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Significance and Implications of Vitamin B-12 Reaction Shema-ETH ZURICH VARIANT: Mechanisms and Insights

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CHEMISTRY THESIS

SIGNIFICANCE AND IMPLICATIONS OF VITAMIN B-12 REACTION SCHEMA- *ETH*

ZURICH VARIANT: MECHANISMS AND INSIGHTS

DAVID JOSHUA FERGUSON

2019

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Chapter 1

A. INTRODUCTION.

Vitamin B-12 otherwise known as cyanocobalamin is a compound with synthetic elegance. Considering how it is composed of an aromatic macrocyclic corrin there are key features of this molecule that are observed either in its synthesis or in the biochemical reactions it plays a role in whether they be isomerization reactions or transfer reactions. In this paper the focus for the discussion will be on the history, chemical significance and total synthesis of vitamin B12. Even more so the paper will be concentrated on one of the two variants of the vitamin B-12 synthesis, namely the ETH Zurich variant spearheaded by Albert Eschenmoser. Examining the structure as a whole it is observed that a large portion of the vitamin B12 is a corrin structure with a cobalt ion in the center of the macrocyclic part, and that same cobalt ion has cyanide ligands. The general macrocyclic portion of the structure is rimmed with either methyl or amide group attachments. One of the amide groups is N-alkylated by a large isopropanol group, then a phosphate, followed by a ribose which is attached to the dimethylbenzimidazole.

However, in terms of history there were some key steps in the process of determining and synthesizing the overall structure of vitamin B-12.

" We made up in our minds that we're going to specialize in research in the field of vitamins.

We're going to isolate every vitamin. We're going to determine their structures if it hasn't already

been done and synthesize them and make them available."- Randolph Major, as told by Mac Tishler, 1983. Our understanding of disease in the modern world was aided by the work of Louis Pasteur and Germ theory. The issue came into being with diseases such as pellagra, anemia and beriberi in which the origin is not pathogenic typically but based in nutrient deficiency and in this case vitamin B. This new category came into being in the 1900. In 1889 **Dutch Physician Christiaan Eijkman** investigated beriberi. He and **Gerrit Grijns** studied the effects of dietary variations on the occurrence of beriberi. After, in 1906 English biochemist **Frederick Gowland Hopkins** suggested a connection between nutrition and diseases such as beriberi and scurvy. Following that in 1911 Casimir Funk, a Polish biochemist working in London, further advanced this idea. University of Wisconsin biochemist Elmer McCollum was able to distinguish two different species of vitamins "fat-soluble factor A" and "water soluble factor B". Moreover, in 1926 Dutch chemists Barend Jansen and Willem Donath, isolated crystal of anti-beriberi factor from extracts of rice polishings. Chemists were arduously working with natural product chemistry, with Merck in 1930 already working on this task.

Williams of Bell Laboratories approached Merck to help isolate and make thiamine. Randolph Major was chosen to head the new research and development laboratory Merck built as a part of its efforts to grow basic research. Eventually, Williams and Cline synthesized thiamine. As was seen in 1922 Riboflavin, Vitamin B2, had been discovered in 1922 by Richard Kuhn in Germany and Theodor Wagner-Jauregg in Austria. Moreover in 1933 Riboflavin was isolated by Kuhn and Gyorgy in Germany. As time progressed in 1934, Vitamin B6, pyridoxine, was discovered by Gyorgy and colleagues. Following this in 1938, the active compound of

pyridoxine, was isolated by Samuel Lepovsky of U.C. Berkley. After which in 1939, Folkers and Harris along with Kuhn in Germany determined the structure of pyridoxine. Eventually in 1940, synthesis of vitamin B5 ,pantothenic acid, was reported by Merck.

Discovery of Cobalamin

Cobalamin was discovered through an interesting process. First in 1926 at Harvard University, a team of physicians found out that ingesting a half a pound of liver would prevent pernicious anemia. As time progressed liver extracts were fed to willingly participating patients. Folkers ultimately learned that Mary Shorb a microbiologist found a bacterium that reacted to liver extracts. Also it was determined that the most promising extracts were those with the “pinkish color”, which implied that the vitamin being sought was a red compound. In 1947 ,Folkers and his team isolated vitamin B12 (cobalamin) which resulted in tiny,bright,red crystals of the vitamin.¹

B. The research problem could be outlined in several questions:

1. What is the historical and chemical significance of vitamin B-12 ?

1. What was the total synthesis that was completed and who were the leading scientists?

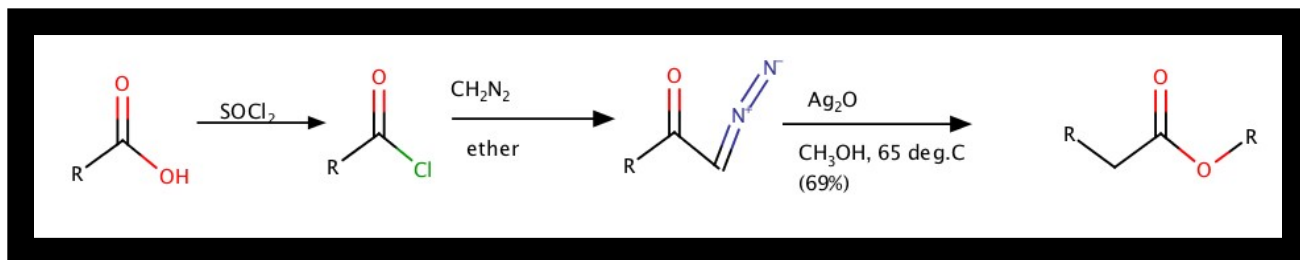
2. *What were the key steps in the synthesis of vitamin B-12?*
3. *What were the key problems associated with the total synthesis of vitamin B-12?*
4. *What are the future initiatives and possible lab applications?*

C. The need for this research and future studies in the area are tied to the critical role vitamins function in the human body as cofactors and catalysts in important reactions and the natural environment.

D. Nominal definitions.

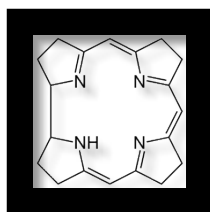
1. Homologation
2. Corrin
3. Ammonolysis
4. Thionation
5. Methanolysis
6. Woodward-Hoffman Rules
7. Protection/Deprotection

Homologation²



Essentially homologation is a reaction that converts the reactant into the next member of the homologous series. In many cases a homologous series is a group of compounds that differ by a constant unit. Homologation occur simply when the repeated structural unit is increased and in reaction above, it is a methylene (-CH₂-).

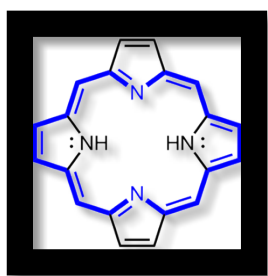
Corrin³



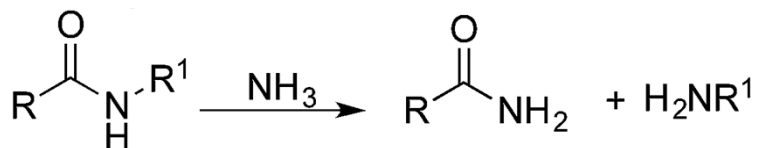
A corrin is a macrocycle. Specifically a corrin is a species consisting of four reduced pyrrole rings joined by three -CH= and one double bond.

A common prefix associated with corrin is “seco-” which refers to a macrocycle in which cleavage of a ring has occurred with the addition of one or more hydrogen atoms at each terminal group thus created is indicated.

One distinction is made between the porphyrin, seen below, and the corrin, seen above, based on size in that the porphyrin is larger.



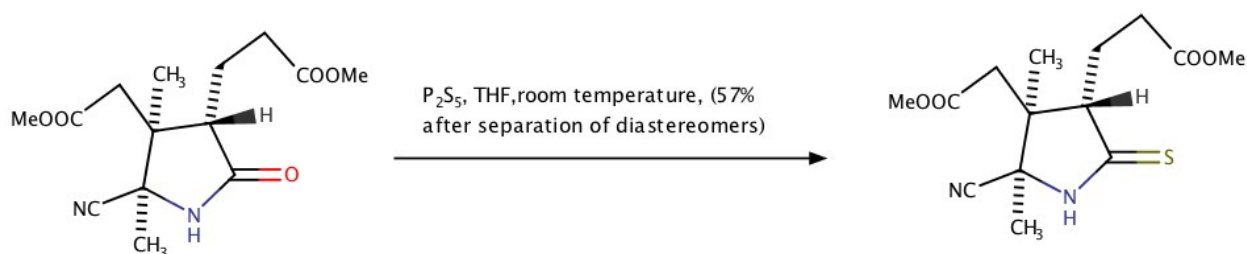
Ammonolysis⁴



Ammonolysis is a reaction similar to hydrolysis in which ammonia reacts with another compound as a nucleophile and often times the solvent usually to result in the formation of an

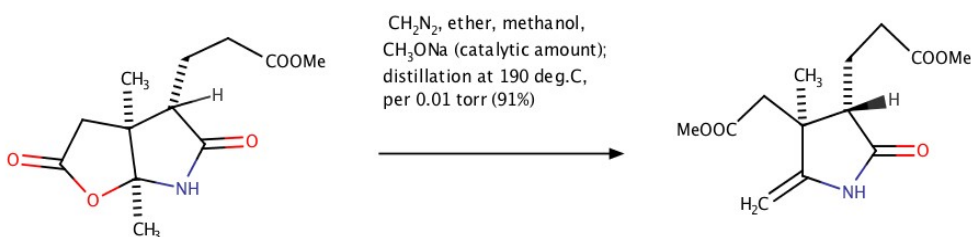
amine functional group of the molecule. An example seen above is the ammonolysis of esters which results in amides.

Thionation



Thionation is a chemical reaction in which the oxygen in a moiety (e.g. carbonyl, hydroxyl) is converted to a sulfur. As seen above is step 12 in the Eschenmoser variant for the total synthesis of vitamin B-12, cyclic carbonyl containing molecule is thionated which results in the precursor to ring A for cobyrinic acid and vitamin B-12.

Methanolysis

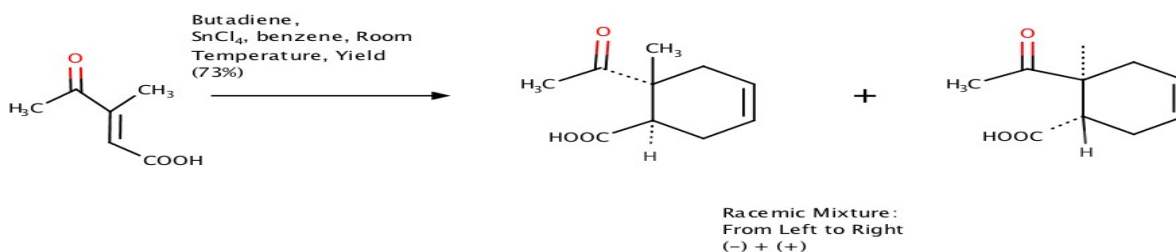


Methanolysis which is similar to hydrolysis, however, instead of water functioning as the nucleophile and solvent, methanol is functioning in that way. Overall it results in the reaction

as seen above, the methanolysis process results in the elimination of the hydroxyl from the ester.

This can also be considered a type of transesterification.

Woodward-Hoffman Rules⁵



The Woodward Hoffman rules were sorted out by Robert B. Woodward and Roald Hoffman, although further work was done by Fukui. These rules involve the use of a simple procedure for determining whether a pericyclic reaction is thermally allowed. Primarily the focus is on the aromaticity of the transition state, which is understood based on orbital topology and electron count. The reaction above shows an example where these rules can be applied in this unique cycloaddition in the form of a Diels- Alder reaction. In short, these rules state that whenever possible reaction go through aromatic transition states.

As Eschenmoser⁶ wrote in his lecture “but I should perhaps propose that we enjoy the figure just from an aesthetic point of view, by watching the corrinoid chromophore system evolve, like a bud blooming into a flower”.

E. For the total synthesis of vitamin B-12 , there are two variants both of which were accomplished in 1972. In 1960, the ETH Zurich variant was started by Albert Eschenmoser and his team. Following that in 1961 the Harvard variant was started, and after 1965 the work was collaboratively pursued. In terms of the amount of collaboration it required the work of 91 post-doctoral fellows, and 12 Ph.D. students from several different nations.⁷

Chapter 2

Synthesis of the Rings:

Within the descriptions, both general and or mechanistic, the numbering of the compounds was based on Eschenmoser's overall schema.⁸

Ring A:

1. Claisen-Schmidt Condensation⁹

2. and 3. Diels-Alder

4. Oxidation

5. Arndt-Eistert

6. Ammonolysis

7. Ring Opening

8. Thionation¹⁰

Ring B:

1. Claisen-Schmidt Condensation
2. and 3. Diels- Alder¹¹
4. Oxidation
5. Arndt-Eistert¹²
6. Ammonolysis
7. Thionation

Ring C:

1. Claisen-Schmidt Condensation
 2. Diels -Alder
 3. Oxidation
 4. Arndt-Eistert
 5. Ammonolysis
-

6. Esterification and Methanolysis

7. Thioesterification¹³

8. Reductive Decarbonylation

Ring D:

1. Claisen-Schmidt Condensation

2. Diels-Alder Reaction

3. Oxidation Reaction

4. Ammonolysis Reaction

5. Ring Opening Reaction

6. Arndt-Eistert Reaction

7. Hydrolysis, Decarboxylation and Esterification

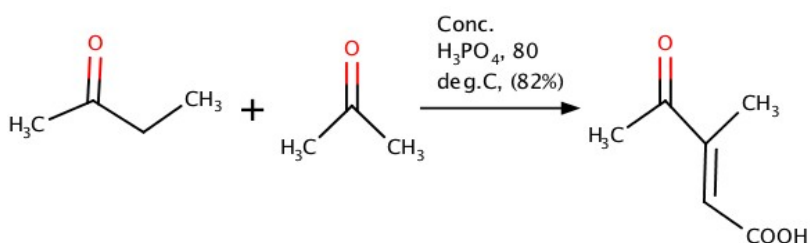
8. Protection/Sulfonation

9. Reduction/Deprotection

10. Protonation

11. Beckmann Fragmentation

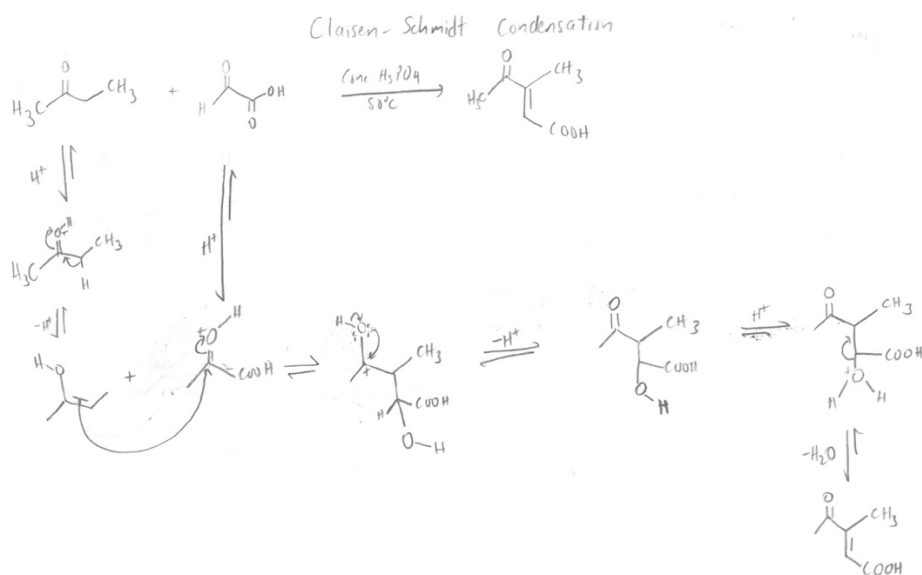
12. Bromination of the Ketimine

Ring A:**1. Claisen-Schmidt Condensation**

This first reaction of the B12 reaction scheme involves a ethyl methyl ketone (Compound 1A) reacting with acetaldehyde (Compound 1B) using reagents which are concentrated phosphoric acid (H₃PO₄) at 80°C and the yield is 82%. The type of reaction that is occurring the Claisen Schmidt condensation in which you have the formation of (2E)-3-methyl-4-oxopent-2-enoic acid. This reaction plays the role of producing the dienophile that will be used in the following reaction.

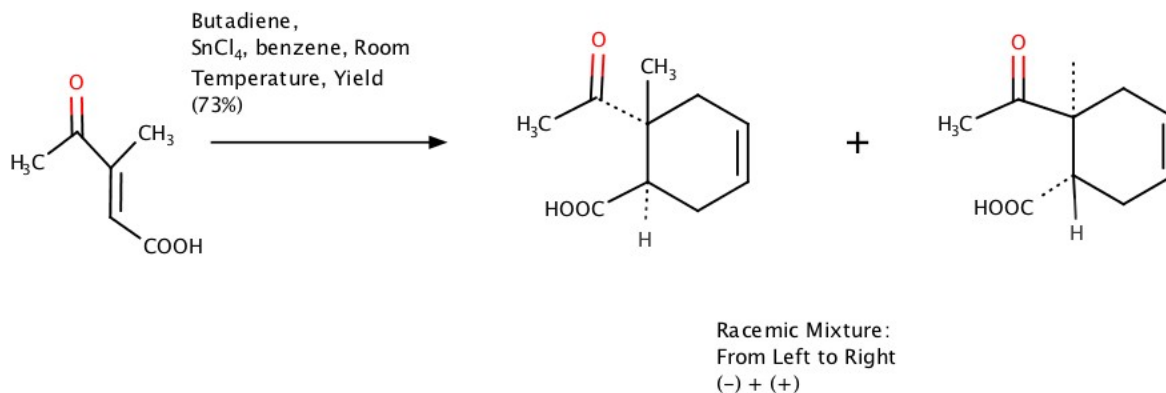
Overall if simplified this reaction is a type of condensation that results in the formation of an “ electron-poor molecule.

Mechanism:



2. and 3. Diels-Alder

2.and 3.

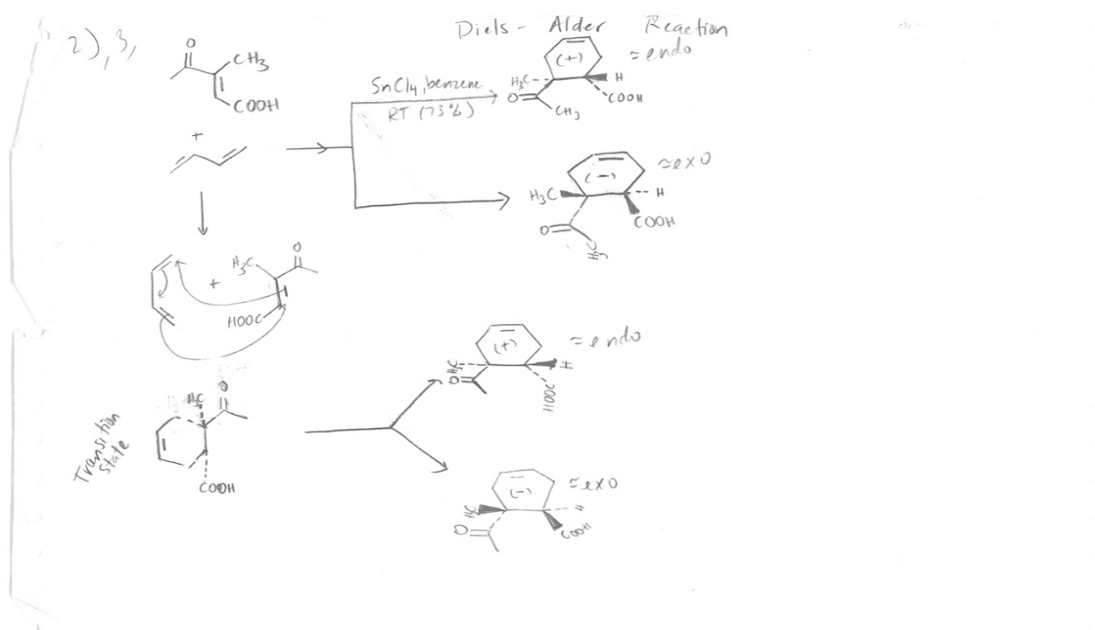


The second reaction of the B12 schema involves (2E)-3-methyl-4-oxopent-2-enoic acid (Compound 2) reacting with butadiene in tin (IV) chloride (SnCl₄) and benzene at conditions of room temperature. This results in a yield of 73%. The type of reaction that is occurring is the Diels Alder reaction which involves the formation of a racemic mixture of two carboxylic acid like molecules with ketone like moieties attached to it. For the purposes of this discussion the products will be labeled compounds 3A (-) and 3B(+).

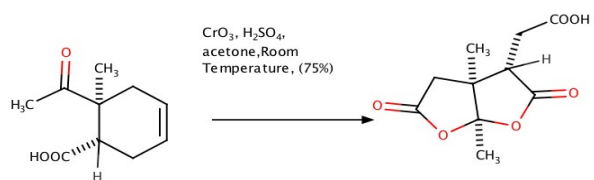
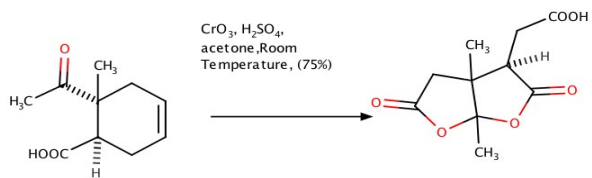
Overall if simplified the type of reaction, Diels Alder, is stereospecific and a type of concerted reaction in that all the bond breaking and bond forming occur at the same time. Moreover addition is syn. Also if this reaction follows the typical Diels Alder format it is a 1-step cyclo-addition or conjugate addition. This resulted in enantiomers which were

resolved using Phenylethylamine in chloroform and hexane. Followed by the use of diluted HCl.

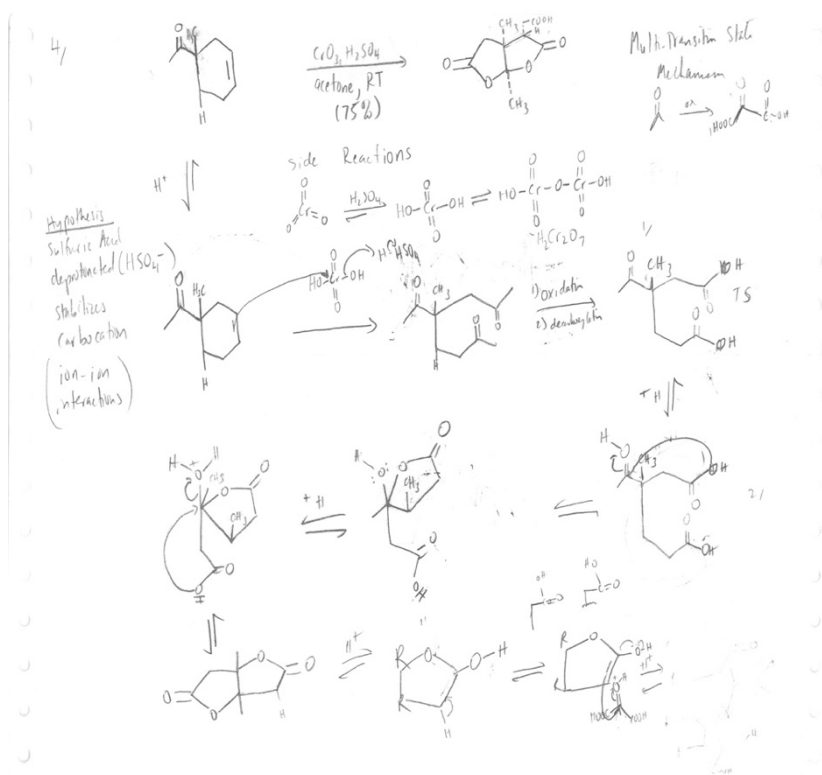
Mechanism:



4. Oxidation



The fourth reaction in the B12 reaction schema involves compounds 3A(-) and 3B(+) reacting with chromate and sulfuric acid in acetone at room temperature to form dilactone carboxylic acids which will be labeled compounds 4B (-) and 4A (+) (from top to bottom), both of which are starting products for B12 ring reactors. This fourth reaction has a predicted yield of 75%. From the reagents and the reactants this appears

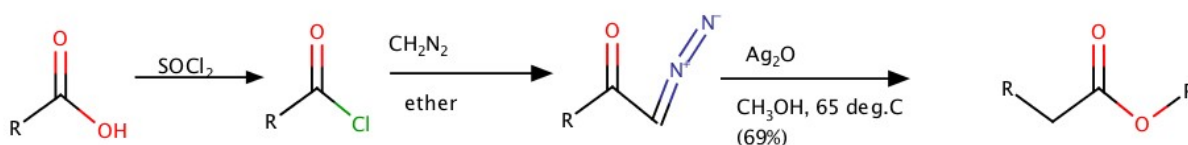


to be an organic redox reaction, possibly a Jones oxidation, in which we have a molecule being oxidized and or gaining hydrogen deficiency in the form of another ring. Stated simply, these reactions involve the oxidation of compounds 3A(-) and

3B(+) into ten carbon dilactone-carboxylic acids using reagents that normally are used in a type of organic redox reaction.

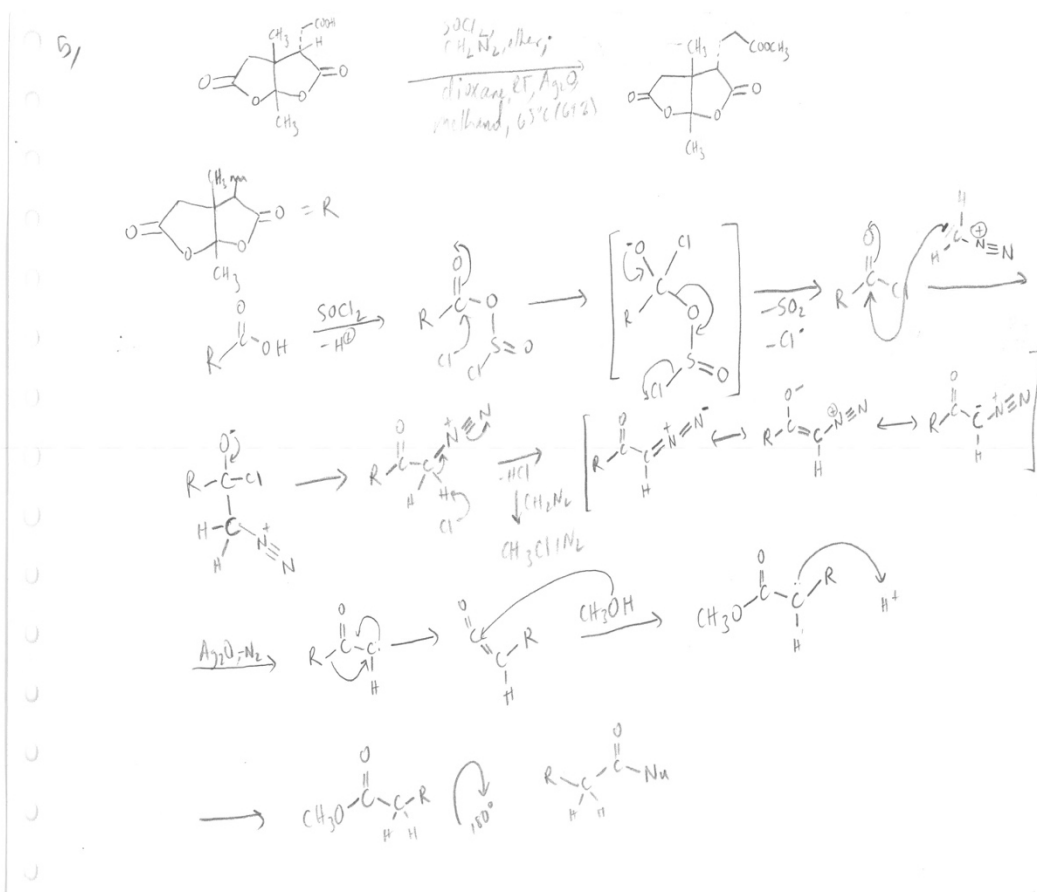
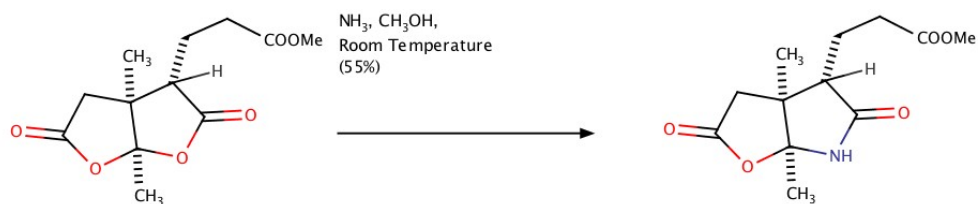
Mechanism

methanol at 65 degrees Celsius. This fifth reaction had a predicted yield of 69%. However the overall name of the reaction that is occurring is an Arndt-Eistert synthesis. Additionally an important step in the Arndt-Eistert reaction is the Wolff rearrangement of diazoketones to ketenes. The overall Arndt-Eistert reaction, excluding the Wolff Rearrangement, can be seen from the reaction drawn below, this sequence involves several steps that result in a higher order or homologated carboxylic acid.

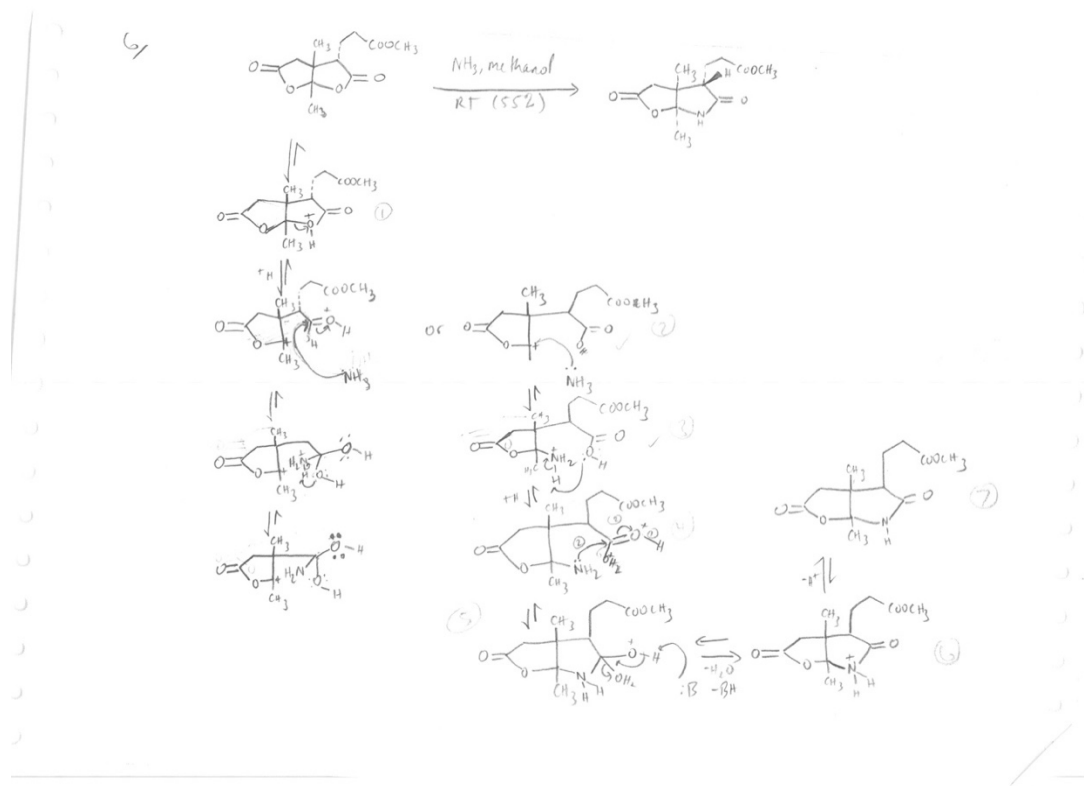
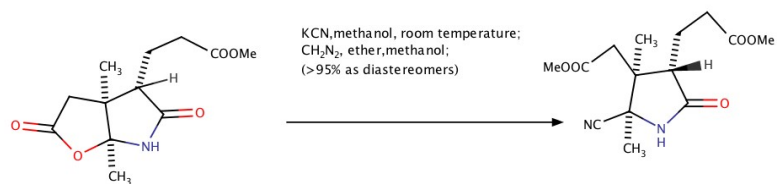


Stated simply this is a multistep reaction step that involves the conversion of a carboxylic acid to an acid chloride, then to diazo-ketone type molecule, and then the ester.

Some key points to note on the reaction, from Eschenmoser's notes for his 1973 German Lecture at ETH Zurich, "the treatment of the acid chloride with methanol/ pyridine at room temperature gives the same methyl ester as obtained by esterification with diazomethane; in the preparation of the acid chloride, there is no other structural change".

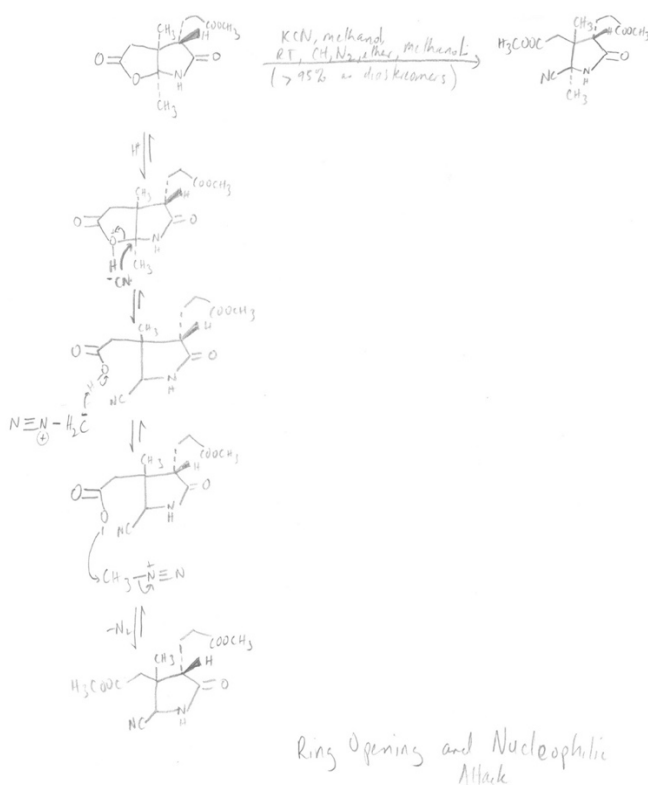
Mechanism:**6. Ammonolysis**

The sixth reaction in the B12 reaction schema involves compound 5A reacting with ammonia in methanol at room temperature. The sixth reaction had a yield of 55%. From this reaction it appears to be an Ammonolysis reaction in the presence of methanolic solvent. Also “the carbonyl groups of the dilactone moiety are much more nucleophilic towards ammonia than normal lactone or ester groups. “Ammonolysis” of this type are much faster in methanol than in non-hydroxyl containing solvents. The constitution assignment for the isomeric lactone-lactams resulted from the identity of compound 6A with the main product of intramolecular NH transfer”. Stated simply this reaction involved a intramolecular NH transfer using ammonia in methanol at room temperature¹⁴.

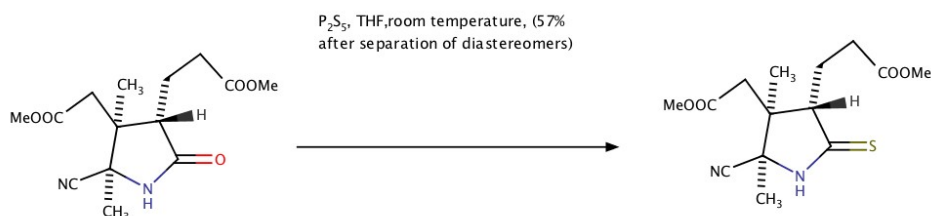
Mechanism:**7. Ring Opening (Step 11 in reference)**

The eleventh reaction in the B12 reaction schema involves compound 6A reacting with potassium cyanide in methanol at room temperature, followed by a reaction with diazomethane in ether and methanol. This resulted in 95% being diastereomers. From this reaction we can see that a lactone ring is opened and a respective ester and cyano group are on the ends. Based on observation this appears to have gone through acid-catalyzed (methanol) ring opening, followed by nucleophilic attack by the cyanide anion from the potassium cyanide. Stated simply this involves the conversion of a 12 carbon- dicarbonyl-bicyclic compound to a cyclic compound with the other ring being cleaved to form an ester and a cyanide at the ends where the ring broke.

Mechanism:

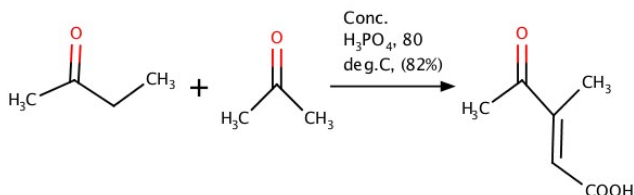


8. Thionation (Step 12 in reference)



The twelfth reaction in the B12 reaction schema involves compound 11A reacting with diphosphorous pentasulfide, and tetrahydrofuran at room temperature to form compound 12 A. Based on observation compound 11A, a 14 carbon-monocyclic compound going through a thionation of the carbonyl to form compound 12A, a 14 carbon-monocyclic compound. Stated simply this involves the conversion of a carbonyl to a thio-carbonyl on a 14- carbon monocyclic compound.

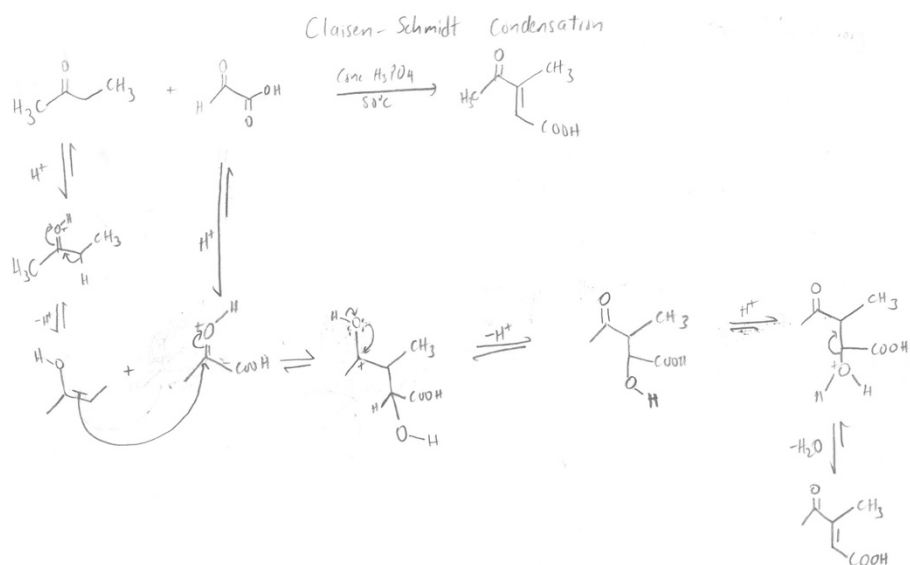
1.



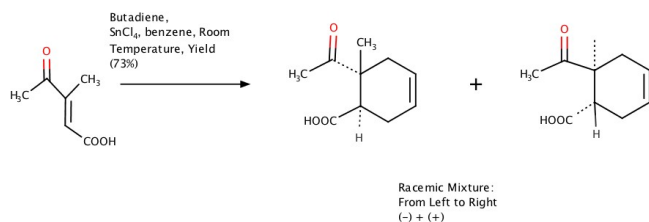
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Overall if simplified this reaction is a type of condensation that results in the formation of an “ electron-poor molecule.

Mechanism:



2. and 3. Diels-Alder



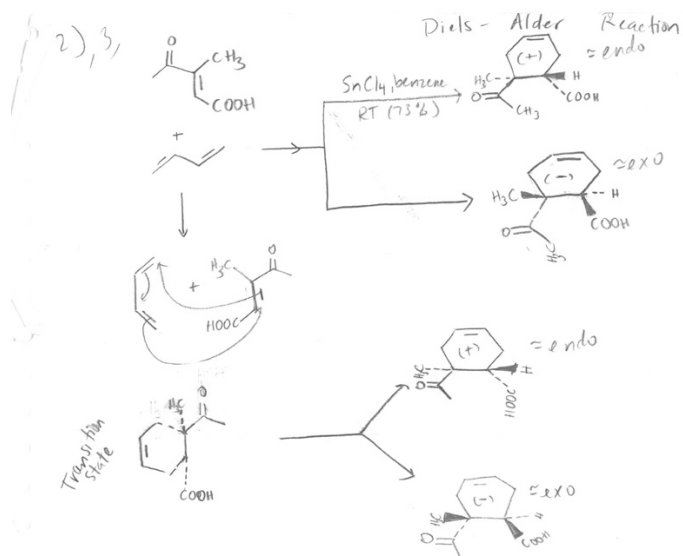
From left to right the products are presumably, exo and endo adducts.

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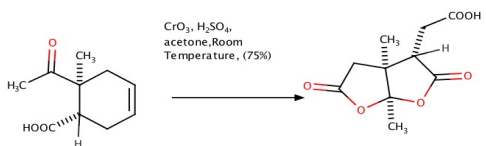
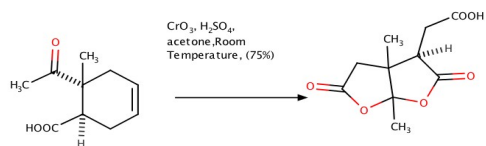
of room temperature. This results in a yield of 73%. The type of reaction that is occurring is the Diels Alder reaction which involves the formation of a racemic mixture of two carboxylic acid like molecules with ketone like moieties attached to it. For the purposes of this discussion the products will be labeled compounds 3A (-) and 3B(+).

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Mechanism:



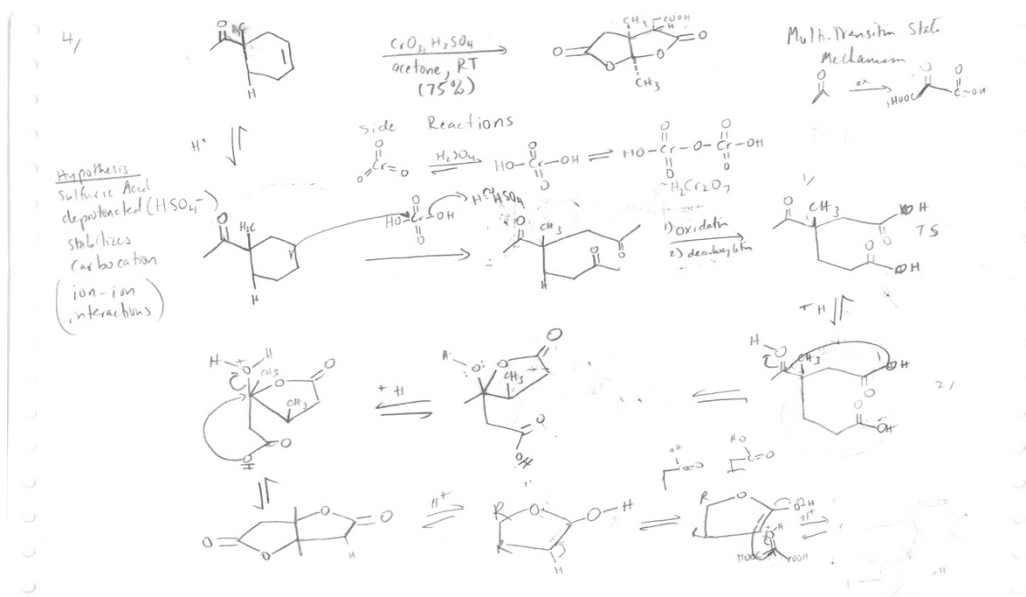
4. Oxidation

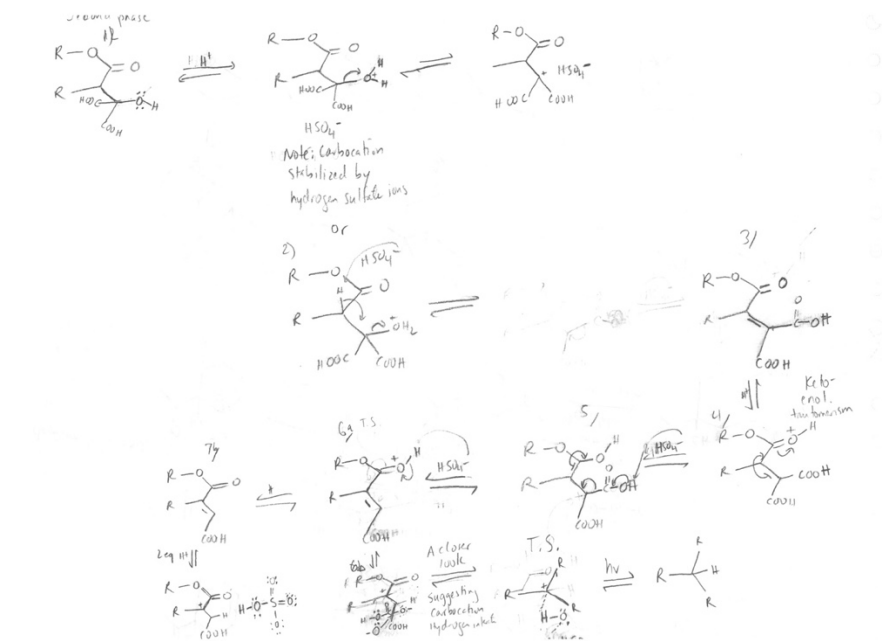


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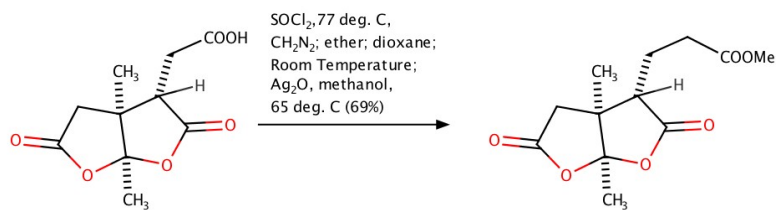
From the reagents and the reactants this appears to be an organic redox reaction, possibly a Jones oxidation, in which we have a molecule being oxidized and or gaining hydrogen deficiency in the form of another ring. Stated simply, these reactions involve the oxidation of compounds 3A(-) and 3B(+) into ten carbon dilactone-carboxylic acids using reagents that normally are used in a type of organic redox reaction.

Mechanism:



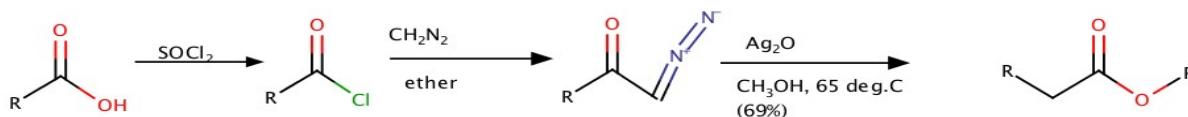


5. Arndt-Eistert



The fifth reaction in the B12 reaction schema involves compound 4A (+) reacting with thionyl chloride at 77 degrees Celsius. This was followed by reacting the acid chloride with diazomethane in ether at room temperature. After which it was reacted with silver dioxide in methanol at 65 degrees Celsius. This fifth reaction had a predicted yield of 69%. However the overall name of the reaction that is occurring is an Arndt-Eistert synthesis. Additionally an important step in the Arndt-Eistert reaction is the Wolff rearrangement of diazoketones to

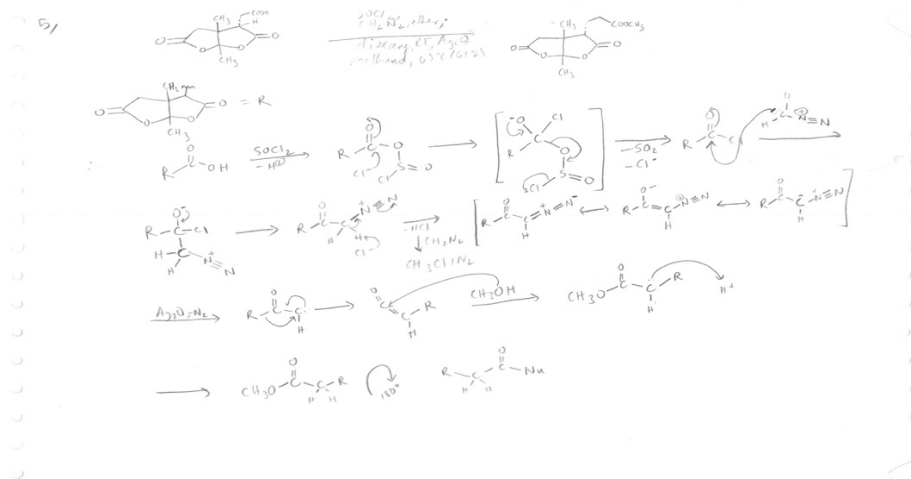
ketenes. The overall Arndt-Eistert reaction, excluding the Wolff Rearrangement, can be seen from the reaction drawn below, this sequence involves several steps that result in a higher order or homologated carboxylic acid.



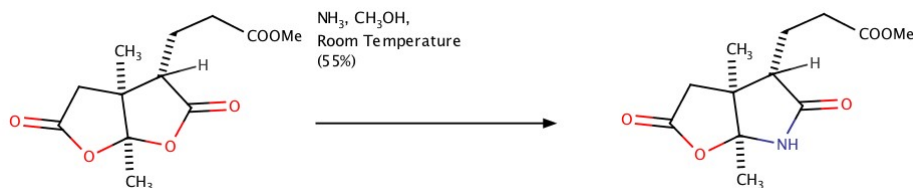
Stated simply this is a multistep reaction step that involves the conversion of a carboxylic acid to an acid chloride, then to diazo-ketone type molecule, and then the ester.

Some key points to note on the reaction, from Eschenmoser's notes for his 1973 German Lecture at ETH Zurich, "the treatment of the acid chloride with methanol/ pyridine at room temperature gives the same methyl ester as obtained by esterification with diazomethane; in the preparation of the acid chloride, there is no other structural change".

Mechanism:



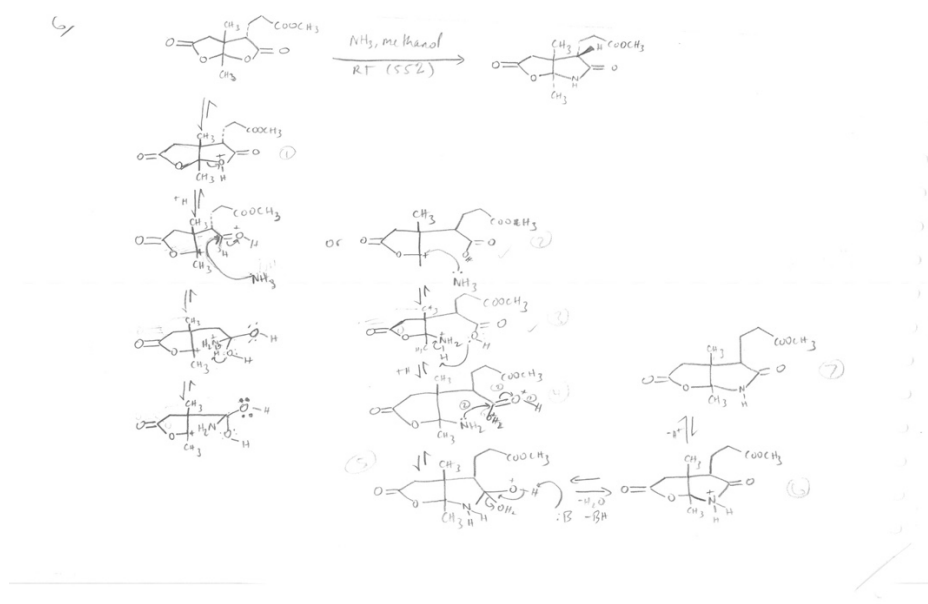
6. Ammonolysis



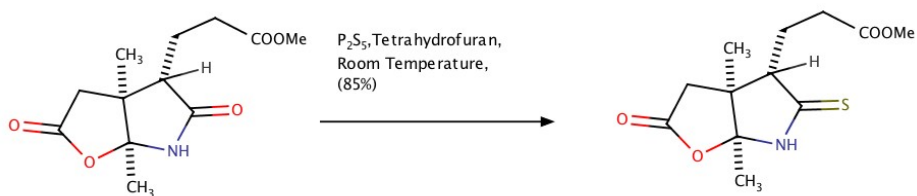
The sixth reaction in the B12 reaction schema involves compound 5A reacting with ammonia in methanol at room temperature. The sixth reaction had a yield of 55%. From this reaction it appears to be an Ammonolysis reaction in the presence of methanolic solvent. (Put in reference to Albert Eschenmoser's German lecture) "The carbonyl groups of the dilactone moiety are much more nucleophilic towards ammonia than normal lactone or ester groups. "Ammonolysis" of this type are much faster in methanol than in non-hydroxyl containing solvents. The constitution assignment for the isomeric lactone-lactams resulted from the identity of compound

6A with the main product of intramolecular NH transfer". Stated simply this reaction involved a intramolecular NH transfer using ammonia in methanol at room temperature.

Mechanism:



7. Thionation

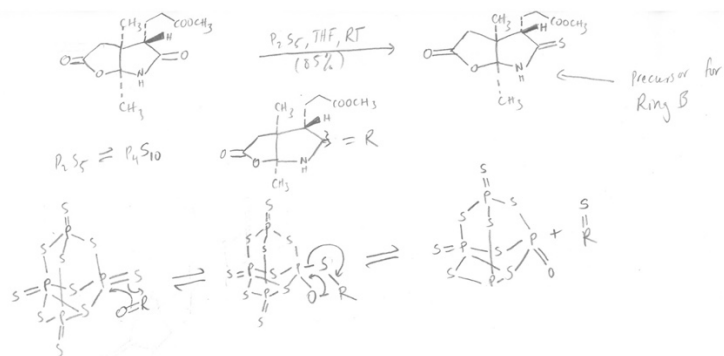


The seventh reaction in the B12 reaction schema involves compound 6A reacting with di-phosphorus pentasulfide in tetrahydrofuran at room temperature. The seventh reaction had a

yield of 85%. From the reaction compound 6A is converted to compound 7A with a subsequent thionation in which the carbonyl is converted to a thiocarbonyl. Thionation, is the conversion of the carbonyl group to thiocarbonyl, which is a commonly used procedure for the preparation of organosulfur compounds. As for many thionations of both the ketone and ester carbonyl groups of the oxoester, can be effected by P_4S_{10} , but typically in rather low yield.ⁱ This thionation was specific in that both carbonyl groups were not thionated to thiocarbonyls. Simply put the seventh reaction involved the conversion of one of the carbonyl in the C10-Dilactone-ester to a thiocarbonyl using thionating reagents at room temperature. An interesting fact to note is that compound 7A was a precursor for ring B of the macrocyclic corrin that composes the cyanocobalamin.

Mechanism:

7.

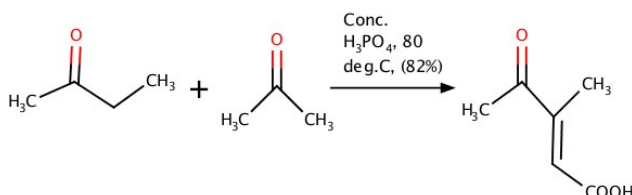


Thionation

Chapter 4

Ring C:

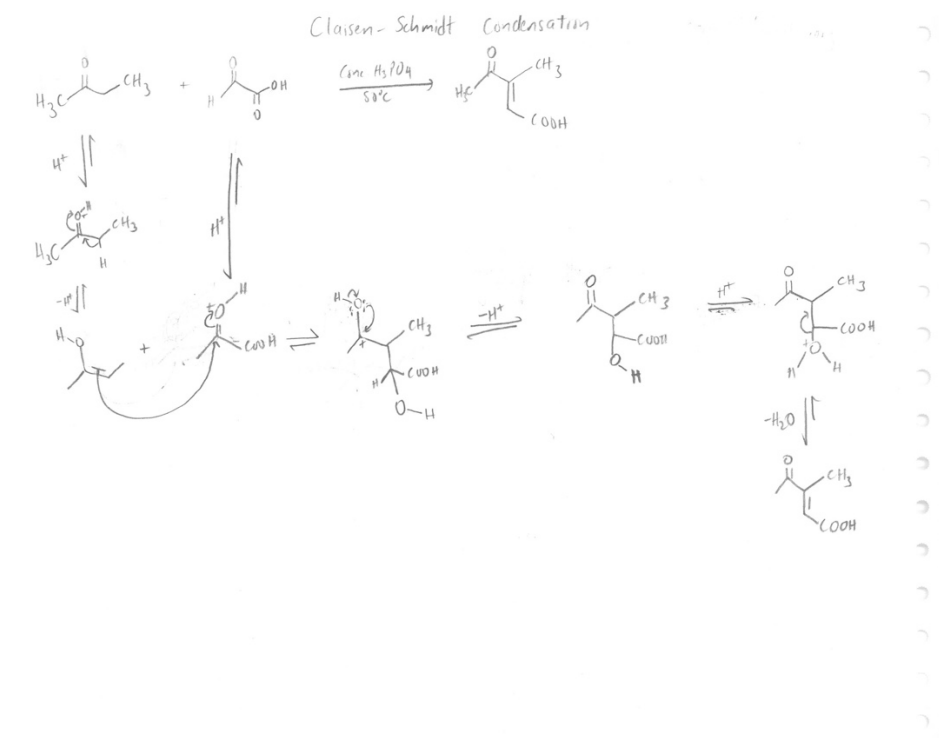
1. Claisen-Schmidt Condensation



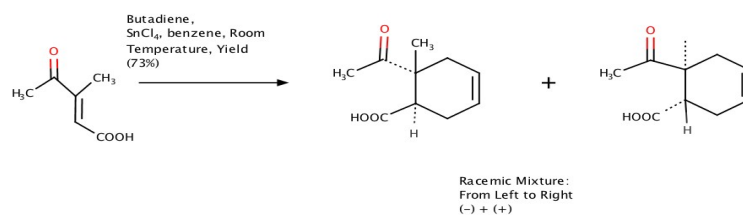
This first reaction of the B12 reaction scheme involves a ethyl methyl ketone (Compound 1A) reacting with acetaldehyde (Compound 1B) using reagents which are concentrated phosphoric acid (H₃PO₄) at 80°C and the yield is 82%. The type of reaction that is occurring the Claisen Schmidt condensation in which you have the formation of (2E)-3-methyl-4-oxopent-2-enoic acid. This reaction plays the role of producing the dienophile that will be used in the following reaction.

Overall if simplified this reaction is a type of condensation that results in the formation of an “ electron-poor molecule.

Mechanism:



2. and 3. Diels -Alder



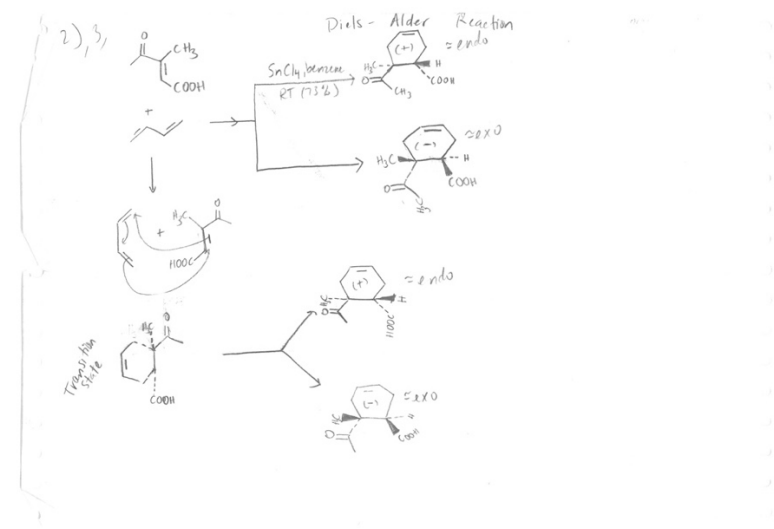
From left to right the products are presumably, exo and endo adducts.

The second reaction of the B12 schema involves (2E)-3-methyl-4-oxopent-2-enoic acid (Compound 2) reacting with butadiene in tin (IV) chloride (SnCl_4) and benzene at conditions of room temperature. This results in a yield of 73%. The type of reaction that is occurring is

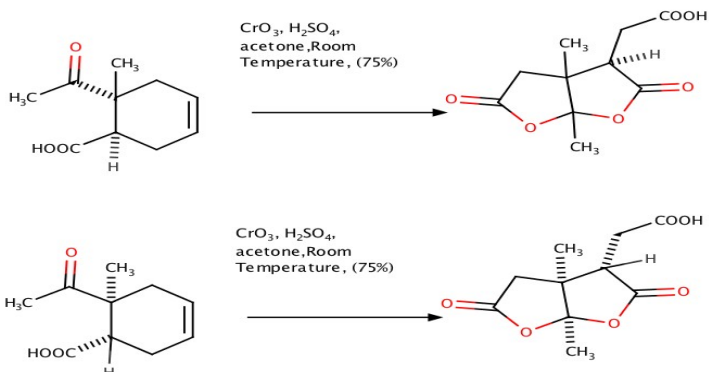
the Diels Alder reaction which involves the formation of a racemic mixture of two carboxylic acid like molecules with ketone like moieties attached to it. For the purposes of this discussion the products will be labeled compounds 3A (-) and 3B(+).

Overall if simplified the type of reaction, Diels Alder, is stereospecific and a type of concerted reaction in that all the bond breaking and bond forming occur at the same time. Moreover addition is syn. Also if this reaction follows the typical Diels Alder format it is a 1-step cyclo-addition or conjugate addition. This resulted in enantiomers which were resolved using Phenylethylamine in chloroform and hexane. Followed by the use of diluted HCl.

Mechanism:



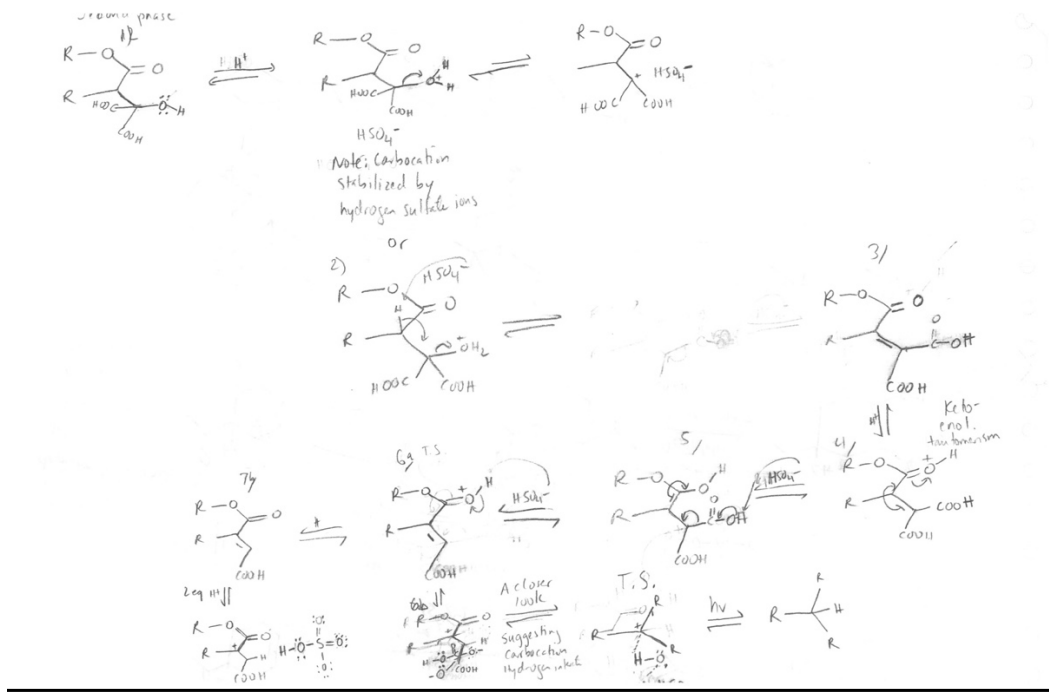
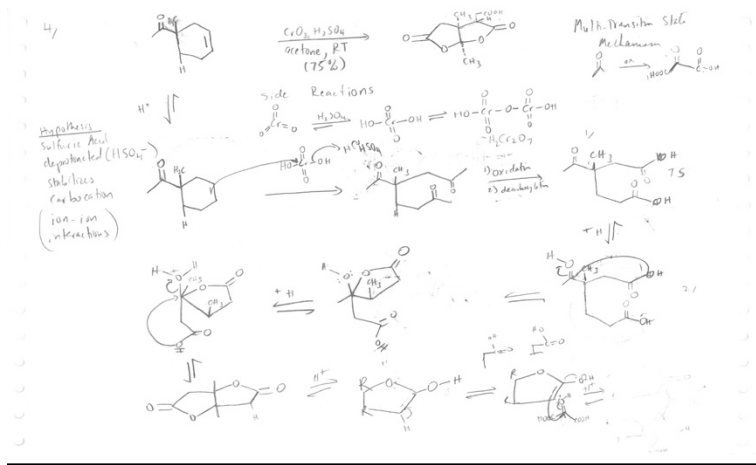
4. Oxidation



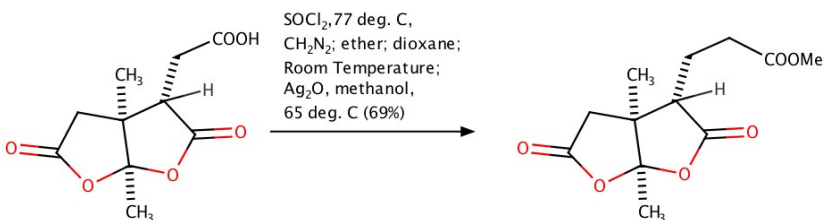
The fourth reaction in the B12 reaction schema involves compounds 3A(-) and 3B(+) reacting with chromate and sulphuric acid in acetone at room temperature to form dilactone carboxylic acids which will be labeled compounds 4B (-) and 4A (+) (from top to bottom), both of which are starting products for B12 ring reactors. This fourth reaction has a predicted yield of 75%.

From the reagents and the reactants this appears to be an organic redox reaction, possibly a Jones oxidation, in which we have a molecule being oxidized and or gaining hydrogen deficiency in the form of another ring. Stated simply, these reactions involve the oxidation of compounds 3A(-) and 3B(+) into ten carbon dilactone-carboxylic acids using reagents that normally are used in a type of organic redox reaction.

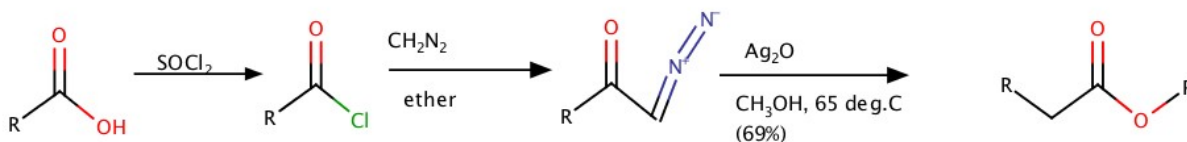
Mechanism:



5. Arndt-Eistert



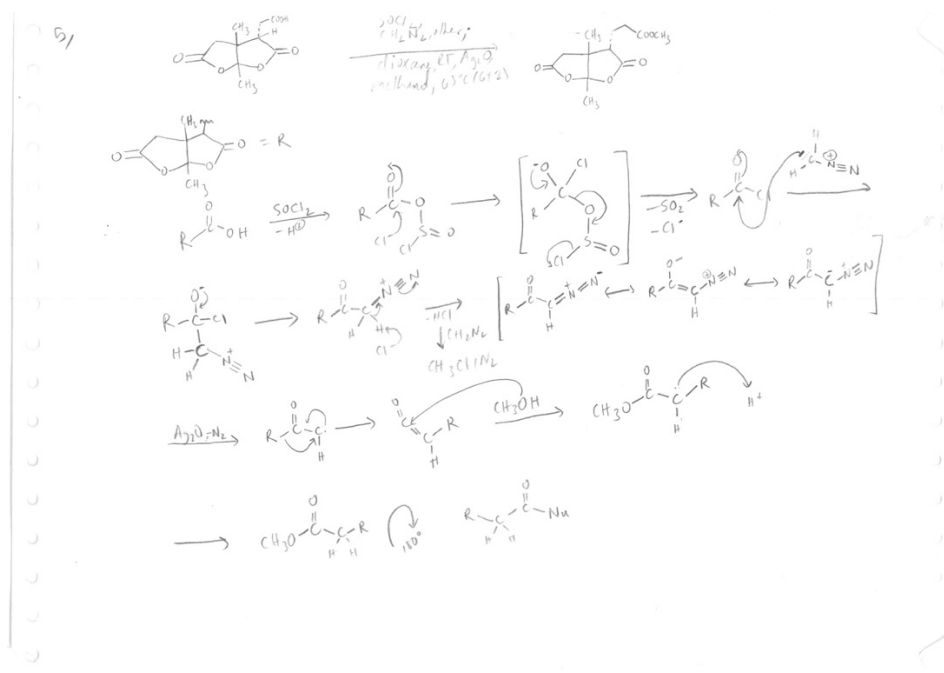
The fifth reaction in the B12 reaction schema involves compound 4A (+) reacting with thionyl chloride at 77 degrees Celsius. This was followed by reacting the acid chloride with diazomethane in ether at room temperature. After which it was reacted with silver dioxide in methanol at 65 degrees Celsius. This fifth reaction had a predicted yield of 69%. However the overall name of the reaction that is occurring is an Arndt-Eistert synthesis. Additionally an important step in the Arndt-Eistert reaction is the Wolff rearrangement of diazoketones to ketenes. The overall Arndt-Eistert reaction, excluding the Wolff Rearrangement, can be seen from the reaction drawn below, this sequence involves several steps that result in a higher order or homologated carboxylic acid.



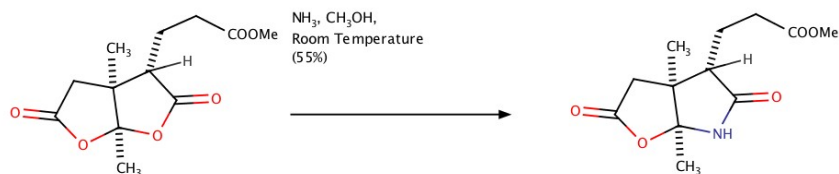
Stated simply this is a multistep reaction step that involves the conversion of a carboxylic acid to an acid chloride, then to diazo-ketone type molecule, and then the ester.

Some key points to note on the reaction, from Eschenmoser's notes for his 1973 German Lecture at ETH Zurich, "the treatment of the acid chloride with methanol/ pyridine at room temperature gives the same methyl ester as obtained by esterification with diazomethane; in the preparation of the acid chloride, there is no other structural change".

Mechanism:

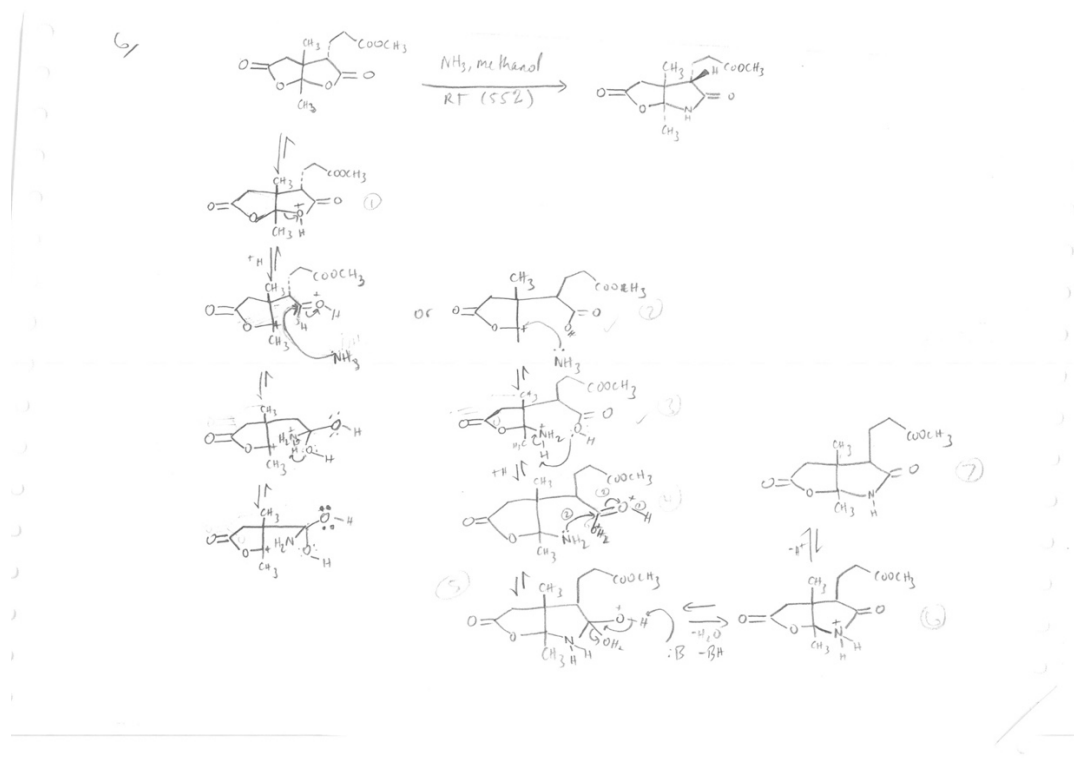


6. Ammonolysis

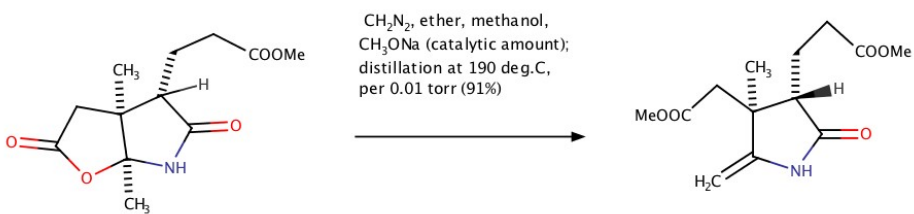


The sixth reaction in the B12 reaction schema involves compound 5A reacting with ammonia in methanol at room temperature. The sixth reaction had a yield of 55%. From this reaction it appears to be an Ammonolysis reaction in the presence of methanolic solvent. (Put in reference to Albert Eschenmoser’s German lecture) “The carbonyl groups of the dilactone moiety are much more nucleophilic towards ammonia than normal lactone or ester groups. “Ammonolysis” of this type are much faster in methanol than in non-hydroxyl containing solvents. The constitution assignment for the isomeric lactone-lactams resulted from the identity of compound 6A with the main product of intramolecular NH transfer”. Stated simply this reaction involved a intramolecular NH transfer using ammonia in methanol at room temperature.

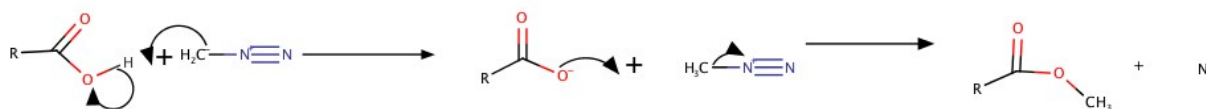
Mechanism:



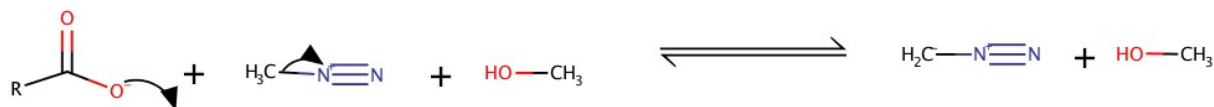
7. Esterification and Methanolysis (Step 8 in the reference)



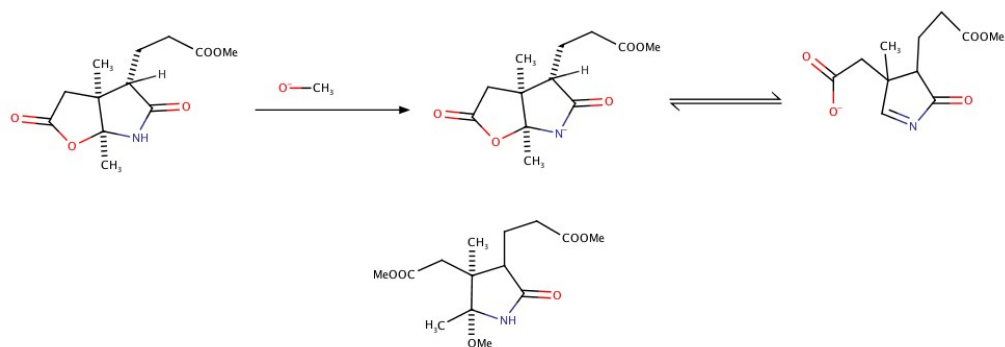
The eighth reaction in the B12 reaction schema involves compound 6A reacting with diazomethane in ether with methanol, and a catalytic amount of sodium methoxide. After which distillation at 190 degrees Celsius at a pressure of 0.01 torr. This reaction had a yield (are the yields predicted or are they resultant) of 91%. From the reaction compound 6A is converted to compound 8A in which an esterification occurs resulting in the formation of a methoxy-ester, and the formation of a double bond with a methene. Through the use of Dr. Albert Eschenmoser's 1973 ETH Zurich, German lecture notes we gain a better understanding. It states that "Normally when diazomethane is esterified, the free carboxylic acids are transformed with an ethereal solution of CH_2N_2 , and the hypothetical mechanism can be seen below:



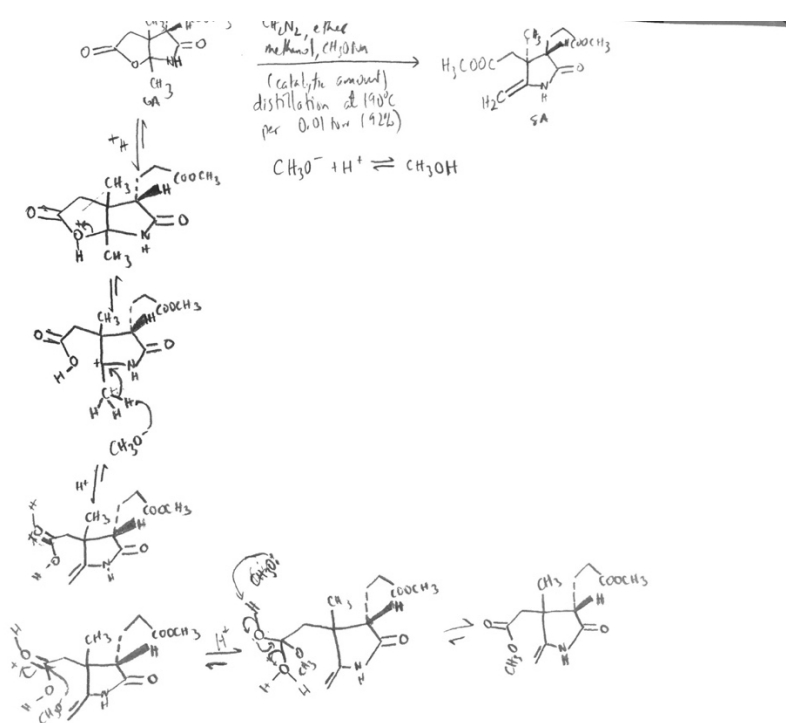
Conversion of compound 6A to compound 8A is "one of the rare examples of esterification in a basic mechanism".



The catalytic amount of sodium methoxide serves to adjust the following equilibrium.

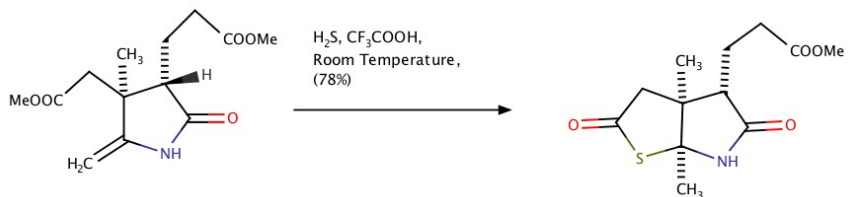


8



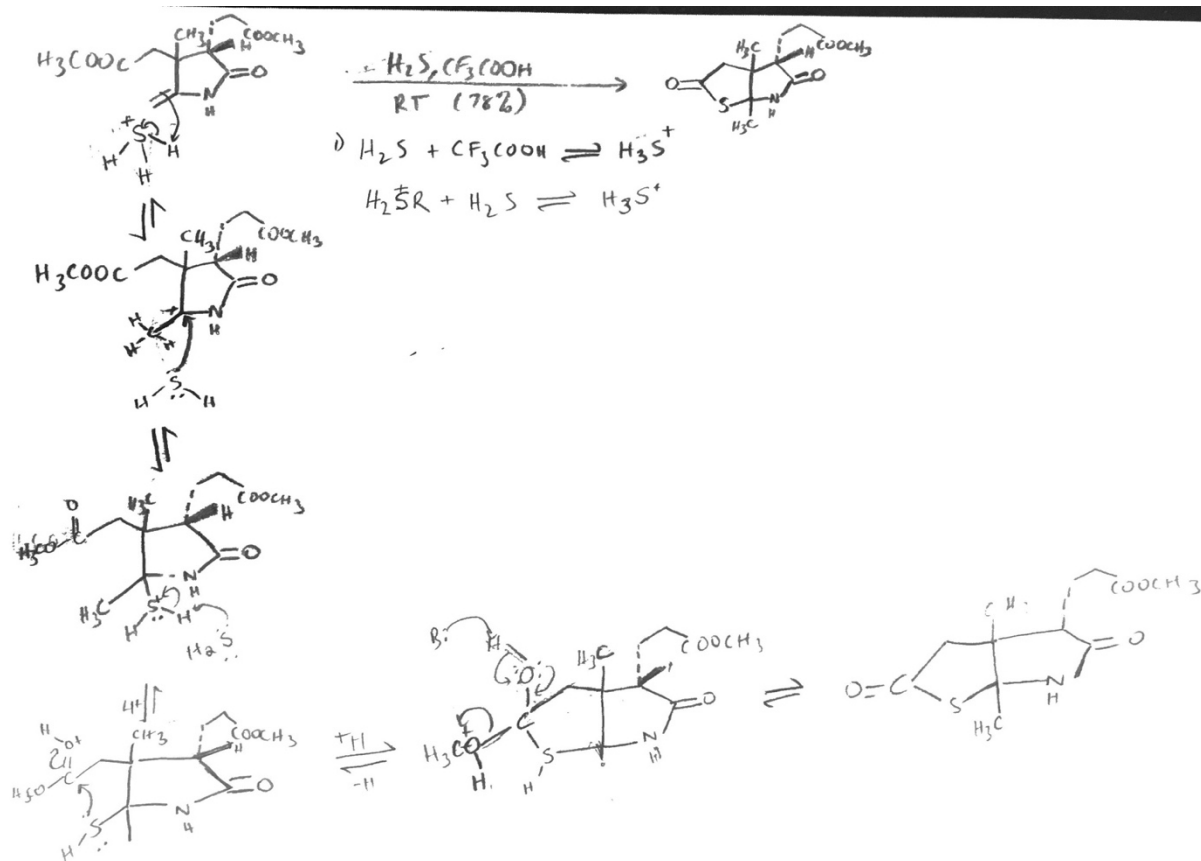
Esterification and Methanolysis.

8. Thioesterification (Step 9 in reference)

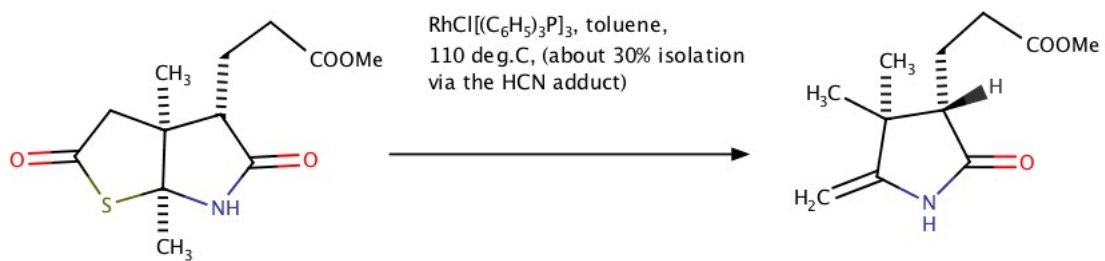


The ninth reaction in the B12 reaction schema involves compound 8A reacting with hydrogen sulfide, in trifluoroacetic acid at room temperature resulting in a yield of 78%. From the reaction compound 8A is converted to compound 9A with a subsequent elimination of the methoxy moiety and the conversion of an ester to a thioester. This thioester conversion results in the formation of a thiolactam portion on the bicyclic compound 9A. In many cases thioesters can be prepared by the condensation mechanisms however, in this case a different mechanism is occurring. States simply this is a thioesterification in which an ester and a carbene are condensed to form a cyclic structure joined by a thiolinkage.

Mechanism:



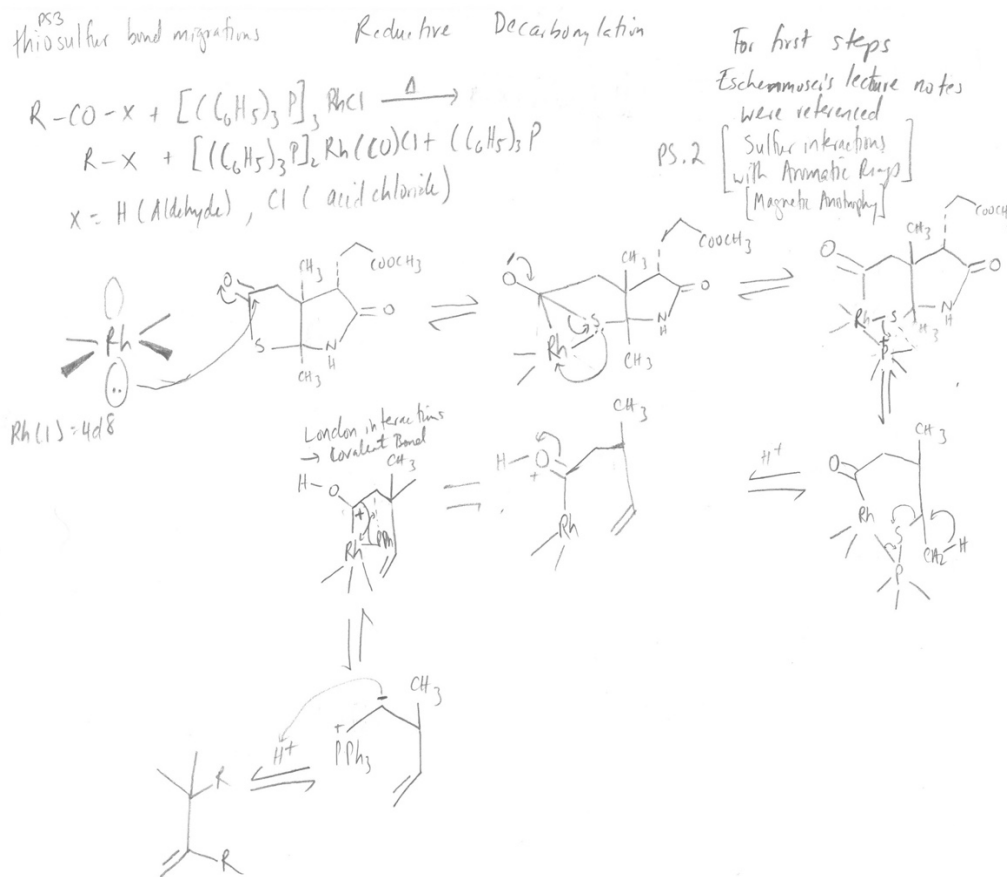
9. Reductive Decarbonylation (Step 10 in reference)



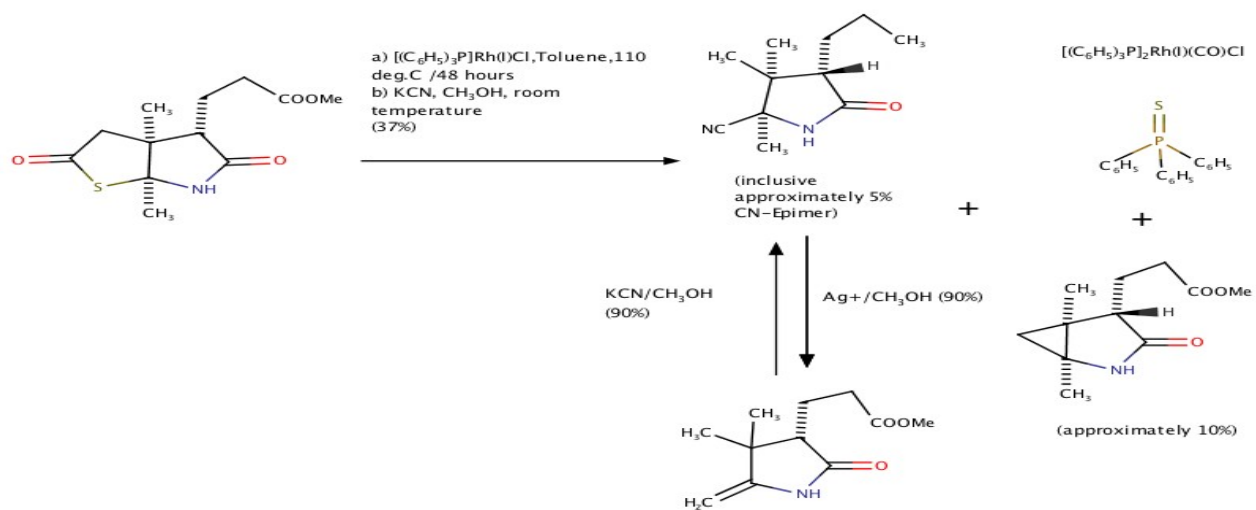
The tenth reaction in the B12 reaction schema involves compound 9A reacting with a rhodium based catalyst in toluene at 110 degrees Celsius, which resulted in about 30% isolation through the use of an HCN adduct. From the reaction a thiolactam ring is opened resulting in a separate methyl and ethylene. As seen the remainder of the bicyclic reactant structure remains the same. However it is worth noting that in Eschenmoser's 1973 lecture notes it includes that there are several products including the two cyclic structures, a phosphor-sulfuryl, and a rhodium based compound, all of which are reflective of the reagents, the reactants collides or reactants with. Added to that, one of the group of products is reacted again with silver ions in the presence of methanol ($\text{Ag}^+ / \text{CH}_3\text{OH}$) to form the final pyrrolidine like product, which is a precursor to ring C for the Vitamin B12 synthesis. Additionally the ring precursor can be converted back to the reactant is by the use of potassium cyanide in methanol (KCN, methanol). Both the conversion of Ring C from the intermediate group of products to the final product and the reversed conversion back to the reagent in the group of products have yields of 90%. Stated simply this reaction involves the conversion of a bicyclic dicarbonyl- 12- carbon ester to a cyclic 8 carbon pyrrolidine like molecule using a catalyst in organic solvent. In other words, the "corresponding thiolactone is ran through reductive decarbonylation brought about by the chloro-tris-

trisphenylphosphine complex of rhodium (I)¹⁵. Then through the use of HCN there was about 30% isolation. The entire reaction scheme for this step can be seen below:

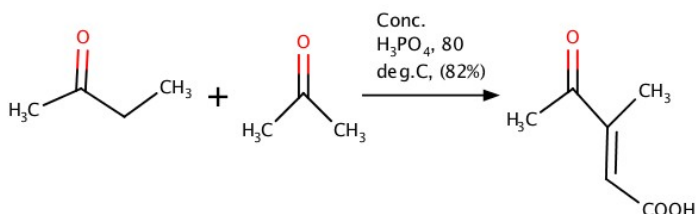
Mechanism:



Note: In mechanism ten some insight was gained from Eschenmoser's German lecture



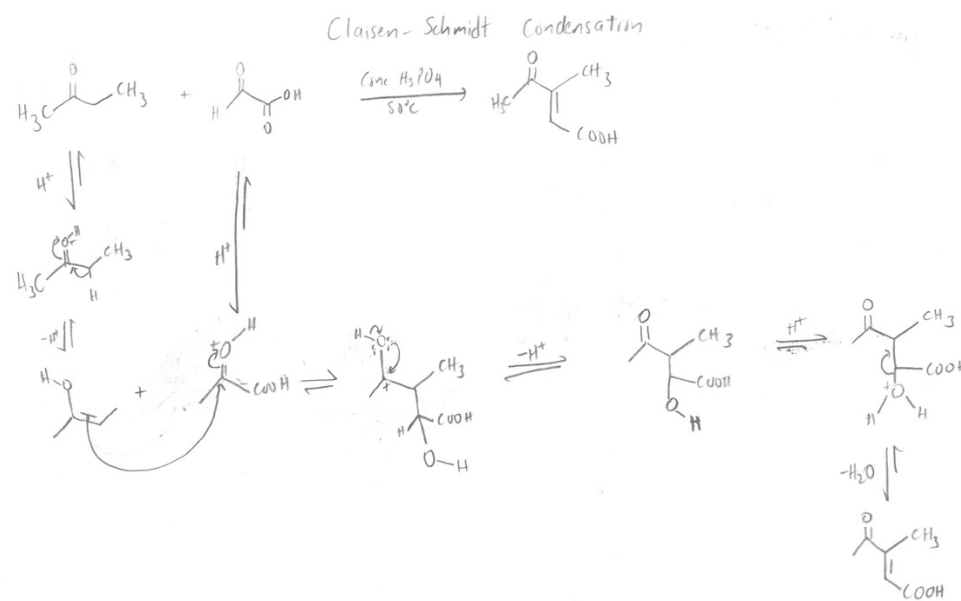
Chapter 5

Ring D:**1. Claisen-Schmidt Condensation**

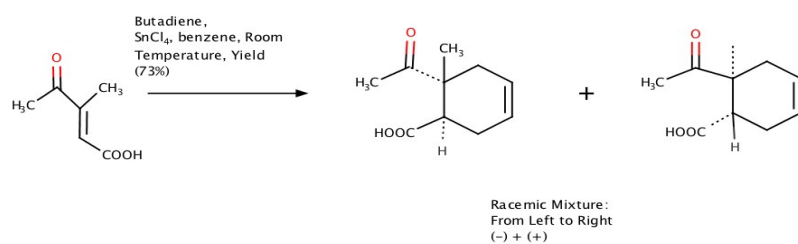
This first reaction of the B12 reaction scheme involves a ethyl methyl ketone (Compound 1A) reacting with acetaldehyde (Compound 1B) using reagents which are concentrated phosphoric acid (H₃PO₄) at 80°C and the yield is 82%. The type of reaction that is occurring the Claisen Schmidt condensation in which you have the formation of (2E)-3-methyl-4-oxopent-2-enoic acid. This reaction plays the role of producing the dienophile that will be used in the following reaction.

Overall if simplified this reaction is a type of condensation that results in the formation of an “ electron-poor molecule.

Mechanism:



2. and 3. Diels-Alder Reaction

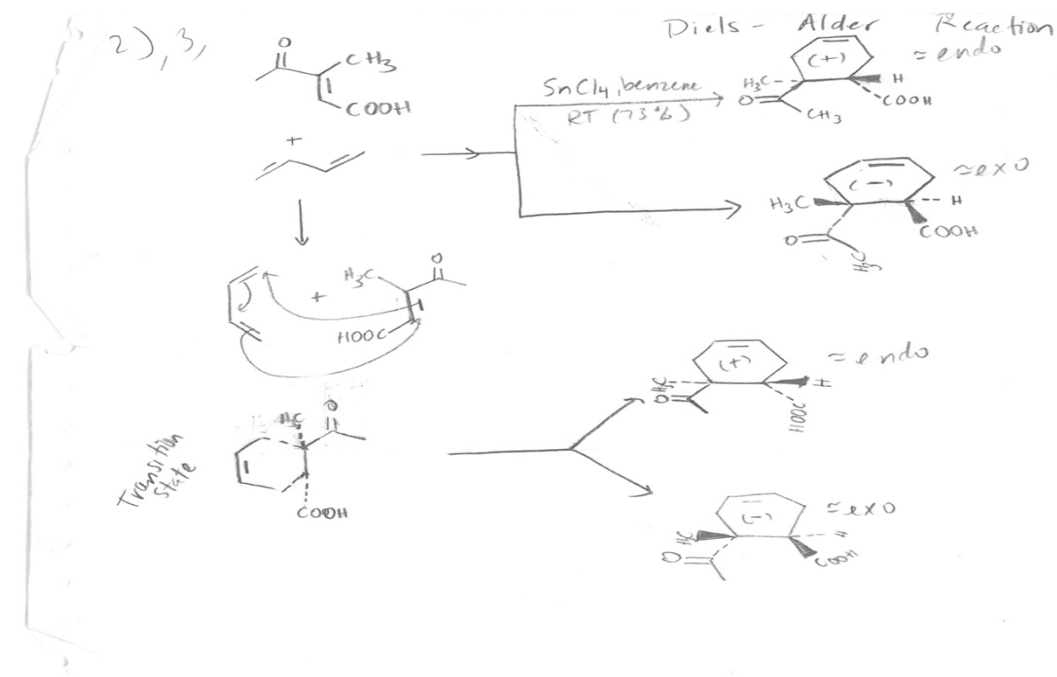


From left to right the products are presumably, exo and endo adducts.

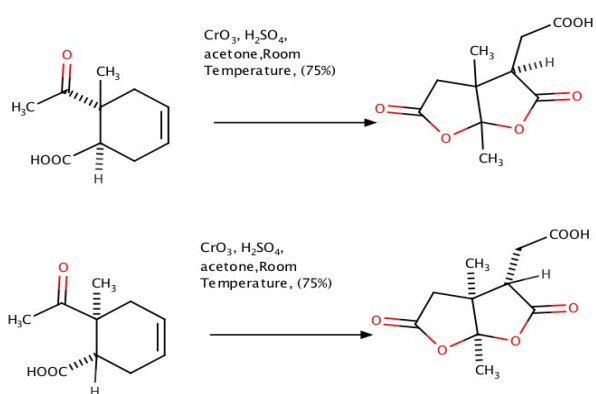
The second reaction of the B12 schema involves (2E)-3-methyl-4-oxopent-2-enoic acid (Compound 2) reacting with butadiene in tin (IV) chloride (SnCl_4) and benzene at conditions of room temperature. This results in a yield of 73%. The type of reaction that is occurring is the Diels Alder reaction which involves the formation of a racemic mixture of two carboxylic acid like molecules with ketone like moieties attached to it. For the purposes of this discussion the products will be labeled compounds 3A (-) and 3B(+).

Overall if simplified the type of reaction, Diels Alder, is stereospecific and a type of concerted reaction in that all the bond breaking and bond forming occur at the same time. Moreover addition is syn. Also if this reaction follows the typical Diels Alder format it is a 1-step cyclo-addition or conjugate addition. This resulted in enantiomers which were resolved using Phenylethylamine in chloroform and hexane. Followed by the use of diluted HCl.

Mechanism:



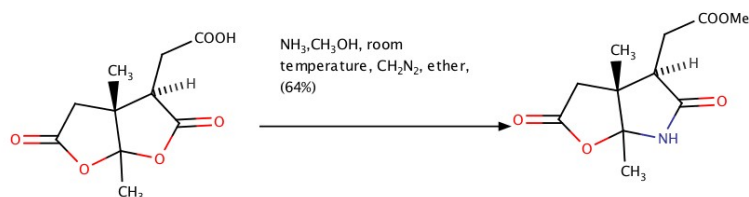
4. Oxidation Reaction



The fourth reaction in the B12 reaction schema involves compounds 3A(-) and 3B(+) reacting with chromate and sulfuric acid in acetone at room temperature to form dilactone carboxylic acids which will be labeled compounds 4B (-) and 4A (+) (from top to bottom), both of which are starting products for B12 ring reactors. This fourth reaction has a predicted yield of 75%. From the reagents and the reactants this appears to be an organic redox reaction, possibly a Jones oxidation, in which we have a molecule being oxidized and or gaining hydrogen deficiency in the form of another ring. Stated simply, these reactions involve the oxidation of compounds 3A(-) and 3B(+) into ten carbon dilactone-carboxylic acids using reagents that normally are used in a type of organic redox reaction.

Mechanism:

5. Ammonolysis Reaction (Step 13 in the reference)



The thirteenth reaction in the B12 reaction schema involves compound 4B reacting with ammonia, in methanol at room temperature, followed by a reaction with diazomethane in ether and methanol. This resulted in product 13B with a 64% yield.

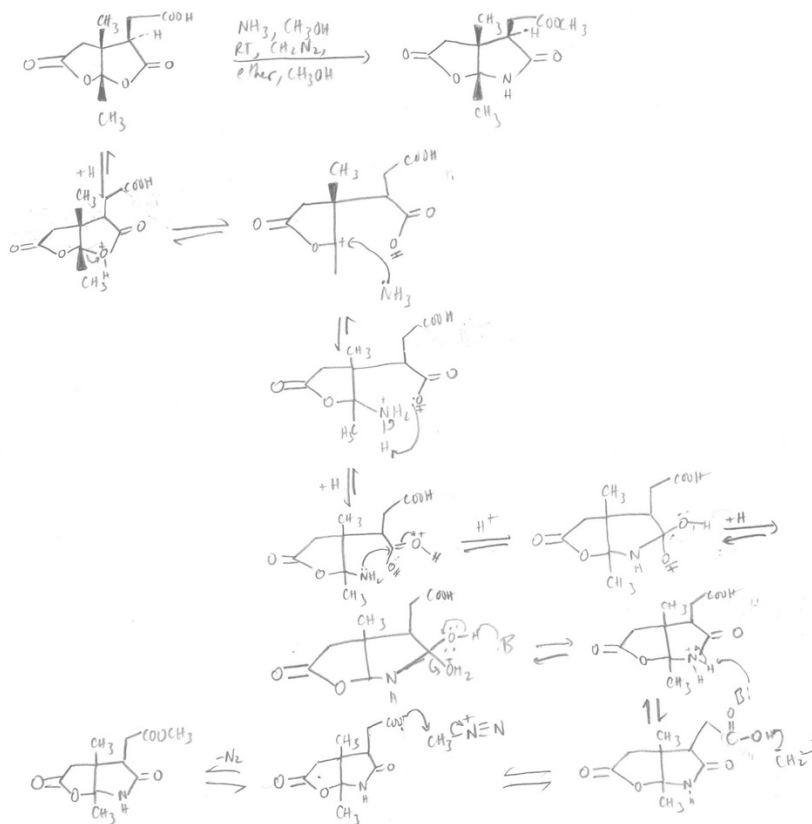
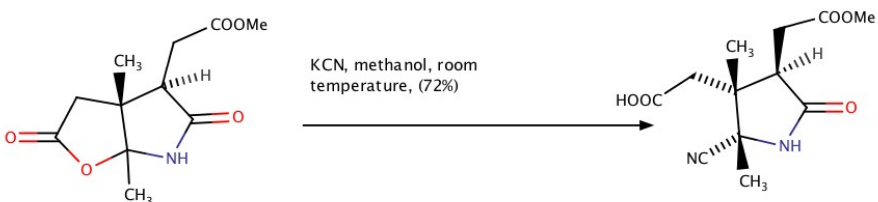
From this reaction we can see that a right lactone ring is N-substituted, going through a selective amidation. Based on the observation a plausible mechanism is that the right lactone oxygen ring is probably protonated by the ammonia then the ring is opened. The amine attacks the carbocation formed at the bridge carbon. Then the nucleophilic amide portion of the molecule attacks the nearest terminal carboxylic acid's carbonyl, resulting in temporary loss of the carbonyl, forming a tetrahedral intermediate moiety. The carboxylic acid's hydroxyl then becomes protonated from the ammonium formed. After which the carbonyl is reformed, resulting in water being eliminated. The terminal carboxylic acid that remains goes through Fischer esterification to form the corresponding methyl ester.

Selective amidation of the right lactone ring in the 10 carbon bicyclic lactone molecule

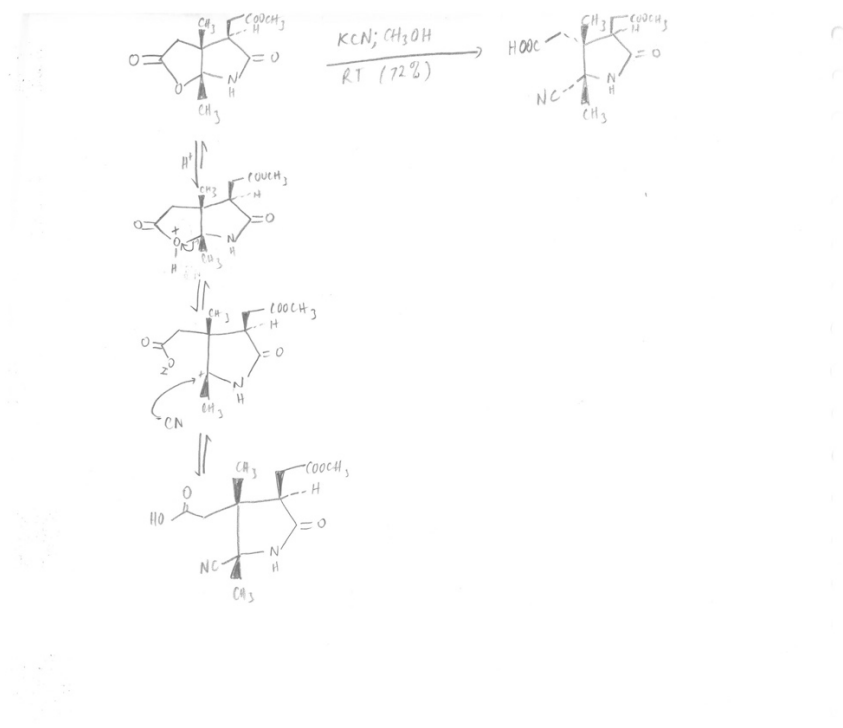
(reference that confirms observation : <https://pubs.acs.org/doi/pdf/10.1021/acsomega.7b01540>)

Mechanism:

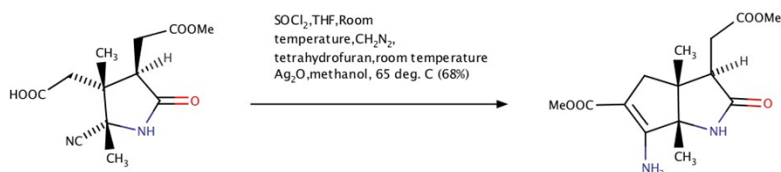
13

**6. Ring Opening Reaction (Step 14 in the reference)**

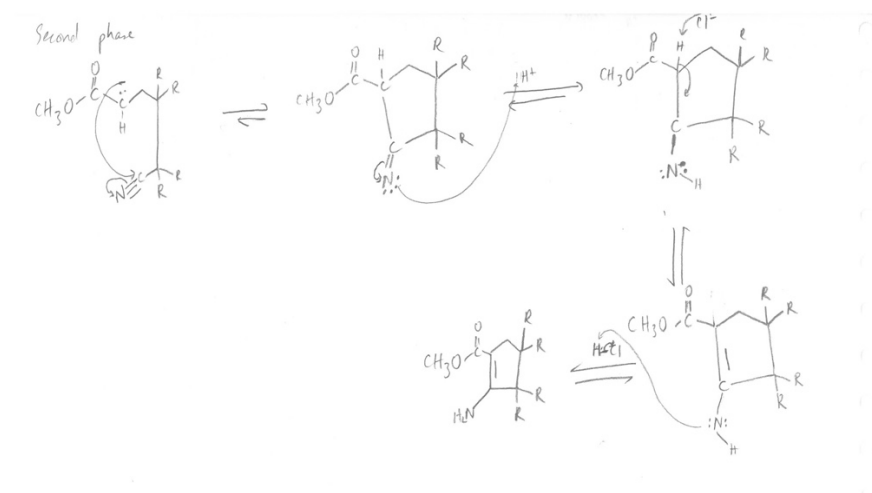
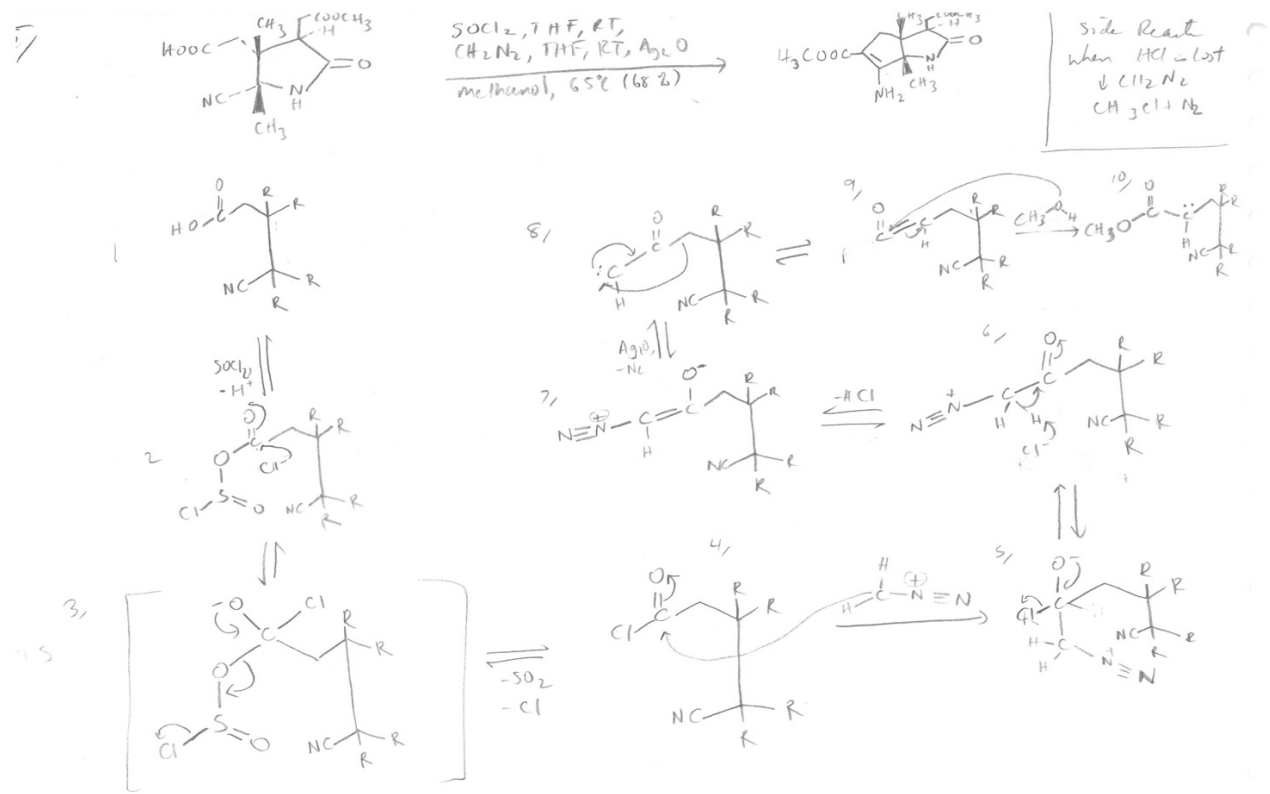
The general reaction involves compound 13B reacting with potassium cyanide in methanol at room temperature with a resultant 72% yield. On compound 14 B there are respective ester and cyano group are on the ends. Based on observation this appears to have gone through acid-catalyzed (methanol) ring opening in which the left lactone was protonated and the connecting oxygen resulting in the formation of a oxonium . After which the bond connecting one of the lactones to the ring breaks in order to donate the electron pair to the oxygen. In the presence of the resultant carbocation at the bridge the cyanide ion, which is very nucleophilic, attacks the carbocation at the bridge. Stated simply this involves the conversion of a 12 carbon- dicarbonyl-bicyclic compound to a cyclic compound with the other ring being cleaved to form a carboxylic acid and a cyanide at the ends where the ring broke.

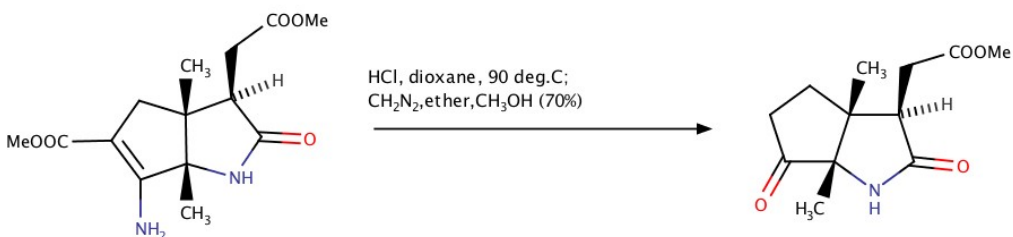


7. Arndt-Eistert Reaction (Step 15 in the reference)



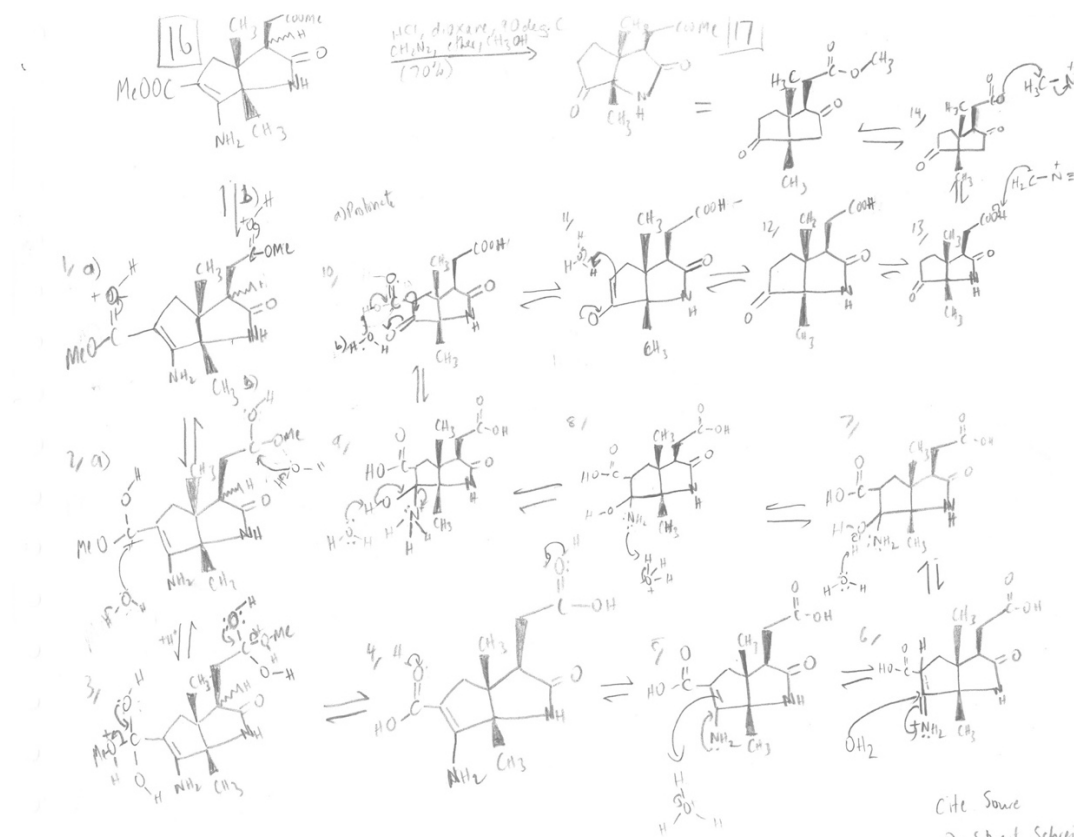
The general reaction involves compound 14B reacting with thionyl chloride in tetrahydrofuran at 65 degrees celsius. Compound 15B is a 12 carbon-bicyclic system with one of the cycles having a unit of unsaturation i.e. a double bond. Attached to the cycle with the unit of unsaturation is an ester moiety and an amine moiety. Based on observation this appears to have gone through an Arndt-Eistert reaction. However the lecture notes states that it was an unsuccessful Arndt-Eistert reaction. Therefore some things can be presumed, either it may have been an entirely different reaction other than Arndt -Eistert reaction or it was due to the low yield that the reaction was classified as unsuccessful. With than in mind, we continue the discussion. First the carbonyl on the terminal carboxylic acid would have been protonated following the nucleophilic attack on thionyl chloride, removal of Cl as a leaving group, nucleophilic attack on the carbonyl, and then the departure of the leaving group. After the acid chloride has been formed it goes thorough the Arndt-Eistert reaction in which the cyclic group with the unit of unsaturation is formed. Stated simply this involves the conversion of a 11 carbon- cyclic compound to a 12 carbon bicyclic compound with a ring with a double bond and an ester moiety and an amine group.

Mechanism:**8. Hydrolysis, Decarboxylation and Esterification (Step 16 in the reference)**



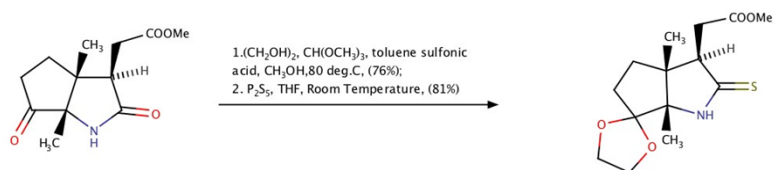
The general reaction involves compound 15B reacting with hydrochloric acid in dioxane at 90 degrees Celsius. This is followed by the reaction with diazomethane in ether and methanol . Compound 15B is a 12 carbon-bicyclic system with one of the cycles having a unit of unsaturation i.e. a double bond and attached to the cycle with the unit of unsaturation ,is an ester moiety and an amine moiety. Based on observation the two esters on compound 15B are hydrolyzed to acids, followed by the hydrolysis of an examine to a ketone, then the decarboxylation of the beta-ketoacid, and finally the diazomethane is used to convert the remaining acid to an ester. Stated simply, this involves the conversion of a 12 carbon- cyclic compound to an 11 carbon di-carbonyl- bicyclic compound , by hydrolysis, decarboxylation and then esterification.

Mechanism:



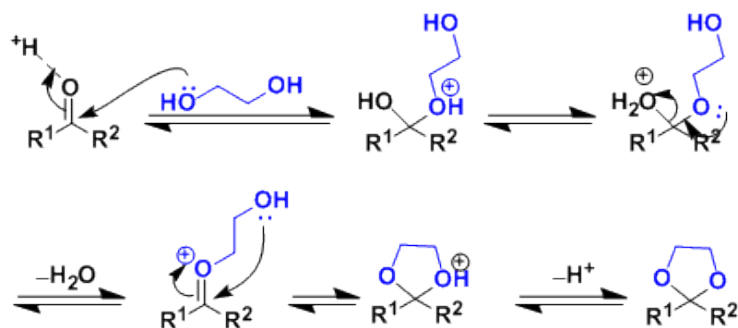
Note: Some insight was gained from Dr. S. Schreiber.

9. Protection/Sulfonation (Steps 17 and 18 in the reference)

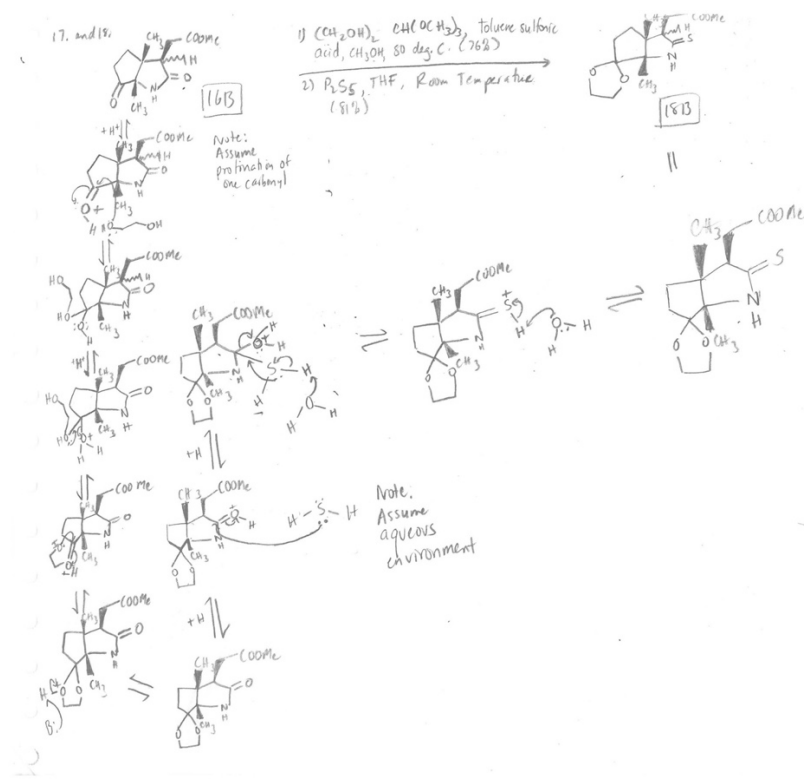


The general reaction involves compound 16B reacting with ethylene diol in triethyl-orthoformate, toluene sulfonic acid and methanol at 80 degrees Celsius. This reaction has a 76 % yield. This is followed by the reaction of the compound made by the previous step with diphosphorus

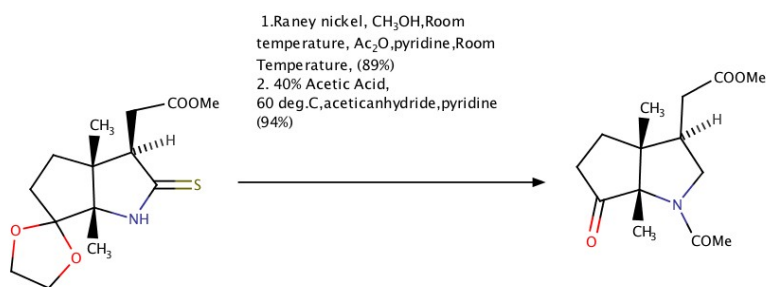
pentasulfide in tetrahydrofuran at room temperature . This reaction resulted in a yield of 81%. Compound 16B is an 11 carbon- dicarbonyl bicyclic system that is converted to a 13- carbon thicarbonyl-tricyclic system having similar substituents and ring components. Based on observation the carbonyl on the left ring is protected by the ethylene diol. Followed by the thioylation of the other carbonyl by the diphosphorus pentasulfide. Stated simply, this involves the conversion of a 11 carbon- cyclic compound to a 13- carbon thicarbonyl-tricyclic system, by protection and then thioylation.



Mechanism:



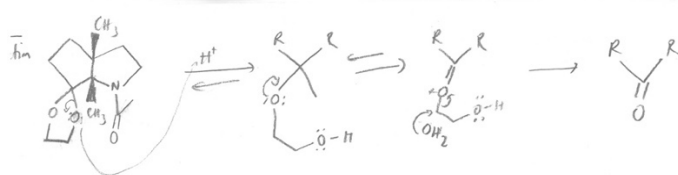
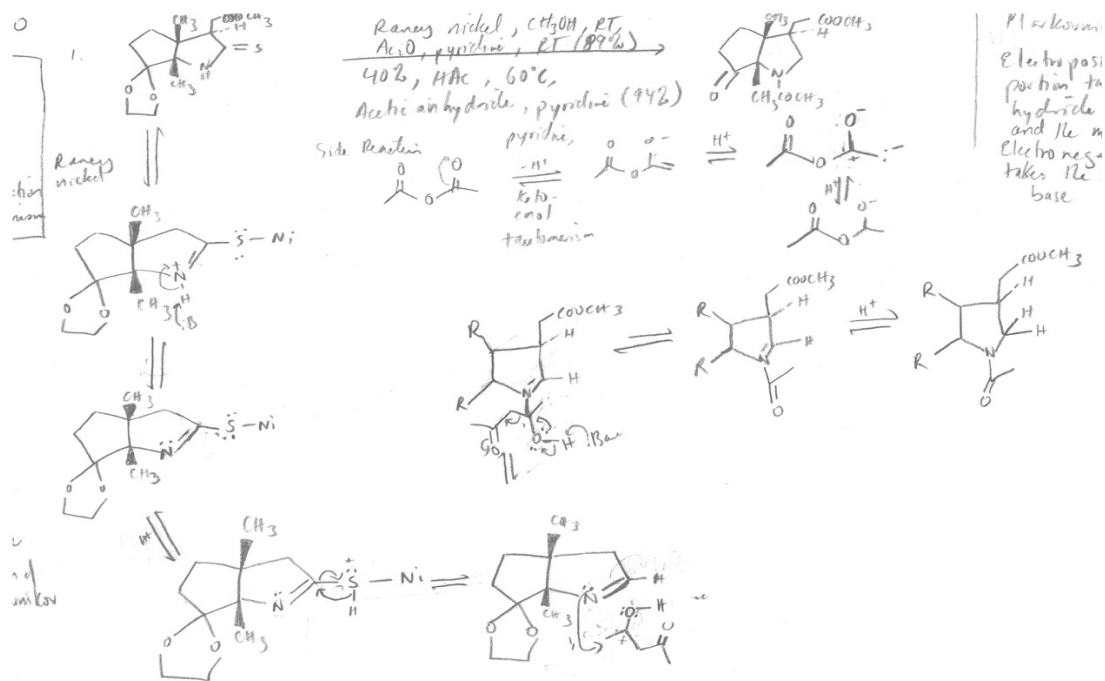
10.Reduction/Deprotection (Steps 19 and 20 in the reference)



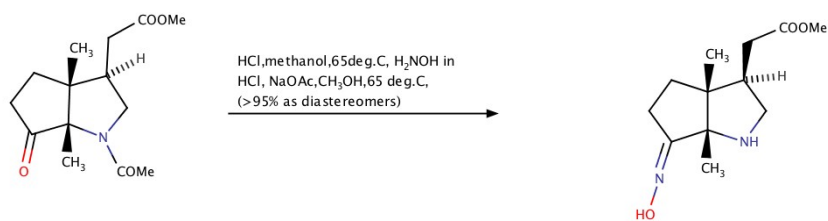
The general reaction involves compound 18B reacting with Raney nickel in methanol at room temperature. This was followed by a reaction with acetic anhydride in pyridine at room temperature. The overall yield of the reaction was 89%. After this the resultant compound was

reacted with acetic acid at 60 degrees Celsius in acetic anhydride and pyridine. The overall yield of this reaction was 94%. The resulting compound was 20B. The overall reaction involved the conversion from a 13-carbon thicarbonyl-tricyclic system to a 12-carbon bicyclic system. Based on observation this reaction involved the reduction of the thicarbonyl using the Raney nickel . Followed by the removal of the protecting group using the acetic acid resulting in the reformation carbonyl on the left ring of the bicyclic system. From reading Eschenmoser's German lecture notes it shows that " 1) the product is isolated in N-acetylated form because amino ketals are hydrolysable and 2) free alpha-amino ketones are less stable". Stated simply, this involves the conversion of a 13-carbon thiocarbonyl-tricyclic system to 12-carbon bicyclic system, using reduction followed by deprotection.

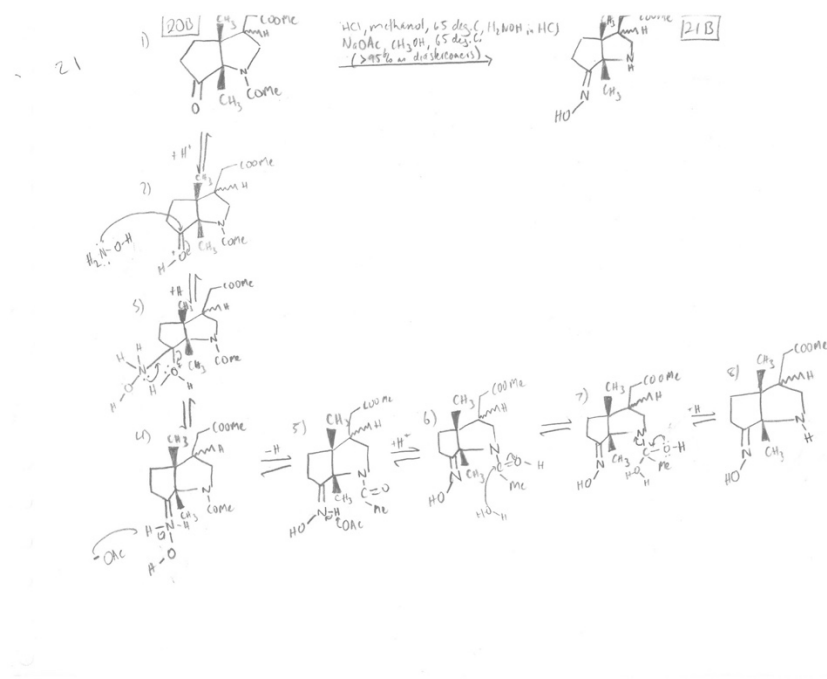
Mechanism:



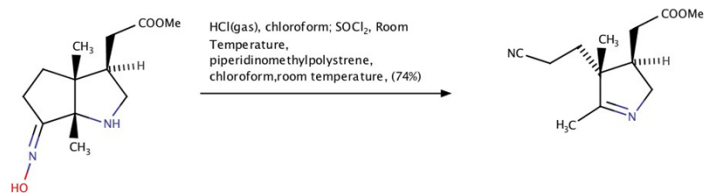
11. Protonation (Steps 21 in the reference)



The general reaction involves compound 20B reacting with hydrochloric acid in methanol at 65 degrees Celsius, followed by the reaction of hydroxylamine in hydrochloric acid. After which the product of the previous reaction reacts with sodium acetate in methanol at 65 degrees Celsius. This results in a product mixture with greater than 95% yield made of diastereomers. The overall reaction involved the 12 carbon bicyclic system being converted to an 11 carbon-bicyclic system. Based on observation, we assume the environment is aqueous the ketone is protonated, then nucleophilic attack occurs with the nitrogen of the hydroxylamine. Following this the protonated hydroxyl is eliminated and the double bond forms between the carbon and the nitrogen. After which the acetate anion takes off the protons off of the nitrogen successively. Then a water molecule attacks the carbon of the N-methyl-carbonyl, then the N-methyl-carbonyl is eliminated, and the nitrogen is protonated. Stated simply, this involves the 12 carbon bicyclic system being converted to an 11 carbon-bicyclic system using protonation, elimination and then protonation again.



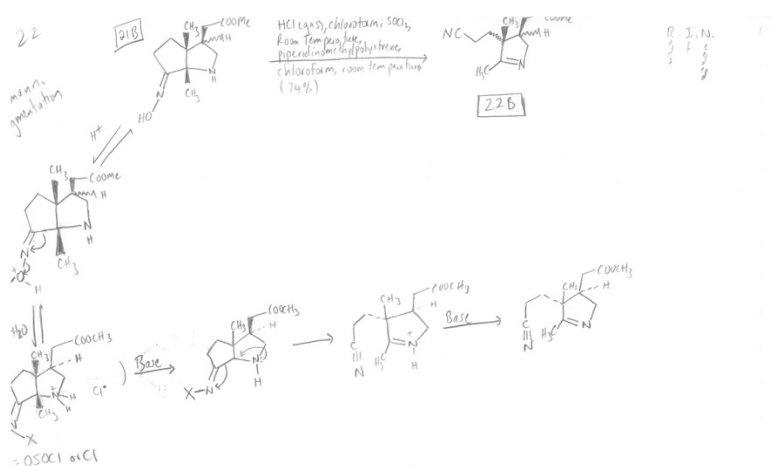
12. Beckmann Fragmentation (Steps 22 in the reference)



The general reaction involves compound 21B reacting with hydrogen chloride gas in chloroform, and thionyl chloride at room temperature to form 22B. Followed by the reaction with piperidinomethylpolystyrene in chloroform at room temperature. This reaction results in a product yield of 74%. The overall reaction involves the conversion of an 11 carbon-bicyclic system to a single cyclic system with several substituents. Based on observation the 12-carbon-

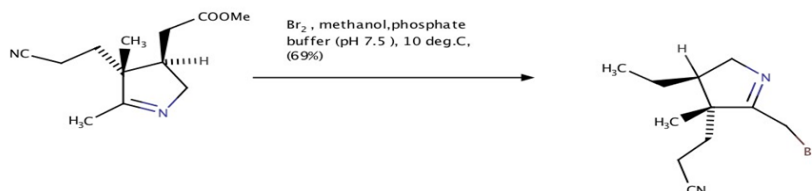
bicyclic system goes through Beckmann Fragmentation resulting in the fragmentation of one of the cycles. On reading Eschenmoser's German lecture notes it shows that in this step fragmentation occurs instead of rearrangement, when the potentially migrating group can form a stabilized cation. At the same time the lecture notes elaborate on what is happening mechanistically with there being first "protonation of the amino group (with hydrogen chloride gas), otherwise it would react with thionyl chloride". Stated simply, this involves the conversion of an 11 carbon-bicyclic system to a single cyclic system with several substituents using Beckmann Fragmentation.

Mechanism:



Note: Some insight was gained from Eschenmoser's German lecture.

13. Bromination of the Ketimine (Step 23 in the reference)



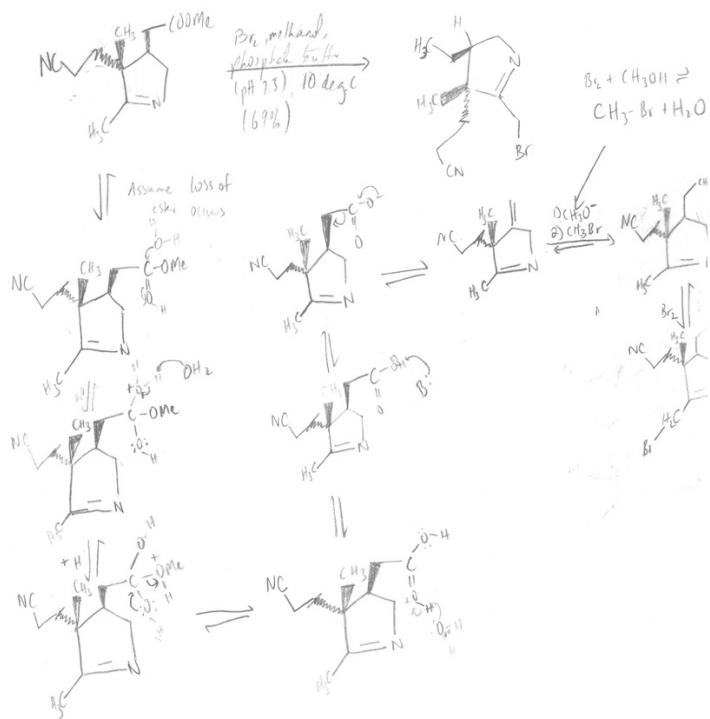
The general reaction involves compound 22B reacting with bromine gas in methanol, in a phosphate buffer (pH 7.5), and at a temperature of 10 degrees Celsius to yield 23B . This reaction results in a product yield of 69%. The overall reaction involves the conversion of an 11 carbon-cyclic system to a brominated 11- carbon single cyclic system with several substituents.

Based on observation the reaction involves the careful bromination of the ketimine. Possibly: going through the mechanism of hydrolysis of the ester, followed by deprotonation of the double bond, addition of the alkyl bromide and finally N-beta-bromination (double check terms).

Stated simply, 11 carbon-cyclic system to a brominated 11- carbon single cyclic system with several substituents using careful bromination.

Mechanism:

23



Chapter 6

Common steps in the entire synthesis :

The first four steps in the synthesis :

- The Claisen-Schmidt Condensation
- Diels-Alder (Steps 2 and 3)
- Oxidation

Common Problems in the synthesis of cobyric acid:

- Introduction of cobalt
- Closure of the macrocyclic ring
- Ester differentiation
- Introduction of methyl groups at bridges
- Restoration of lost stereochemistry¹⁶

General Approaches to Problems:

Collaboration with other scientists

Exhaustive study of the relationships between thioethers

Purifications using analytic instrumentations such as High Performance Liquid Chromatography

Use of pure reagents, exclusion of oxygen and moisture ¹⁷

Possible Future Studies:

The role of sulfur-aromatic interactions in certain mechanistic steps

Carbocation-conjugate base interactions or stabilization

Variations of Markovnikov rules in the context of heterocycles

Hypothesized Organic Chemistry Mechanism Testing (Experimental)

Given that the plausibility of the mechanism is tied to the reality of the reaction

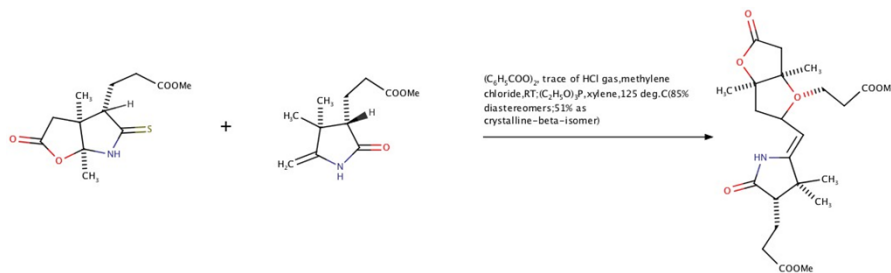
Chapter 7

Final Steps in the synthesis of cyanocobalamin

- 1. Iminoester Condensation and Sulfide Contraction (Step 24 in reference)**
- 2. Thionation (Step 25 in reference)**
- 3. Sulfide Contraction via alkylative coupling (Step 26 in reference)**
- 4. Ammonolysis (Step 27 in reference)**
- 5. Iodination (Step 28 in reference)**
- 6. Elimination (Step 29 in reference)**
- 7. Photochemical A/D cycloisomerization (Step 30 in reference)**
- 8. Metal Complexation (Step 31 in reference)**
- 9. Lactonization (Iodolactonization), (Step 32 in reference)**
- 10. Alkylation (Step 33 in reference)**
- 11. Reduction and Esterification (Step 34 in reference)**
- 12. Reduction (Step 35 in reference)**
- 13. Hydrolysis and Ammonolysis (Step 36 and 37 in reference)**

14. Final Step: Cobyric acid to Cyanocobalamin (Step 38 from K. Bernhauer and Eschenmoser lecture)

24.

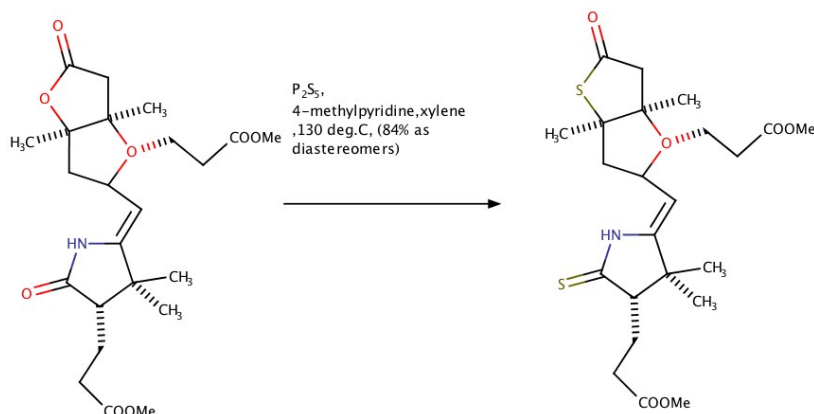


The general reaction involves compounds 7A and 10A reacting with dibenzoylperoxide and hydrogen chloride gas in methylene chloride at room temperature. Followed by the reaction of the intermediate with triethylphosphite at 125 degrees Celsius.

Based on observation and review of Eschemoser's lectures (put in citation) there are some key ideas worth noting. First the thiolactam and enamide (or enamine) partners are linked together to form a sulfur bridge intermediate. Second due to it being thioiminoester and an enamide (or enamine) derivative, the intermediate can undergo intramolecular carbon-carbon condensation to form via the hypothetical episulfide, the vinylogous amidine. Third, "this contraction process is susceptible to acid and base catalysis and is carried out in the presence of a trivalent phosphorus compound as thiophilic reagent". Fourth, "the identified intermediate in this oxidative coupling process is the bisimidoylsulfide which, in turn, undergoes an acid catalyzed electrophilic substitution at the methyldene carbon of the enamide; the equivalent of thiolactam thereby

liberated is recycling through further oxidation”. Fifth, “the initial intermolecular coupling can be achieved by either an oxidative or an alkylative pathway, depending on whether the enamine partner is led to act as a nucleophile on the pre-oxidized thiolactam sulfur atom or alternatively, whether the coupling is brought about by alkylation of the thiolactam sulfur atom by a halogenated enamine derivative”. The overall reaction involves the thiolactam and enamide (or enamine) partners linking together to form a sulfur bridge intermediate, which undergoes a contraction process. Stated simply, two molecules condensing to form a new molecule composed of the two reactants.

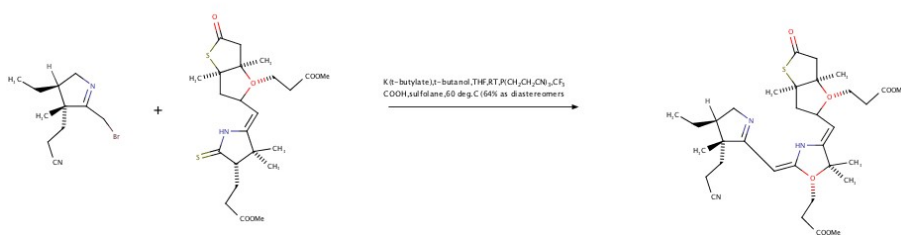
25.



The general reaction involves compounds 24 and reacting with diphosphorus pentasulfide, in methylpyridine and xylene at 130 degrees Celsius to result in the formation of a thionated top ring and a thiocarbonyl on the bottom ring.

Based on observation and review of Eschenmoser's lectures (put in citation) this reaction proceeds through a thionation mechanism in which there is selective thionation of the top ring and the bottom carbonyl. The overall reaction involves the thionation of the top ring and of the bottom carbonyl. Stated simply, two parts of the molecule are selectively thionated.

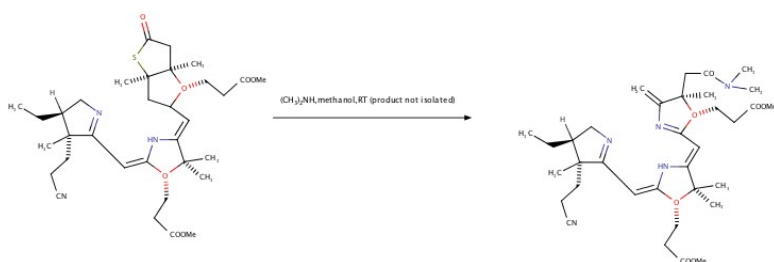
26.



The general reaction involves Ring D reacting with compound 25 in basic potassium tert-butyrate in tert-butanol, and tetrahydrofuran at room temperature. This reaction was followed by the product of the previous reaction, reacting with tris-(beta- cyanoethyl)-phosphine in trifluoroacetic acid and sulfolane at 60 degrees Celsius. Based on observation and reading from Eschenmoser's lecture notes, this appears to be an "iminoester-enamine condensation followed by an S-transfer to thiophile (phosphine or phosphite)", all including a sulfide contraction. The overall reaction is composed of a condensation and sulfide contraction reactions. As noted in Eschenmoser's notes "Studies on organic synthesis", "The ring D bromide condenses very smoothly with the thiolactam form of the B/C component after deprotonation with the potassium tert.butoxide". He then adds "subsequently, sulfide contraction is cleanly induced by

trifluoroacetic acid and tris-(beta- cyanoethyl)-phosphine in sulfolane”. It is worth noting that given the two variants of synthetic approaches to vitamin B-12, at this point, as noted in Eschenmoser’s “ Studies on Organic Synthesis” , “ the B/C components in the two synthetic approaches to cobyrinic acid are, in fact, identical”. The overall reaction involves an iminoester-enamine condensation and sulfide contraction via alkylative coupling. Stated simply this involves a condensation reaction and a sulfide contraction reaction.

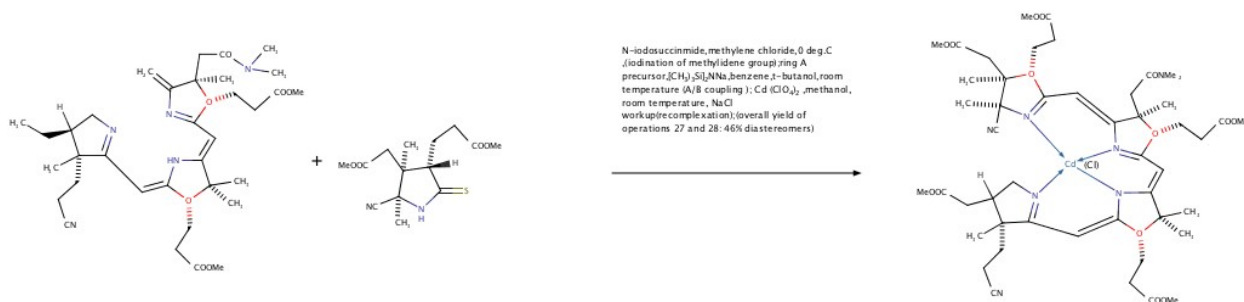
27.



The general reaction involves compound 26 reacting with dimethylamine in methanol at room temperature with the product not being isolated. This reaction resulted in the formation of compound 27. Based on observation and reading from Eschenmoser’s lecture notes “ Studies on Organic Synthesis” this reaction has several key features worth noting. It involved “ mild aminolysis of the tetracyclic thiolactone with dimethylamine in methanol”. Moreover “ this introduction of the exocyclic methylidene double bond does, very fortunately indeed, avoid a dangerous precipice, namely, the thermodynamically highly favored formation of the endocyclic enaminoisomer which has been found to be a dead-end compound with respect to our synthetic aim”. Added to this the “dimethylamine is presumed to attack specifically the

thiolactone carbonyl function to form the tertiary mercapto intermediate which loses the mercapto group by a dissociation driven by the (NH)-electron lone pair of the enaminoid chromophore. The highly electrophilic tris-ketimine intermediate stabilizes itself by enamination and it seems to do this faster by going to the exocyclic methyldiene isomer than to the endocyclic analog. The latter, on the other hand, is formed exclusively under equilibrating conditions". The overall reaction involves the mild aminolysis of the tetracyclic lactone. Stated simply this is an aminolysis reaction of compound 26.

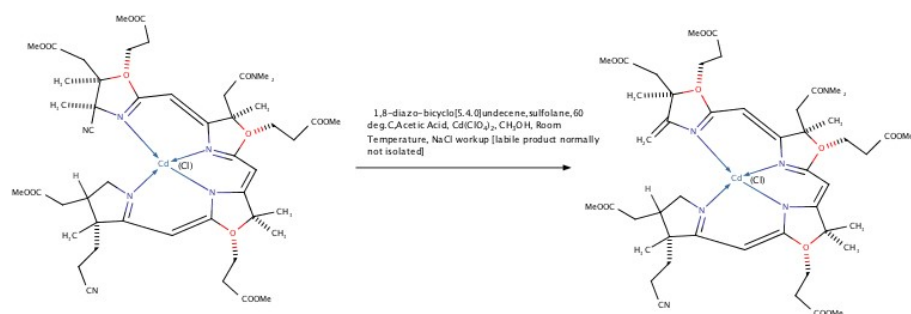
28.



The general reaction involves compound 27 reacting with N-iodosuccinimide, in methylene chloride at 0 degrees Celsius. This reaction resulted in the formation of compound 28. Based on observation and reading from Eschenmoser's lecture notes "Studies on Organic Synthesis" this reaction has several key features worth noting. It involved "iodination of the methyldiene group with iodosuccinimide, alkylative coupling with the cyano-protected thiolactam form of ring A in the presence of sodium-hexamethylsilazane, complexation with zinc perchlorate, and finally, acid-catalyzed contraction in the presence of triphenylphosphine leads (after recomplexation and

chromatography) to a material which has, as we firmly believe, the constitution of the A/D-seco-corrinoid zinc complexes". The overall reaction involves iodination, alkylative coupling and complexation to form compound 28. Stated simply this is compound reaction in which several chemical processes are occurring to form the product.

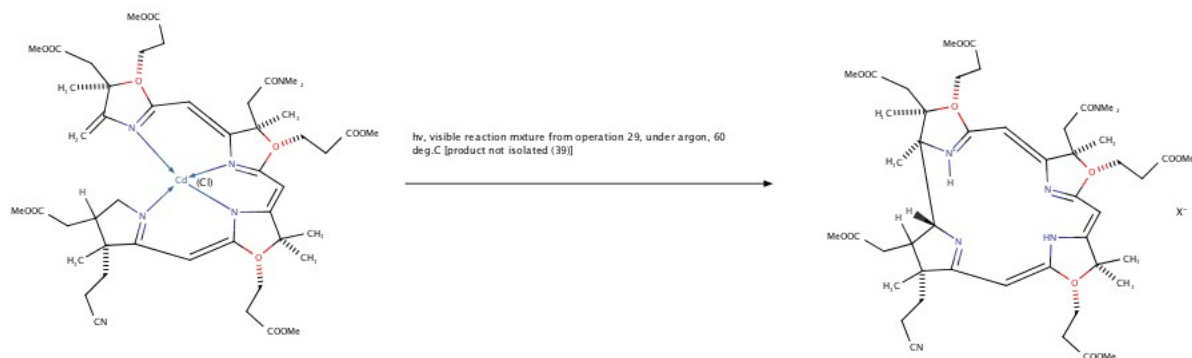
29.



The general reaction involves the reaction of compound 28 with 1,8-diaza-bicyclo[5,4,0]-7-undecene, in sulfolane at 60 degrees. Followed by the reaction of the intermediate and acetic acid with cadmium perchlorate, in methanol at room temperature with a sodium chloride workup. It was also noted that the labile product was not normally isolated. Based on observation and looking at Eschenmoser's lecture notes specifically "Studies on organic synthesis", "the solution looks simple in retrospect: 1,8-diaza-bicyclo[5,4,0]-7-undecene converts the protected zinc complex, on heating in sulfolane, into a solution of a horribly unstable material which, on the basis of its electronic spectrum, we presume to contain primary elimination products". After which the solution was transferred into strictly degassed methanol, and "buffered with zinc-chloride, under carefully defined conditions". The overall reaction involves the elimination of the

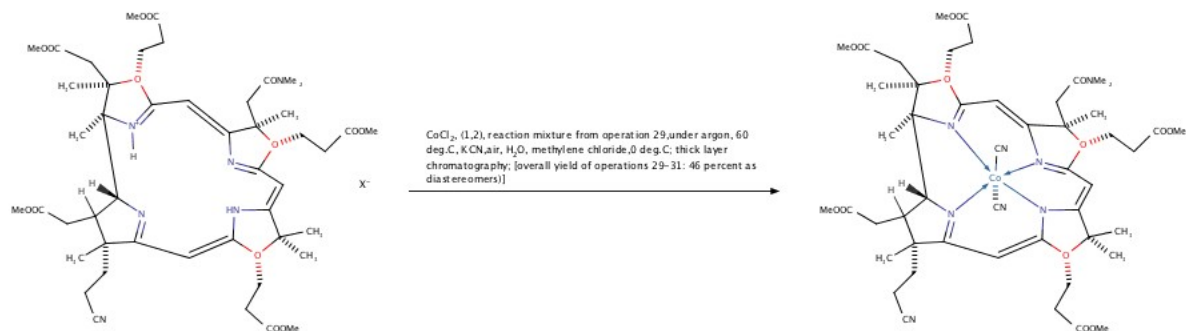
cyano group on ring A, and the conversion of the methyl ,next to the cyano group, to a methyldiene. Stated simply this reaction involves the careful formation of a methyldiene on ring

30.



The general reaction involves the reaction of the mixture from reaction 29 with a specific frequency of light, under argon at 60 degrees Celsius to form compound 30. It was also noted that the product was not isolated. Based on observation and looking at Eschenmoser's lecture notes specifically, "Studies on organic synthesis", there are some things worth noting. First the results of this reaction were complemented by Walter Fuhrer in which another solution came about which "now shows the UV- and VIS-spectral behavior of the desired methyldiene form of the A/D-seco-corrinoid zinc-complex". Moreover the discussion continued in that "attempts to isolate the material led to serious losses, so one hastens to execution of the next step, the really crucial one, the photochemical A/D-cyclo-isomerization". The overall reaction involves the photochemical A/D-cyclo-isomerization. Stated simply this reaction in the presence of lights leads to the closure of the macrocyclic corrin ring which is compound 30.

31.



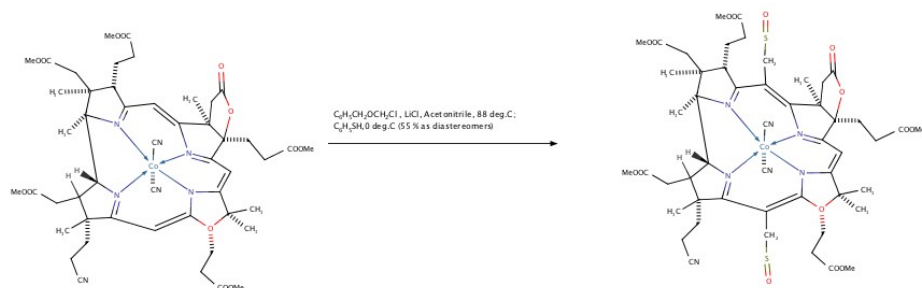
The general reaction involves the reaction of the mixture from reaction 30 with cobalt chloride, at 58 degrees Celsius. Furthermore the products of the first reaction react with potassium cyanide, air, water and methylene chloride at 0 degrees Celsius. Based on observation, this reaction involves the complexation of cobalt in the center of the macrocyclic core. Looking at the product the cobalt forms four coordinate covalent bonds. Additionally the cobalt goes through oxidative addition in which the ionic potassium cyanide dissociated in the water and donates the cyanide ligand to the metal center forming two more covalent bonds with specific stereochemistry. This overall reaction is significant since the cobalt center is important in the chemistry of B-12 as well as a basis of its name (“cobalamin”), involving the complexation of cobalt and the attachment of the cyanide ligands to the metal center. Stated simply, this reaction involves the cobalt being complexed into the center with cyanide ligands being bonded to it.

32.



The general reaction involves the product from reaction 31 reacting with iodine gas, in acetic acid and N,N-dimethylacetamide at 95 degrees Celsius using high performance liquid chromatography as the form of instrumentation to separate the diastereomers. Based on observation this reaction involves iodolactonization at ring B, and the cleavage, rearrangement and reduction of the ether at ring A. The overall reaction involves the formation of a lactone and the reduction of cyclic ether to a cycloalkane. Stated simply this reaction involved reduction and rearrangement to form compound 32.

33.



The general reaction involves the product from reaction 32 reacting with benzyl chloromethyl ether and lithium chloride in acetonitrile at 88 degrees Celsius. Followed by the reaction of benzyl thiol at 0 degrees Celsius. Based on observation this reaction involves the formation of methyl thionyl attaching to the connecting bridges between rings A and B and C and D. The overall reaction involves the formation of methyl-thionyls at specific locations on the macrocyclic structure. Stated simply this reaction involved the formation of methylated thionyls at specific locations on the macrocyclic ring to form compound 33.

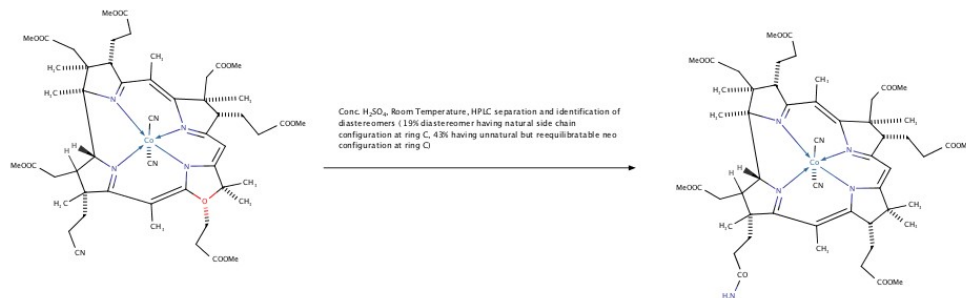
34.



The general reaction involves the product from reaction 33 reacting with zinc amalgam at room temperature. Followed by the reaction of the intermediate with diazomethane in ether and methylene chloride. This reaction resulted in a yield of 40 to 51% Based on the observation this reaction involves the reduction of the methenyl-thionyl to a methyl group. The overall reaction is

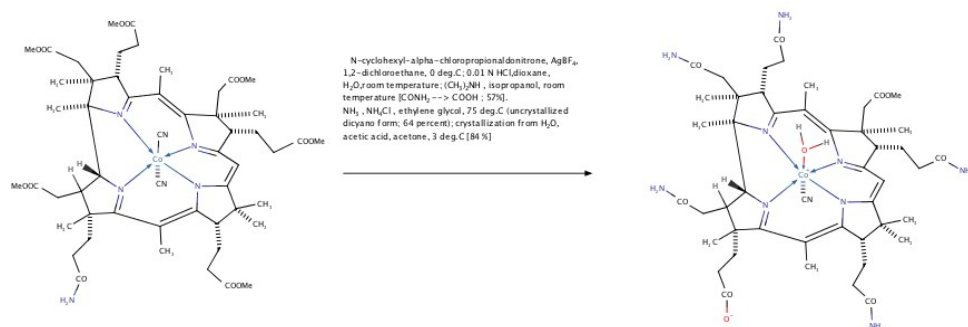
a reduction reaction that results in the loss of the thionyl and the conversion of the methylene to a methyl group. Stated simply this reaction involved reduction and elimination to form compound

35.



The general reaction involves the product from reaction 34 reacting with concentrated sulfuric acid at room temperature. Following this HPLC was done to separate diastereomers. This resulted in 19% of diastereomers having natural side configuration at ring C, and 43 % having the unnatural but equilibrate neo configuration at ring C. Based on observation this reaction involved the ring cleavage, rearrangement and reduction of the cyclic ether to a cyclic alkane ring. The overall reaction involve the formation of cyclic alkane from a cyclic ether.. Stated simply this reaction involved reduction and rearrangement to form compound 35.

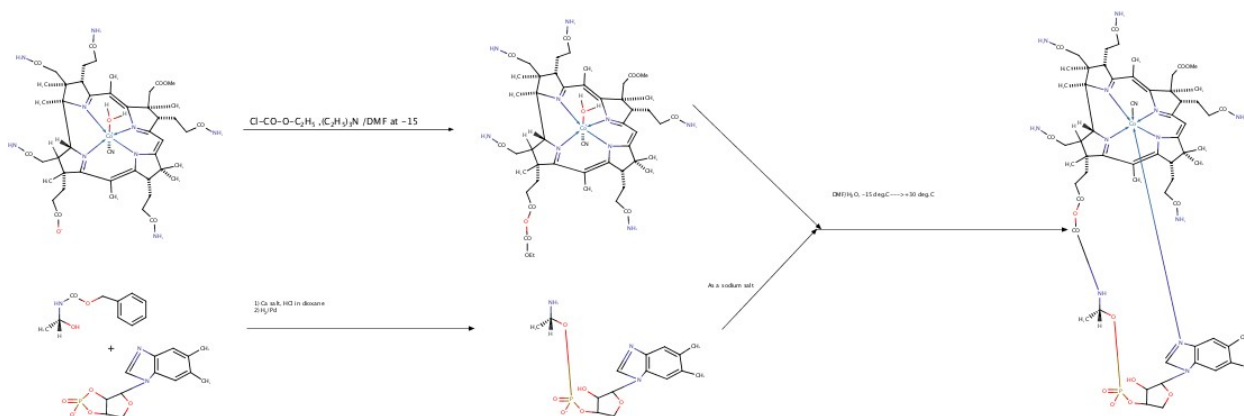
36. and 37.



The general reaction involves the product from reaction 35 reacting with N-cyclohexyl- α -chloropropionaldonitrone, AgBF_4 , 1,2-dichloroethane at 0 deg.C. Followed by the reaction with 0.01 N HCl, dioxane, H_2O at room temperature. After which the product of the previous reaction reacted with dimethylamine ($(\text{CH}_3)_2\text{NH}$), isopropanol at room temperature [CONH₂ \rightarrow COOH ; 57%]. After that the product of the previous reaction was involved the reaction with ammonia, ammonium chloride, and ethylene glycol at 75 deg.C, noting that there was uncrystallized dicyano form in 64 percent of the mixture. Moreover there was crystallization from H_2O and the reaction with acetic acid and acetone at 3 deg.C. This resulted in a product yield of 84 %.

Based on observation this reaction involved the replacement of one of the cyanide ligands with a water ligand resulting in compound 36. Stated simply this reaction involved replacement of cyanide ligand with water as the ligand.

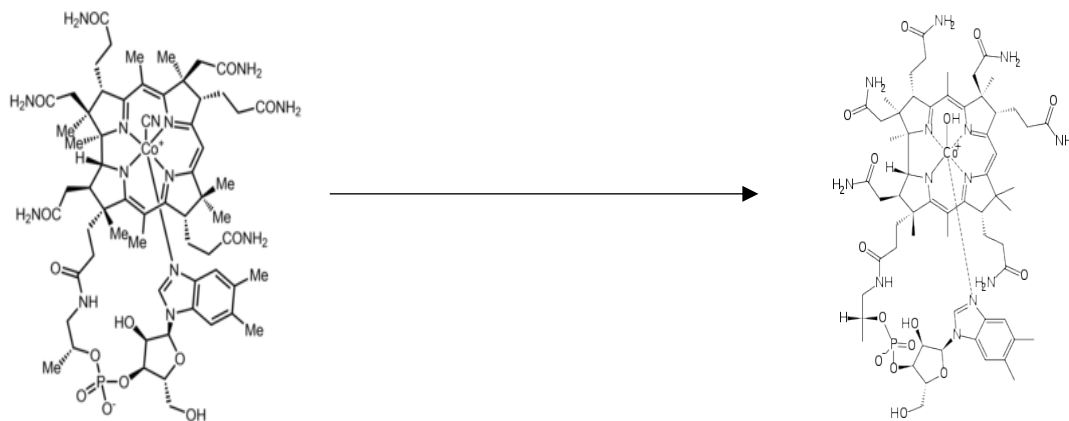
38.



The general reaction involves three main parts. The first part that will be discussed involved the product of reaction 36 reacting with ethyl chloroformate and triethylamine in dimethylformamide at -15 degrees Celsius. The second part involved the formation of the ribofuranosyl-5,6-dimethyl-benzimidazol-phosphoric acid-diester using the reactants as shown above reacting with calcium salt in hydrochloric acid and dioxane and then hydrogen gas on palladium. Following this reactants combined with a particular isomer of the phosphoric acid diester as a sodium salt. The third part of the reaction involved the reactants being combined in dimethylformamide in water at -15 degrees Celsius with the temperature being increased to 30 degrees Celsius. Based on observation this was a combination reaction in which reactants were combined in separate parts first then as a whole molecule afterward specifically in this reaction you had the formation of ether linkages and phosphoester linkages. Stated simply this was a complex biochemical reaction that resulted in the formation of cyanocobalamin.

Laboratory Applications:

1. THE CONVERSION OF CYANOCOBALAMIN TO HYDROXYCOBALAMIN:



This can occur under specific frequencies of light namely UV light as well as under oxidative conditions such as with ascorbic acid. The confirmation of the success of the reaction can be gained through the use of UV spectra. There is a difference in the third peak of cyanocobalamin and hydroxycobalamin. The difference comes into play in that cyanocobalamin has a third peak at 550nm and hydroxycobalamin has a third peak at 526nm. Additionally reagents are easily obtainable.

Appendix

Some Notable Quotes:¹⁸

“The emergence of the Woodward-Hoffman rules out of such a situation is an extreme example (2) and its impact on chemistry as a whole is beyond any comparison.... The very existence of these rules had stimulated, encouraged and assisted experimental involvement in a research project which eventually led to a new type of corrin synthesis.”

“The photochemical ring-closure reaction has been intensively investigated in our and other laboratories during the last three years.”

“The overall process is formally envisaged as a thermally forbidden, antarafacial sigmatropic 1,16-hydrogen transfer merged with, or followed by, a thermally allowed 1,15 ($\pi \rightarrow \sigma$) isomerization, all taking place in a helical conformation of the seco-corrinoid ligand system.”

“ Both the constitution, as well as the trans-configuration of the cyclization products have been rigorously proved by independent syntheses and, in some cases, by X-ray analyses”

“However the occurrence or non-occurrence of the cycloisomerization reaction depends on more than ground state geometry”

“Nevertheless, no luminescence of seco-corrinoid complexes with metal ions, such as palladium and zinc, could be observed by these workers. However, the corresponding cyclized, structurally more rigid corrin complexes do show luminescence”

“ In short, those transition-metal ions that quench luminescence of the excited corrin chromophore by virtue of their unfilled d-shells, also seem to thwart photochemical cycloisomerization of the corresponding A/D-seco-corrinoid complexes”

“Out of all these studies it has become increasingly clear that the A/D-seco-corrin → corrin system offers an optimal opportunity to study relationships between the nature of the metal ion complexation centers and the photochemical behavior of excited porphyrinoid ligand chromophores”

“Clearly as a reflection of underlying structural regularities in the (essentially still unknown) biosynthesis of the natural corrinoids from the monocyclic pyrrolic precursor porphobilinogen, it seems possible to convert a single starting material (the C10-dilactone acid of figure 7) into three optically active, chirally correct, monocyclic intermediates that can serve as the precursors of all four rings of the cobyrinic acid molecule.”

“ the precursors of ring A and B are structurally identical, whereas the precursor of ring C differs by having the carboxy function of the acetic acid side chain replaced by hydrogen. In the ring D precursor, the attachment of the two carboxy side chains appears both constitutionally and configurationally interchanged, presenting an arrangement obviously related to the biosynthetically puzzling ring D pattern of natural type III porphyrins”.

Characteristics of the cobyrinic acid molecule complex

“ The cobyric acid molecule contains all peripheral carboxy functions in the primary amide form, except that of the propionic side chain in ring D.”

Problems that had to be solved

For rings A and B they are:

1. Elongation of the free acetic acid chain by one methylene unit
2. Specific replacement of one lactone oxygen by NH
3. Conversion of the potential methylketone group into the enamide form.

Note: An essentially analogous reaction sequence starting from the enantiomeric form of the C₁₀-dilactone acid leads to the skeleton of the ring D precursor, provided that not the free, but the lactonized (-CH₂-COO)- chain is lengthened by one methylene unit”

The conversion of ring B to the precursor of ring C requires a method for specific removal of the carbomethoxy group of acetic acid side chain and its replacement by hydrogen.

End of introduction: As Eschenmoser¹⁹ wrote in his lecture “but I should perhaps propose that we enjoy the figure just from an aesthetic point of view, by watching the corrinoid chromophore system evolve, like a bud blooming into a flower”.

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