LAB 4 – Macromolecules

Objectives

1. Determine the molecular structures and properties of key chemical groups.
2. Build models of several amino acids and assemble them into a polypeptide.
3. Use chemical reagents to determine the carbohydrate and protein content of various foods.

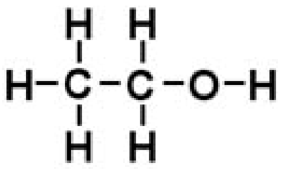
Part 1: BUILDING MACROMOLECULES

The Structure of Organic Molecules

All living things consist of both **organic** and **inorganic** molecules. Organic molecules contain the elements carbon (C) and hydrogen (H), and more specifically, carbon–hydrogen bonds. Molecules lacking C–H bonds are considered to be inorganic. For example, oxygen gas (O_2), water (H_2O) and carbon dioxide (CO_2), despite their obvious importance for life, are inorganic molecules. Methane (CH_4), ethanol (C_2H_6O) and glucose ($C_6H_{12}O_6$) on the other hand, are all organic. In general, organic molecules are derived from living organisms, hence the association of the word *organic* with natural, living things.

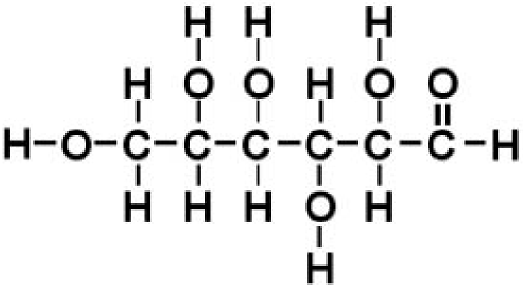
It is helpful to think of organic molecules as skeletal carbon structures (**carbon skeletons**) to which various chemical groups are attached. To illustrate this, let's take a look at the structure of a simple organic molecule that we are all familiar with, *ethanol*:

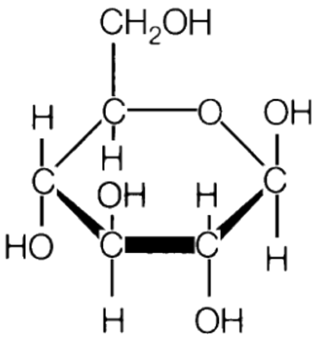
ethanol



Notice that a molecule of ethanol contains a core carbon structure or skeleton consisting of 2 carbon atoms connected to each other by a single covalent bond. The remaining unpaired electrons in the carbon atoms are involved in covalent bonds with individual *hydrogen atoms* or a *hydrogen-oxygen* combination known as a **hydroxyl** group. The single hydrogen atoms and the hydroxyl group are examples of common **functional groups** (though a hydrogen atom technically is not a "group") that are attached via covalent bonds to carbon skeletons. Let's look at another slightly larger organic molecule, the simple sugar *glucose*:

glucose
(a monosaccharide)





If you look carefully you'll notice that the glucose molecule above (shown in both its linear and ring forms) is simply a 6-carbon skeleton to which numerous hydrogens and hydroxyl groups are attached (as well as a double-bonded oxygen).

Hydrogen atoms and hydroxyl groups are by no means the only functional groups found in organic molecules, so let's get acquainted some other common functional groups in addition to these two and take note of their chemical properties when bound to a carbon framework:

functional group	**molecular formula**	**property (at pH 7)**
hydrogen	**$-H$**	*non-polar*
hydroxyl	**$-OH$**	*polar*
methyl	**$-CH_3$**	*non-polar*
amino	**$-NH_2$**	*basic* (binds H^+)
carboxyl (or carboxylic acid)	**$-COOH$**	*acidic* (releases H^+)

Exercise 1A – Constructing functional groups

Much like you did in a previous lab, diagram the *structural* formulas for the functional groups shown above on your worksheet and then build them with your molecular model kit. In your structural formulas, represent the bond that will connect to a carbon skeleton as a line sticking out from your function group. In your models, each functional group should have a covalent bond connector that is not connected to anything on one side.

Here is a key to the components of your kit:

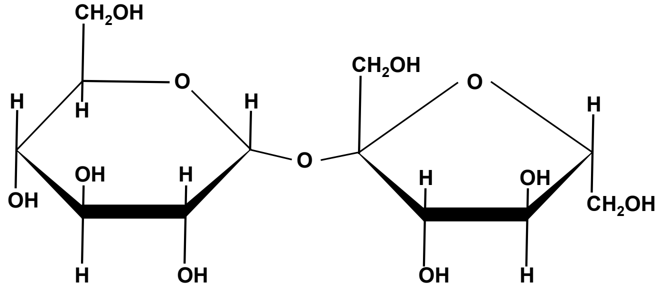
○	**WHITE = *hydrogen atom***	🔴	**RED = *oxygen atom***
●	**BLACK = *carbon atom***	🔵	**BLUE = *nitrogen atom***

short connectors (use for single covalent bonds)
long connectors (use in double & triple covalent bonds)

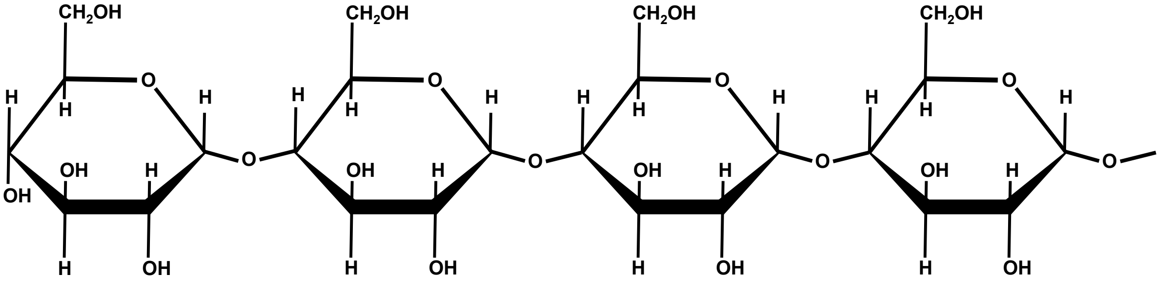
Monomers and Polymers

The organic molecules we classify as carbohydrates, proteins, lipids and nucleic acids include single unit ***mono*mers** (*one* unit molecules) as well as chains of monomers called ***poly*mers** (*many* unit molecules). Terms like ***di*mer** (*two* unit molecule) and ***tri*mer** (*three* unit molecule) are also used. For example, carbohydrates can be monomers (such as glucose and fructose), dimers (such as sucrose and lactose), and polymers (such as starch and glycogen). For carbohydrates, such molecules are more specifically referred to as ***mono*saccharides**, ***di*saccharides**, and ***poly*saccharides** (*saccharide* is Greek for “sugar”).

sucrose
(a disaccharide)

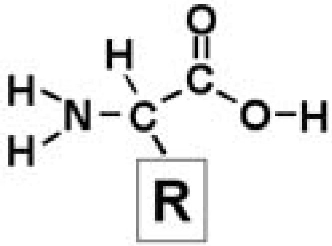


starch
(a polysaccharide)



Proteins are another important type of biological polymer. The monomers from which proteins are assembled are amino acids. In a moment you will use some of the functional groups you just made to construct an amino acid. The general structure of an amino acid is shown below:

amino acid structure

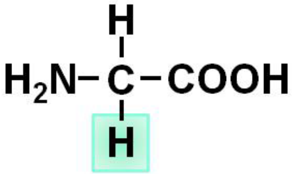


Notice that there is a central carbon atom (essentially a carbon skeleton consisting of one carbon atom) connected via covalent bonds to the following:

- a hydrogen atom
- an amino group (–NH_2)
- a carboxyl group (–COOH)
- a variable “R” group

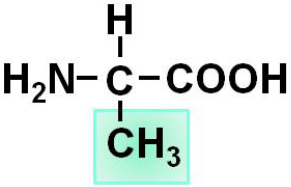
All amino acids have in common the first 3 functional groups: the hydrogen, amino and carboxyl groups. Proteins are constructed from up to 20 different amino acids, and the “R” group is different for each giving each amino acid its unique properties. Let’s examine the “R” groups (highlighted in green) of six different amino acids, after which you and a partner will assemble one amino acid using a molecular model kit:

glycine



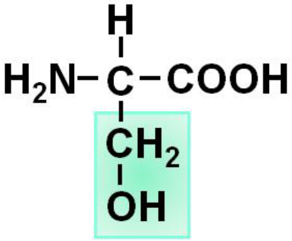
$H_2N-\overset{H}{\underset{H}{C}}-COOH$

alanine



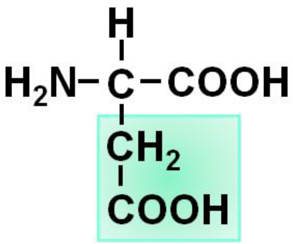
$H_2N-\overset{H}{\underset{CH_3}{C}}-COOH$

serine



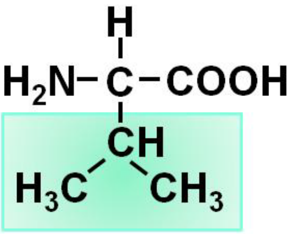
$H_2N-\overset{H}{\underset{\underset{OH}{CH_2}}{C}}-COOH$

aspartic acid

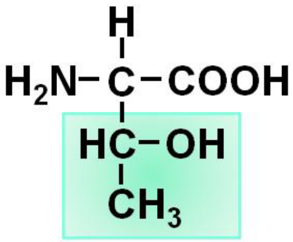


$H_2N-\overset{H}{\underset{\underset{COOH}{CH_2}}{C}}-COOH$

valine



threonine



$H_2N-\overset{H}{\underset{\underset{CH_3}{HC-OH}}{C}}-COOH$

<u>Exercise 1B</u> – Building an amino acid

1. On your worksheet, diagram the *structural* formula for the amino acid you are assigned to build.

   *Note: the diagrams shown are <u>partial</u> structural formulas to help guide you in determining the <u>complete</u> structural formula (with **<u>all</u>** covalent bonds shown)*

2. Working in pairs, build the amino acid with your molecular model kit as follows:

   a) to a central carbon atom, attach the following functional groups you’ve already made:

   - a hydrogen atom
   - an amino group
   - a carboxyl group

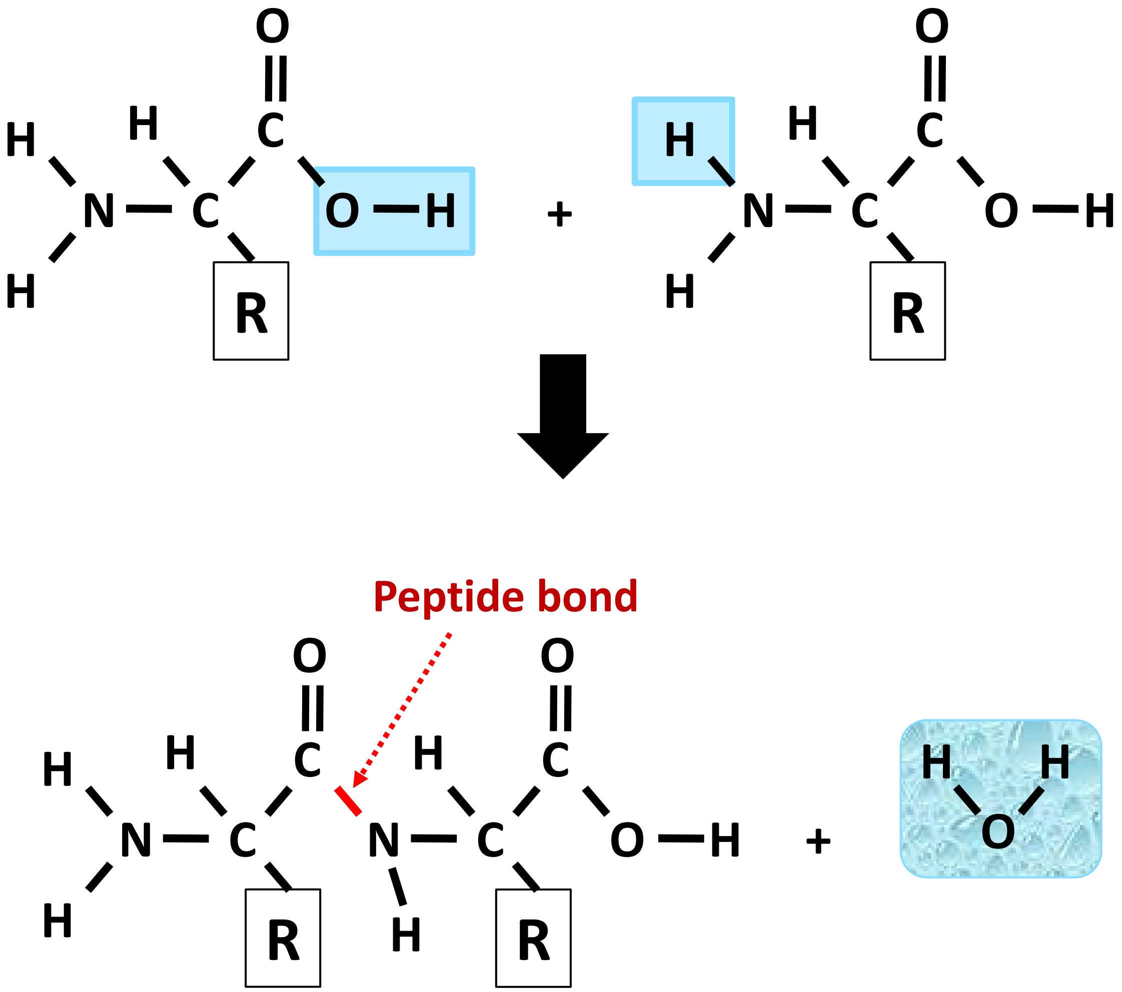
   b) construct the “R” group for your amino acid separately

   c) attach your “R” group to the remaining bond in your central carbon atom

Assembling and Breaking Down Polymers

Living organisms such as yourself are continuously building polymers and breaking them down into monomers. For example, when you eat a meal you ingest large amounts of polymers (proteins, starch, triglycerides) which are subsequently broken down into monomers (amino acids, glucose, fatty acids) within your digestive system. Within your cells there is a continuous cycle of building new protein, carbohydrate, lipid and nucleic acid polymers, and breaking down "old" polymers into their respective monomers (amino acids, sugars, fatty acids, nucleotides).

There is a common theme to the building and breaking down of biological polymers. Whenever a monomer is added to a growing polymer, a molecule of water (H_2O) is released in a process called **condensation** or **dehydration**. For example, when two amino acids are joined in a growing polypeptide, the –OH of the carboxyl group of the first amino acid will combine with an H from the amino group of the second amino acid. This results in the simultaneous formation of a covalent bond between the two amino acids, a **peptide bond**, and release of a water molecule:

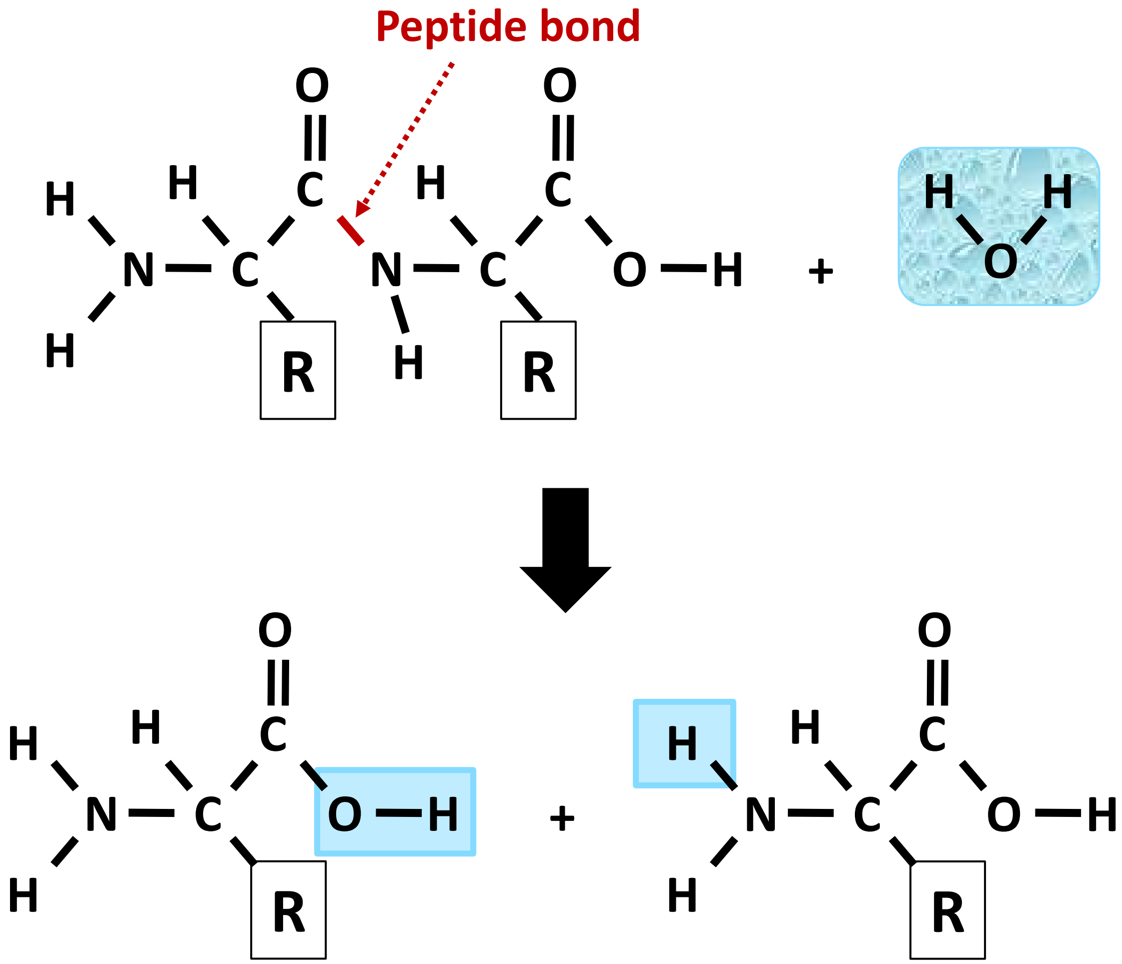


Exercise 1C – Assembling a polypeptide

You will work with other members of your group to assemble a small polypeptide containing each of the amino acids your group has just built. Assemble the polypeptide in alphabetical order (e.g., ***alanine*** *before* ***glycine****), and the amino acid you have built will be added at the appropriate position. To reproduce how polypeptides are actually assembled in living cells, the polypeptide should be assembled as follows (AA = amino acid):*

1. Position AA1 and AA2 so that the –COOH of AA1 is next to the –NH_2 of AA2.
2. Remove the –OH from the –COOH of AA1, and an H from the –NH_2 of AA2.
3. Combine the –OH and H to form H_2O and set it aside (you will use it in Exercise 1D).
4. Connect the carbon from the original –COOH in AA1 to the amino group of AA2.
5. Repeat steps 1 to 4 with each additional amino acid until the polypeptide is assembled.

The breakdown of a polymer into monomers essentially reverses the process of its assembly. Whenever a monomer is removed from a polymer, a water molecule must be inserted in a process called **hydrolysis**. This is illustrated below for the breakdown of a *di*peptide into individual amino acids:



Exercise 1D – Breaking down a polypeptide

Break down the polypeptide your group has just assembled as follows:

1. Break the peptide bond between the last 2 AAs in your polypeptide.
2. Use your water molecule to restore the H on the amino group of AA just removed and the –OH on carboxyl group of the other AA.
3. Repeat steps 1 and 2 for each successive peptide bond until the polypeptide is completely broken down into its original amino acid monomers.

Part 2: DETECTING MACROMOLECULES

In the exercises to follow, you will test various food items for the presence of simple sugars, starch and protein using chemical reagents specific for each. When doing such tests it is always important to include control reactions. As you learned in the first lab, a control experiment is one in which the independent variable (e.g., the source of sugar, starch or protein in test samples) is "zero" or some background level. For example, if you are testing for starch you want to be sure to include a sample that you know does NOT contain starch. The perfect negative control for this and other such experiments is plain water since it does not contain starch or anything else. This sort of control is referred to as a ***negative* control** since it is *negative* for what you are trying to detect. The importance of performing a negative control is two-fold:

1) *To verify that a known negative sample actually gives a negative result with the materials you are using.*

2) *To allow you to see what a negative result looks like for the sake of comparing with your other test samples.*

You will also want to include a ***positive* control** for each of your experiments, i.e., a sample that DOES contain the substance you are testing. For example, when you test various foods for the presence of starch you will want to include a sample that you know contains starch. The ideal positive control in this case would be simply a starch solution (water with starch and nothing else). The importance of performing a positive control is also two-fold:

1) *To verify that a known positive sample actually gives a positive result with the materials you are using.*

2) *To allow you to see what a positive result looks like for the sake of comparing with your other test samples.*

If either control fails to give the predicted outcome in a given experiment, then the results for *all* of your test samples are suspect. If your controls give the expected outcomes, then you can be confident that the results for your test samples are reliable.

Now that you understand the importance of performing positive and negative controls, you are ready to test the following foods for the presence of **simple sugars**, **starch** and **protein**:

cow's milk
rice milk
almond milk
chicken broth
fruit juice
flour
soda
diet soda

Exercise 2A – Detection of simple sugars

Benedict's reagent is a chemical reagent that will reveal the presence of any monosaccharide as well as the disaccharides lactose, maltose or mannose (not sucrose). The reagent itself is blue, however when it reacts with monosaccharides (or the disaccharides indicated) it will change to a green, yellow, orange or reddish brown color depending on how much sugar is present (green to yellow if low levels, orange to reddish brown if high levels). Materials you will need include:

Benedict's reagent
10 test tubes and a rack
8 food samples to test
deionized water (negative control)
glucose solution (positive control)
boiling water

NOTE: Before you start, remove the hot plate from your drawer and plug it in. Half fill a large beaker with water, place it on the hotplate and turn on the heat dial ~halfway.

Test each of your 10 samples as follows:

1. Label each of your 10 test tubes accordingly (e.g., A1, A2, A3…).
    - *label the upper part of each tube so it won't come off when you boil!*
2. Add 1 ml of Benedict's reagent (1 squeeze of dropper) to each tube.
3. Add 0.5 ml of the appropriate test sample to each tube, mix.
4. Boil all samples for 5 minutes.
    - *be sure beaker is no more than half full with boiling water to avoid overflowing!*
5. Record the colors for each tube and determine whether or not sugars are present.

Exercise 2B – Detection of starch

Iodine solution will reveal the presence of starch. The reagent itself is a light brown color, however when it reacts with starch it will change to a dark blue or black color. Materials you will need include:

iodine solution
10 test tubes and a rack
8 food samples to test (use solid pieces of potato, banana, coconut, peanut)
deionized water (negative control)
starch solution (positive control)

Test each of your 10 samples as follows:

1. Label each of your 10 test tubes accordingly (e.g., B1, B2, B3…).
2. Add 2 ml of the appropriate liquid test sample to each tube (water, starch solution, milk, soda).
3. Add 3 ***drops*** of iodine solution to each tube, mix **(do NOT boil!)**, determine color immediately**.**
4. Record the colors for each sample and determine whether or not starch is present.

Exercise 2C – Detection of protein

Biuret reagent will reveal the presence of protein. The reagent itself is blue, however when it reacts with protein it will change to a dark purple color. Materials you will need include:

Biuret reagent
10 test tubes and a rack
8 food samples to test
deionized water (negative control)
albumin solution (positive control)

Test each of your 10 samples as follows:

1. Label each of your 10 test tubes accordingly (e.g., C1, C2, C3…).
2. Add 0.5 ml of Biuret reagent to each tube.
3. Add 1 ml of the appropriate test sample to each tube, mix **(do NOT boil!).**
4. Record the colors for each tube and determine whether or not protein is present.

Exercise 2D – Analysis of an unknown sample

The unknown sample you've been given may contain any combination of sugars, starch, protein or just plain water. You are to test the sample for all three macromolecules as you did in Exercises 2A, 2B and 2C. Record the results and your conclusions on your worksheet.

PLEASE be sure to dispose of all tube contents in the chemical disposal jug in the flow hood and wash all of your tubes with soap and hot water leaving them upside in your test tube rack in the sink.

We don't have the staff to wash so many tubes and your cooperation will be greatly appreciated. THANK YOU!

Before you leave, please make sure your table is clean, organized, and contains all supplies listed below so that the next lab will be ready to begin. Thank you!

Supply List

- Molecular model kit
- 30 test tubes in a rack
- Marker pen or China marker
- Deionized water bottle
- 400 ml beaker
- Hotplate and gloves
- Test tube holder

Also, please wash your unknown tube with soap and water using the test tube brush and return it to your instructor before you leave.

LABORATORY 4 WORKSHEET

Name ________________________

Section________________________

Exercise 1A – Constructing functional groups

Draw the structural formulas for the following functional groups:

–OH **$-CH_3$**

$-NH_2$ **–COOH**

Match each functional group below with the correct chemical property on the right (*choices may be used more than once*).

____ amino group

____ carboxyl group

____ hydrogen

____ hydroxyl group

____ methyl group

A. acidic

B. basic

C. polar

D. non-polar

Exercise 1B – Building an amino acid

Draw the structural formula for the amino acid you built with your molecular model kit.

amino acid _______________

- *Circle and label the **amino, carboxyl** and **R groups**; mark the **central carbon** with an asterisk (*).*

Exercises 1C & 1D – Assembling and hydrolyzing a polypeptide

*Draw the complete structural formula for the dipeptide your group assembled, and **circle the peptide bond**. Be sure to show your instructor your dipeptide and demonstrate its hydrolysis.*

Exercises 2A, 2B & 2C – Detecting macromolecules

- For each test below, place a checkmark (✓) next to the foods you predict will be positive for the test. This is your hypothesis.

Record your data below (use –, +, ++ or +++ to indicate the amounts of each substance detected):

Benedict's reagent

sample	color	sugars?
cow's milk		
rice milk		
almond milk		
chicken broth		
fruit juice		
flour		
soda		
diet soda		
glucose		
water		

Iodine solution

sample	color	starch?
cow's milk		
rice milk		
almond milk		
chicken broth		
fruit juice		
flour		
soda		
diet soda		
starch		
water		

Biuret reagent

sample	color	protein?
cow's milk		
rice milk		
almond milk		
chicken broth		
fruit juice		
flour		
soda		
diet soda		
albumin		
water		

- For each experiment above, **circle the *positive* control** and **underline the *negative* control**.

Indicate below the foods that have simple sugars, starch, and protein based on ***your test results*** *and put an "**X**" next to those that did not match your hypothesis:*

Foods with sugars	**Foods with starch**	**Foods with protein**

- List the 4 major categories of biological macromolecules below (refer to chapter 3 of your textbook if necessary) and circle any categories that are ***NOT*** detected by any of the tests you just performed.

- Compare your results with the nutrition facts and ingredient listings for each food (this should be on the refrigerator), and indicate below all results that are not consistent with the food labels.

- Considering your results, comment on the accuracy of the food labels.

Exercise 2D – Unknown # ______

test	color	substance detected
Benedict’s		
Iodine		
Biuret		

- Summarize below what types of macromolecules are contained in your unknown.