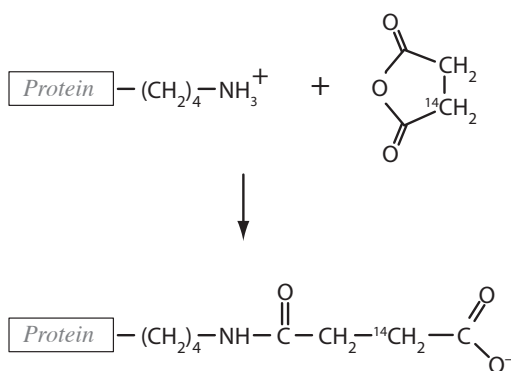


Carboxylic Acid Derivatives Practice Items

1. What type of linkage is a peptide bond?

- A. ester
- B. amino
- C. anhydride
- D. amide

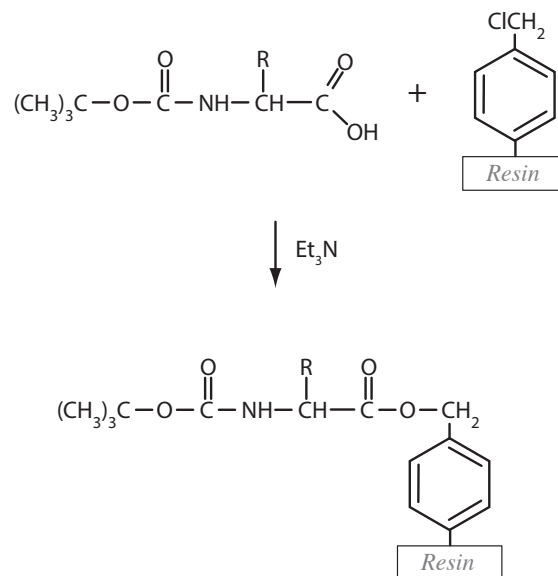
2. A method was devised to quantify free ε-amino groups of proteins using [¹⁴C]succinic anhydride for measurement in scintillation counting.



In the depiction above, what type of reaction mechanism has occurred between [¹⁴C]succinic anhydride and the lysine side chain?

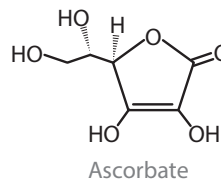
- A. SN2 substitution
 - B. imine formation
 - C. nucleophilic acyl substitution
 - D. cyanohydrin formation
3. Which of the following general class of carboxylic acid derivatives is most reactive towards nucleophiles in acyl substitution?
- A. amides
 - B. thioesters
 - C. carboxylates
 - D. carboxylic acids

4. A technique for solid phase polypeptide synthesis begins with anchoring a chemically protected C-terminal amino acid to a chloromethyl substituted polystyrene resin.



What type of reaction is depicted in the above figure?

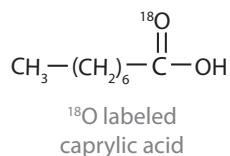
- A. SN2 substitution
 - B. Michael addition
 - C. nucleophilic acyl substitution
 - D. oxidation
5. Vitamin C (ascorbate) functions as a cofactor in many enzymatic reactions in humans.



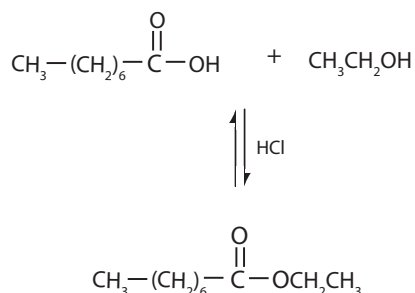
Vitamin C is a . . .

- A. β-lactone
- B. γ-lactone
- C. β-lactam
- D. δ-lactone

6. To form ethyl caprylate, Fischer esterification was carried out upon ^{18}O labeled caprylic acid, a medium-chain saturated fatty acid.



Caprylic acid is sparingly soluble in water. The reaction was carried out with excess ethanol in aqueous solution.

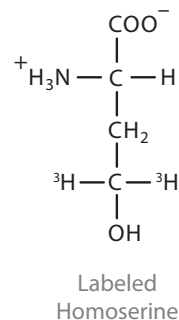


Upon completion of the reaction the supernatant was evaporated and the ethyl caprylate and unreacted caprylic acid were recovered as precipitates. The supernatant was separately recovered by means of a condensing column.

The various components were subsequently analyzed to confirm the presence of the ^{18}O label. In which of the following reaction components were ^{18}O labeled molecules confirmed to be present?

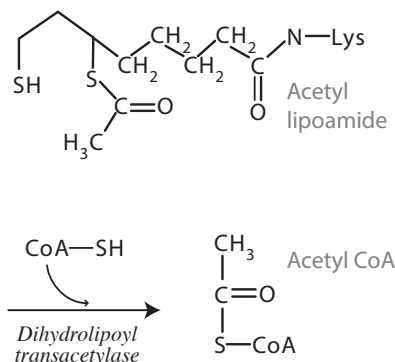
- I. ethyl caprylate
 - II. caprylic acid
 - III. water
 - IV. ethanol
- A. I and II
 B. II and III
 C. I, II and III
 D. III and IV

7. A method for characterization of phosphorylated aspartate residues on enzymes involves cleavage of the acyl phosphate bond with sodium $[^3\text{H}]$ borohydride. Analysis of the acid hydrolysate of the reacted protein will show labeled homoserine if phosphorylated aspartate residues had been present.



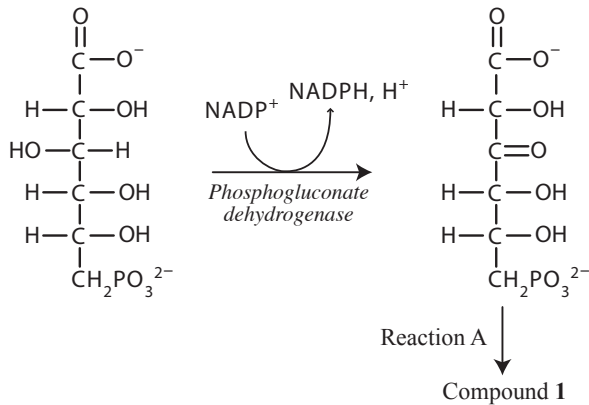
This technique is possible only because

- A. NaBH_4 is a strong acid.
 - B. NaBH_4 does not reduce carboxylic acids.
 - C. Acyl phosphates are strong oxidizers.
 - D. Tritide is more nucleophilic than hydride.
8. The figure below depicts a step in the mechanism of dihydrolipoyl transacetylase, a component of the pyruvate dehydrogenase complex. Which of the following describes this reaction?



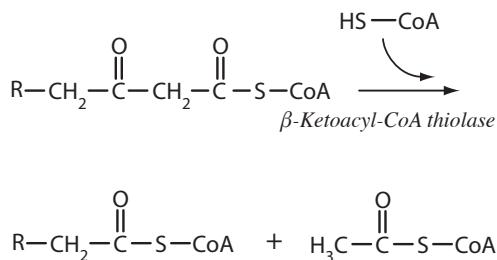
- A. transthioesterification
- B. transsulfuration
- C. transthioation
- D. transcarboxylation

9. 6-Phosphogluconate dehydrogenase is an enzyme in the pentose phosphate pathway. The enzyme converts 6-phospho-D-gluconate into the intermediate 6-phospho-2-dehydro-D-gluconate. Still bound to the enzyme, 6-phospho-2-dehydro-D-gluconate then undergoes Reaction A to form Compound 1. What is reaction A?



- A. oxidation
 B. reduction
 C. decarboxylation
 D. esterification

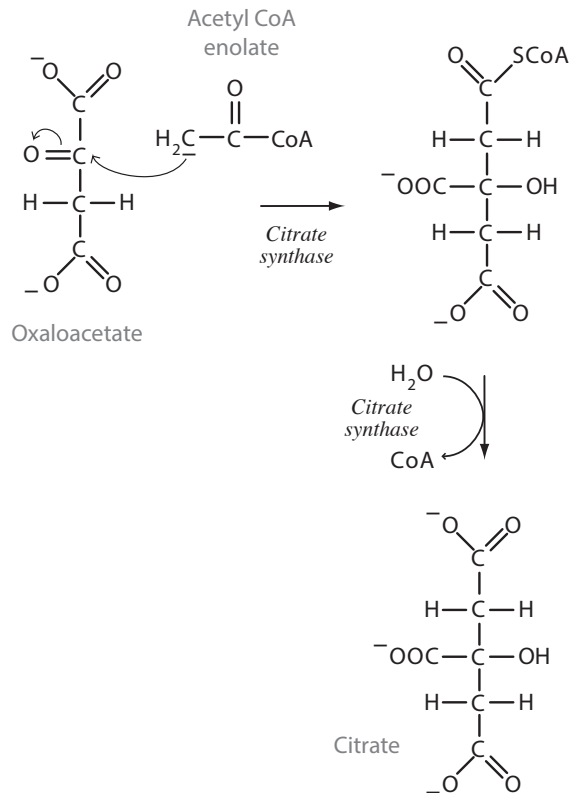
10. β -Ketoacyl-CoA thiolase catalyzes the final step of fatty acid oxidation in which acetyl-CoA is released and the CoA ester of a fatty acid two carbons shorter is formed.



What is the best description of the mechanism of this reaction?

- A. retro-Claisen condensation
 B. SN2 substitution
 C. aldol cleavage
 D. thioesterification

11. Citrate synthase catalyzes the first reaction of the citric acid cycle: the condensation of acetyl-CoA and oxaloacetate to form citrate.

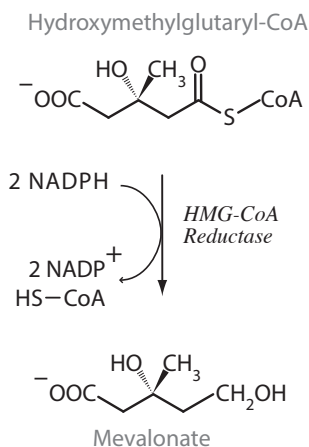


The standard free energy change (ΔG°) for the citrate synthase reaction is -31.5 kJ/mol , and the enzyme functions far from equilibrium under physiological conditions.

What is the primary factor determining the negative standard free energy change of the citrate synthase reaction?

- A. hydration of the tri-carboxylate citrate product
 B. resonance stabilization of the acetyl Coa enolate
 C. hydrolysis of the thioester
 D. electrostatic repulsion between the carboxylate groups of oxaloacetate

12. HMG-CoA reductase catalyses the conversion of HMG-CoA to mevalonate, a necessary step in the biosynthesis of cholesterol and other isoprenoid lipids.



What change has occurred to the oxidation state of the thioester carbon of HMG-CoA as a result of this reaction?

- A. $-3 \rightarrow +1$
 B. $+2 \rightarrow 0$
 C. $+2 \rightarrow -1$
 D. $+3 \rightarrow -1$
13. Glutamine synthetase (GS) catalyzes the ATP-dependent condensation of glutamate with ammonia to yield glutamine. The hydrolysis of ATP drives the first step of a two-part mechanism. In the first step (Step 1), ATP phosphorylates glutamate to form γ -glutamyl phosphate. In the second step (Step 2), γ -glutamyl phosphate reacts with ammonia, forming glutamine and inorganic phosphate.

Which of the two respective steps in the glutamine synthetase mechanism described above has a negative standard free energy change (ΔG°)?

- A. Step 1
 B. Step 2
 C. neither Step 1 nor Step 2
 D. both Step 1 and Step 2

14. In polypeptide synthesis, the C-terminal residue of the growing polypeptide is sequentially esterified to the 3' hydroxyl of a t-RNA adenosine ribose (peptidyl-tRNA). In chain termination, a release factor binds to the stop codon leading to hydrolysis of peptidyl-tRNA by the peptidyl transferase ribozyme forming polypeptide and free tRNA. If an experiment were conducted with ^{18}O labeled water (H_2^{18}O) employed for hydrolysis, after liberation of the free polypeptide, ^{18}O label would be located on

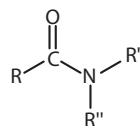
- A. the polypeptide
 B. the tRNA
 C. neither the polypeptide nor the tRNA
 D. both the polypeptide and the tRNA

Carboxylic Acid Derivatives

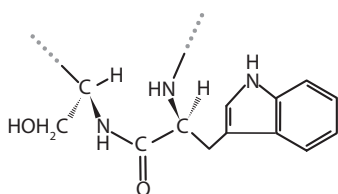
Answers and Explanations

1. D

The peptide bond is an amide linkage. Amides are carboxylic acid derivatives with the general structure below, where R, R', and R'' represent organic groups or hydrogen atoms. The peptide bond is a 2° amide (R'' is a hydrogen).

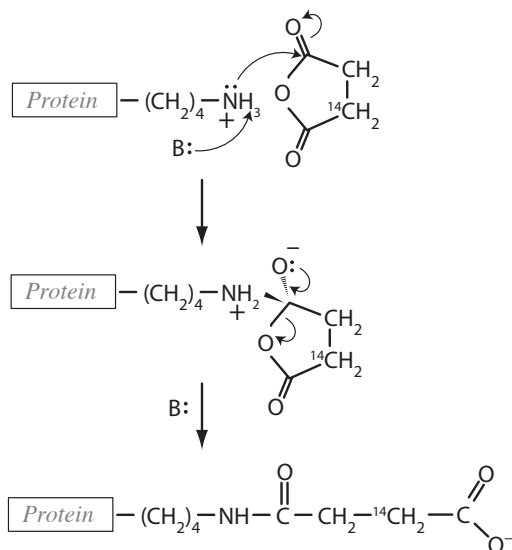


The figure below depicts the peptide bond between serine and tryptophan residues within a polypeptide:



2. C

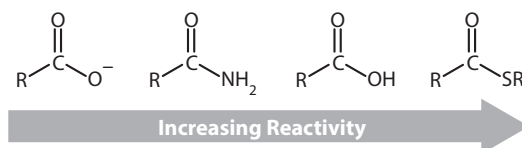
Aminolysis of an anhydride is a type of nucleophilic acyl substitution reaction. In this type of reaction, a nucleophile – such as an alcohol, amine, or enolate – displaces the leaving group of an acyl derivative – such as an acid halide, anhydride, or ester. In the resulting product, the nucleophile has taken the place of the leaving group present in the original acyl derivative. In the mechanism, the nucleophile attacks the carbonyl carbon, forming a tetrahedral intermediate. The tetrahedral intermediate of an acyl compound contains a substituent attached to the central carbon that can act as a leaving group. After the tetrahedral intermediate forms, it collapses, recreating the carbonyl C=O bond and ejecting the leaving group in an elimination reaction.



3. B

Among the choices, thioesters are the most reactive towards nucleophiles, followed by esters and carboxylic acids. Carboxylate ions are essentially unreactive towards nucleophilic substitution, since they possess no leaving group.

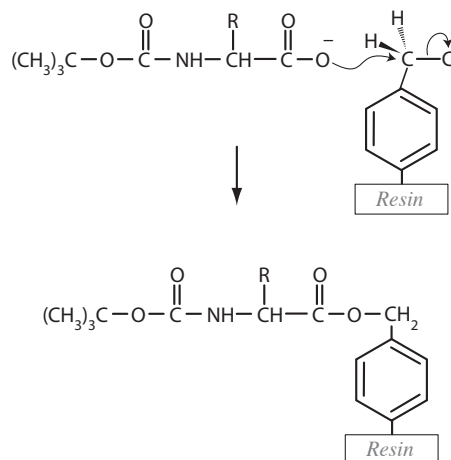
A major factor in determining the reactivity of acyl derivatives is leaving group ability, which is related to basicity. This is a thumbnail heuristic for comparing the reactivity of various carboxylic acid derivatives. Weak bases are better leaving groups than strong bases. Thiolate will be a better leaving group than amide or hydroxide. Therefore, thioesters are more reactive than esters or carboxylic acids.



The reactivity of thioesters (and phosphate anhydrides) towards nucleophilic acyl substitution is a *major* theme in biochemistry. For example, the role of coenzyme A as a carrier of 'activated' acyl groups depends on the reactivity of thioesters for nucleophilic acyl substitution. Thioesters are involved in the synthesis of many biomolecules including triglycerides, fatty acids, sterols, terpenes, porphyrins, and others.

4. A

The deprotonating agent triethylamine (Et₃N) transforms the α-carboxyl of the amino acid into its carboxylate form. The carboxylate anion then serves as a nucleophile in an S_N2 reaction upon the chloromethyl substituent to form an ester.

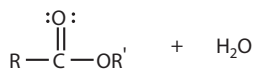
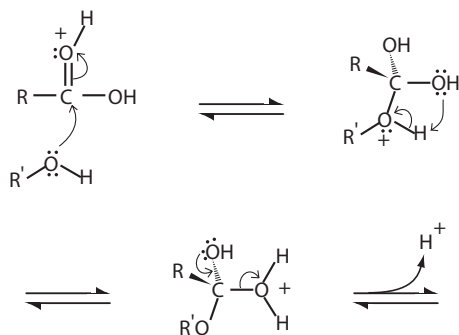


5. B

Lactones are cyclic esters. (Lactams are cyclic amides.) The Greek letter prefixes indicate the size of the ring: α -lactone = 3-membered ring, β -lactone = 4-membered, γ -lactone = 5-membered, and δ -lactone = 6-membered.

6. C

Fischer esterification is, basically, acid catalyzed nucleophilic acyl substitution. The acid catalyst protonates the carbonyl group to increasing its electron withdrawing character. In the mechanism, the alcohol nucleophile attacks the carbonyl carbon, forming a tetrahedral intermediate. The tetrahedral intermediate then collapses, recreating the carbonyl C=O bond and ejecting the water leaving group in an elimination reaction.



To keep track of the ^{18}O label, it's important to understand that *the two carboxyl group oxygens are equivalent*. Acidic protons come and go many thousands of time per second. Even under acidic conditions, the identity of the protonated oxygen changes frequently, so it will be a 50% chance whether the water leaving group carries the ^{18}O label, so there will be label present in the water, the ethyl caprylate product, as well as the unreacted caprylic acid (also some of the caprylate reformed at equilibrium through the reverse reaction).

7. B

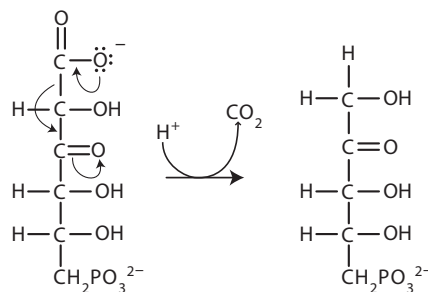
NaBH_4 is not powerful enough to reduce carboxylic acids but will reduce phosphate anhydrides (acyl phosphates). The presence of the alcohol product of reduction, homoserine, confirms the presence of phosphorylated aspartate residues in the enzyme being tested.

8. A

Transthioesterification is the process of exchanging the organic group SR'' of a thioester with the organic group SR' of a thiol. The reaction is nucleophilic acyl substitution with $\Delta G \sim 0$. The reaction is driven forward by mass action as the acetyl CoA product is removed for citric acid cycle in the mitochondrion or shuttled to the cytosol for fatty acid synthesis.

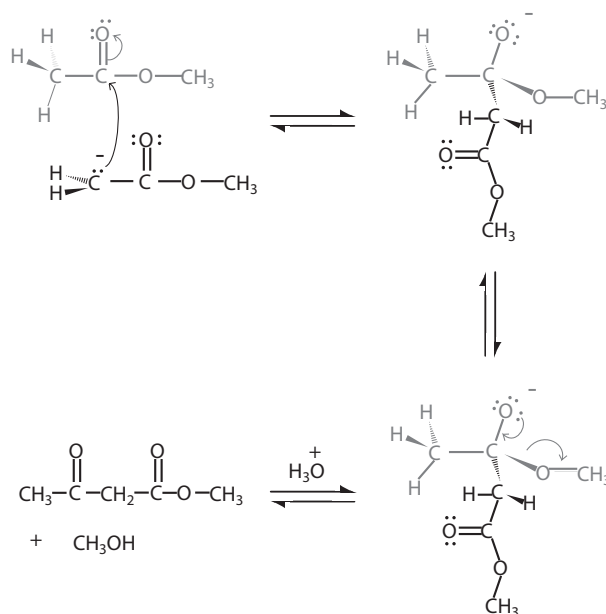
9. C

6-phospho-2-dehydro-D-gluconate is a β -keto acid, a molecule in containing a carbonyl group on the β -carbon of a carboxylic acid. β -Keto acids are very unstable and readily undergo decarboxylation with elimination of carbon dioxide under mild conditions.



10. A

Claisen condensation is like aldol addition for the world of esters. Instead of the nucleophilic addition pattern you see with aldehydes and ketones, the reaction follows the pattern of nucleophilic acyl substitution.



There are a number of reactions that appear frequently in biochemistry that did not appear on the AAMC MCAT topic outline. Familiarity with these is a figure of merit for the exam. These reactions include E1 & E2 elimination, Michael addition, and Claisen condensation. There may be others. The appearance of things like this in an MCAT passage from outside the scope is part of the exam. Prior knowledge is better, but the test won't necessarily expect it.

11. C

Thioesters are among the more reactive carboxylic acid derivatives. Hydrolysis of a thioester is thermodynamically favorable. To see thioesters (and phosphate anhydrides) as a form of metabolic energy is a big theme in biochemistry.

12. D

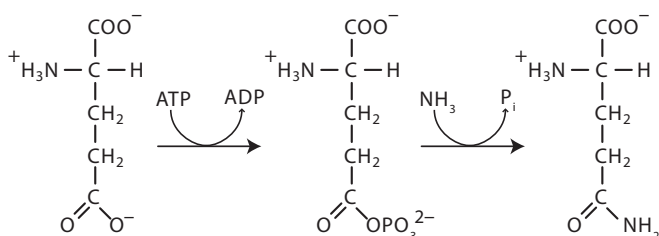
Assign oxidation numbers by deciding which atom has 'control' of the electrons in the bonds. Control goes to the more electronegative atom.

In redox accounting, the thioester carbon at the start has lost two electrons to the double bond to oxygen and an additional electron to sulfur, which is slightly more electronegative than carbon (2.6 vs. 2.5). Therefore, the oxidation state of the thioester carbon in HMG-CoA is +3. This is the universal oxidation state of the carboxylic acid derivatives.

After the reaction, the carbon will now have one electron invested in a bond with oxygen while it gained two from the bonds to hydrogens, so carbon's oxidation state in malonate has become -1.

13. D

Both steps have a negative standard free energy change (ΔG°). Phosphorylation of glutamate is powered by ATP cleavage. The second step, aminolysis of phosphate anhydride, transforms a higher energy carboxylic acid derivative into a lower energy carboxylic acid derivative. This is one of the most basic functions of ATP, activating a carboxylate for nucleophilic acyl substitution by first transforming it into a phosphate anhydride.



14. A

After the reaction the ^{18}O label will be on the polypeptide. This type of question, which tests understanding of the nucleophilic acyl substitution mechanism, is a long-standing tradition with AAMC.

