Exercise 2:
Carbohydrates

Question 1:

Which of the following statements are TRUE, and which are FALSE? Why?

a. Carbohydrates are exclusively composed of C, H and O atoms with an overall formula $C_nH_{2n}O_n$.

b. Carbohydrates always have one or more chiral centers and different number of epimeric carbons.

c. Carbohydrates can form linear or branched polymers through hydrolysis reaction.

d. Carbohydrates can form complex molecules by covalent binding to proteins, lipids, and nucleotide bases.

e. The primary type of non-covalent interaction in the majority of carbohydrates are hydrogen bonds.

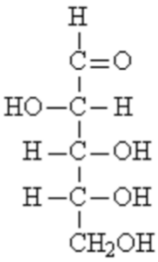
f. sp3-hybridized carbons in carbohydrate building blocks make them convert between different states which in turn makes the oligosaccharides and polysaccharides very flexible.

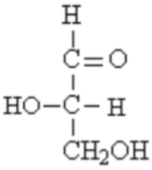
Answers:

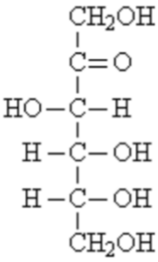
a. **FALSE**: There are carbohydrates that have other atoms and different general formula.
b. **FALSE**: Some carbohydrates do not have a chiral center (e.g., dihydroacetone)
c. **FALSE**: Their bonds are broken through hydrolysis reaction.
d. **TRUE**: Examples are: nucleotides, glycolipids, glycoproteins and proteoglycans.
e. **TRUE**: The hydroxil (OH) and aldehyde/keto groups provide a wealth of hydrogen bond donors and acceptors. Electrostatic interactions can also occur but require special chemical groups so they are not as frequent. Van der Waals interactions can also occur and contribute to the packing of monosaccharide rings (e.g., in cellulose sheets) but their energy contribution compared to hydrogen bonding is lower.
f. **TRUE**: The chair-boat-chair conformational changes can occur spontaneously in most monosaccharide units, and this becomes amplified when multiple units are bound together, leading to a range of different states/conformations for oligo and polysaccharides.

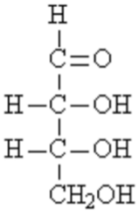
Question 2:

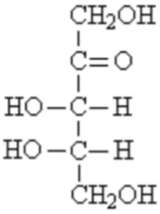
Below you will find linear structures of several monosaccharides. Classify them based on the (1) length of carbon chain, (2) functional group and (3) stereochemistry (handedness).



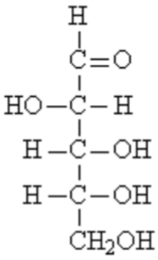
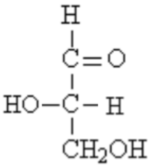
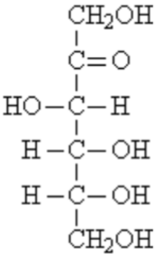
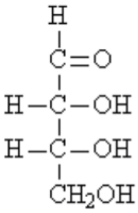
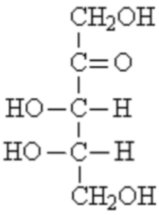






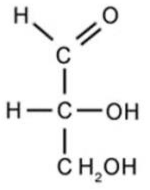


Answer

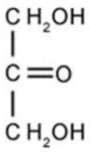
				
Pentose	Triose	Hexose	Tetrose	Pentose
Aldose	Aldose	Ketose	Aldose	Ketose
D	L	D	D	L

Question 3:

The smallest monosaccharides are trioses, glyceraldehyde and dihydroxyacetone shown below:



Glyceraldehyde



$$\begin{array}{c}CH_2OH\\ |\\ C{=}O\\ |\\ CH_2OH\end{array}$$

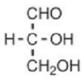
Dihydroxyacetone

a) Can you draw the stereoisomeric versions of each monosaccharide? Include the D- or L-annotation.

b) Can these monosaccharides assemble into cyclic forms in aqeuous solutions? Explain.

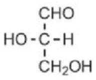
Answers:

a)



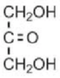
$$\begin{array}{c}CHO\\ H-C-OH\\ CH_2OH\end{array}$$

D-Glyceraldehyde



$$\begin{array}{c}CHO\\ HO-C-H\\ CH_2OH\end{array}$$

L-Glyceraldehyde



$$\begin{array}{c}CH_2OH\\ C{=}O\\ CH_2OH\end{array}$$

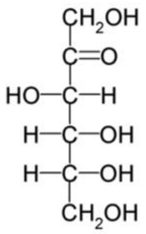
Dihydroxyacetone

Dixydroxyacetone does not have an asymetric chiral center which is why there are no stereoisomers.

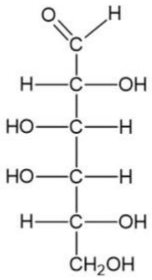
b) They cannot assemble spontaneously as pentose and hexose sugars. The length of the carbon backbone does not allow sufficient flexibility for the conversion to cyclic form to take place. To readily convert to cyclic forms the sugars need to have minimally 5 carbons.

Question 4:

Below are the linear structures of D-fructose and D-galactose.



D-Fructose



D-Galactose

a) Draw the cyclic structures of each sugar in alpha (α) stereoisomeric form and indicate the location of anomeric carbon. How does the beta (β) form differ from the alpha?

b) Lactulose (galatose-β(1→4)β-fructose) is a disaccharide used in the treatment of constipation and hepatic encephalopathy. It is assembled from **1 D-galactose** and **1 D-fructose** building block through **β(1-4)β O-glycosidic linkage**. Draw the structure of this disaccharide based on the cyclic forms of each monosaccharide building block.

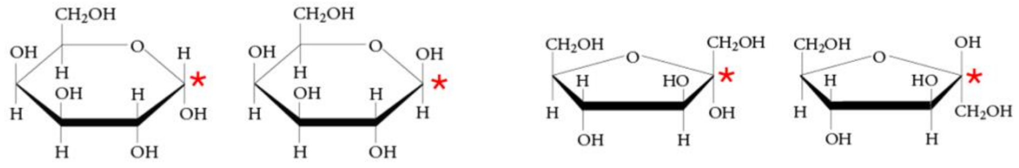
c) Melibiulose (galactose-α(1→6)β-fructose) is another disaccharide assembled from the same buidling blocks using the **α(1-6)β O-glycosidic bond**. Draw the structure of this disaccharide based on the cyclic forms of each monosaccharide building block.

Answers:

a)

Galactose

Fructose



Alpha

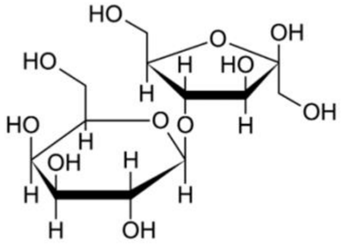
Beta

Alpha

Beta

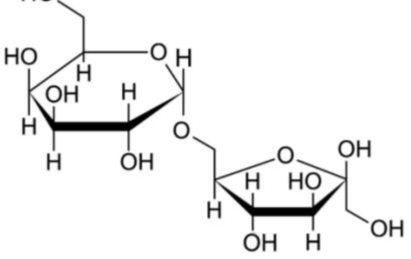
b)

Lactulose



c)

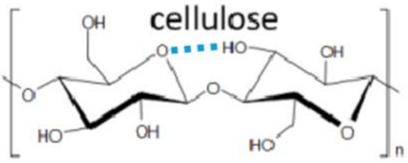
Melibiulose

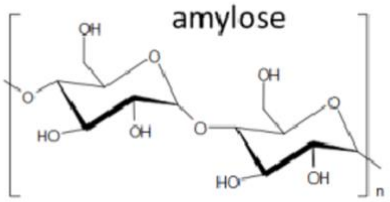


* - anomeric carbon

Question 5:

Below is a structure of cellulose and amylose chain segments. Both are composed of the same building block (glucose) linked via the same carbon atoms (C1 and C4), but using a **β(1-4)** or **α(1-4)** O-glycosidic linkage. In blue we highlighted the hydrogen bond that is essential for intrachain stability of cellulose leading to assembly into sheets. This improves the mechanical properties of cellulose (making it a great material for cell wall) but excess rigidity of such large polymers reduces the solubility in water.





a) What effect does the α(1-4) linkage have on the capacity of amylose to form intrachain hydrogen bonds? How does this influence the macroscopic properties (stability/flexibility and solubility)?

b) Cellulose is often chemically or enzymatically modified to optimize some of its properties. One of the modifications includes the substitution of accessible hydroxyl groups in glucose with negatively charged phosphate groups (R-O-PO$_3^{2-}$). What would be the impact of this modification on cellulose packing and mechanical properties? What about the solubility in water?

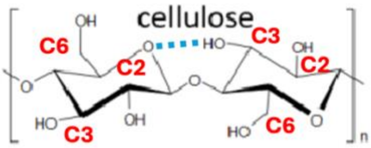
c) In the above structure of cellulose, label all the hydroxyl groups that can in theory be substituted with phosphate groups (use the sugar ring carbon numbering to label the group locations). Modification of which single group do you think would cause the greatest destabilizing effect? Why?

Answers:

a) α(1-4) linkage changes the relative orientations of the two rings such that various hydroxyl groups (= potential hydrogen bond donors) in either glucose subunit are not in an optimal position to create a stable hydrogen bond. There are certainly degrees of freedom around different bonds (e.g., chair-boat conversion) and while hydrogen bonds can certainly occur at least transiently, they cannot be perfectly maintained from one glucose subunit to the next. This makes the amylose polymer more flexible (less rigid), and more soluble since it can accommodate water molecules better and make more hydrogen bonds with them.

b) Regular display of negative charges from a phosphate group would [i] increase repulsion within and between the cellulose strands through electrostatic interactions, [ii] increase the capacity to interact with water through providing extra hydrogen bond acceptors (4 oxygens in phosphate vs only one in hydroxyl group) and [iii] increase the capacity to create van der Waals (dipole–dipole and ion–dipole) interactions with water and other polar groups. Altogether, the effect would be reduced packing of the cellulose strands and better interaction with the solvent, thereby increasing solubility.

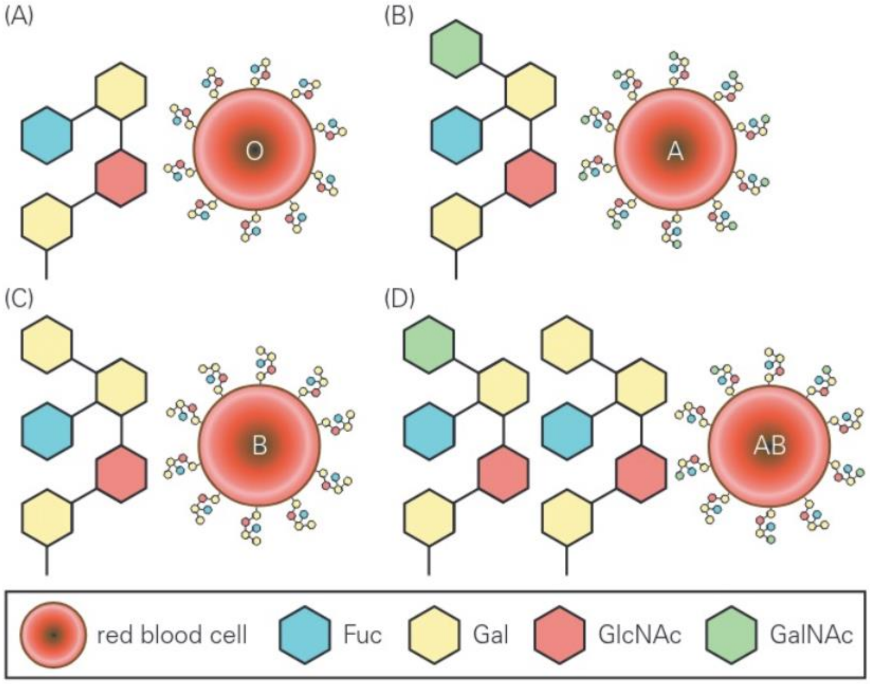
c) The hydroxyl groups are located on carbons C2, C3 and C6. The greatest destabilizing effect on cellulose would come from modifications on C3 hydroxyl group since this group is essential for the hydrogen bond network that rigidifies the cellulose strand and allows packing into sheets. By substituting this group with the phosphate the change will disrupt the hydrogen bonding pattern and incorporate the additional “charge” effects discussed in answer for (b).



Of course, the greatest destabilizing effect would come from simultaneous modification of all 3 hydroxyl groups (i.e., C2, C3 and C6), but the question here was to select the one that would have the strongest impact.

Question 6:

The O blood type group is known as the universal donor. That means that people having this blood type can give their red blood cells to other people, regardless of their group, without eliciting an immune response (at least to first approximation; more complex in medical practice).



a) Considering the glycosylation pattern of the O blood type versus all other groups, can you explain why that is?

b) Using the same rationale, can you explain why AB group is considered a universal acceptor for red blood cell transfusion purposes?

c) If O-type red blood cells can be donated universally, why can't O-type individuals receive blood from any donor?

d) Imagine that you have an endless supply of enzymes to precisely modify carbohydrates on cell surface in any way you wish. How could you use those enzymes to make any red blood cell sample serve as universal donor?

Answer:

a) O-type has the core glycan configuration that are also present in the A, B and AB groups. It does not have any additional groups, compared to A and B glycans that would make it distinct. Hence this is not recognized as "foreign" by the immune system.

b) On the surface, AB-type will have both A and B glycan types individually, so it will tolerate the transfusion of A- or B-type cells. Given that both A and B glycans in the core also have the O-type glycan, then O-type red blood cells will also be tolerated by the immune system.

c) Given that A- and B-type glycans are longer by a single monosaccharide residue, the immune system in people with O-type glycans will recognize the configuration with extra Gal or GalNAc residues as foreign.

d) There are several strategies here but the most universal and straightforward would be to have enzyme mix that cleaves (hydrolyzes) the extra GalNAc in A-type and extra Gal in B-type leaving just the core O-type glycan configuration on the surface of red blood cells from any donor.

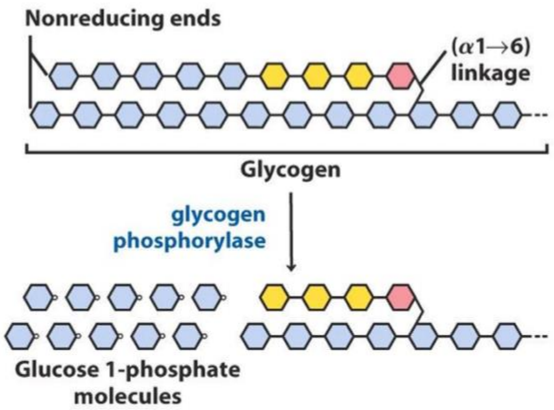
Since 1980s, when it was first proposed, this idea actually developed into an active field of research with several enzyme candidates discovered over the years. They are typically found in bacteria and their official names are:

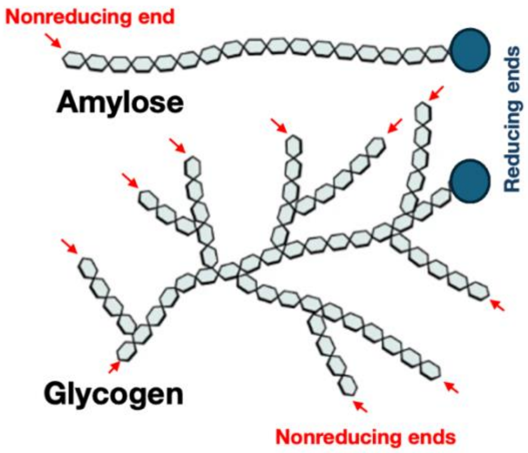
- α-N-acetylgalactosaminidases (removes GalNAc)
- α-galactosidases (removes Gal)

These enzymes have been tested in the lab, demonstrating feasibility, but are yet to be applied in clinic.

Question 7:

Glycogen phosphorylase is an enzyme that catalyzes the phosphorylation and release of the glucose residue from the termini of glycogen branches (“nonreducing ends”, see image below). Assume glycogen phosphorylase can release one molecule of glucose from each nonreducing end in glycogen at a rate of 10 residues per second.





a) If a glycogen molecule has 2’000 nonreducing ends, how many glucose residues can be released per second from this single molecule?

b) If an amylose molecule of the same size has only 1 nonreducing end (no branching), how many glucose residues can be released per second?

c) Calculate the fold-difference in mobilization rate between glycogen and amylose.

Answer:
a) Each nonreducing end will be processed at 10 residues per second which makes the total glucose release (mobilization) rate:

V (glycogen) = 2’000 x 10 glucose/second = 20’000 glucose/second

b) Using the same approach as above we get:

V (amylose) = 1 x 10 glucose/second = 10 glucose/second

c) The ratio of release rates is:

V (glycogen) / V (amylose) = 2’000

In this scenario glucose will be released 2’000-times faster from glycogen, which is why this molecule is superior as short- and intermediate-term energy storage in animal cells.