

Vitamin B12 for Human Body: Synthesis and Function

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Abstract. Vitamin B12, a vital trace element, plays a pivotal role in maintaining nerve and blood health in the human body. It has found extensive use in the medical field, serving as a key component in the treatment of a myriad of health conditions. This paper seeks to provide a concise yet comprehensive overview of vitamin B12, particularly focusing on its synthesis process, thereby serving as a valuable resource for novices in the field. The study delineates the foundational knowledge pertaining to vitamin B12, subsequently delving into the intricate process of its total synthesis, a groundbreaking development orchestrated by Robert Burns Woodward and Albert Eschenmoser in 1972. This seminal work marked a significant milestone in the field of chemistry, paving the way for further advancements in understanding and utilizing this essential vitamin. By offering a succinct overview of vitamin B12 and its synthesis, this paper aims to facilitate a swift yet thorough understanding for beginners, fostering a deeper appreciation for the complex processes underlying the synthesis of this critical nutrient. Through this exploration, readers are expected to gain a nuanced understanding of the vital role and synthesis of vitamin B12, equipping them with the foundational knowledge necessary to delve deeper into this fascinating study area.

Keywords: Total synthesis, vitamin B12, cobalamin, stereochemistry, human health.

1. Introduction

Vitamin B12, scientific name is cobalamin. The standard state of vitamin B12 is a solid, red crystalline powder. Vitamin B12 is odorless, tasteless, and water-soluble [1, 2]. Vitamin B12 is one of the most complex vitamins. Its chemical formula is $C_{63}H_{88}N_{14}O_{14}PCo$. Vitamin B12 consists of a cobyrinic acid as the main body, followed by the addition of a coenzyme. George Whipple discovered that patients with severe anemia could rely on eating animal liver to relieve their symptoms [3].

Vitamin B12 was noticed in the 1920s by George Whipple and William Murphy, and intervention studies determined it to be present in the animal liver. They were awarded the 1934 Nobel Prize for their work. In 1964, British scientist Dorothy Hodgkin and her team determined the chemical structure of vitamin B12 and successfully extracted it from animal livers. In 1973, the total synthesis of vitamin B12 was developed by Woodward and Eschenmoser, respectively. People study vitamins so much because vitamins are natural organic compounds necessary for the growth and maintenance of organisms.

Each vitamin has its function [4]. The two main functions of vitamin B12 are as a cofactor of methyltransferase and to protect folate transfer and storage in cells. Vitamin B12 is an essential micronutrient for growth and is involved in protein synthesis, neurolipoproteins synthesis, and red blood cell synthesis [4]. Lack of vitamin B12 will cause deficiency disease of slowed growth, decreased thinking ability, anemia, etc. [5]. In most cases, natural vitamin B12 is synthesized by bacteria, and complex plants and animals cannot synthesize vitamin B12 on their own. Fortunately, humans and animals cannot produce vitamin B12, but it can be obtained through food or supplements. Vitamin B12 is commonly found in meat, such as animal liver and sardines, and vitamin B12 supplements are often synthesized in chemistry.

People can usually get enough vitamin B12 through a normal diet. People mainly use vitamin B12 supplements to treat the symptoms of deficiency or anemia caused by various causes [6]. It is also occasionally used to treat peripheral neuroinflammation and peripheral paralysis, a condition in which the hands and feet are partially painful due to peripheral neuropathy [7].

2. Effects of Vitamin B12 on Human Health

Vitamin B12 helps transfer methyl in the body to help produce proteins. Vitamin B12 can act as a cofactor of methyltransferase, which helps the methyl receptor become a methyl derivative, such as Methionine (C₅H₁₁NO₂S), an important building block of proteins. A lack of vitamin B12 can cause a baby or child to grow slower [2]. Vitamin B12 works with folic acid (C₁₉H₁₉N₇O₆, aka Vitamin B9, Vitamin Bc, and Vitamin M) to promote the development and maturation of red blood cells. Vitamin B12, as a coenzyme, increases the efficiency of folate. Vitamin B12 deficiency may lead to megaloblastic anemia due to insufficient folate [2, 5].

Vitamin B12 activates amino acids to facilitate the synthesis of nucleic acids, a process that also helps form proteins and blood [5]. Vitamin B12 is a cofactor of two major enzymatic reactions, which can help the formation of DNA and metabolize useless by-products. The vitamin helps synthesize methionine. Vitamin B12 can also use adenosyl-cobalamin form as a cofactor to help convert methyl malonyl CoA to succinyl CoA, which can help hemoglobin formation. Many practical studies demonstrate that vitamin B12 is associated with promoting the treatment of nerve damage. Vitamin B12 can treat some neurological conditions by treating or repairing the myelin sheath [8-12], which is the substance that protects the axons of neurons. Vitamin B12 is now used in the clinical treatment of many neurological disorders [13-16].

3. Synthesis Strategies of Vitamin B12

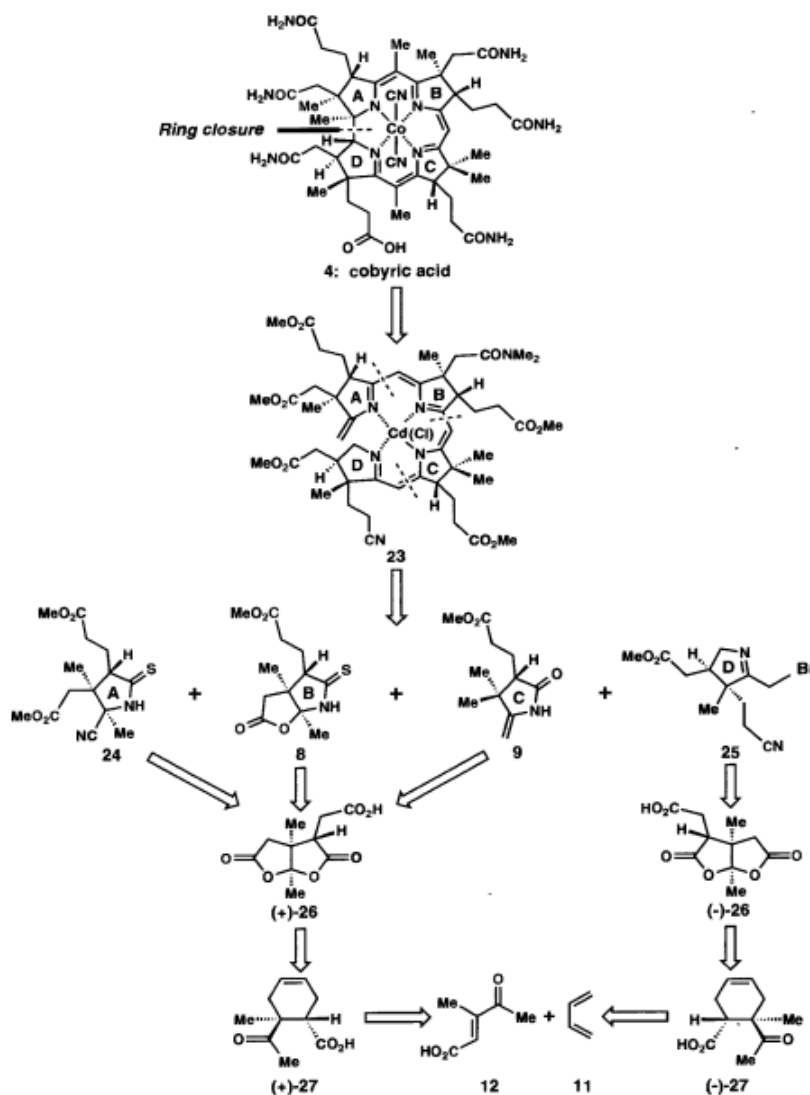


Figure 1. Eschenmoser's retrosynthesis [1]

The total synthesis of vitamin B12 was invented in 1972 by Robert Burns Woodward and Albert Jakob Eschenmoser. Woodward's Harvard and Albert Jakob Eschenmoser's ETH group worked for 11 years and developed two very different synthesis strategies. However, the final part of the total synthesis of vitamin B12 was the Harvard team and ETH team chose to complete together. So, the preparatory intermediate produced by their macrocyclization is the same as the following chemical transformations. Eschenmoser's total synthesis was first synthesizing A, B, C, and D rings independently. Macrocyclization then occurs to form preparatory intermediates. Finally, chemical transformations occur to form cobyrinic acid, which is synthesized with coenzymes to form vitamin B12. Furthermore, the difficulties encountered during the development of vitamin B12 led scientists to discover stereochemistry. The illustration of Retsosynthesis gives us a good perspective to understand Eschenmoser's roughly total synthesis approach (Fig. 1).

Woodward's synthesis strategy was first to synthesize the AD ring, cyanobromide, and the BC ring, thiodextrolin. The AD and BC rings then react with an S_N2 substitution, creating a single carbon-sulfur bond between the two rings to form thioether type I. After a series of chemical macrocyclization reactions, AD ring and BC are joined by two bonds to form a preparatory intermediate bisnorcobyrinic acid abdeg pentamethyl ester.c dimethylamide f nitrite. At this point, the intermediate is similar to cobyrinic acid, the main component of vitamin B12. Finally, the intermediate undergoes some chemical conversion steps to become cobyrinic acid, which is synthesized with coenzymes to form vitamin B12 (Fig. 2).

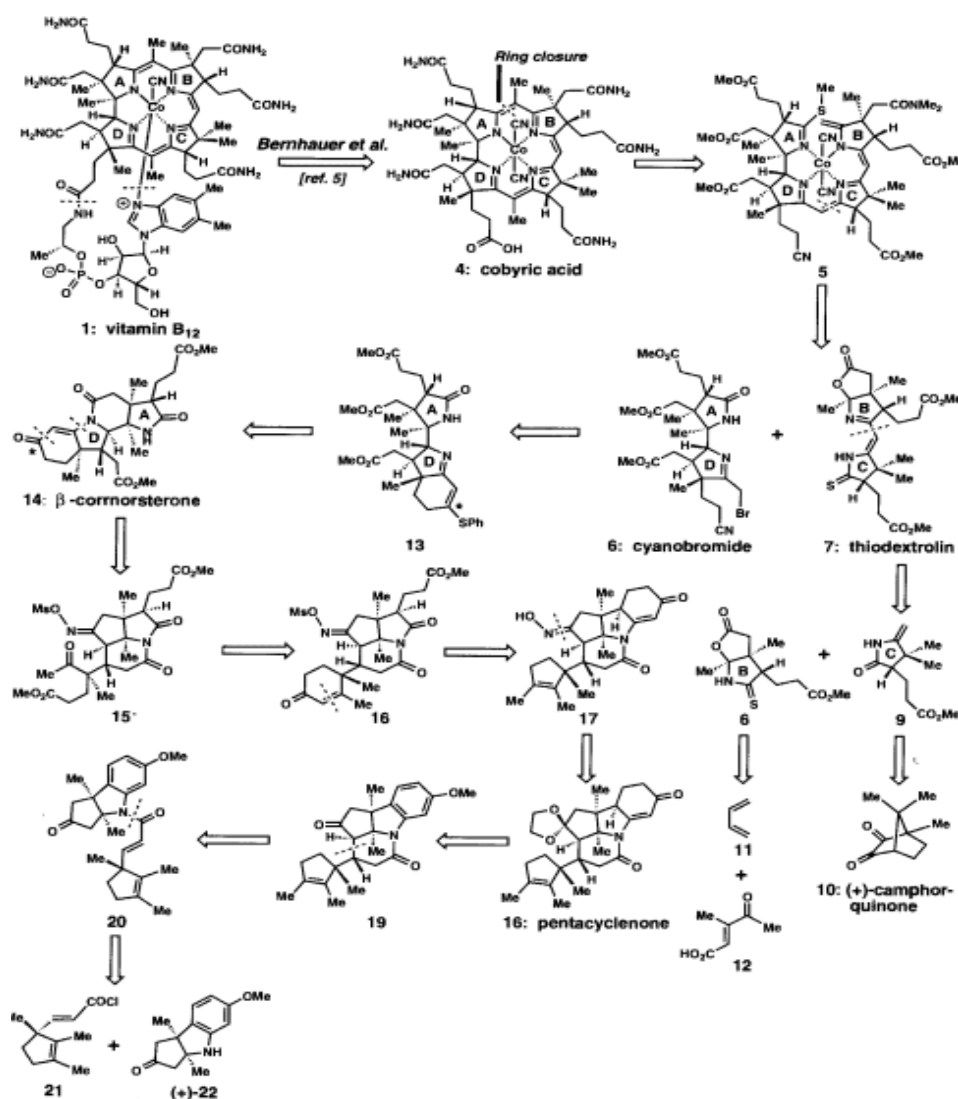


Figure 2. Woodward's retrosynthesis [1]

4. Total Synthesis of Vitamin B12

4.1. Synthetic Strategy of Cyanobromide

Woodward's Harvard group mainly develops the synthesis strategy of AD ring, that is, the synthesis strategy of cyanobromide. Woodward's group provided valuable, instructive comments and explanations for developing the synthesis [10]. Although Eschenmoser's ETH team also found a new AD ring development strategy and a special circularization method later. However, because of the discovery and research of stereochemistry by the Woodward group, it still needs to be found and solved from Woodward's synthesis strategy to understand the selection and synthesis of some chemical solvents fully.

4.1.1 Synthesis of material 21 and 22

All steps are shown in Fig. 3. The synthesis of cyanobromide starts with material 21 and material 22. Material 21 (1, 2, 3-trimethylcyclopentene) can be prepared from L-camphor by substitution reaction and Wittig reaction. Material 22, starting with three additional methyl oxindoles (methoxydimethylindol, 28), was treated with a magnesium salt to give intermediate 29. A chiral center is obtained at this step. To obtain tricyclic ketone 22, regarding chemical space selectivity, BF_3 and HgO with special properties must be used as solution conditions. At this time, the obtained tricyclic ketone is a racemic mixture, which means that it is a mixture of L-tricyclic ketone and D-tricyclic ketone. However, only D-tricyclic ketone is required, so treatment with α -phenyl ethyl-isocyanate is also required for purification.

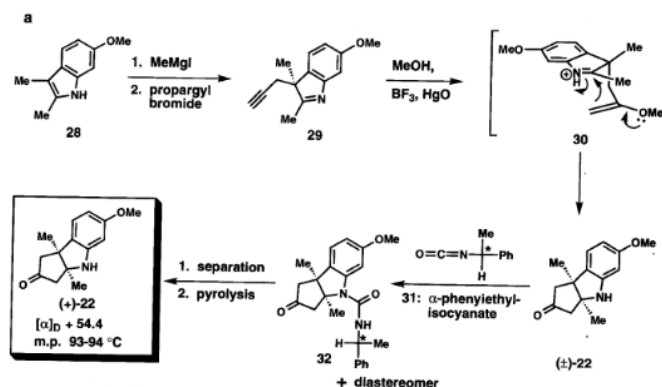


Figure 3. Synthesis of tricyclic ketone [1]

4.1.2 Convert β -cornerstone

An acylation reaction occurs between the hydrogen atom of D-tricyclic ketone and the aliphatic acyl group of trimethyl-cyclopentane (Fig. 4), and the two compounds are connected in the form of ketone. In intermediate 20, the intermediate expects the ring to connect and try to get as many correct products as possible. Then, in the chemical space selection, people need to use macromolecular solvents. In this process, *t*-BuOK and *t*-BuOH are not consumed.

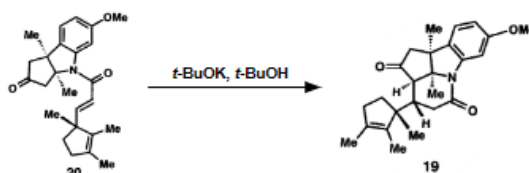


Figure 4. Acylation reaction [1]

In a two-step reaction, the ketone in intermediate 19 was reacted with alcohols and then alkylated by Meerwein salt (Et_3OBF_4). Subsequently, a simple $\text{S}_{\text{N}}2$ reaction and proton transfer occur. The amine cation from the previous step is replenished. These steps mainly liberate the aromatic rings for the Birch reduction reaction, previously used to stabilize the molecule. After the conditions are met, metal dissolution and Birch reduction occur. Then, add mild acid form pentacyclenon 18 (Fig. 5).

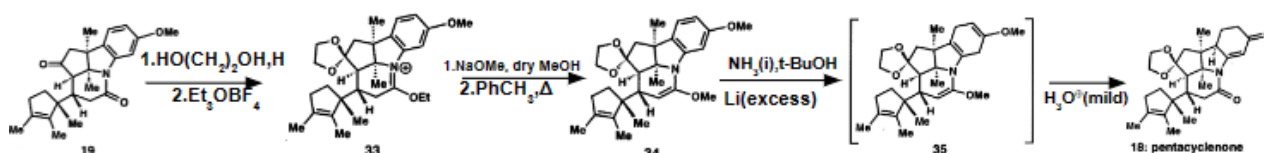


Figure 5. Convert intermediation 19 to 18 [1]

The pentacyclenone is also subjected to aqueous acid cleavage followed by oximation to form a suitable intermediate to produce β -cornorsterone, which generates the oxime nitrogen attached to the central cobalt atom. In this step, the bis-oxime product is inevitably produced. Therefore, one deoxime is required to form a monoxime product (Fig. 6).

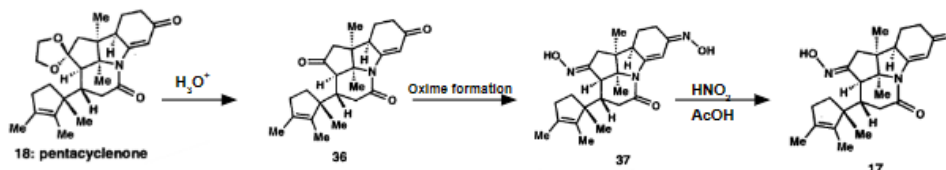


Figure 6. Convert intermediation 18 to 17 [1]

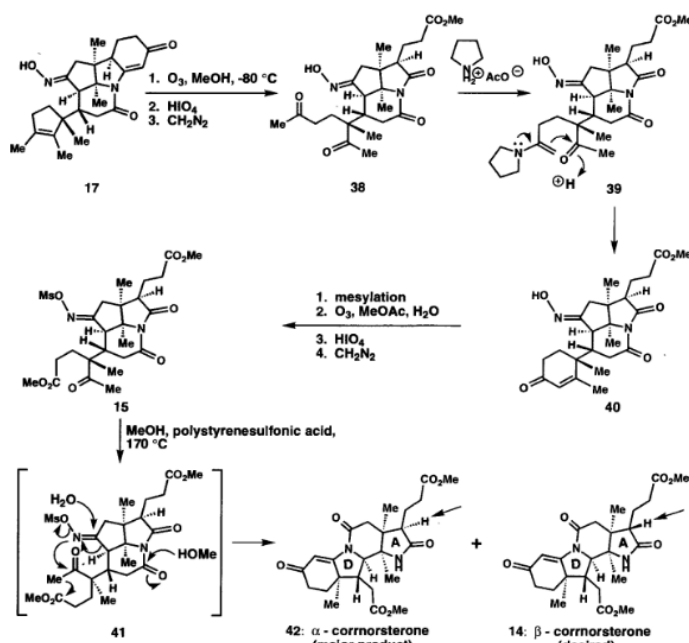


Figure 7. Convert intermediation 17 to 14 [1]

Multiple ozone cleavages are required to convert pentacyclic ketones to cornorsterones with AD rings. The main purpose of the first ozonolysis is to change the bottom ring to cyclohexenone. The second ozone cracking is to esterify the lower ring to induce the connection of cyclohexenone to the nitrogen atom to form the D ring as well as cleavage of the ketone adjacent to the A ring, which allows the nitrogen atom on the A ring to form an amine. So far, materials 21 and 22 have been successfully transformed into a racemic mixture of cornorsterone 14, which is very similar to cyanobromide. However, regarding chemoselectivity, we need β -cornorsterone as the conversion cyanobromide rather than the main product α -cornorsterone. Unfortunately, few efficient ways exist to convert the main product to β -cornorsterone (Fig. 7).

4.1.3 Formation of cyanobromide

β -cornorsterone has two redundant rings. To leave only A ring and D ring in the final product, it must be cleaved with alcohol and ozone. Finally, after some simple substitution reactions, cyanobromide 6 was prepared. The key parts of cyanobromide are the bromide attached to the D ring and the ketone on the A ring because they provide the initial ability to bind to thiodextrolin, as shown in Fig. 8.

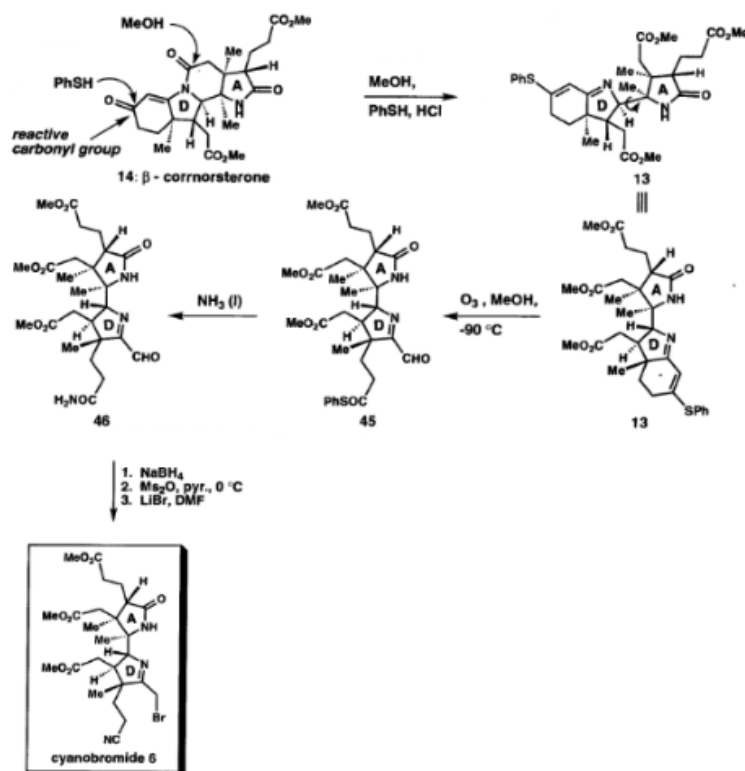


Figure 8. Convert 14 to cyan bromide [1]

4.2. Synthesis Strategies of Thiodextrolin

The synthetic development of thiodextrolin was mainly developed by the ETH group of Eschenmoser. Woodward's Harvard group had only alternatives for synthesizing the C ring. In this part, the synthesis strategy of Eschenmoser will be the main one. Different from the synthesis of AD rings, thiodextrolin is synthesized by ring B and ring C directly binding. The first part will be how to synthesize the material of thiodextrolin.

4.2.1 B ring synthesis

Eschenmoser used the Diels-Alder reaction to select the B ring synthesis strategy of vitamin B12. This is an extremely convenient way to form six-membered rings using conjugated dienes and dienophiles. Moreover, this reaction will not destroy the stereochemical structure to a great extent. The activated dienophile undergoes a Diels-Alder reaction with butadiene, and the double bond on the butadiene is transferred and unfolded to form a cyclohexene. According to the stereochemistry, treating the racemic intermediate (\pm)-27 by α -phenylethylamine is necessary to separate the enantiomeric dextrorotatory intermediate (+)-27 (Fig. 9).

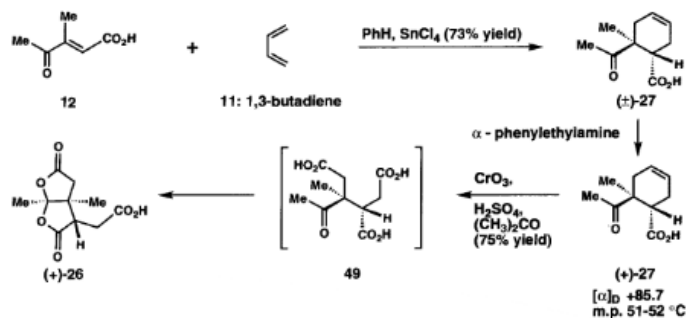


Figure 9. Synthesis of intermediation 26 [1]

Subsequent oxidative cleavage of the ring with chromic acid and adding $(\text{CH}_3)_2\text{CO}$ under sulfuric acid conditions forms two rings. The lower ring is the B ring, the upper ring will be kept until the macrocyclization, and it will be the part connecting the A ring. The addition of Arndt-Eistert

homologation to dextrorotatory intermediate 26 gives intermediate 50. The arndt-Eestert reaction is the process of conversion of carboxylic acids to their homologs (Fig. 10).

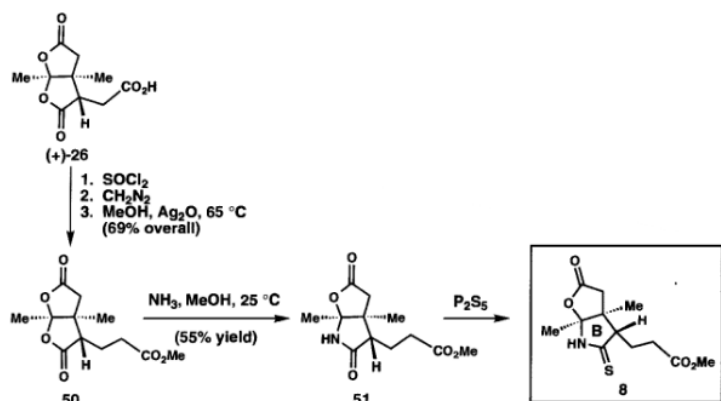


Figure 10. Synthesis of ring B [1]

In the final step, compounds require chemical moieties that can be linked to other compounds. The first step uses methanol as an auxiliary leaving group to help the oxygen atom leave the ring, followed by using an amine as a nucleophile to attack the carbocation, and finally, two hydrogen atoms leave the nitrogen atom. Hydrogen nitrogen replaces the oxygen atom, and this moiety is used to link the cobalt in the macrocyclization step. Another place where a substitution reaction occurs is when the underlying oxygen atom is replaced with a sulfur atom. This part is used to connect with the C ring. After completing the synthesis of the B ring, there are three connecting parts on the B ring: the first is the upper ring, which is used to connect the AD ring, and the second is the NH in the B ring, which is used to connect the cobalt atom, and the third place is the sulfur atom below, which is used to connect the C ring.

4.2.2 C ring synthesis

Eschenmoser found that the precursor 51 of the B ring intermediate 8 can be used to synthesize the C ring, and the steps are very simple. In the first step, intermediate 51 was treated with diazomethane and sodium methoxide under a diethyl ether and methanol mixture to obtain intermediate 57. This step disassembled the six-membered ring, and in a subsequent step, the ring was reconstituted with the addition of bicyclor octan. Finally, Wilkins catalyst is converted to intermediate 9, the precursor of the C ring (Fig. 11).

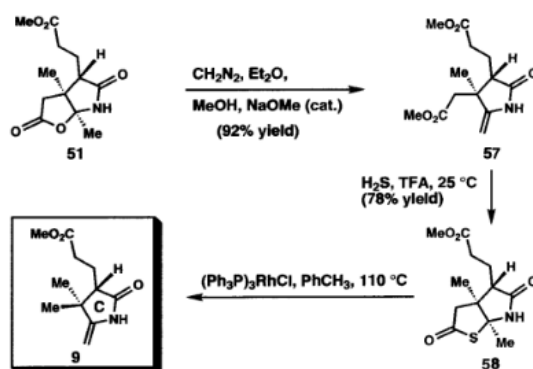


Figure 11. Synthesis of ring C [1]

4.2.3 Synthesis of thiodextrolin

The BC ring synthesis is much simpler than the AD ring synthesis. The intermediate 69 can be formed between the B and C rings using a benzoyl proxy catalyst to establish a sulfur bridge under dichloromethane. After the addition breaks the sulfur bridge of the solvent, the carbon atoms on both sides of the sulfur atom will combine because they are too close to form intermediate 70. The reason may be that carbon and sulfur atoms have the same valence electrons and similar properties. Finally, after O-alkylation by Meerwein salt, the O-C double bond is replaced by the S-C double bond. The

sulfur atom here will be connected to the bromine atom of the AD ring in the subsequent macrocyclization, providing the initial connection between the AD ring and the BC ring (Fig. 12).

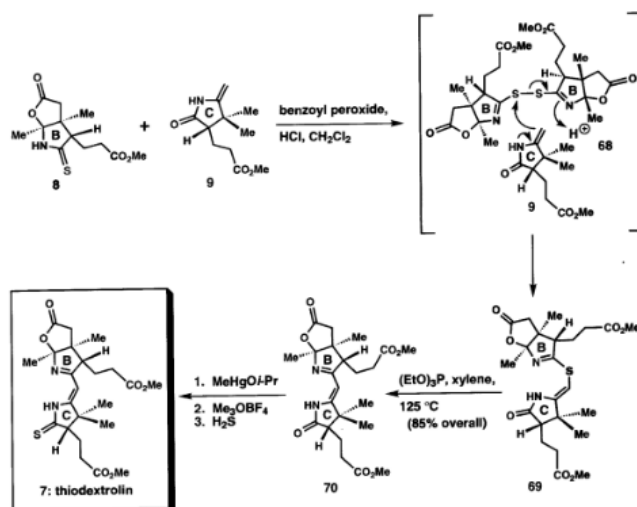


Figure 12. Synthesis of thiodextrolin [1]

4.3. Macrocyclization

Woodward and Eschenmoser developed two macrocyclizations. Woodward's and Eschenmoser's invented a way to connect the AD ring to the BC ring. Later, Eschenmoser's group developed another method to directly connect the A, B, C and D rings. It took Woodward and Eschenmoser several years to develop the macrocyclization scheme, which is quite difficult (Fig. 13).

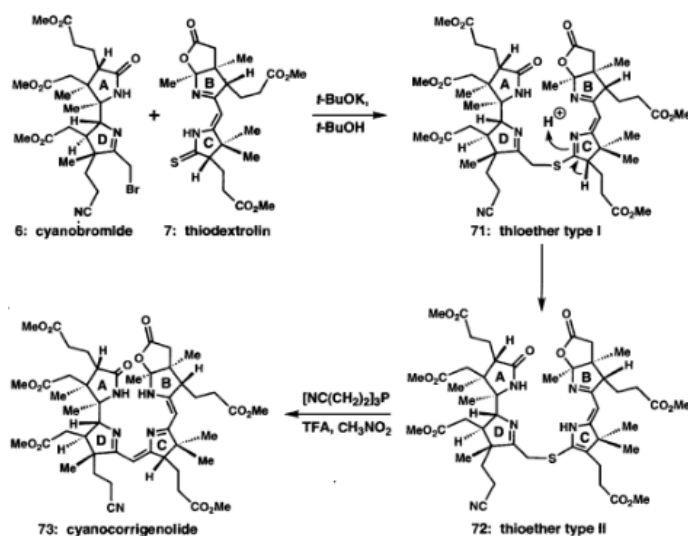


Figure 13. Synthesize cyanobromide and thiodextrolin [1]

During the initial conjugation, cyanobromide 6 on the left wing of vitamin B12 acts as an alkylating agent because of its single-bonded bromine atom. The right-wing thiodextrolin of vitamin B12 is a nucleophile. However, the thioamide group is not a good leaving group and needs the help of potassium tert-butoxide to form the ion, forming thioether type I 71. Thioether type I 71 will spontaneously form thioether type II 72. This transformation destroys the original chiral center, requiring additional combined reactions of tris- β -cyanoethylphosphine and cyanocorrigenolide to repair the chiral center and form cyanocorrigenolide 73 (Fig. 14).

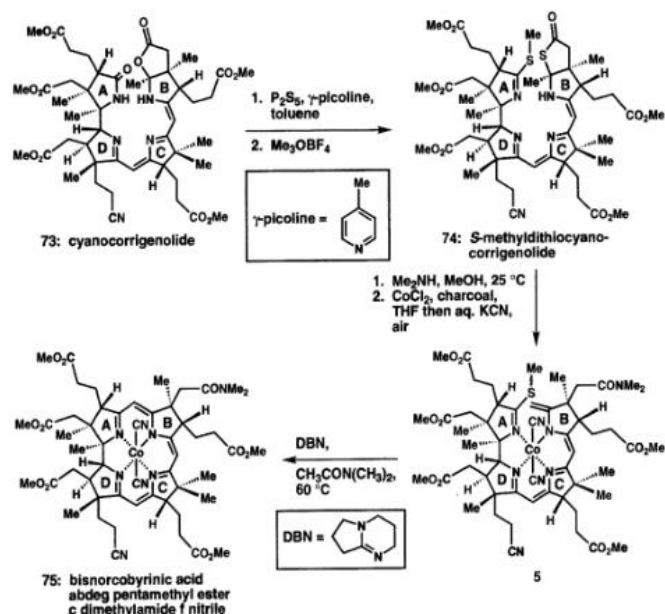


Figure 14. Convert intermediation 73 to 75 [1]

The cyanocorrigenolide 73 is still not the most suitable state for cyclization, and the acyl groups of ring A and ring B are replaced by thiocarbonyl to form S-methyldithiocyanocorrigenolide 74. Phosphorus pentasulfide was used here, and this compound also appeared frequently in the previous synthesis. The five-membered ring above the B ring is now cleaved by methanol, providing a double bond and a carbon atom. Finally, THF was used to stabilize the compound to obtain intermediate 5. The cyanocorrigenolide 73 is still not the most suitable state for cyclization, and the acyl groups of ring A and ring B are replaced by thiocarbonyl to form S-methyldithiocyanocorrigenolide 74. Phosphorus pentasulfide is used here, and this compound also often appears in the previous synthesis. S-methyldithiocyanocorrigenolide 74 is still not suitable for cyclization. The five-membered ring above the B ring is now cleaved by methanol, providing a double bond and a carbon atom. Then, add cobalt chloride to make the compound obtain the central cobalt atom, and the cyclization conditions are satisfied. The compound was then stabilized with THF to obtain intermediate 5. At this time, the upper bond has not been completely cyclized. Using DBN as a solvent under the conditions of dimethylacetamide and 60 degrees Celsius, the sulfur and the methyl group leave, and the single bond of the A ring is bonded with the double bond of the B ring to form an intermediate 75.

4.4. Final chemical convert

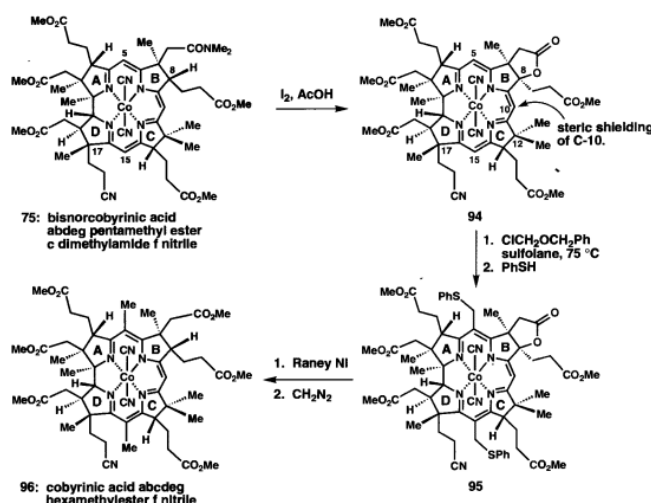


Figure 15. Convert intermediation 75 to 96 [1]

The macrocyclization part has been completed, but some compound transformations and introductions are still required. The first is the addition of methyl groups at the 15, 17 positions.

Intermediate 75 is first converted to intermediate 94 by iodine and acetic acid (Fig. 15). According to intermediate 94, it can be found that the ring at 8 merges and 10 and 12 are protected from nucleophiles under the protection of steric hindrance. Nucleophiles selectively attacked 15 and 17. Finally, using Raney Ni and CH₂N₂, the ring at 8 was cleaved, a methyl group was introduced at 15, and the intermediate 96 formed.

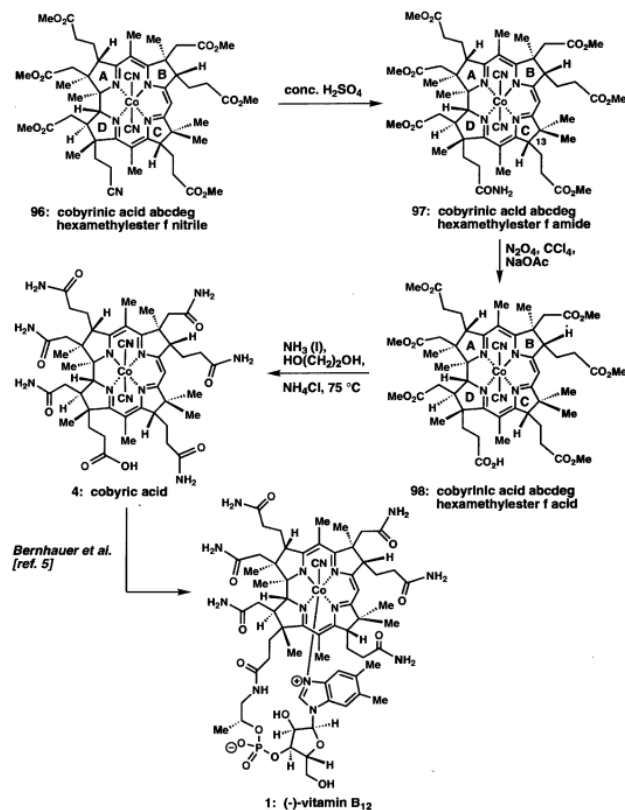


Figure 16. Final synthesis of vitamin [1]

The remaining chemical transformation is to convert the nitrile group in intermediate 96 to a primary amide group using concentrated sulfuric acid to form intermediate 97 (Fig. 16). Under strong acid conditions, a by-product can easily be produced. It differs very little from intermediate 17, with only one more chain. The proportion of this stereoisomer in the final mixture is high. Therefore, it is necessary to use the High-Pressure Liquid Chromatograph method for separation to obtain the pure form of compound 97 [1]. The synthesis still expects the compound on the 7 side chains to be correct. Therefore, in the transformation from intermediate 97 to intermediate 98, the amide is deaminated in a solution of nitrogen tetrachloride using dinitrogen tetroxide. This step is to prepare for the synthesis of intermediate 99.

Woodward's group invented another method to solve this problem (Fig. 17). Woodward's method is more efficient and productive. In the conversion of intermediate 98 to cobyric acid, all seven side chains of intermediate 98 were changed. This includes converting the ester groups at the end of the six side chains into primary amide groups and the deaminyl group at the end of the side chain below the D ring into a ketone with a carbonyl group. This step can be accomplished by heating liquid nitrogen and ethylene glycol to 75 degrees Celsius in the presence of ammonium chloride [1]. At this point, the synthesis of cobyric acid 4 is completed. Cobyric acid can be easily converted into various forms of vitamin B12 by adding coenzymes.

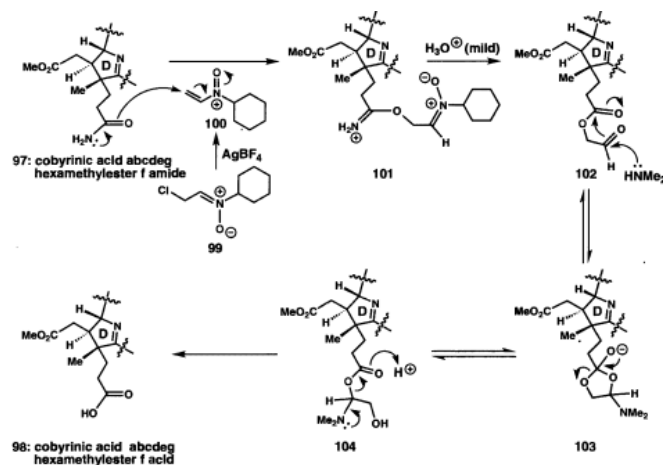


Figure 17. Final synthesis of vitamin [1]

5. Conclusion

Based on other papers and research, this thesis briefly expounds on the basic properties of vitamin B12, the chemical application of vitamin B12, the total synthesis strategy and the synthesis of vitamin B12. Vitamin B12 is undoubtedly important. The development of the total synthesis of vitamin B12 has great significance and progress for medicine and chemistry. On the medicinal level, vitamin B12 can treat common symptoms such as neurological diseases and anemia. On the chemical level, synthetic methods developed during the synthesis of vitamin B12, such as the Eschenmoser sulfide contraction reaction, and a new branch of chemistry, stereochemistry, were created.

However, many working mechanisms and practical applications of vitamin B12 are not fully understood, and there are still many studies and experiments to be done on vitamin B12. Vitamin B12 is one of the vitamins with the most complex structure and the most synthesis steps. There are certain thresholds and difficulties for beginners to understand vitamin B12 quickly. This review paper can help beginners quickly understand the role of vitamin B12 and the basic principles of the total synthesis of vitamin B12. In the future, researchers may find more facts and applications about vitamin B12. For example, many studies make people think that vitamin B12 may be related to an increased risk of cancer, but there is no definite evidence or hypothesis explaining why vitamin B12 increases cancer risk. There is also research data that vitamin B12 and folic acid may reduce the risk of dementia in the elderly, but there is no definite evidence to prove a clear relationship between them. There will be more to be discovered about the medicinal properties and facts about vitamin B12.

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