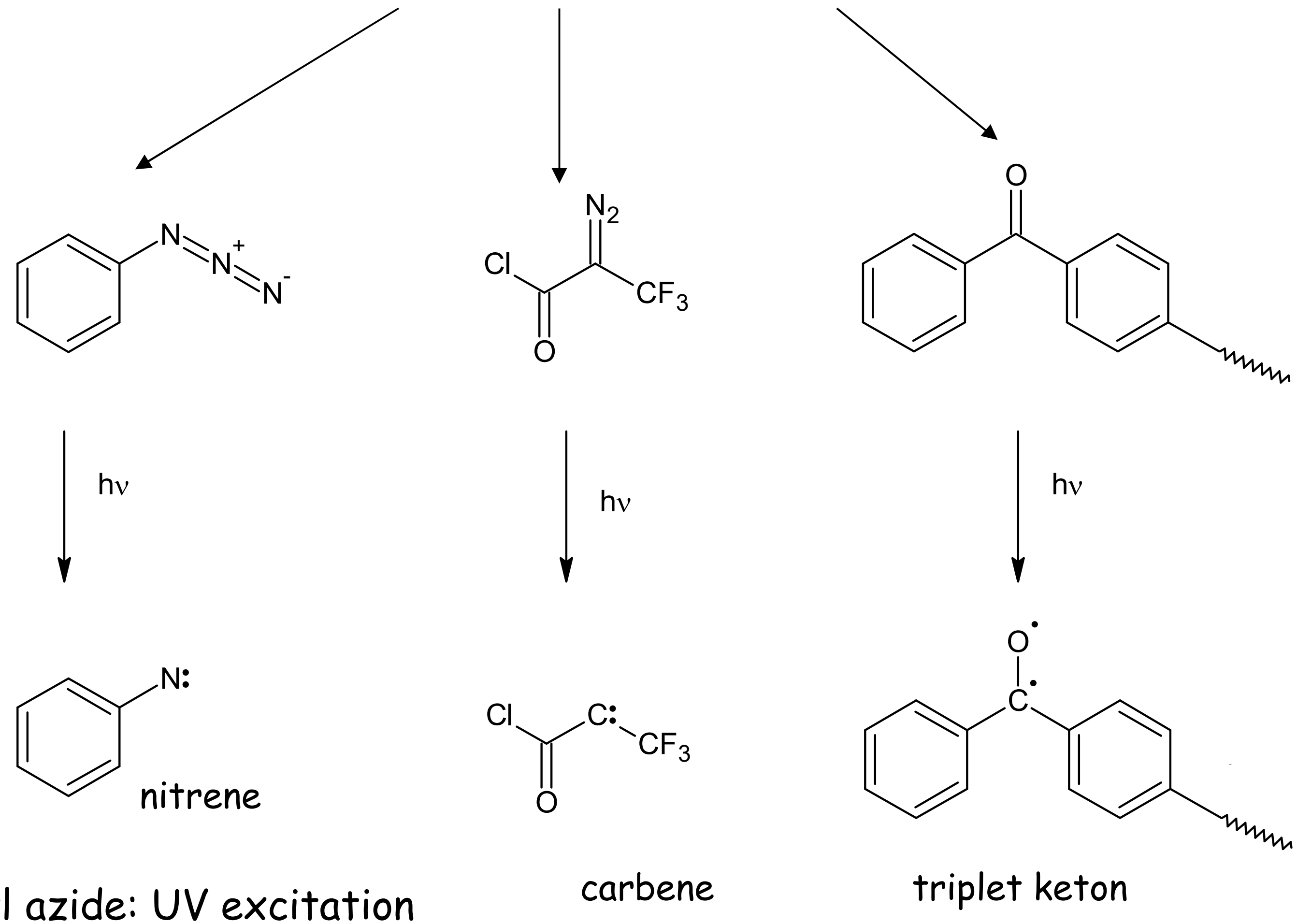
b



4-(4-N-Maleimidophenyl)butyric acid hydrazide

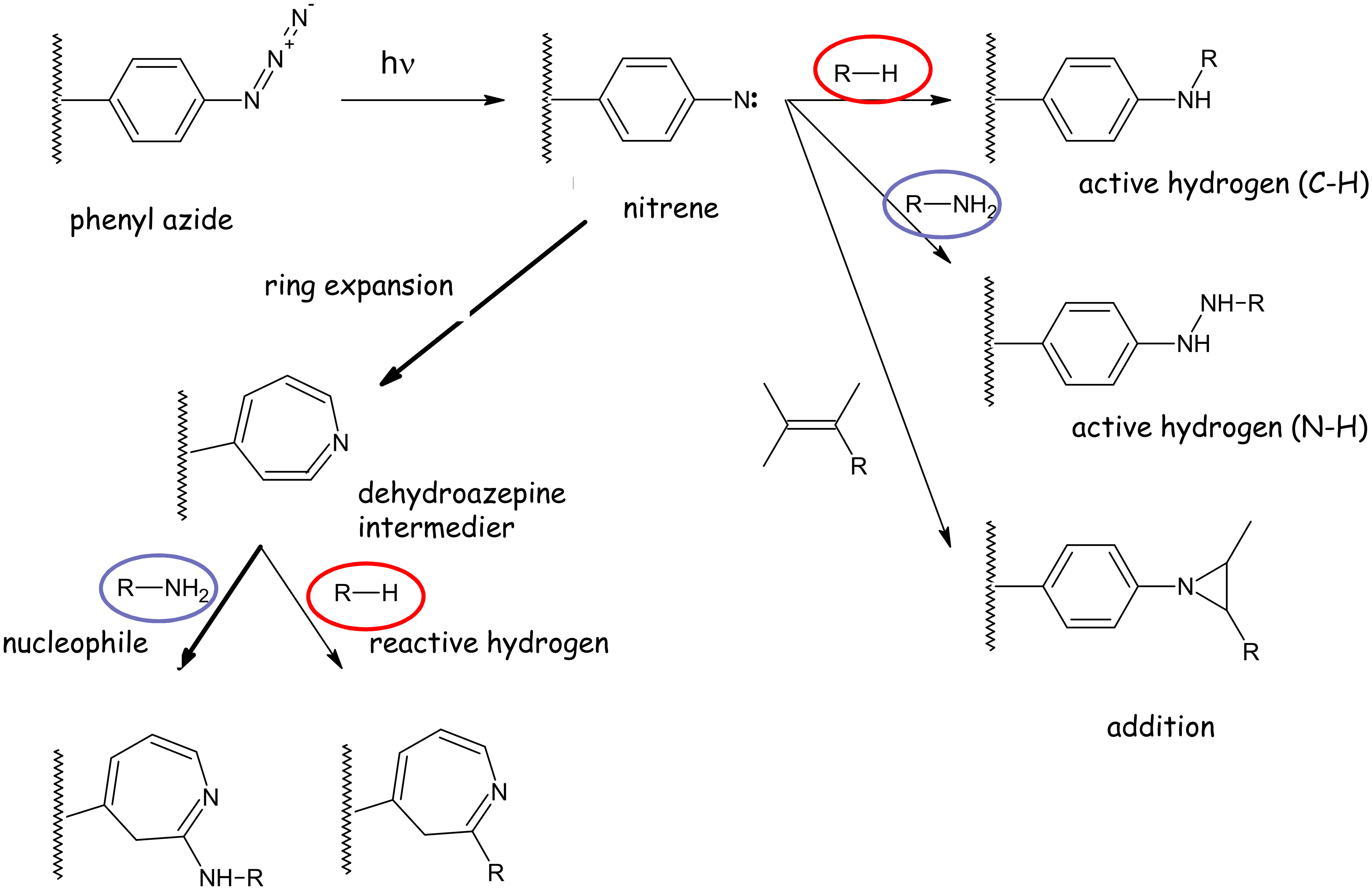
Chamov SM. Et al. J Biol Chem 267 15916 (1992)

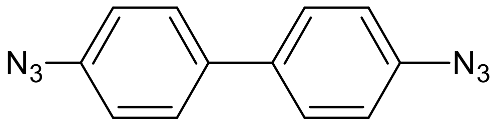
Photoactive groups



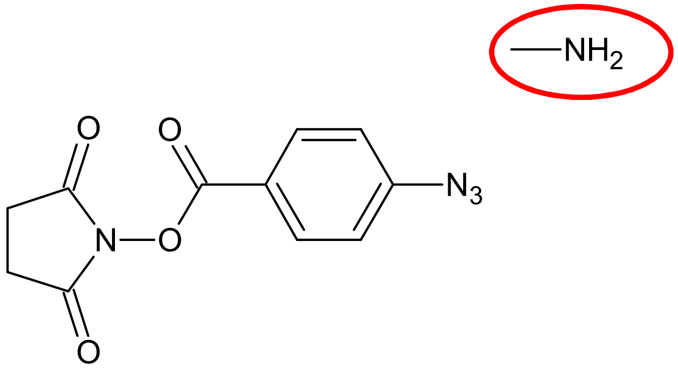
1. Alkyl azide: UV excitation
2. Aryl azide: λ = 300 – 400 nm
3. Acyl azide: reactive in dark too

A. Activation of azide function and its transformation





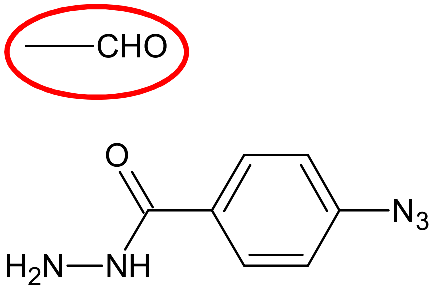
4,4′-diazodo biphenyl



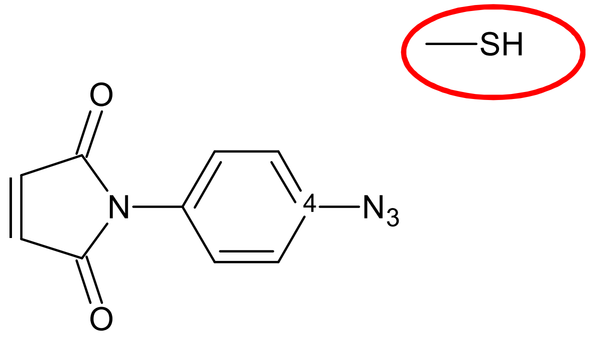
4-Azidobenzoic acid N-hydroxysuccinimide ester

Mikkelsen, RB et al. J Biol Chem <u>251</u> 7413 (1976)

Ji, T. Anal Biochem <u>121</u> 286 (1982)



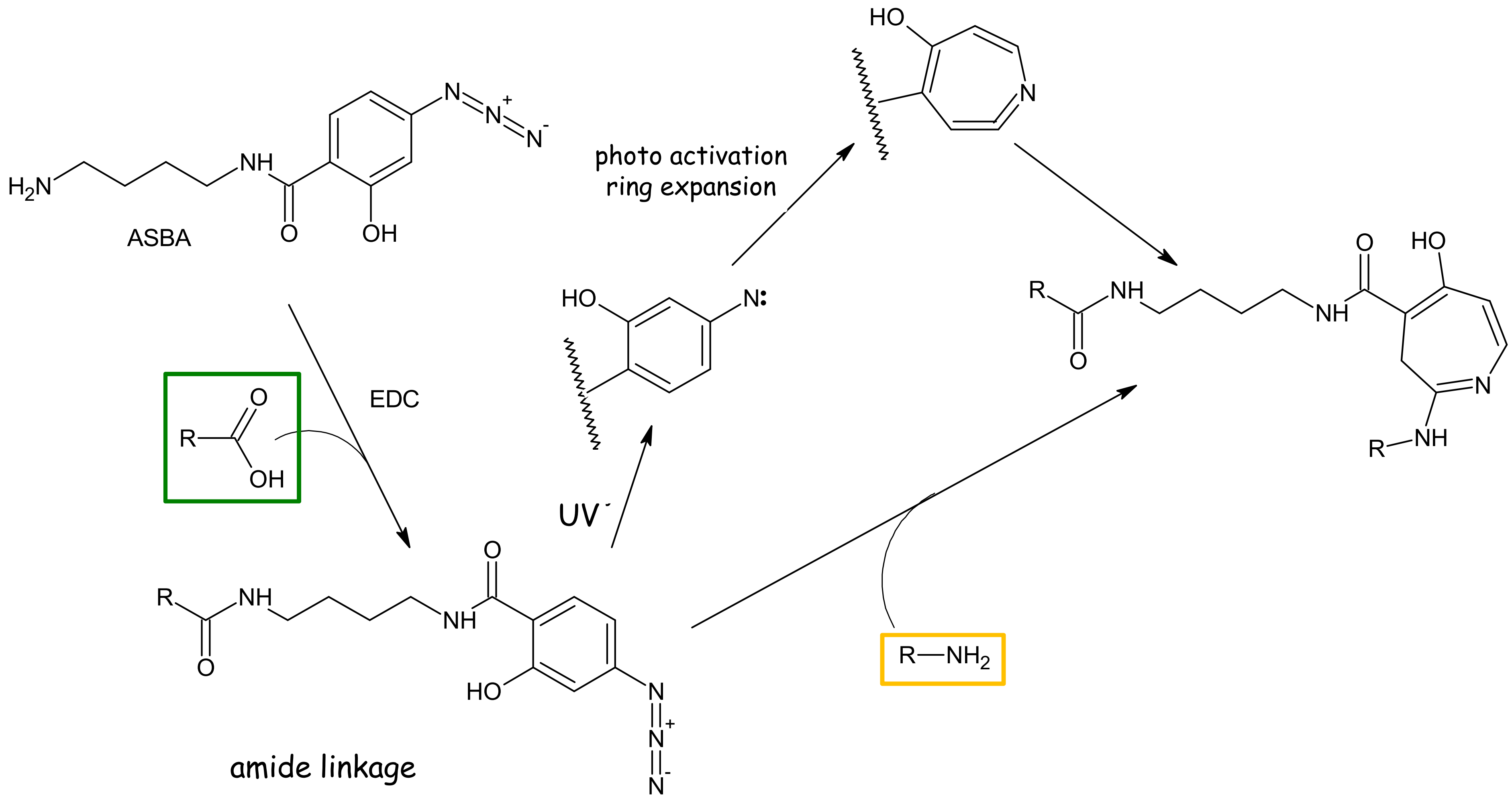
p-azidobenzoic acid hydrazide



4-Azidophenyl maleimide

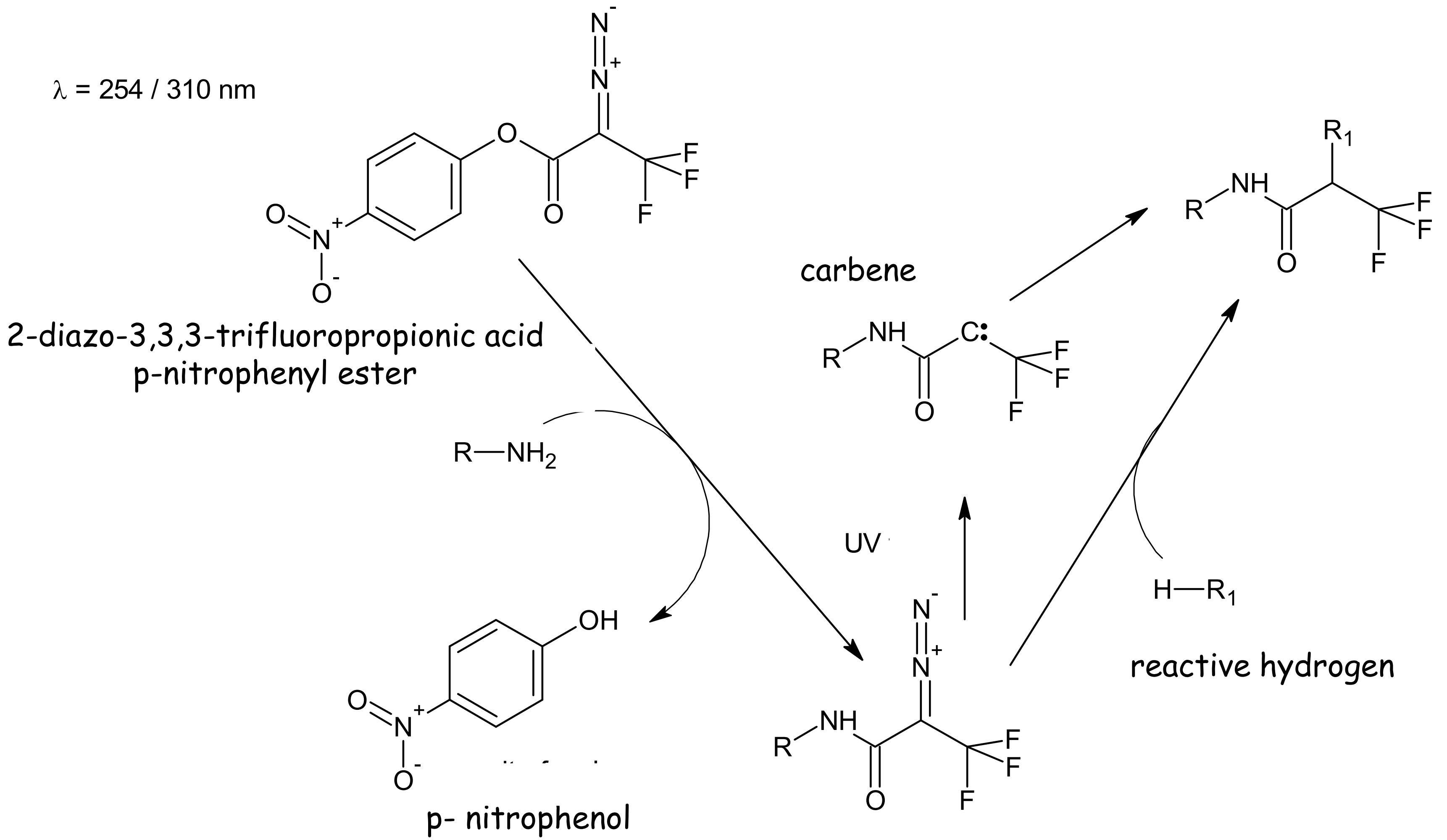
Trommer WE et al. Hoppe-Seyleis Z Physiol Chem <u>356</u> 1455 (1975)

-COOH



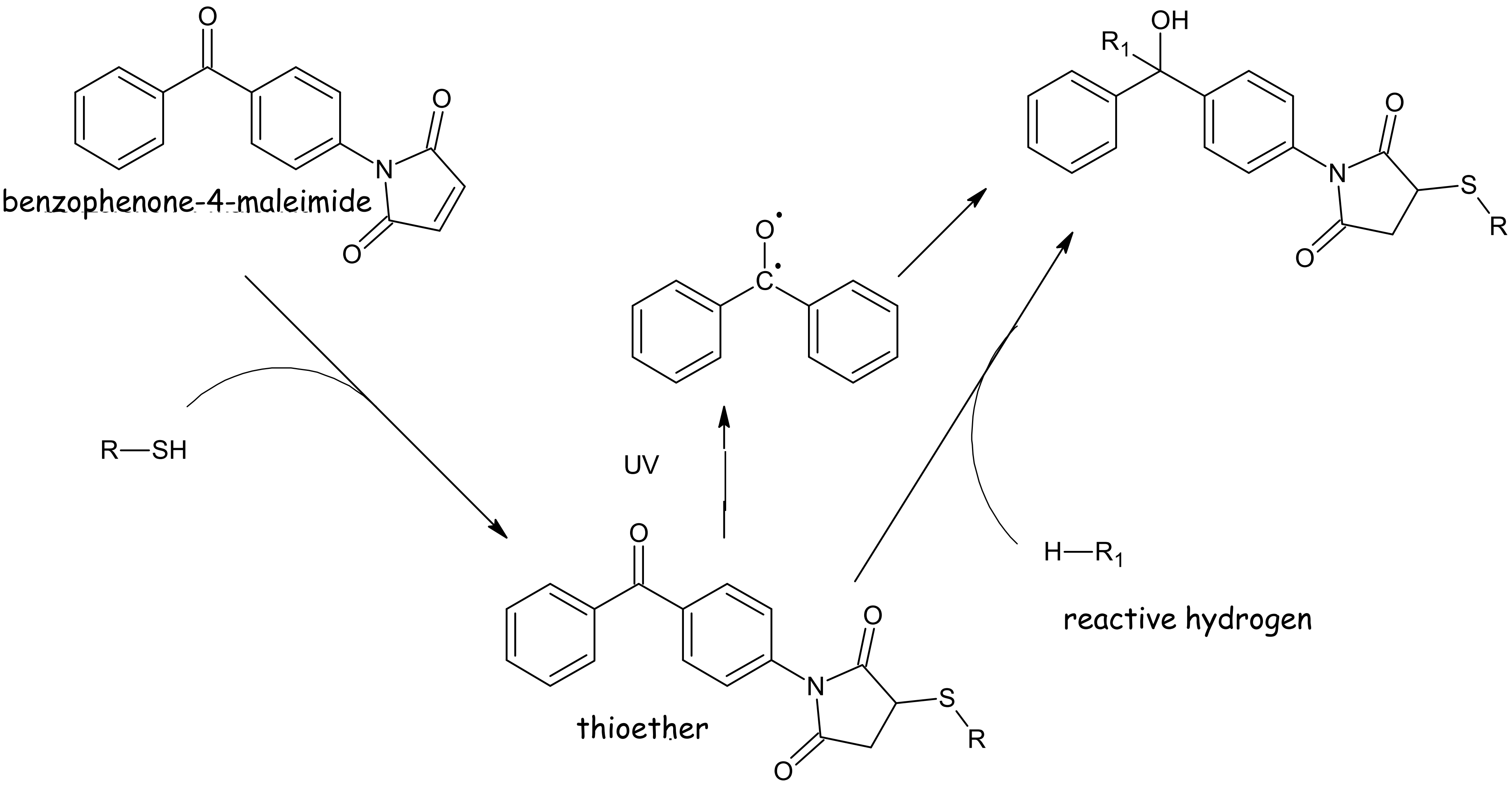
H_2N
NH
O
OH
ASBA
N
N+
N-
R
O
OH
EDC
NH
HO
photo activation
ring expansion
N:
UV
amide linkage
R—NH_2

B. Activation of diazo function and its transformation



Chowdhry et al. PNAS 73 1406 (1976)

C. Activation of function with benzophenone moiety



Walling C, Gibian MJ, JACS <u>87</u> 3361 (1965)

Conjugates of radioactive isotopes

Biosynthetic approach

^{15}C, ^{3}H, ^{14}C, ^{32}P, ^{35}S

Chemical synthesis

- **Direct**

  e.g. modification of Tyr/ His residues

- **Indirect**

  a) Covalent linkage
  b) Complex formation

Direct incorporation of radioactive isotope

Example: ^{125}I, ^{131}I

(17 iodo-, 13 bromo-, 6 chloro-, 2 fluoro-isotope $t_{\frac{1}{2}}$ > 3 perc)

- In vitro ^{125}I
  - long lifetime
  - low energy photon emission (no particle)
  - Price
- In vivo „IMAGING" ^{125}I, ^{131}I (β-), ^{123}I (γ – 159 keV)
  - X-ray or γ-emitting ^{18}F (97% β+)
  - „Single photon emission tomography" (SPECT)
  - „Positron emission tomography" (PET)
  - γ-camera

^{131}I (^{82}Br)

- In vivo THERAPY
  - Not optimal (high energy: danger)

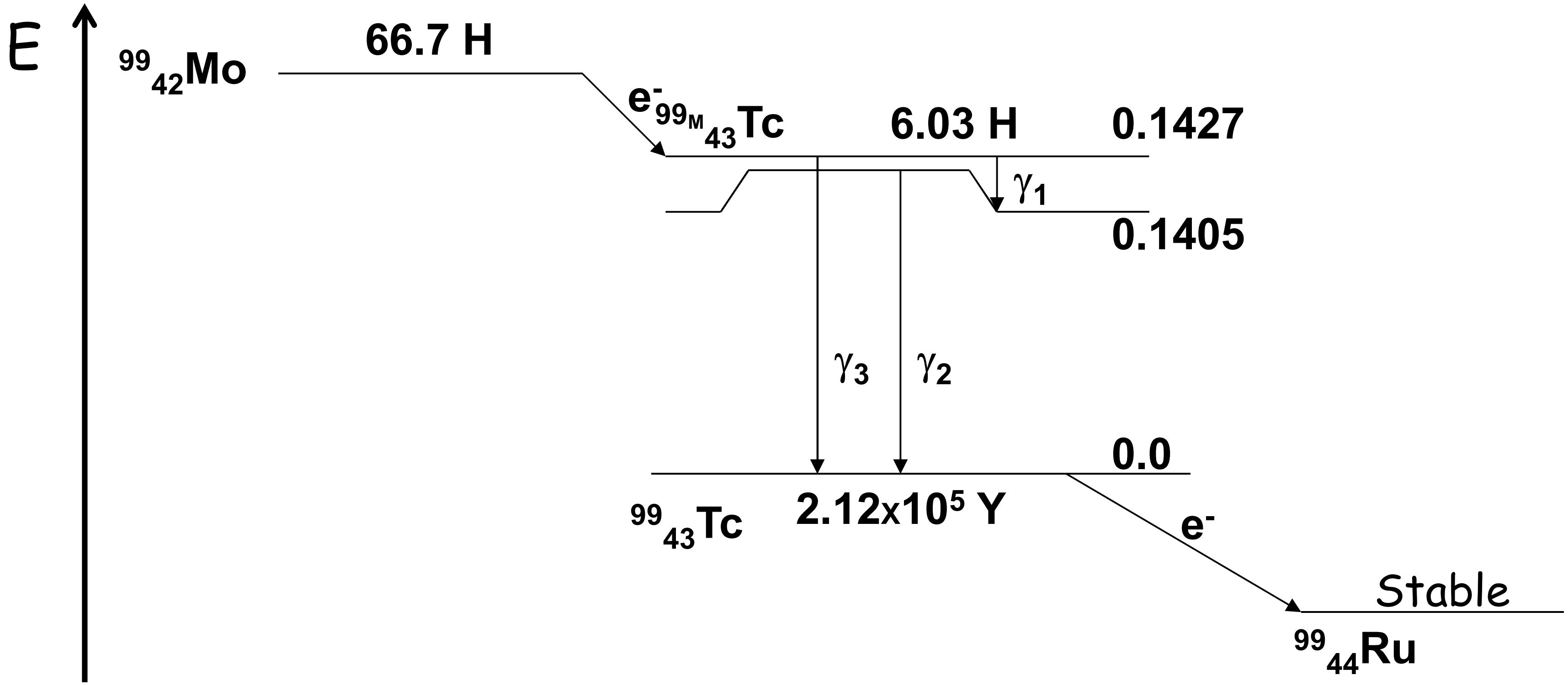
Characterisctics of the most important radioactive isotopes

Sensitivity: $^{131}I > ^{125}I > ^{14}C, ^{32}P, ^{35}S, ^{3}H$

Atomic number		Izotope	Half life	Decay	Highest particle energy (MeV)	γ-energy (MeV)
H	1	^{3}H	12,33 y	β^-	0,0186	-
C	6	^{11}C ^{14}C	20,4 min 5760 y	β^+ β^-	0,96 0,155	- -
N	7	^{13}N	10 min	β^+	1,19	-
O	8	^{15}O	2 min	β^+	1,73	-
F	9	^{18}F	109,8 min	β^+	0,633	-
Na	11	^{24}Na	15,02 h	β^-, γ	1,392	2,754 1,369
P	15	^{32}P	14,28 day	β^-	1,710	-
S	16	^{35}S	87,2 day	β^-	0,167	-
K	19	^{40}K ^{42}K	$1{,}28^{*}10^{9}$ y 12,36 h	β^-, K β^-, γ	1,31 3,52 (75%) 1,99 (25%)	1,46 1,525
Ca	20	^{45}Ca	163 day	β^-	0,257	-
Cr	24	^{51}Cr	27,7 day	K, e^-, γ	0,315 (e^-)	0,320
Fe	26	^{52}Fe ^{59}Fe	8,2 h 44,6 day	β^+, γ β^-, γ	0,8 1,566	0,5 1,30 1,10

Atomic number		Izotope	Half life	Decay	Highest particle energy (MeV)	γ-energy (MeV)
Co	27	^{60}Co	5,272 y	β^-, γ	0,318	1,33
						1,17
Cu	29	^{64}Cu	12,74 h	β^- (39%)	0,575	
				β^+ (19%)	0,656	
				K (42%)		
				γ (1%)		1,34
Ga	31	^{67}Ga	78 h	γ	-	0,185
						0,30
Kr	36	^{85}Kr	10,73 y	β^-, γ	0,687	0,514
Ru	37	^{81}Rb	4,7 h	β^+, γ	0,99	1,93
		^{86}Rb				0,95
			18,65 day	β^-, γ	1,78	1,078
Sr	38	^{90}Sr	29 y	β^-	0,546	-
It	39	^{90}Y	64 h	β^-, γ	2,29	1,761
Tc	43	^{99m}Tc	6,02 h	γ	-	0,140
In	49	^{111}In	67,0 h	γ	-	0,173
						0,247
		^{113m}In	1,658 h	γ	-	0,391
I	53	^{123}I	13,3 h	γ	-	0,16
		^{125}I	59,7 day	K, γ	-	0,0355
		^{131}I	8,04 day	β^-, γ	0,606	0,364
					0,25	0,080
					0,81	0,723

Formation and decay of ^{99M}Tc



(Dillman, LT; Von der Lage, FC: Radionuclide decay schemes and nuclear parameters for use in radiation dose estimates (MIRD Pamphlet No. 10), Society of Nuclear Medicine, 1975)

- α-decay [in biological medium: mm]

$$ {}^{A}_{Z}Y \longrightarrow {}^{A-4}_{Z-2}X + {}^{4}_{2}He $$

- β-decay
  - e^- emission

$$ {}^{3}_{1}H_2 \longrightarrow {}^{3}_{2}He + e^- + \bar{\nu}_e $$

neutron $\longrightarrow$ proton + electron + antineutrino

  - e^- capture

$$ {}^{40}_{19}K \longrightarrow {}^{40}_{18}Ar $$

electron + proton $\longrightarrow$ neutron

  - e^+ emission

$$ {}^{11}_{6}C_5 \longrightarrow {}^{11}_{5}B_6 + e^+ + \nu_e $$

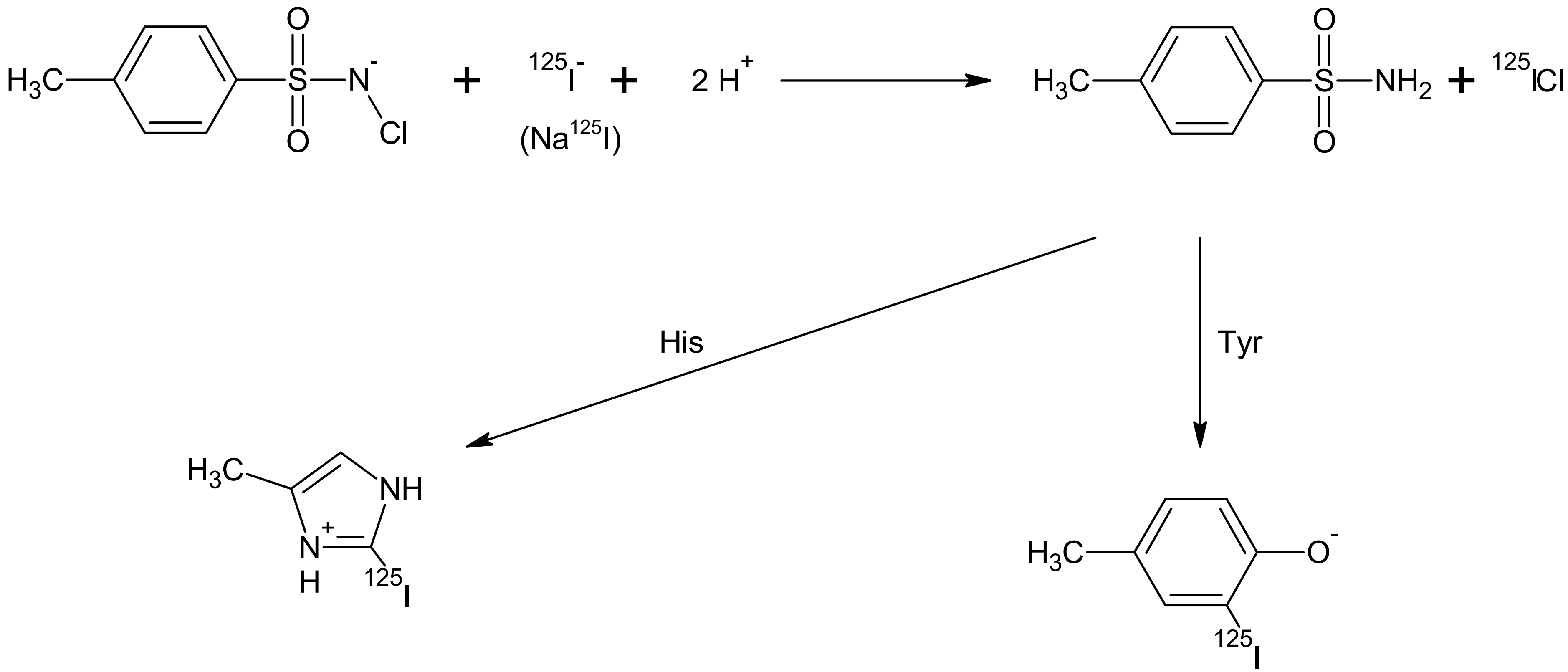
proton $\longrightarrow$ positron + neutrino

- γ-decay

$$ {}^{A}Y^* \longrightarrow {}^{A}Y + \gamma $$

1. Chloramine-T method

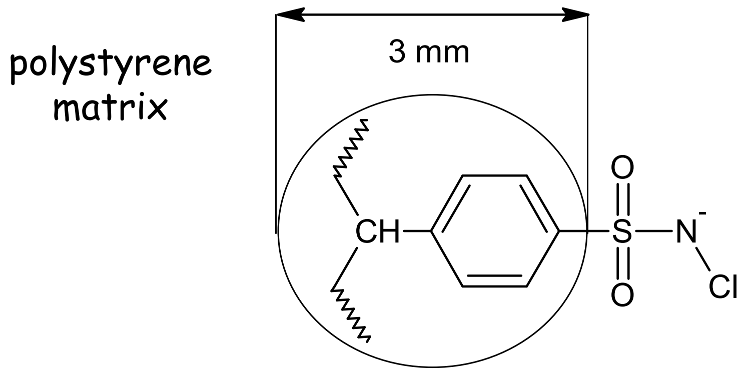
Greewood, FC et al. Biochem J <u>89</u> 114 (1963)
Wilbur, DS Bioconjugate Chem <u>3</u> 433 (1992)



- 30 s – 30 min
- water soluble
- pH 7 phosphate buffer, 0.05 M

2. Immobilised chloramine-T (IODO-BEADS)

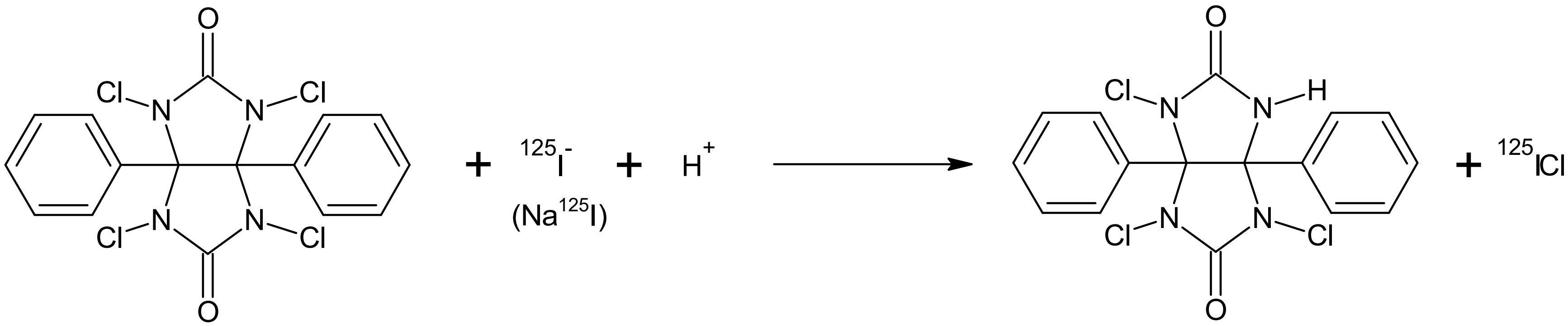
U.S. Patent 4448764   4436718



- 2 - 5 min
- good protein recovery
- mild conditions
- pH 7.2 - 8.4

3. IODO-GEN

Fraker, PJ és Speck, JC BBRC <u>80</u> 849 (1978)



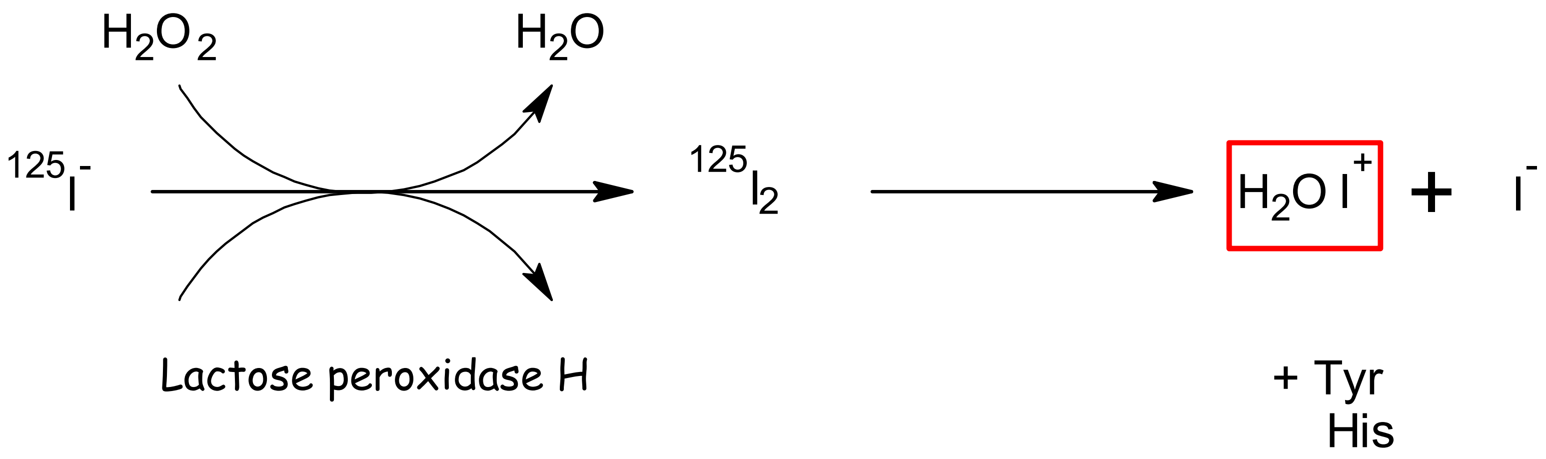
1,3,5,6-tetrachloro-3a,6a-diphenyl glycoluril

- non-water soluble
- surface adsorbed
- speedy
- termination by solvent withdrawal

Enzyme catalysis

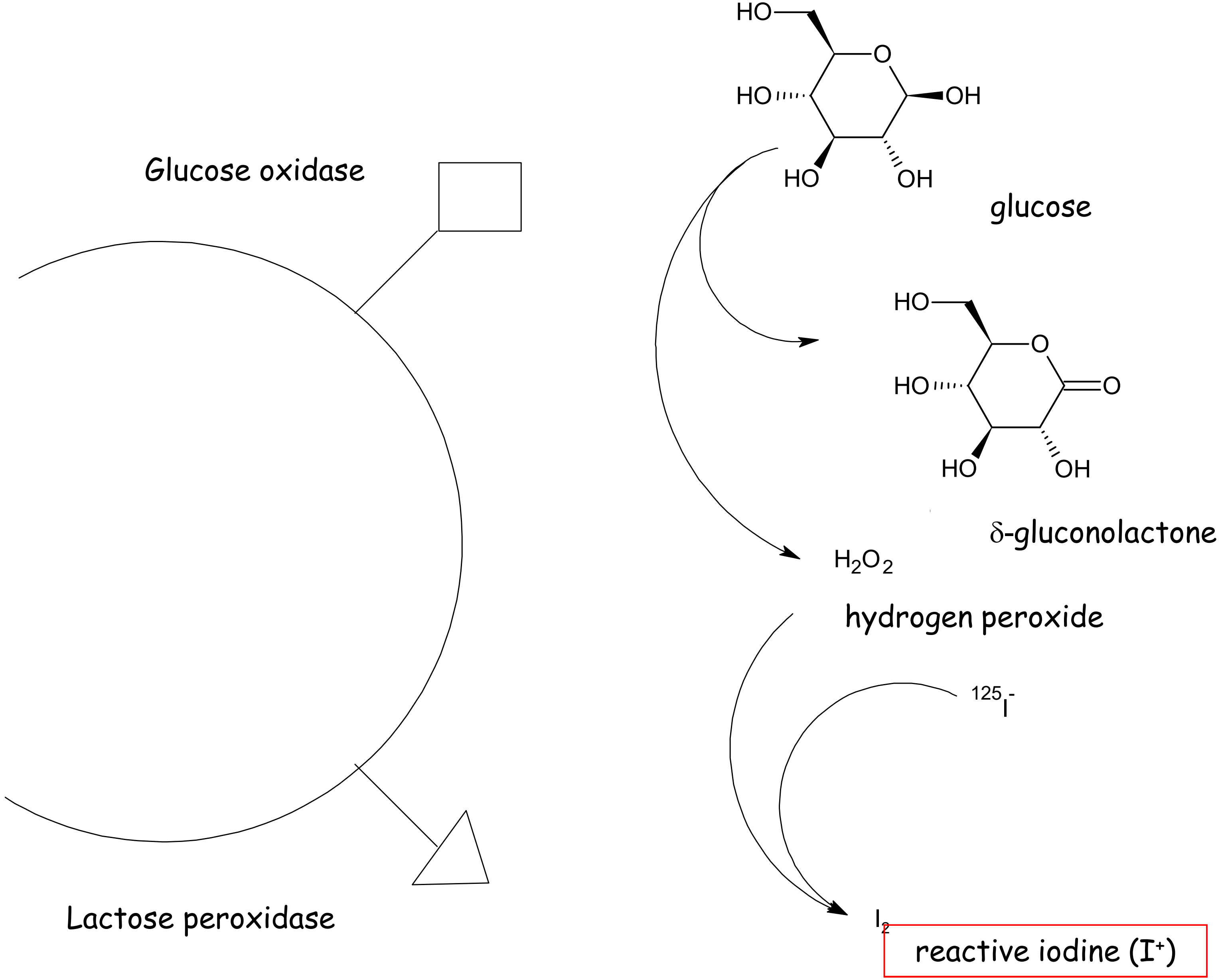
Marcholonis JJ Biochem J 113 299 (1969)

a)

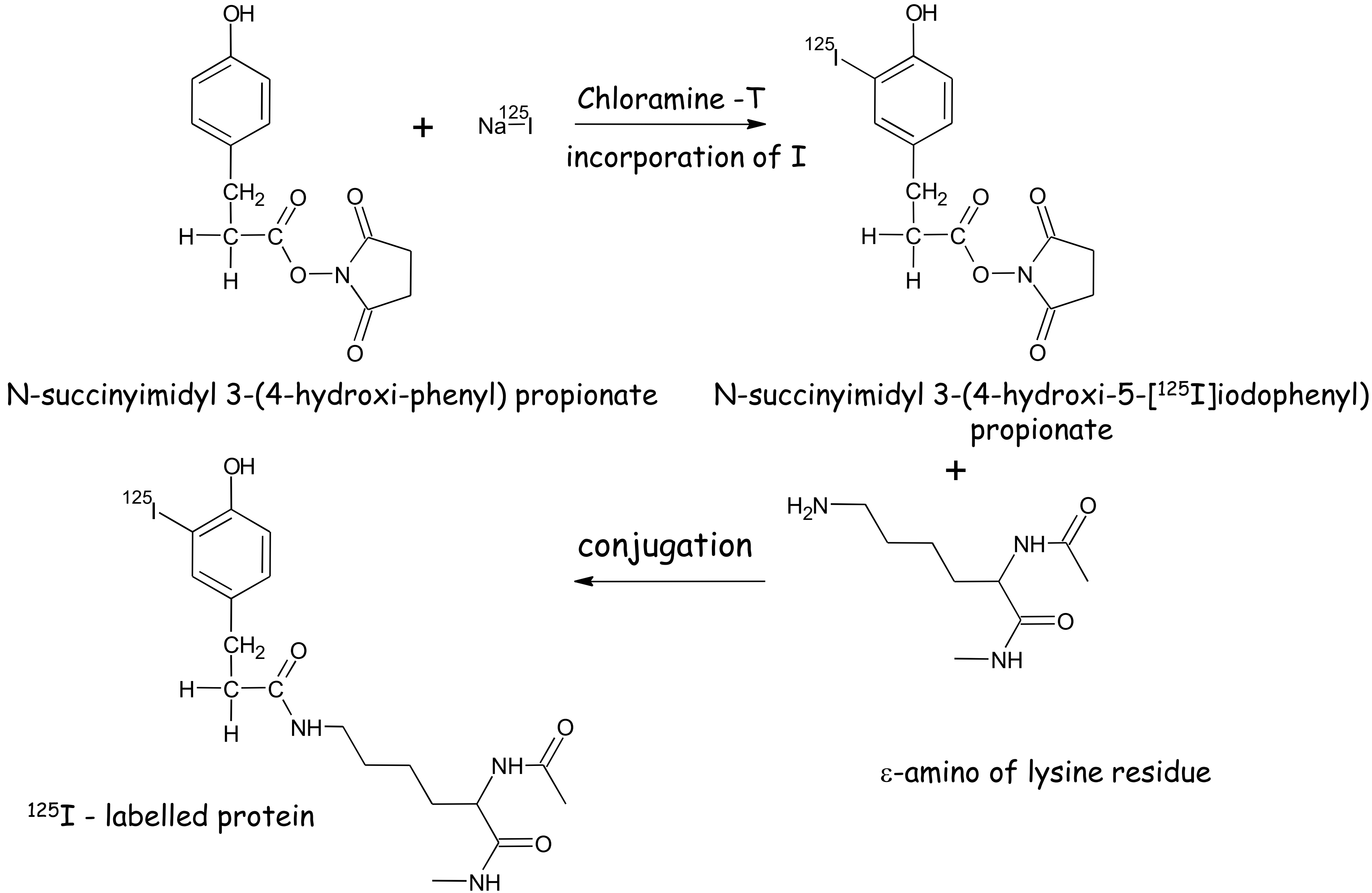


- pH dependent (pH 6 – 7)
- purity of H_2O_2 !

b) In situ preparation of H_2O_2 : immobilised enzymes



N-succinyimidyl 3-(4-hydroxi 5-[^{125}I]iodophenyl) propionate (Bolton and Hunter reagent)



Selection of radionuclide

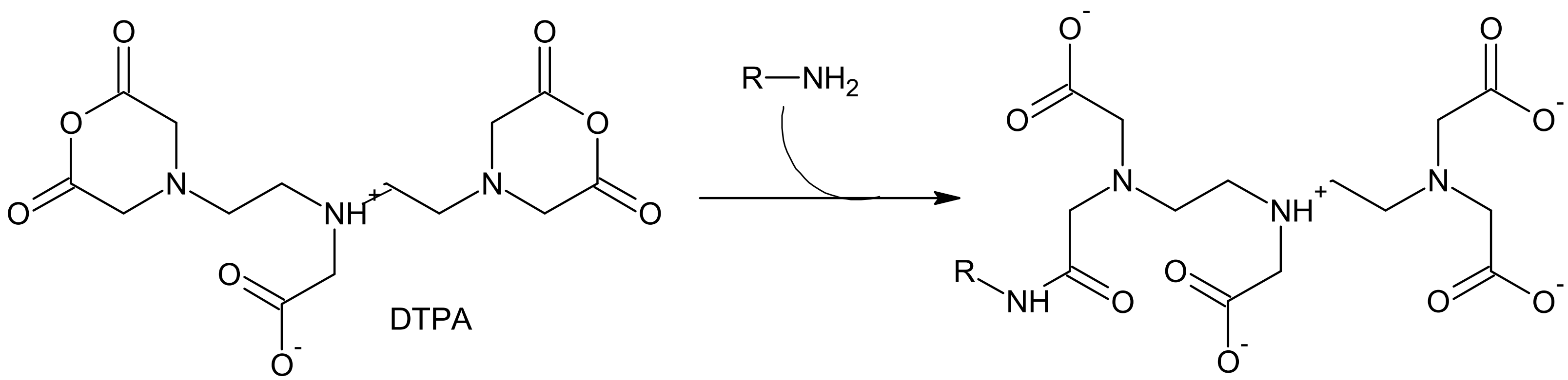
Radioactive atom	Availability	Price	Half-life	Most important γ energy (keV)
^{123}I	Limited	High	13 h	159
^{131}I	Good	Low	8 days	364
^{111}In	Good	Medium	67 h	173 247
^{67}Ga	Good	Medium	78 h	185 300
^{99m}Tc	Good	Low	6 h	141

Incorporation of radionuclide by chelator

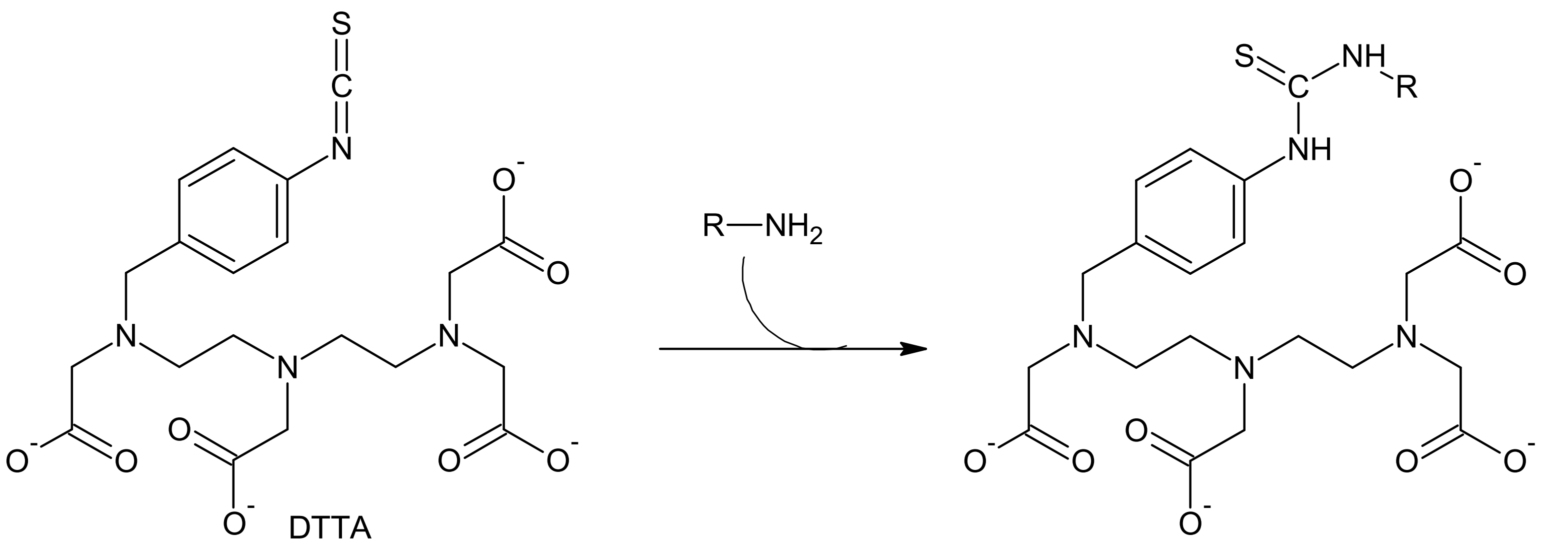
- Application: 1. biodistribution studies (diagnosis, therapy)
  2. localization in cells
- Priciple: complex formation → high stability
  indirect method

Types: 1. linear chelator
2. cyclic chelator

Linear chelator

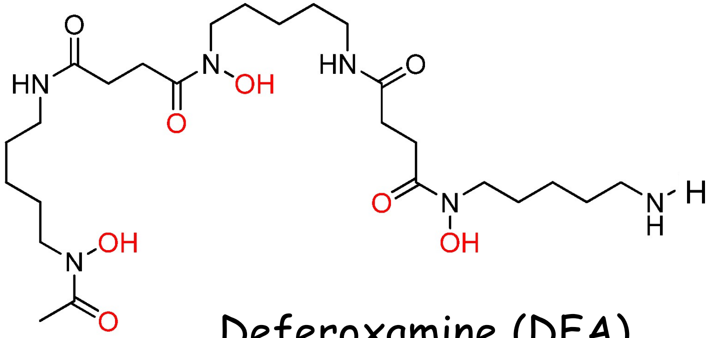


amide linkage

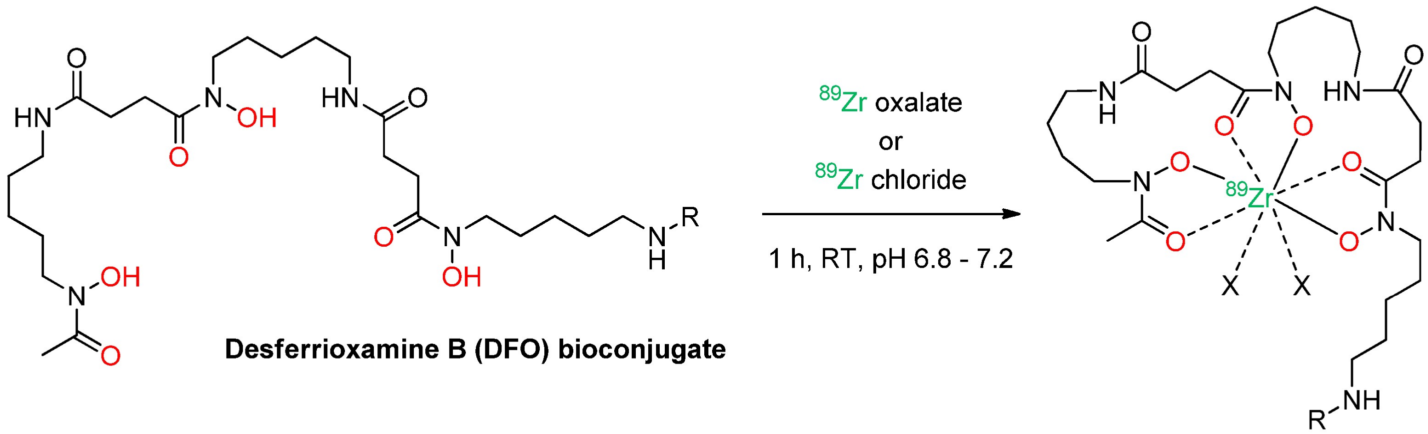


thiourea linkage

Linear chelator



Deferoxamine (DFA)



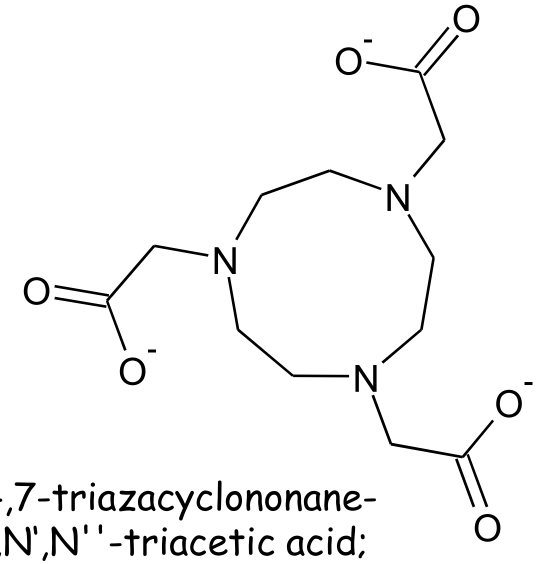
Desferrioxamine B (DFO) bioconjugate

^{89}Zr-DFO bioconjugate

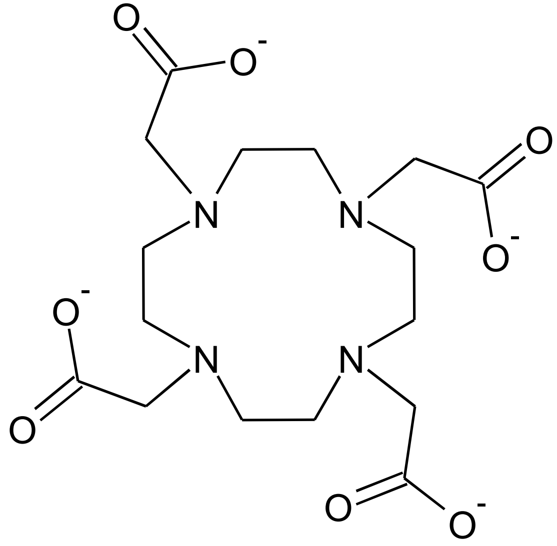
X = H_2O or anion
R = biomolecule

Molecules **2013**, *18*(6), 6469-6490; doi:10.3390/molecules18066469
http://www.mdpi.com/1420-3049/18/6/6469/htm

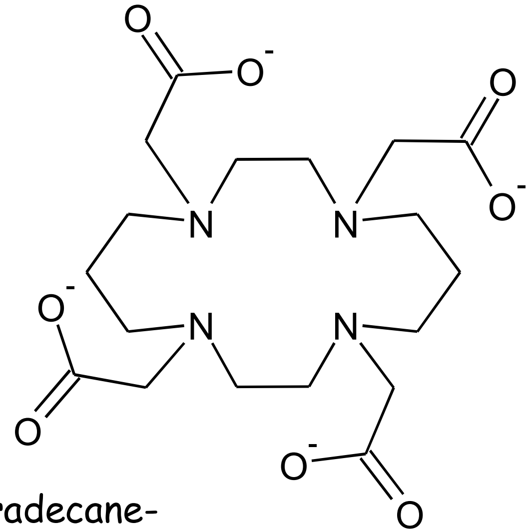
Cyclic chelator



1,4,7-triazacyclononane-
N,N',N''-triacetic acid;
(NOTA)

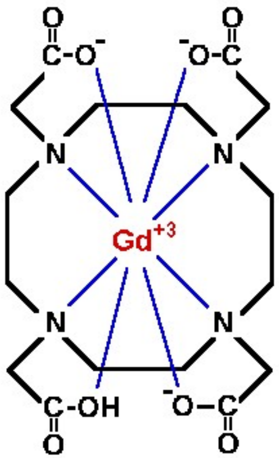


1,4,7,10-tetraazacyclododecane-
1,4,7,10-tetraacetic acid (DOTA)

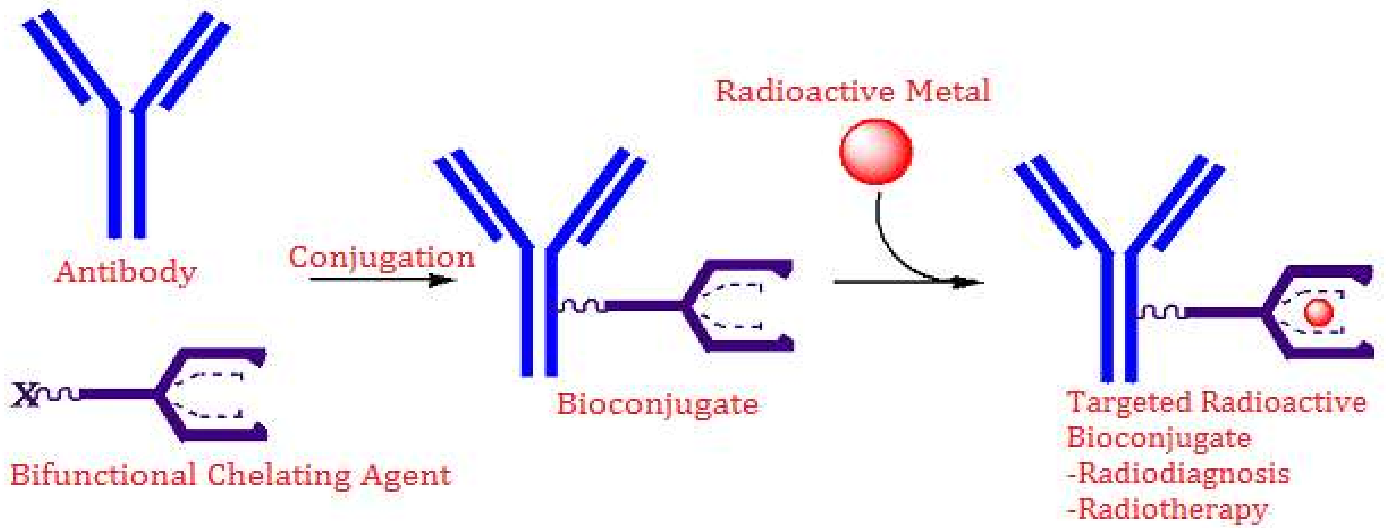


1,4,8,11-tetraazacyclotetradecane-
N,N',N'',N'''-tetraacetic acid
(TETA)

Gd-DOTA



The effect of substitution level on immunological reactivity of antibodies



Prepared under
two different conditions