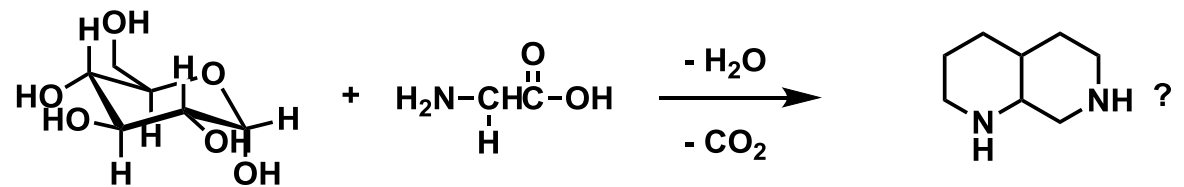


The Maillard Reaction: Radicals and Flavor

Noah Marcus

History: The first report

- First investigation of reaction between sugars and amino acids by Louis Camille Maillard
- Reported reaction of excess d-glucose with glycine in aqueous conditions.
 - Liberation of CO₂ and loss of H₂O
 - Postulated incorporation of amino acid into sugar, forming heterocycles



Some polycyclic molecules with nitrogen incorporated

Blackish substance²

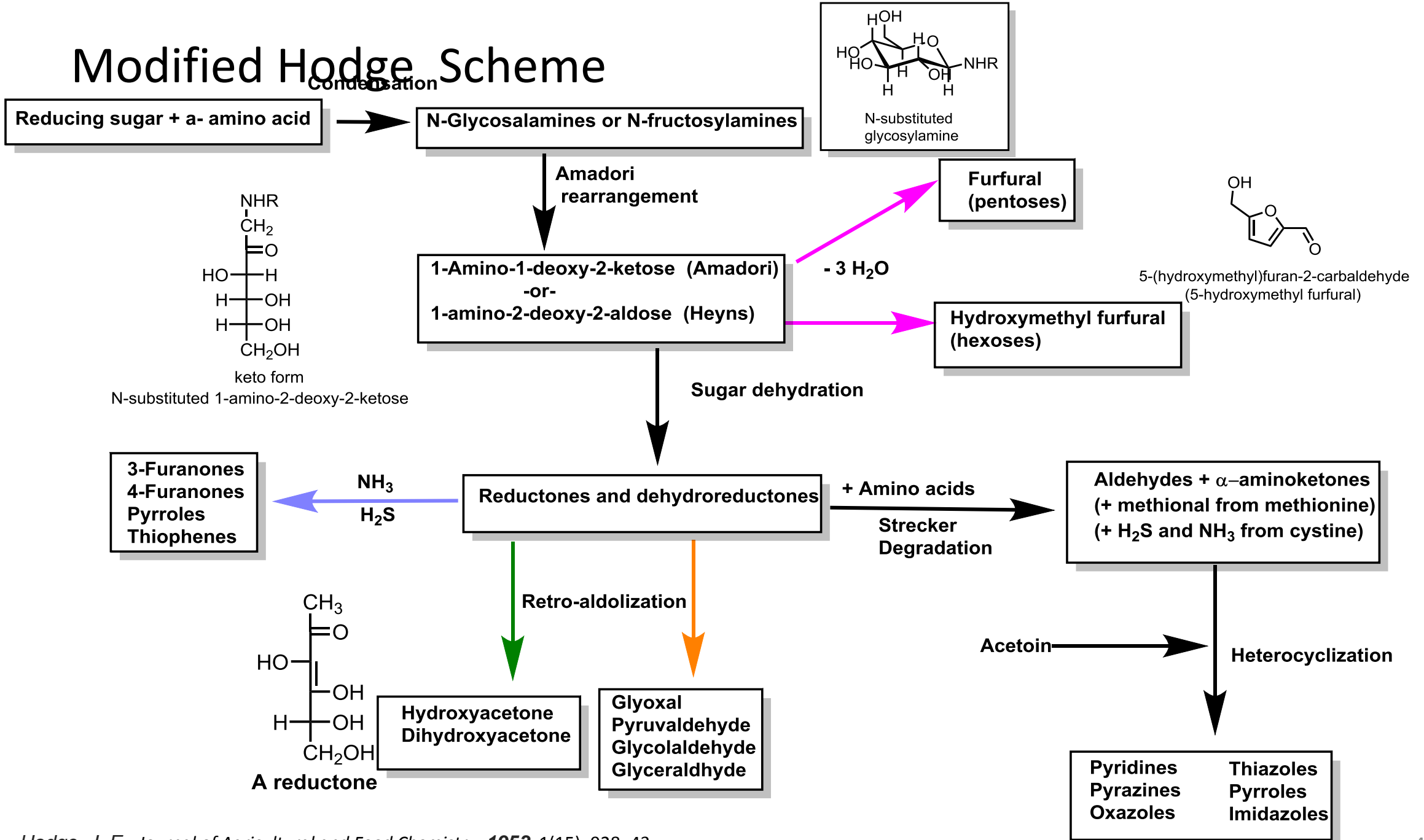
Maillard, L. C., *Compt. Rend.* 1912, 154, 66.

Picture: This work is in the [public domain](#) in its country of origin and other countries and areas where the [copyright term](#) is the author's life plus 70 years or less.

History: First Overall Reaction Pathway

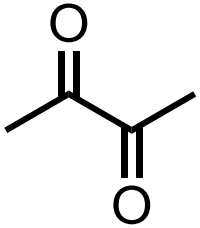
- First overall transformation pathway was proposed in 1953 by John E. Hodge
 - Based on previous work with Carl Rist
- Proposed scheme “Hodge Scheme” is still currently accepted
- In 1979 Science Citation Index named “Chemistry of Browning Reactions in Model Systems” a Citation Classic.

Modified Hodge Scheme



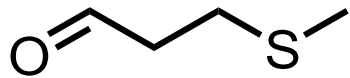
Common flavor compounds: Acyclic

Found in:



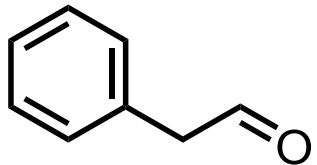
butanedione

Coffee, bread crusts, stewed/ roasted meats, beer, wine



Methional

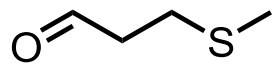
Coffee, bread crusts, tea, stewed/ roasted meats, baked potato, chips, beer



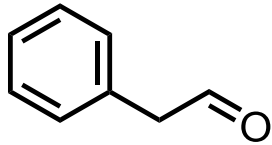
phenylacetaldehyde

Coffee, bread crusts, stewed meats, chocolate, wine

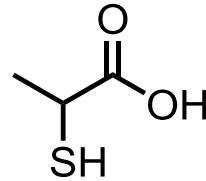
Acyclic products from Maillard reaction



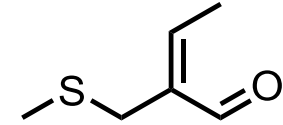
Methional



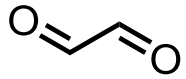
phenylacetaldehyde



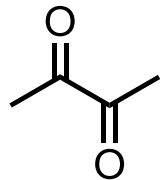
2-mercaptopropionic acid



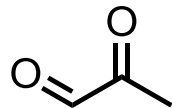
(E)-2-((methylthio)methyl)but-2-enal



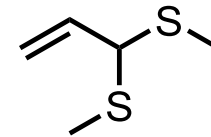
glyoxal



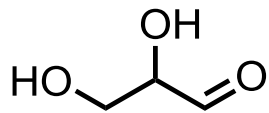
butanedione



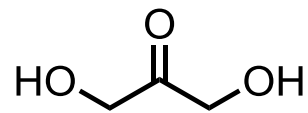
pyruvaldehyde



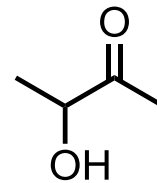
prop-2-ene-1,1-diylbis(methylsulfane)



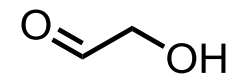
glyceraldehyde



1,3-dihydroxyacetone

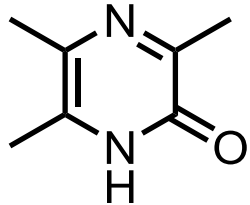


acetoin



glycolaldehyde

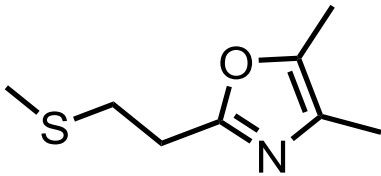
Common flavor compounds: Cyclic



3,5,6-trimethylpyrazin-2(1H)-one

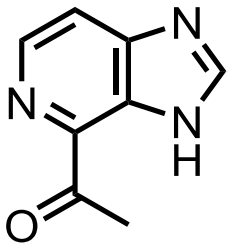
Found in:

Coffee, bread crusts, stewed/ roasted meats, toasted sesame, popcorn



4,5-dimethyl-2-(2-(methylthio)ethyl)oxazole

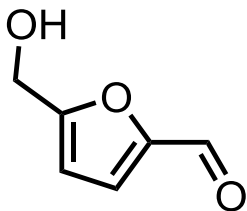
Cocoa, coffee, meats, roasted barley, baked potato, roasted peanuts, French fries



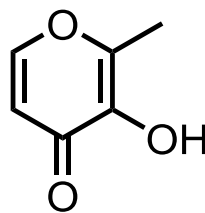
1-(3H-imidazo[4,5-c]pyridin-4-yl)ethan-1-one

Coffee, roasted barley, stewed/ roasted meats,

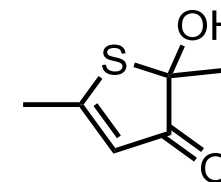
Heterocyclic products



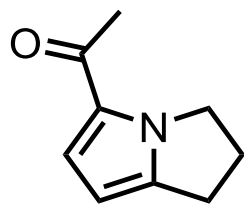
5-(hydroxymethyl)furan-2-carbaldehyde
(5-hydroxymethyl furfural)



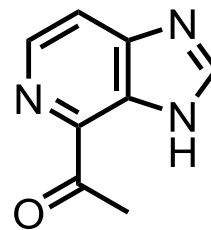
3-hydroxy-2-methyl-4*H*-pyran-4-one



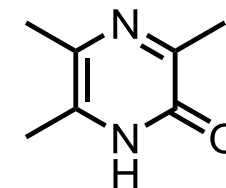
2-hydroxy-2,5-dimethyl-3(2*H*)-thiophenone



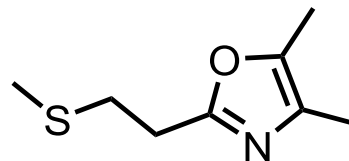
1-(2,3-dihydro-1*H*-pyrrolizin-5-yl)ethan-1-one



1-(3*H*-imidazo[4,5-*c*]pyridin-4-yl)ethan-1-one

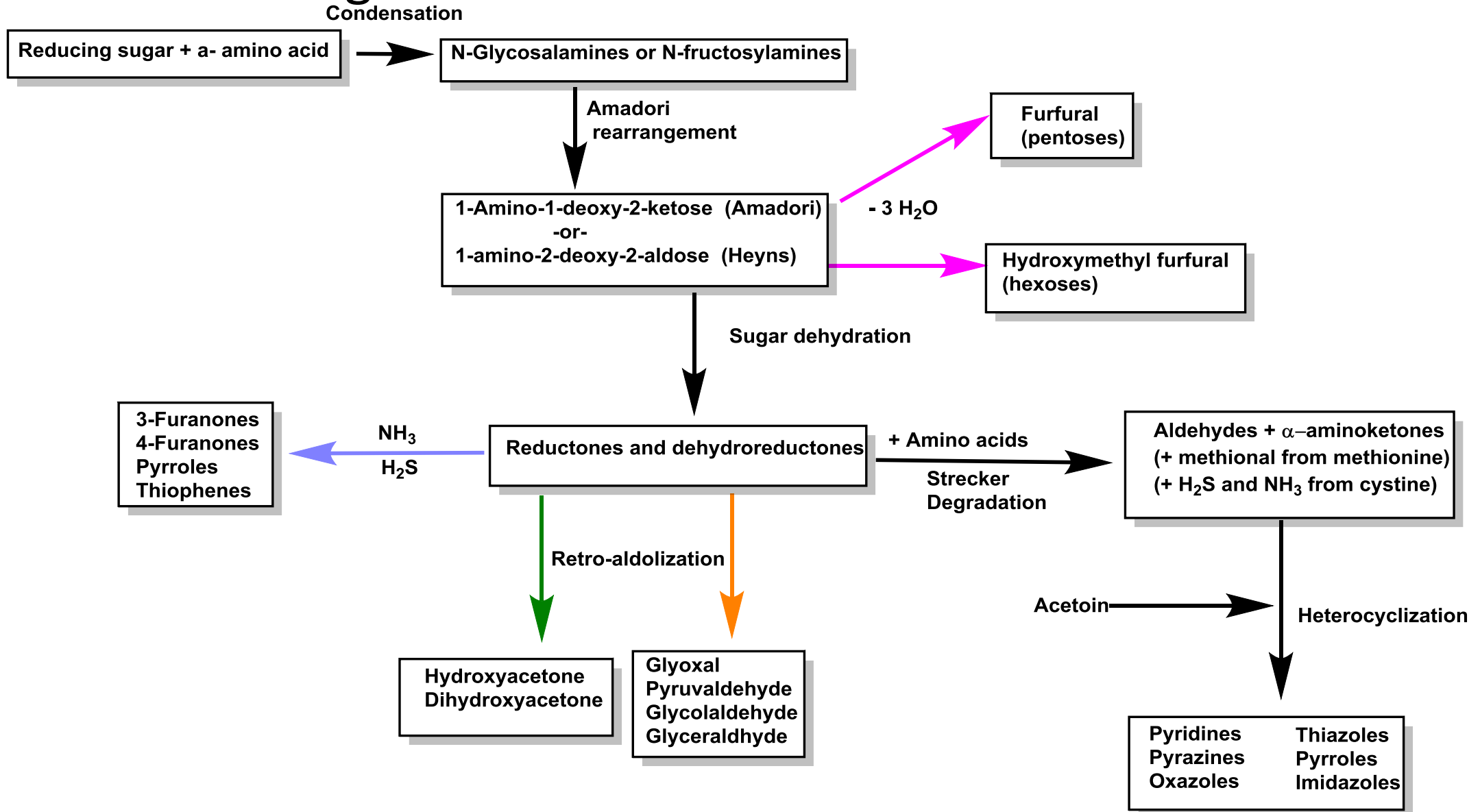


3,5,6-trimethylpyrazin-2(1*H*)-one

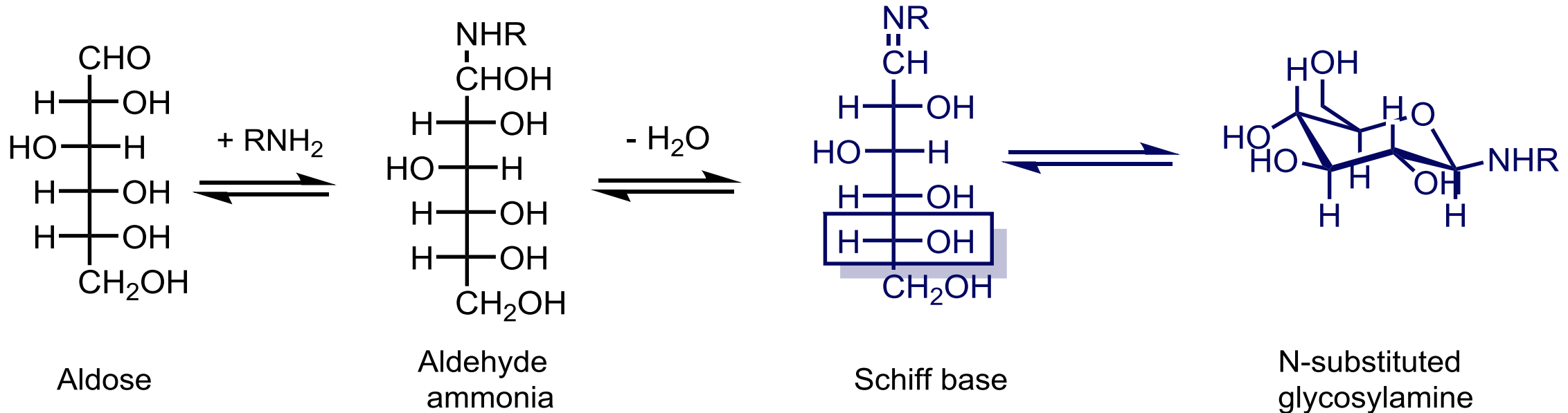


4,5-dimethyl-2-(2-(methylthio)ethyl)oxazole

Modified Hodge Scheme



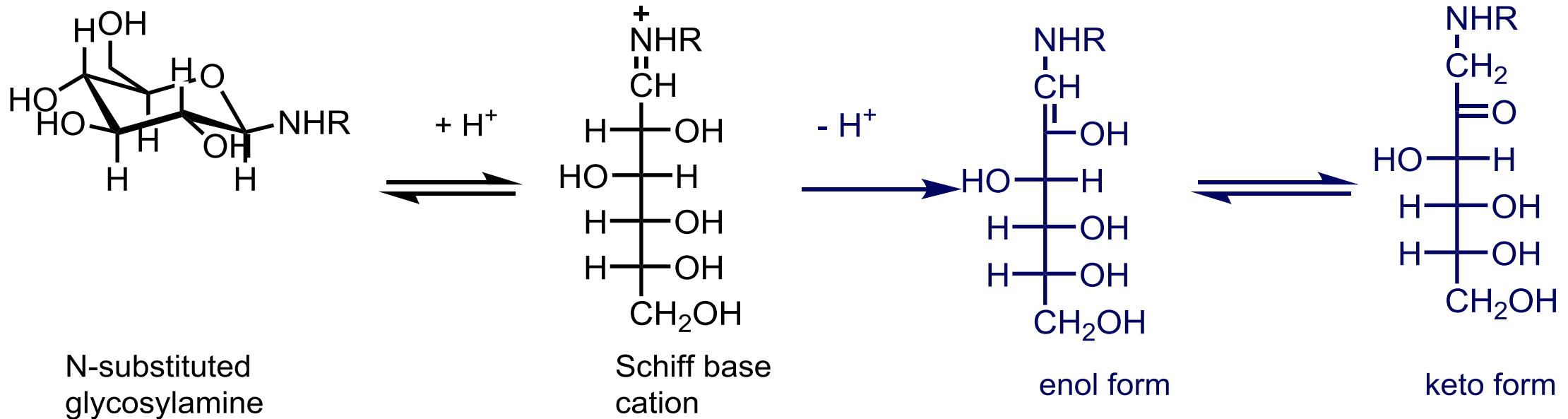
Sugar-Amine condensation



Hodge, J. E., *Journal of Agricultural and Food Chemistry*, **1953**, 1(15), 928–43

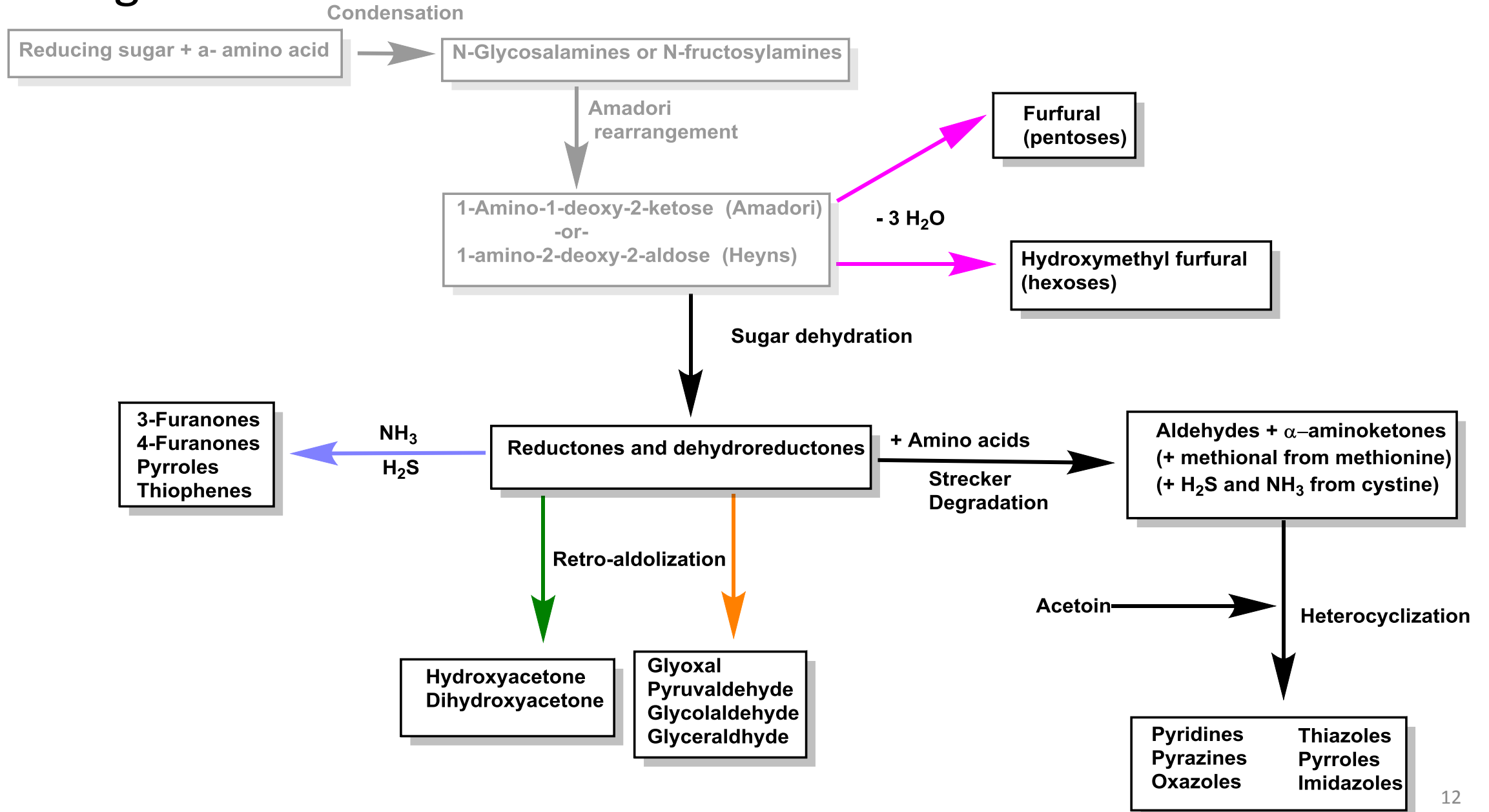
Nursten, H., *The Maillard Reaction Chemistry, Biochemistry and Implications*; Royal Society of Chemistry: Cambridge, **2005**

Amadori rearrangement

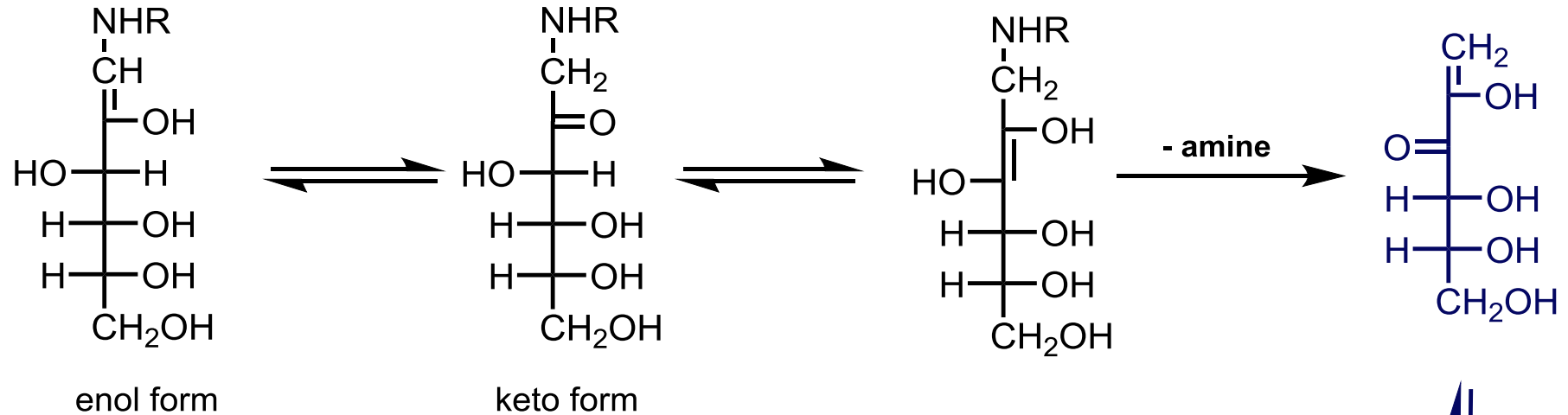


N-substituted 1-amino-2-deoxy-2-ketose

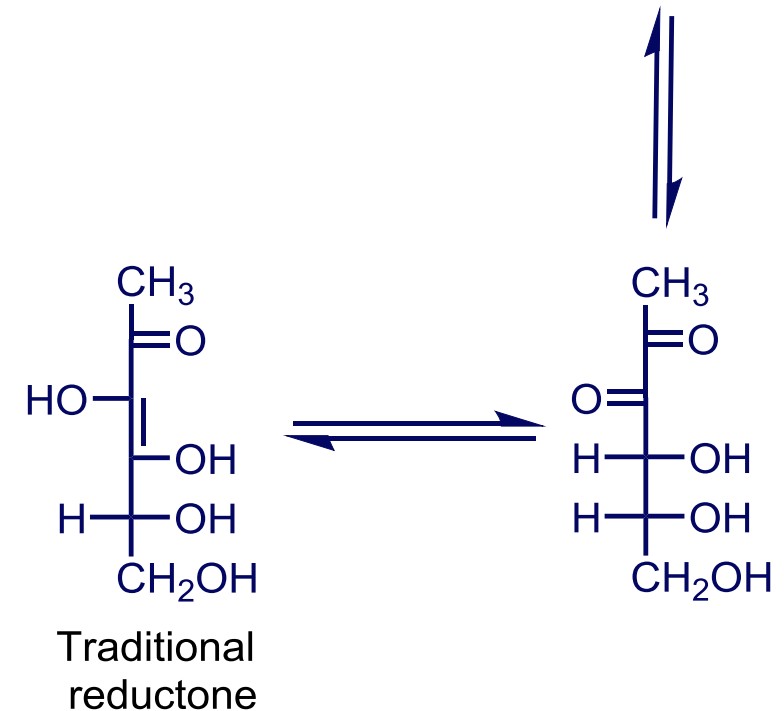
Progress



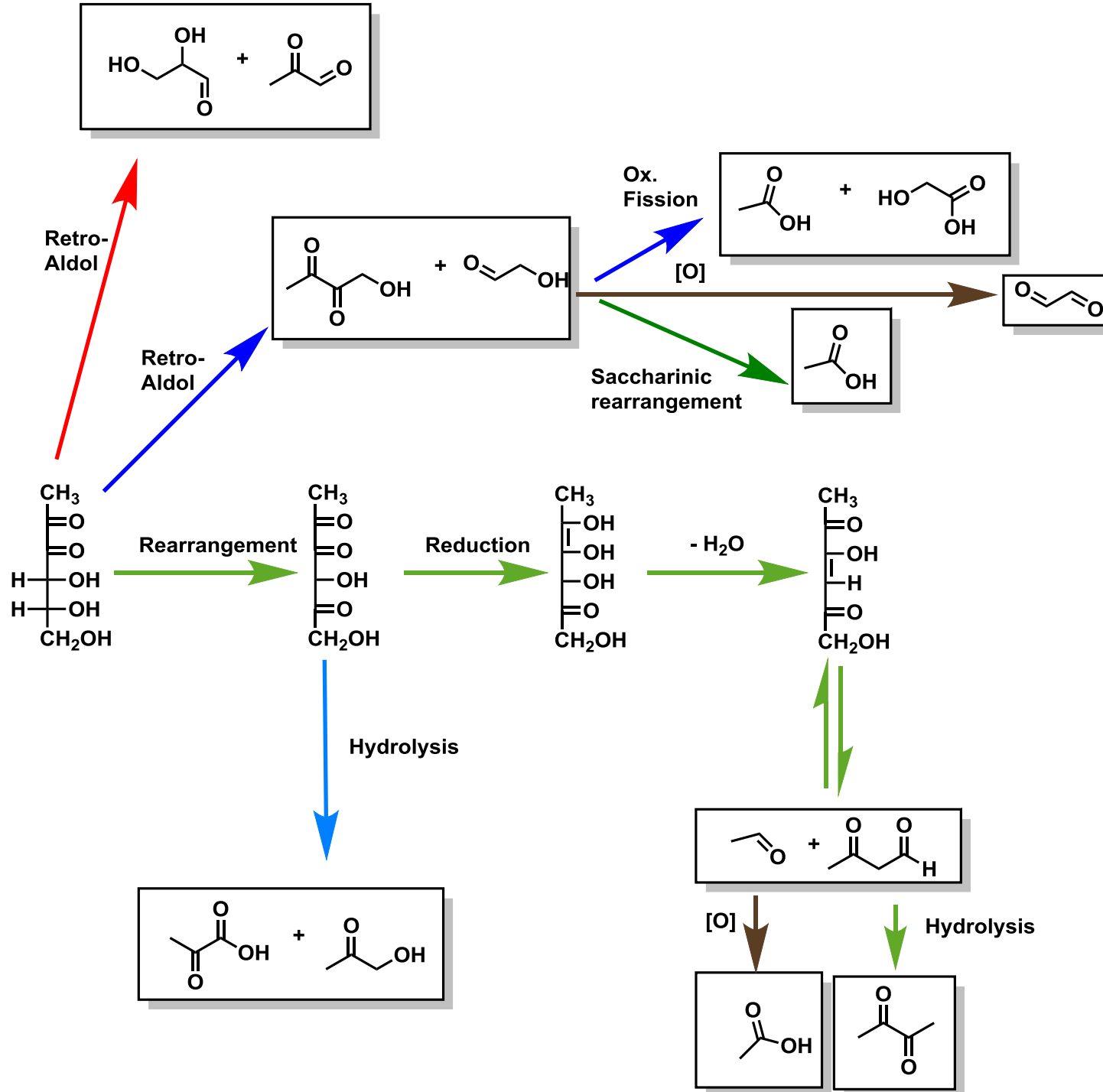
Secondary reductone path



- This pathway is favored under neutral and basic conditions
- May form furones or go on to sugar fragmentations



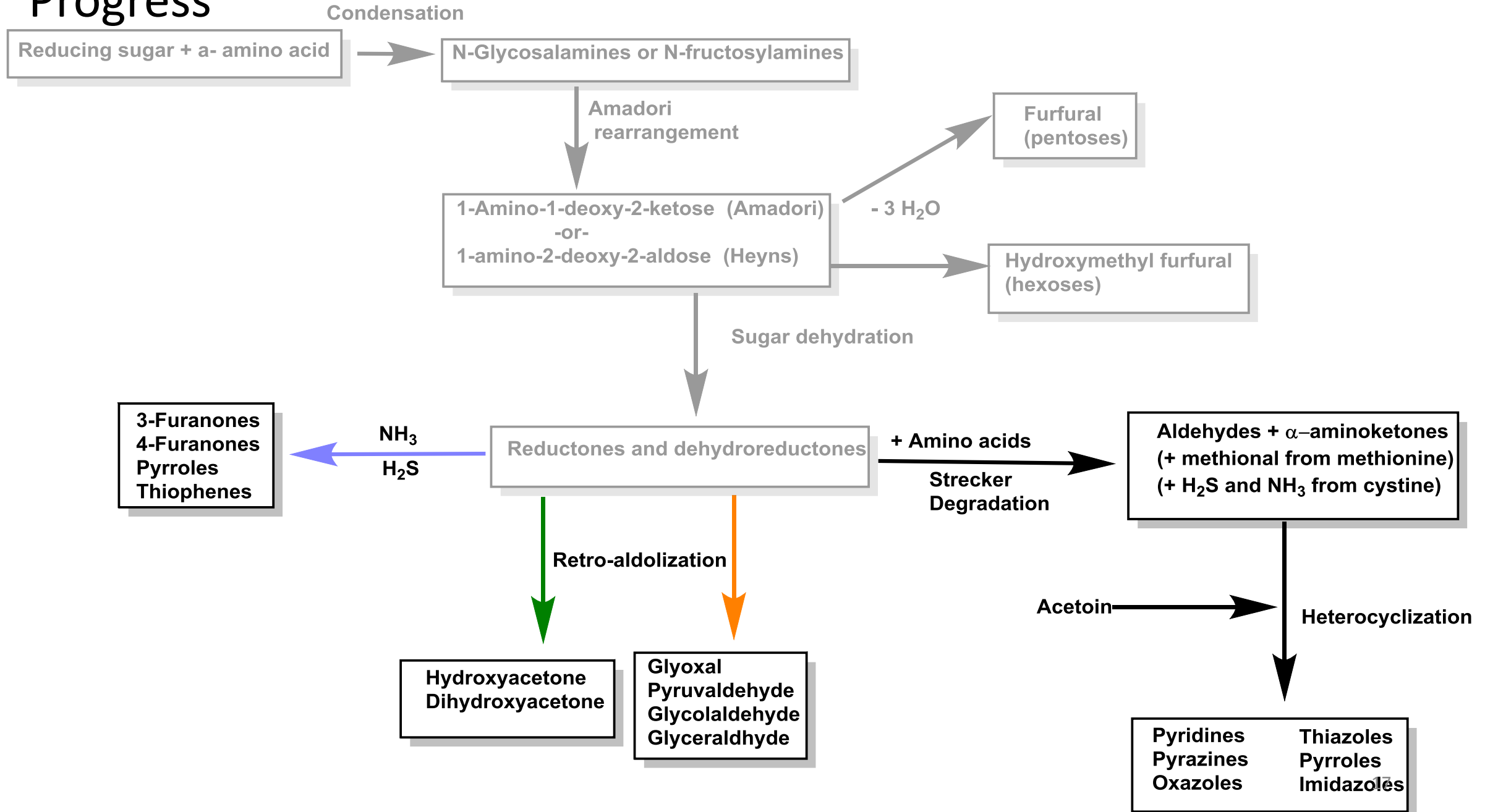
Sugar Fragmentation



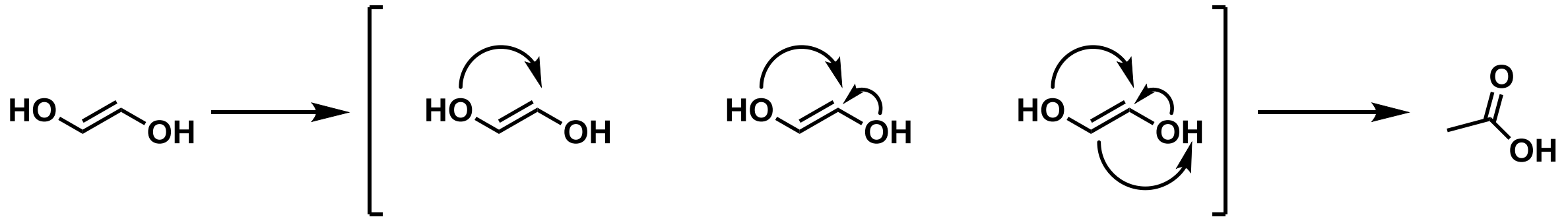
- Only half of possible fragmentation paths shown
- Multiple pathways, all giving different products

H. Weenen and W. Apeldoorn, Carbohydrate cleavage in the Maillard reaction. In *Flavour Science: Recent Developments*, Vol. 197, A. J. Taylor and D. S. Mottram (eds), Royal Society of Chemistry, Cambridge, **1996**, 211–216.
 Nursten, H., *The Maillard Reaction Chemistry, Biochemistry and Implications*; Royal Society of Chemistry: Cambridge, **2005**

Progress

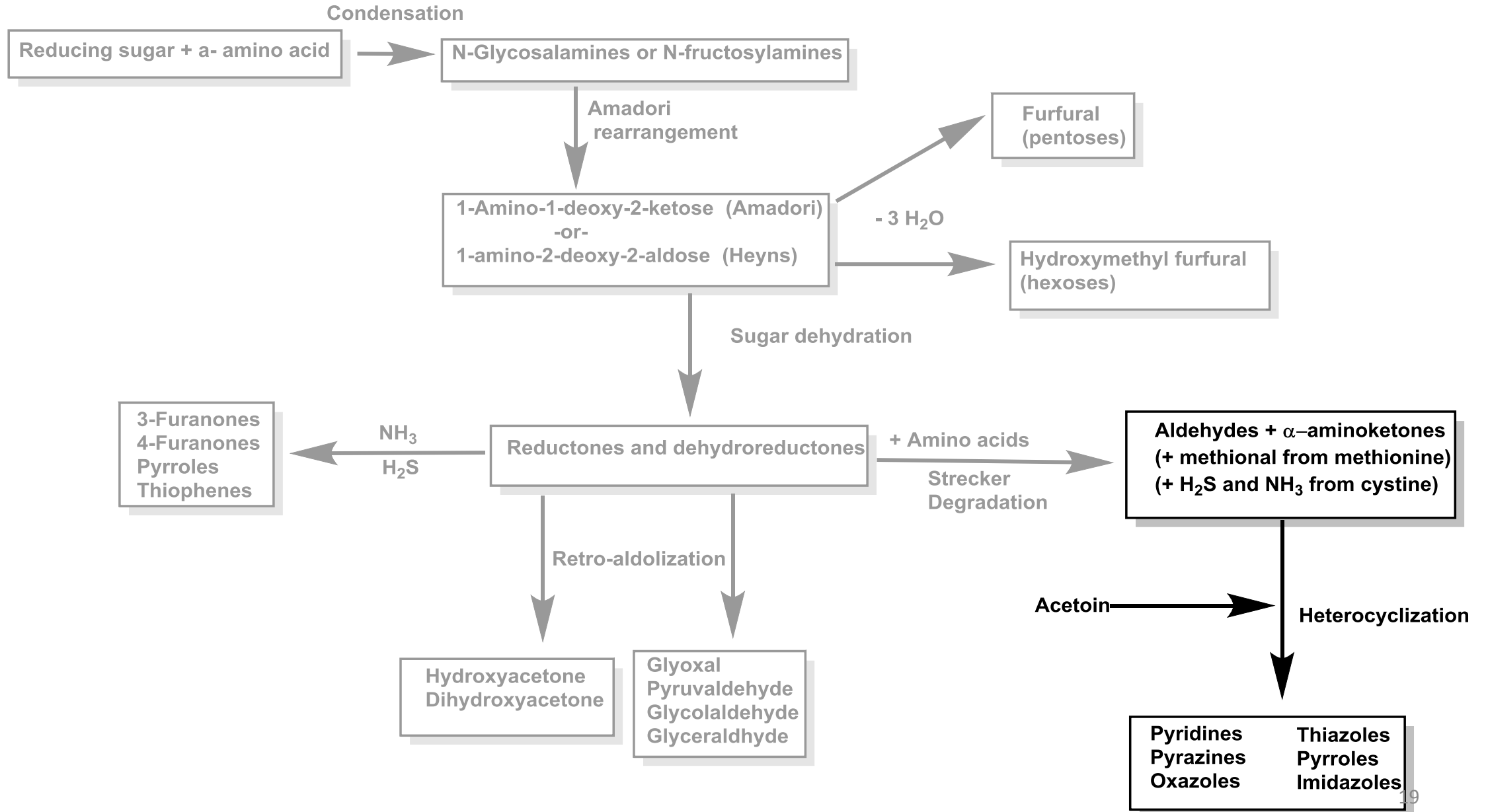


Saccharinic Rearrangement

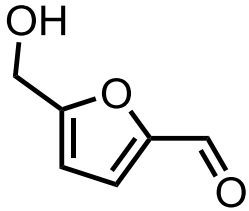


- Enolize into enediol
- One alcohol jumps onto other carbon, carbon losing OH gains H other carbon
- At same time carbonyl reforms
- As carbonyl reforms proton is transferred
- OH migration, carbonyl reformation and proton transfer are one concerted step

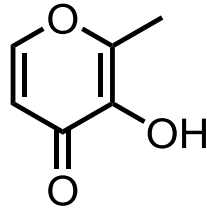
Progress



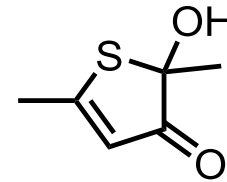
Heterocyclic products



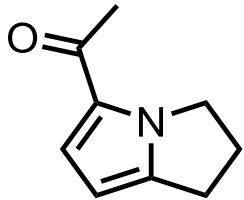
5-(hydroxymethyl)furan-2-carbaldehyde
(5-hydroxymethyl furfural)



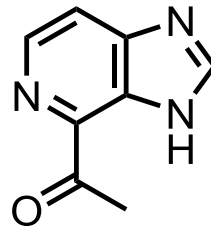
3-hydroxy-2-methyl-4*H*-pyran-4-one



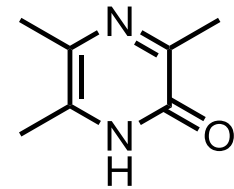
2-hydroxy-2,5-dimethyl-3(2*H*)-thiophenone



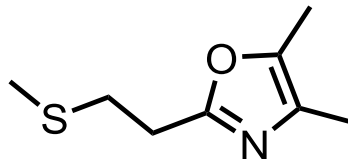
1-(2,3-dihydro-1*H*-pyrrolizin-5-yl)ethan-1-one



1-(3*H*-imidazo[4,5-*c*]pyridin-4-yl)ethan-1-one

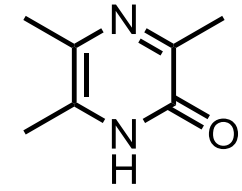


3,5,6-trimethylpyrazin-2(1*H*)-one

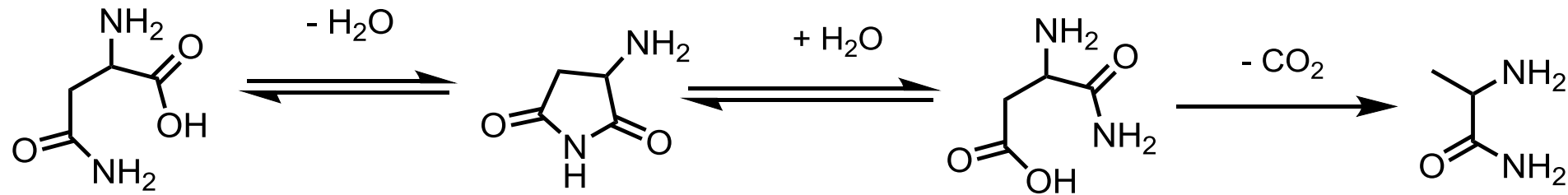


4,5-dimethyl-2-(2-(methylthio)ethyl)oxazole

Pyrazines



3,5,6-trimethylpyrazin-2(1H)-one

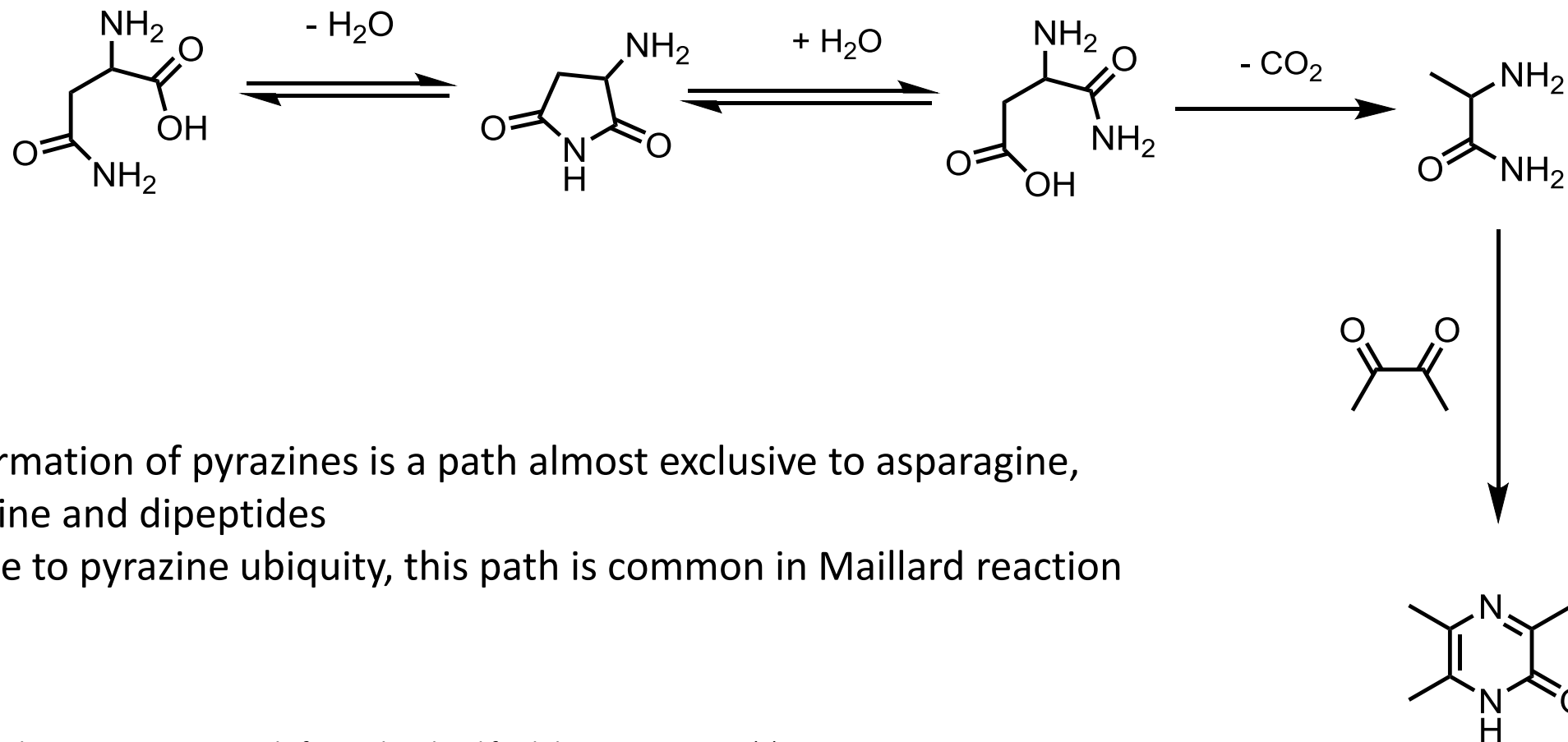
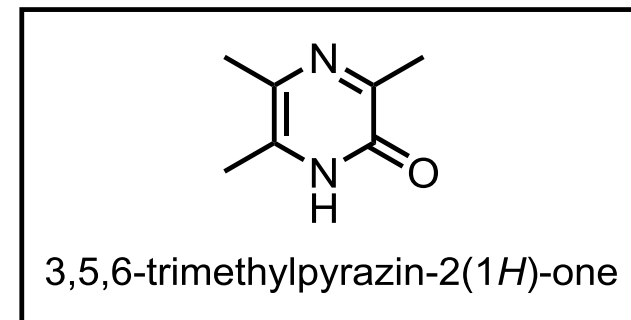


Formation of pyrazines is a path almost exclusive to asparagine, lysine and dipeptides

C.K. Shu and B.M. Lawrence, *Journal of agricultural and food chemistry*, **1995**, 43(3), 779-781

Vernin, G. and Parkanyi, C., Mechanisms for the formation of heterocyclic compounds in Maillard and pyrolysis reactions, in *The Chemistry of Heterocyclic Flavoring and Aroma Compounds*, Vernin, G. Ed; Ellis Horwood Publishers, Chichester, **1982**, pp 151- 207

Pyrazines

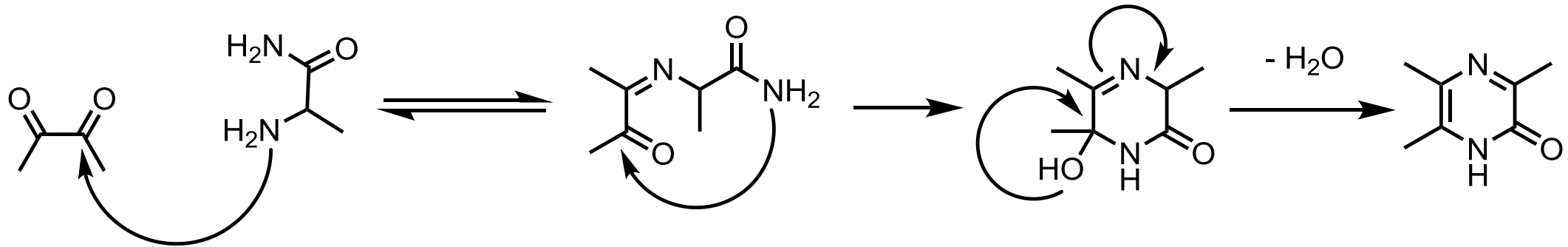


- Formation of pyrazines is a path almost exclusive to asparagine, lysine and dipeptides
- Due to pyrazine ubiquity, this path is common in Maillard reaction

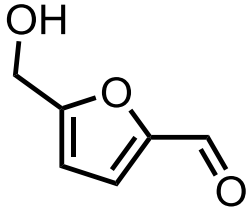
C.K. Shu and B.M. Lawrence, *Journal of agricultural and food chemistry*, **1995**, 43(3), 779-781

Vernin, G. and Parkanyi, C., Mechanisms for the formation of heterocyclic compounds in Maillard and pyrolysis reactions, in *The Chemistry of Heterocyclic Flavoring and Aroma Compounds*, Vernin, G. Ed; Ellis Horwood Publishers, Chichester, **1982**, pp 151- 207

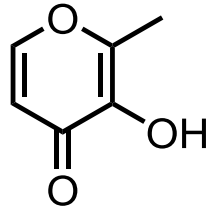
How butanedione combines with amine



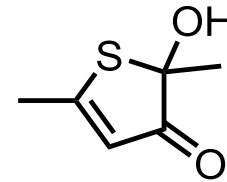
Heterocyclic products



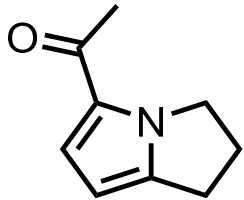
5-(hydroxymethyl)furan-2-carbaldehyde
(5-hydroxymethyl furfural)



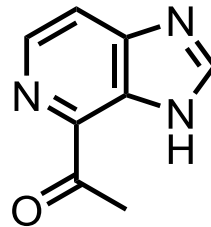
3-hydroxy-2-methyl-4*H*-pyran-4-one



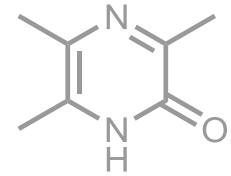
2-hydroxy-2,5-dimethyl-3(2*H*)-thiophenone



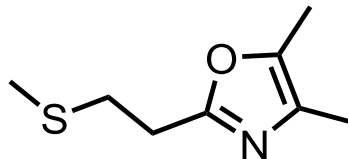
1-(2,3-dihydro-1*H*-pyrrolizin-5-yl)ethan-1-one



1-(3*H*-imidazo[4,5-*c*]pyridin-4-yl)ethan-1-one

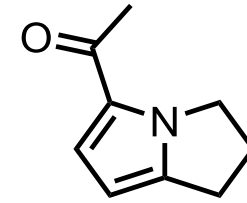


3,5,6-trimethylpyrazin-2(1*H*)-one

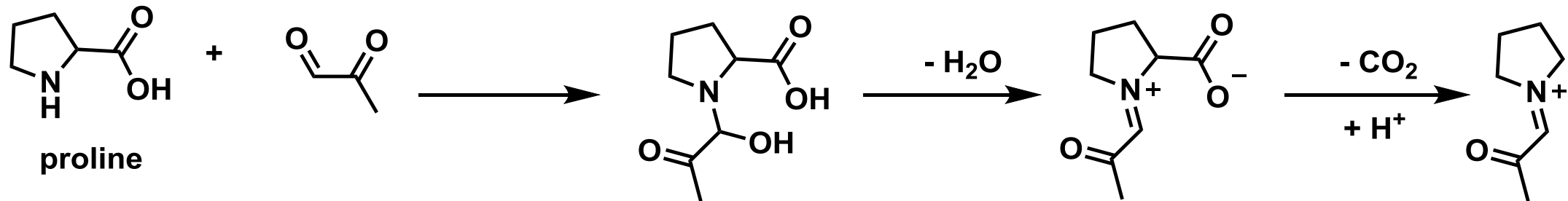


4,5-dimethyl-2-(2-(methylthio)ethyl)oxazole

Pyrroles and Pyrrolizidines



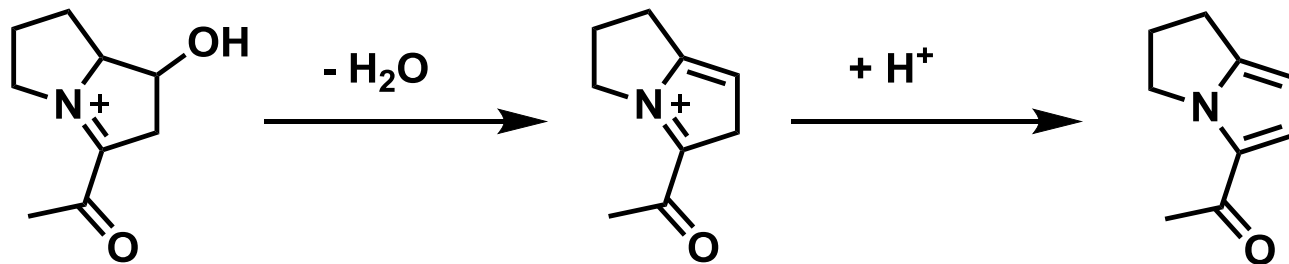
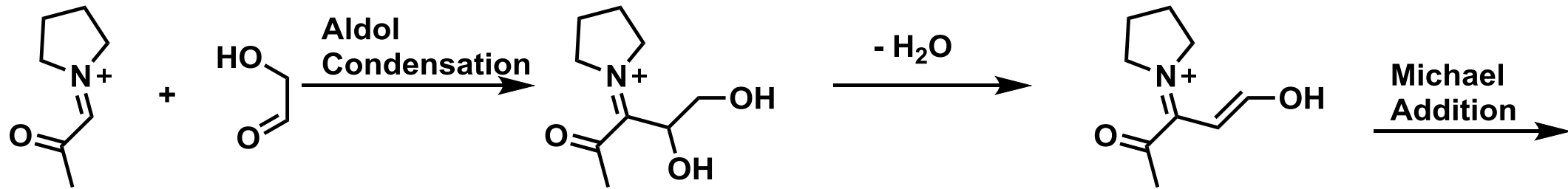
1-(2,3-dihydro-1H-pyrrolizin-5-yl)ethan-1-one



Tressel, R., Helak, B., Rewicki, D., Kampershoer, H., Martin, N., *J. Agric. Food Chem.*, **1985**, 33, 919-23

Tressel, R., Grunewald, K. G., Helak, B., Formation of flavor components from proline and hydroxyproline with glucose and maltose and their importance to food flavor, in *Flavour '81*,
Screier, P. Ed., Walter de Gruyter, Berlin, **1981**, pp 397-416

Addition of a second component

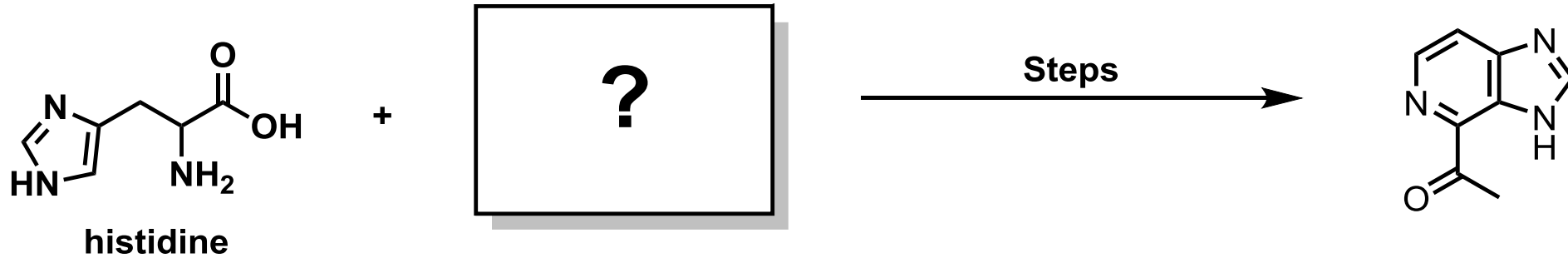


Example of how viable molecules are still present after they have "left" the reaction

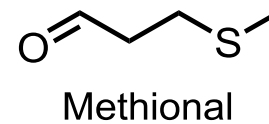
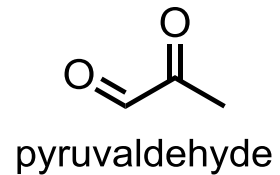
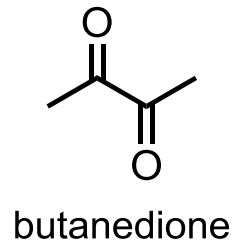
Tressel, R., Helak, B., Rewicki, D., Kampersroer, H., Martin, N., *J. Agric. Food Chem.*, **1985**, 33, 919-23

Tressel, R., Grunewald, K. G., Helak, B., Formation of flavor components from proline and hydroxyproline with glucose and maltose and their importance to food flavor, in *Flavour '81*,
Screier, P. Ed., Walter de Gruyter, Berlin, **1981**, pp 397-416

Group Problem



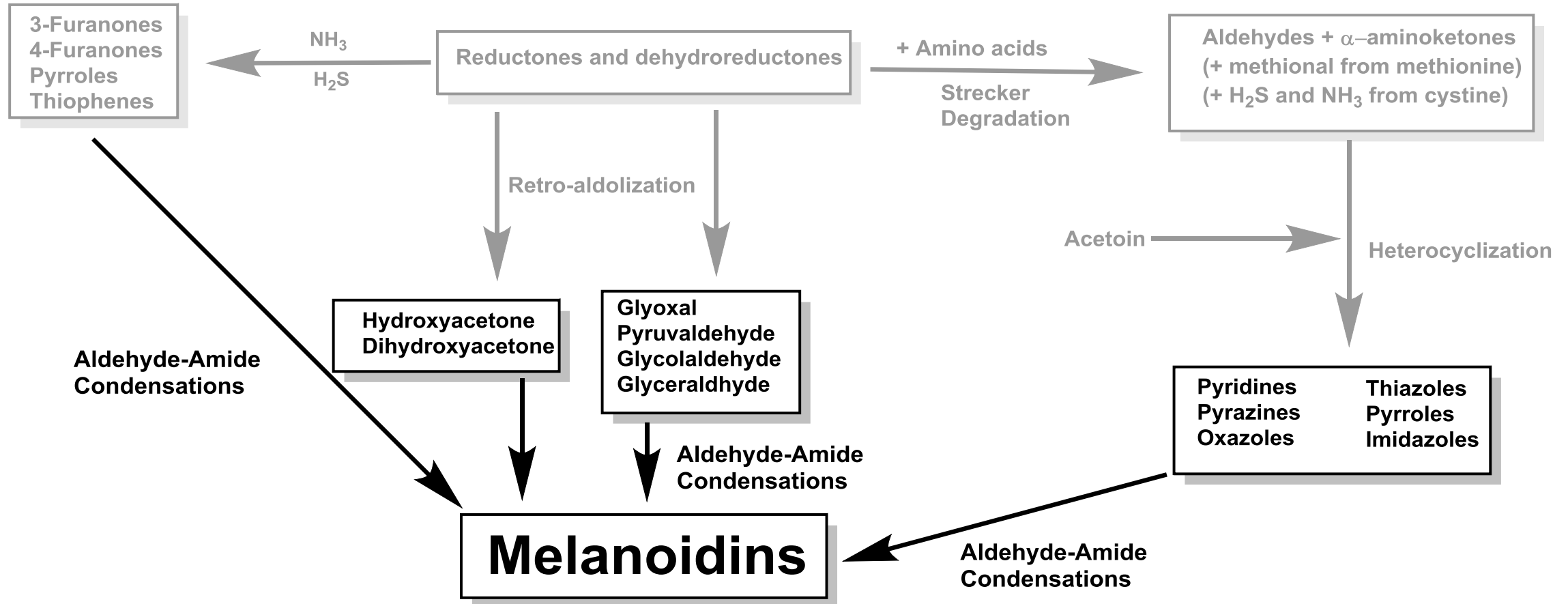
Choices:



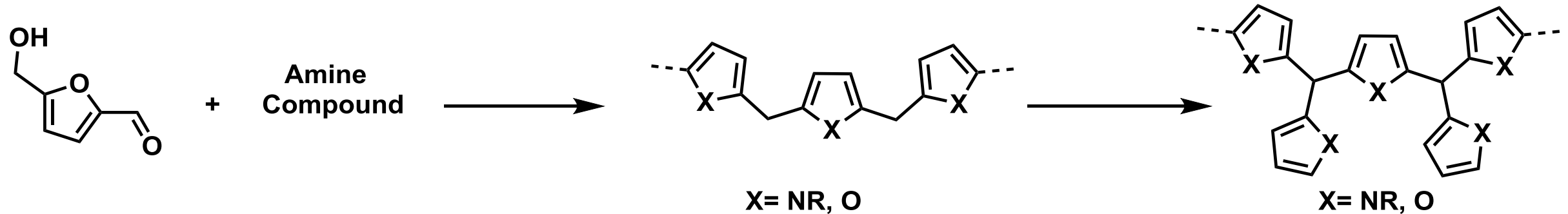
- Determine which fragmentation product combines with histidine
- Propose a pathway for how your chosen starting material forms the shown product
(Hint: No secondary fragmentation products combine in later stages)

Answer:

Browning potential: melanoidins

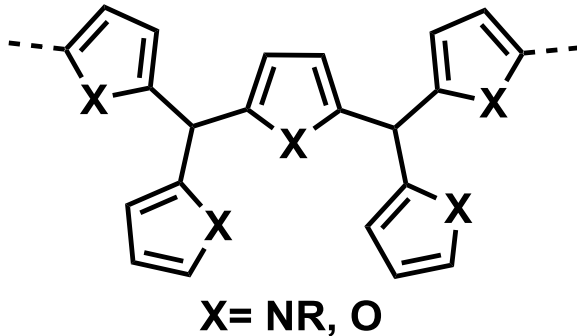


Melanoidins



- High molecular weight brown polymers, with unknown overall structure
- Masses identified from 1 kda to >24 kda
- Oligomers of heterocyclic compounds and/or sugar fragments
- ^{13}C - , ^{15}N -NMR, MALDI-TOF mass spec and IR have determined presence of pyridines, pyrazines, pyrroles and imidazoles
- Oligomers from 14 to >30 identified
- Normally 3-4% nitrogen

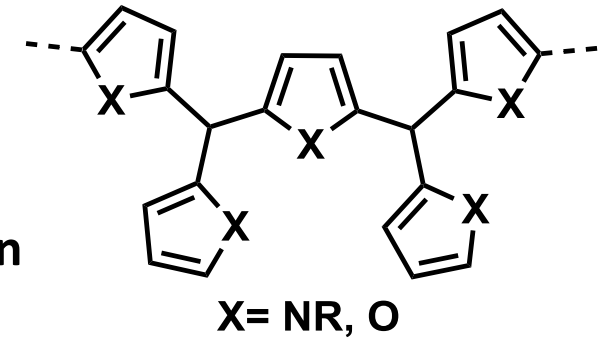
Melanoidin constitution



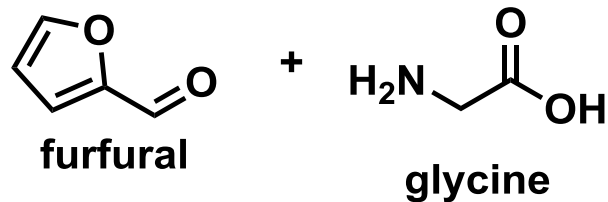
- Constitution of melanoidins vary depending on aldehyde and amine condensing
- Rate of melanoid production is dependent on temperature

Investigation toward melanoidin structures

- Constitution of melanoidins vary depending on aldehyde and amine condensing

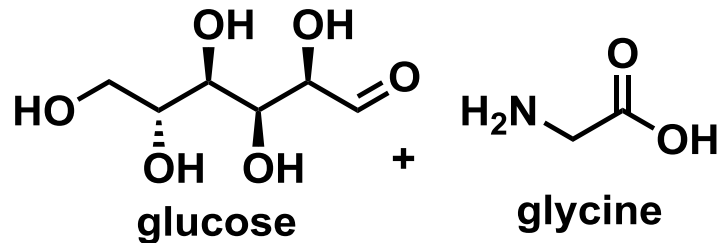


Aldehyde and amine condensed

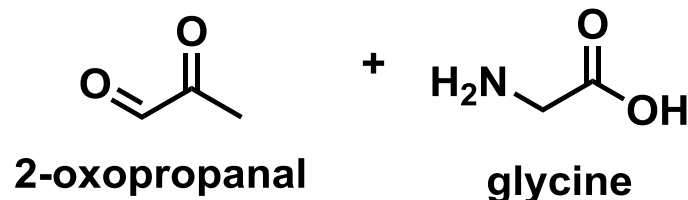


Observed content in melanoidin

High ether content



High alcoholic hydroxyl content



High enolic hydroxyl content, with low ether content

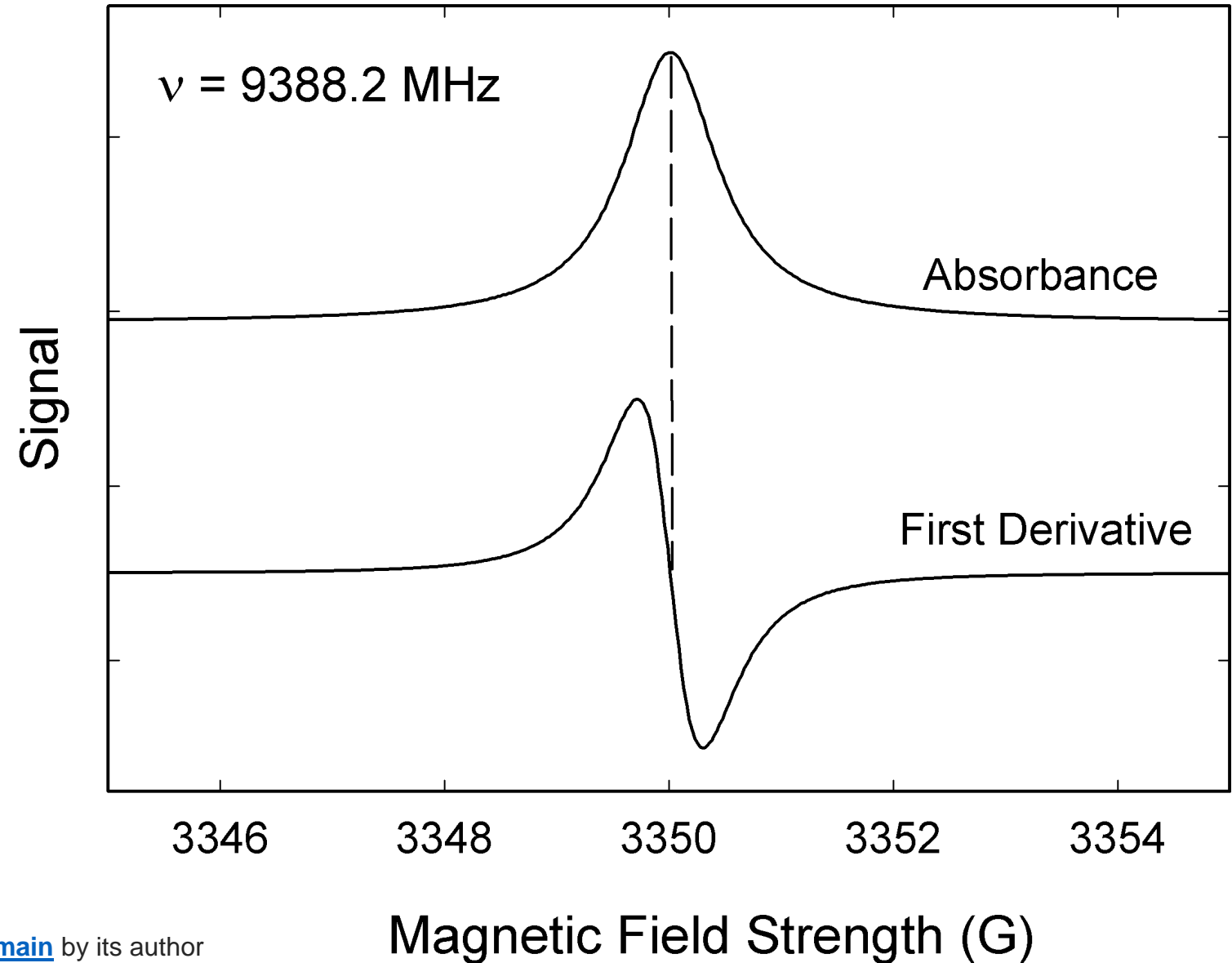
Electron spin resonance spectroscopy (ESR)

- In the presence of a magnetic field (H), the charged dipoles associated with the unpaired electrons line up either parallel or antiparallel to the direction of H and to precess about the field axis at a frequency proportional to the strength of H
- The difference in energy between the two state can be measured by the equation: $\Delta E = h\nu = g\mu_b H$
- h = Planck's constant
- μ_b = Bohr magneton constant
- ν = precession frequency
- H = magnetic field strength
- g = spectroscopic splitting factor and changes with an electron's proximity to magnetic nuclei (in absence of magnetic nuclei $g = 2.0023$ MHz/gauss)

ESR data

- In practice the difference in energy is reported as an absorption and plotted against field strength
- Spectra are normally of the first derivative of this absorption and entire area is measured
 - May be a second derivative

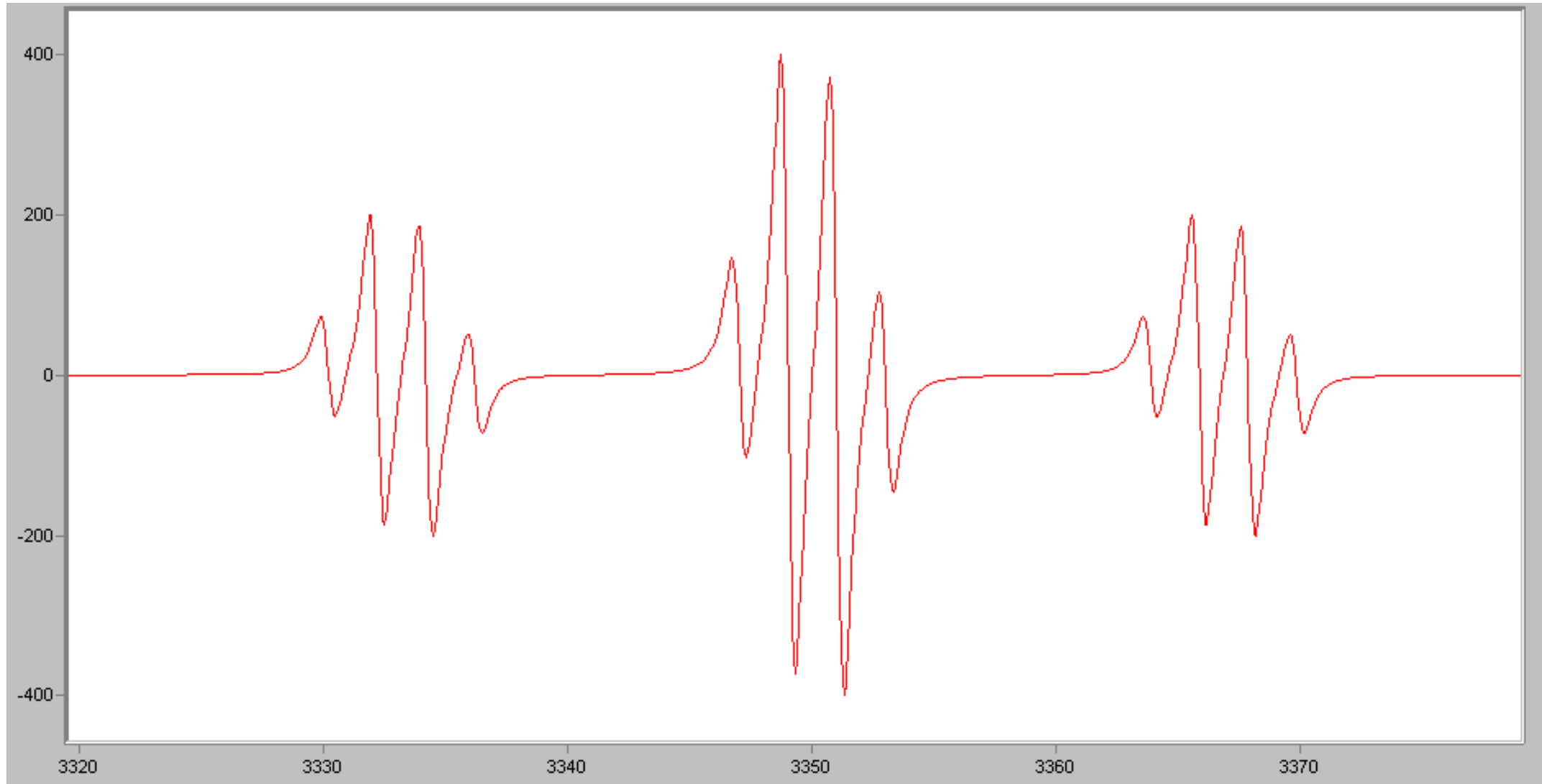
ESR spectra



Gathering structural information

- If an electron is near a magnetic nuclei then there is hyper-fine splitting
- The signal of the electron is split by the magnetic nuclei by $2I+1$
 - I = spin quantum number of the magnetic nucleus
- Think of ESR as NMR for electrons and hyperfine splitting as J-values

Hyperfine splitting



Radicals measured in ESR

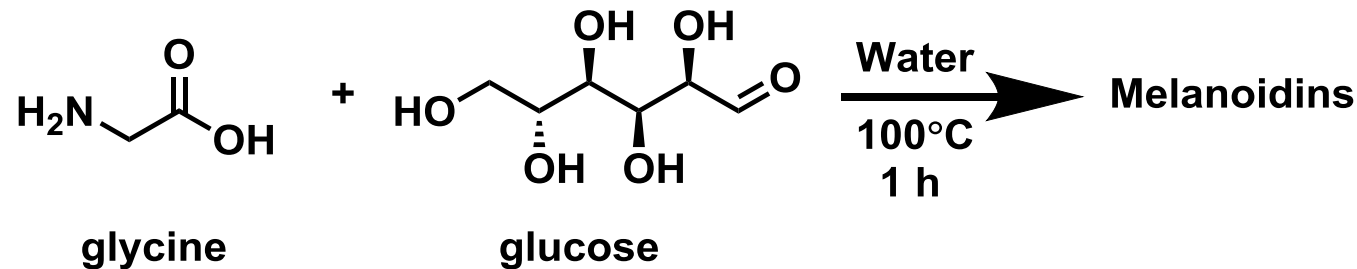
- Electrons have a “spin” that describes the magnetic field (magnetic moment) developed by their rotation as charged particles
- Ordinary organic molecules have all their electrons paired up therefore all spins cancel
 - Non-magnetic or diamagnetic moments
- Free radicals are unpaired and not cancelled causing a paramagnetic moment that can be measured
- To measure a radical it must be stable enough to exist at a concentration of $>10^{-9}$ M at a time

Observation of radicals

- Stable free radicals first observed by Electron Spin Resonance spectroscopy (ESR) in sample of roasted coffee
- The concentration of free radicals is dependent on roasting times
- Radicals found to be present in grounds after brewing process with no change in concentration
 - Radical carrying species must not be water soluble

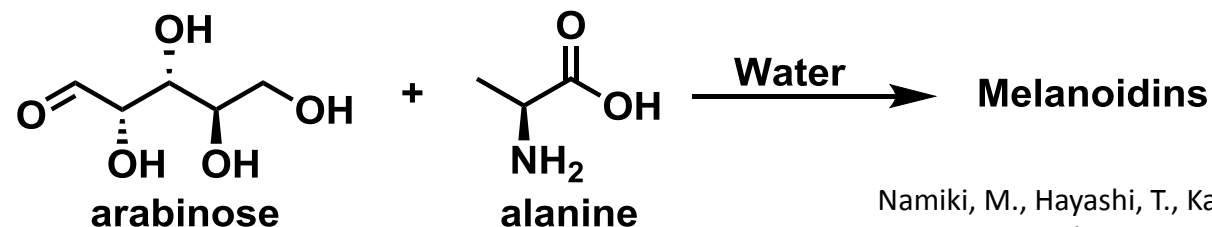
Studying radical stability

- The melanoidin's original radical concentration was measured as 1.9×10^{17} spin/gram
- After two weeks the radical concentration declined by only one third
 - Stored under dry air at room temperature



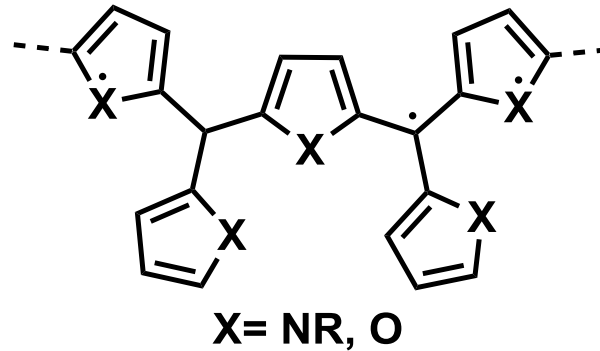
Following radicals through the reaction

- ESR and UV-Vis was used to follow radicals as the Maillard reaction progressed from sugar to melanoidin
- Two distinct radicals were observed
 - One novel radical with a hyper-fine peak structure
 - One radical corresponding to known melanoidin radical character
- α - and β -amino acids gave significantly different splitting patterns
 - β -amino acids splitting pattern was found to be analogous to when n-butylamine was used



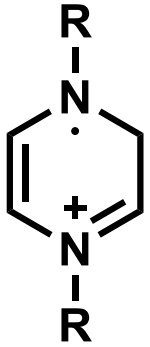
Namiki, M., Hayashi, T., Kawakishi, S., *Agr. Biol. Chem.*, **1973**, 37(12), 2935–2936.
Rizzi, G.P., *Food Rev. Intern.*, **2003**, 19, 375–395.

Melanoidin free radicals



- Model reactions of glucose with glycine, glutamine and lysine gave different melanoidins all with unique radicals
- Higher weight melanoids gave higher concentration of radicals
- All radicals were stable including carbon centered radicals
 - Radicals were observable with no appreciable decrease in concentration after 1 year of storage at 4°C

The novel radical peak



- Hyper-fine peaks in ESR spectra were found to be due to 1,4-disubstituted pyrazinium radical cations
- Structure deduced from ESR studies on various Maillard condition
 - Found as a result of aliphatic amines, and alanine reacting with any group containing an enediol group or potential for an endiol group

Hayashi, T., Ohta, Y., Namiki, M., *J. Agric. Food Chem.* **1977**, 25(6), 1282–1287.

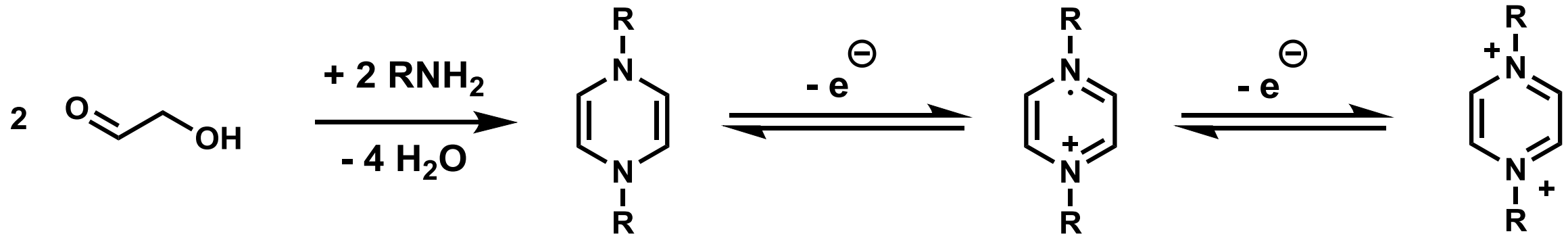
Hayashi, T., Namiki, M., *Agric. Biol. Chem.*, **1981**, 45(4), 933–939.

Rizzi, G.P., *Food Rev. Intern.*, **2003**, 19, 375–395.

Structure Deduction

- Almost all hyperfine structures gave splitting pattern shown
- Coupling to neighbors was shown and structure was elucidated from spectra
- Compared and matched to spectra of synthesized compound

The novel radical peak: creation



- Progression of radical followed via ESR
- Radical cation observed half-life of 10 minutes
- Dication rapidly converted into dihydrohydroxy and dihydrodihydroxy derivatives in water
 - Presence confirmed via LC-MS
- Amine must be a primary amine

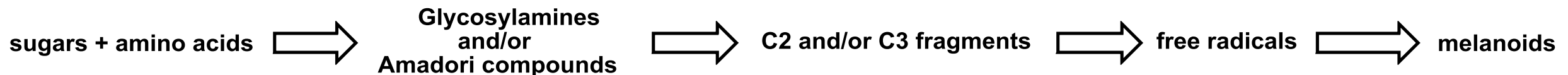
Radicals in the Maillard pathway

- Two different reaction paths emerge: 1) Classic Hodge route; 2) Radical path

Hodge route:

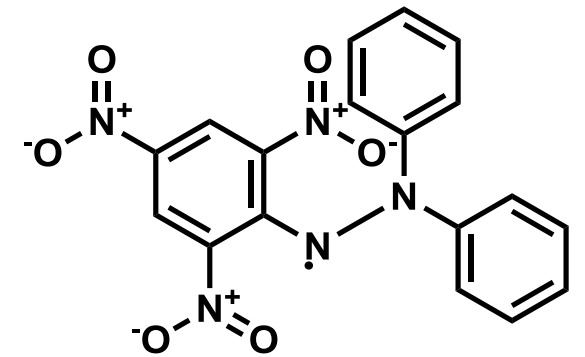


Radical Path:



Determination of dominant path

- Relative proportion of ionic (Hodge) and radical involvement investigated
- DPPH used to quantify both ionic and radical content via UV-Vis
 - Upon reaction with ions or radicals absorbance of DPPH (529nm) is lost
- Ionic intermediates also quantified with ferric ion transformed into ferrous ion
 - Formation of colored (505nm) phenanthroline-ferro(II) complex followed by UV-Vis
- Screened various amino acid and sugar combinations and pH: 5, 7 and 8



**2,2-diphenyl-1-picrylhydrazinyl
(DPPH)**

Radical pH dependence

- Ionic path is generally dominant at low pH
- Radicals present at 60-80% in early reaction stages at pH 7 and above

Radical dependence on amino acids

- Structure of amino acids had small effect on ion/radical ratios

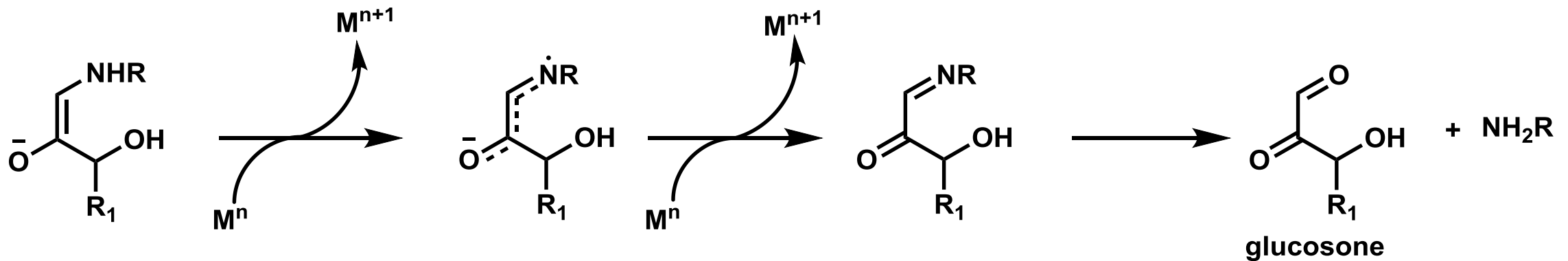
Radical dependence on sugar

- Sugar structure plays a key role in radical content
- Disaccharides give lowest amount of radicals: cleavage of glycoside bond is rate limiting
- Monosaccharides with higher proportion of acyclic structures lead to higher radical formation

(at pH=7)

glucose > fructose >> maltose

Proposed radical mechanism



R= amino acid residue
R₁= sugar residue

- Mechanism investigated in systems with iron and copper
 - Either metals are available in most food systems
- Electron transfer is observed in reaction systems under metal and oxygen free environments

Free radicals and coffee: redux

- Unroasted coffee beans have a concentration of free radicals (CFR) of 10^{15} spins/gram
 - Possibly due to sun exposure during bean drying process or metal ions in the bean
- Roasted coffee has increased CFR to 10^{16} to 10^{17}
- CFR values increased as degree of roast increased
 - Dark roast has more free radicals than light roast
- Radicals found to be especially stable
 - Long lived even after multiple aqueous extractions
 - High proportion of carbon centered radicals to hydroxyl radicals

A look back at coffee's radicals

- Radical extinction of dry coffee with CFR of 7.5×10^{16} was observed
- Over the course of 10-15 min hydroxyl radical singlet rapidly disappeared
- Secondary radical signals persisted with only slow changes
- Secondary signals proposed to correspond to the lysine derived radical cation involved in protein cross-linking and melanoidin formation
 - CROSSPY radical
- Only hydroxyl radicals reacted with radical spin traps

Conclusion

- Maillard reaction: a complex compilation of reactions competing and converging to create the flavors in food
- Hodge path is still accepted, competing radical pathways are possible and probable
- Radical pathways may give insight into formation of heterocycles and antioxidant activity of foods

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