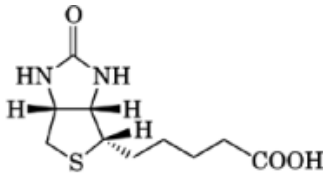
Kirk-Othmer Encyclopedia of Chemical Technology.

BIOTIN

Biotin, [3a*S*-(3a α, 4 β, 6a α)]-hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-4-pentanoic acid [58-85-5] (vitamin H, vitamin B_8, bios IIB, and coenzyme R) (**1**) is a water-soluble B complex vitamin. The name coenzyme R was coined during work on a protective factor, from the liver, for egg white injury. This protective factor was also called factor S, factor W or vitamin B_W (1–4). Biotin is a complex molecule having three stereocenters. There are eight stereoisomers of biotin; only the naturally occurring one is active in metabolism. The richest sources of biotin are yeast, liver, kidney, egg yolks, pancreas, and milk (5–7). The highest content of biotin in cow's milk occurs early in lactation. Plant materials, such as nuts, seeds, cereals such as oats and bulgar wheat, pollen, molasses, rice, soybeans, mushrooms, fresh vegetables such as cauliflower, split peas, cow peas, and legumes, and some fruits, are also good sources. In addition, small amounts of biotin are found in most fish, eg, mackerel, salmon, and sardines.



(**1**)

1. Isolation

In 1936, biotin was isolated from egg yolks (8), in 1939 from beef liver (9), and in 1942 from milk concentrates (10). Biotin-producing microorganisms exist in the large bowel but the extent and significance of this internal synthesis is unknown.

2. Biochemical Function

Biotin forms part of several enzyme systems and is necessary for normal growth and body function. Biotin functions as a cofactor for enzymes involved in carbon dioxide fixation and transfer. These reactions are important in the metabolism of carbohydrates, fats, and proteins, as well as promotion of the synthesis and formation of nicotinic acid, fatty acids, glycogen, and amino acids (5–7). Biotin is absorbed unchanged in the upper part of the small intestine and distributed to all tissues. Highest concentrations are found in the liver and kidneys. Little information is available on the transport and storage of biotin in humans or animals. A biotin level in urine of approximately 160 nmol/24 h or 70 nmol/L, and a circulating level in blood, plasma, or serum of approximately 1500 pmol/L seems to indicate an adequate supply of biotin for humans. However,

Table 1. 1989 Estimated Safe and Adequate Daily Dietary Intake for Biotin

Group	Age, yr	Biotin, μg
infants	0–0.5	10
	0.5–1.0	15
children	1–3	20
	4–6	25
	7–10	30
adolescents and adults	11+	30–100

reported levels for biotin in the blood and urine vary widely and are not a reliable indicator of nutritional status.

3. Nutritional Requirements

Since exact requirements for biotin are uncertain owing to incomplete knowledge regarding biotin availability from food and a lack of definitive studies concerning biotin requirements, the United States National Research Council has established a safe and adequate daily dietary level of intake for biotin rather than a recommended dietary allowance (RDA). The recommended daily intake of biotin in the United States for all persons ages seven years and older is 30–100 μg/d. In France and South Africa, a recommended daily intake for adults of up to 300 μg/d has been established, whereas in Singapore an intake of up to 400 μg/d is recommended. Diets consisting of a daily biotin intake of 28–42 μg/d are considered adequate. A level of 60 μg/d is sufficient for patients under long-term total parenteral nutrition (fed intravenously). An infant's daily intake ranges from 15–20 μg/d and is acquired mostly through human milk containing 3–20 μg/L, or formulas fortified with biotin. An adequate intake level of 10–30 μ/d for infants and young children is recommended. The safe and adequate levels for daily dietary intake of biotin are listed in Table 1 (11). No side effects have been reported with oral doses of biotin as great as 40 mg or parenteral doses of 5–10 mg/d in infants. No toxicity of biotin has been found (5–7).

4. Physiological Significance

4.1. Animal

Dietary biotin deficiencies are extremely rare, perhaps because of the biosynthesis of biotin by intestinal microorganisms. However, biotin deficiency can be easily induced in most animals by ingestion of large amounts of raw egg white, which contain the biotin binding protein avidin. Avidin has a high affinity for biotin and binds with the ureido group to form a complex that is resistant to digestive enzymes. Biotin deficiency in animals causes a decrease in growth rate, loss of weight, alopecia, scaly dermatitis, hyperkeratosis, achromatrichia, and transverse fissures across the soft sole and cracks in the hard horn of the sole and claw wall. Minute amounts of biotin are known to be adequate to support body functions; therefore, biotin requirements for animals may be covered by the natural content of the feed and by the intestinal biosynthesis of biotin. However, biotin-responsive disease conditions not caused by primary biotin deficiency have been observed. One such condition is the fatty liver and kidney syndrome (FLKS). This syndrome has caused heavy economic losses in commercial broiler flocks. FLKS was found to be the result of a suboptimal biotin content in diet, coupled with other nutritional and environmental factors. Although the symptoms of FLKS are not those of classic biotin

deficiency, they can be eliminated by biotin supplements. Supplementation has also been found to reduce the incidence and severity of claw lesions in pigs and weak hoof horn in horses (5–7).

4.2. Human

Biotin deficiencies in humans are extremely rare. The symptoms of deficiency are anorexia, fatigue, nausea, vomiting, hyperesthesia, glossitis, pallor, mental depression, dry scaly dermatitis, alopecia, and localized parasthesias. Both seborrheic dermatitis and Leiner's disease could be the signs of a biotin deficiency in infants (12). Several studies (5–7) have indicated that an erythematous exfoliative dermatitis is the first clinical sign of a biotin deficiency. Infants under six months of age and people who eat large quantities of raw egg whites are probably the groups most susceptible to biotin deficiency. Another susceptible group would be people who lack biotinidase. Biotinidase is the only enzyme capable of catalyzing the cleavage of biocytin (biotinyl- ϵ-lysine), the bound form of biotin. It has also been postulated that there may be a connection between biotin and the etiology of the sudden infant death syndrome (SIDS), which is a common cause of death in the first year of life. Unless biotin is included in their alimentation, patients receiving long-term parenteral nutrition are susceptible to biotin deficiency. All symptoms can be reversed and conditions corrected with biotin treatment. For adults, biotin dosage levels of 150–300 μg per day would be effective treatment. Finally, no drugs have been found to cause a potential biotin deficiency through short- or long-term use. However, low circulating biotin levels have been observed in heavy smokers, alcoholics, and patients under prolonged treatment with anticonvulsant drugs (5–7).

5. Chemical and Physical Properties

The empirical formula for biotin, $C_{10}H_{16}N_2O_3S$, was established in 1941 (13). The full structure of biotin was elucidated in 1942 by two independent groups (14–16). The first total chemical synthesis of biotin was achieved by Harris in 1945; this work confirmed the structure of biotin (17). The configuration of *d*-biotin was definitively established in 1956 by x-ray crystallographic analysis (18, 19). The physical properties of *d*-biotin are found in Table 2. Chemically pure biotin is stable to air and heat. Biotin is also stable for months in mildly acidic and alkaline solutions; however, alkaline solutions, particularly above pH 9, are the least stable. Although biotin is not affected by reducing agents, it is incompatible with formaldehyde, chloramine T, oxidizing agents such as hydrogen peroxide and potassium permanganate, and strong acids such as nitrous acid (20). In most foodstuffs, biotin is bound to proteins, from which it is released in the intestine by protein hydrolysis and the enzyme biotinidase. Biotin is a highly stable, water-soluble vitamin that is resistant to most processing procedures, long-term storage, and normal cooking heat. Most of the biotin losses during cooking are the result of leaching into the cooking water. On the other hand, canning and food processing cause a moderate reduction in biotin content, owing to decomposition.

6. Chemical Synthesis

6.1. Original Synthesis

The first attempted synthesis of *d*-biotin in 1945 afforded racemic biotin (Fig. 1). In this synthetic pathway, L-cysteine[52-90-4] 1 was converted to the methyl ester [5472-74-2] 1. An intramolecular Dieckmann condensation, during which stereochemical integrity was lost, was followed by decarboxylation to afford the thiophanone [57752-72-4] 1. Aldol condensation of the thiophanone with the aldehyde ester [6026-86-4] 1 afforded the conjugated thiophanone [85269-47-2] 1. The aldol condensation allowed for attachment of the properly functionalized

Table 2. Physical Properties of *d*-Biotin

Property	Characteristic
appearance	fine long needles
color	white
molecular weight	244.31
molecular formula	$C_{10}H_{16}N_2O_3S$
elemental analysis, wt %	
carbon	49.16
hydrogen	6.60
nitrogen	11.47
sulfur	13.12
melting point, °C	232–233
α^{21}, specific optical rotation, degrees	+89–91[a]
dissociation constant, pK_A	6.3×10^{-6}
isoelectric point, pH	3.5
solubility, mg/mL	
H_2O, RT	~0.22[b]
95% alcohol, RT	~0.80
common organic solvents	insoluble

[a] $c = 1$ in 0.1 *N* NaOH.
[b] Higher in dil alkali.

C-5 side chain in one step, a weakness in many subsequent syntheses. The introduction of the second nitrogen was achieved by converting the keto group to the oxime [85269-48-3] 1, followed by zinc reduction to the thiophene [85269-49-4] 1. Stereoselective catalytic hydrogenation of the thiophene double bond gave mainly the thiophane [78763-60-7] 1, which was easily transformed to biotin. The nonselective catalytic hydrogenation and the epimerization that occurred during the conversion of 1 to 1 were the major factors leading to formation of racemic biotin (17).

6.2. Original Asymmetric Synthesis

The efficient introduction of the three stereocenters in the all-cis configuration was first accomplished in the elegant synthesis developed by Sternbach (21–23) in 1949. This process, the Hoffmann-La Roche industrial synthesis of biotin, is still (ca 1997) the basis of industrial preparations. An improved version of the original Sternbach synthesis is shown in Figure 2 (24). The fixed cis position of the carboxyl groups of the imidazolidine dicarboxylic acid (cycloacid) 2 and the stereochemistry of the chiral carbons 4 and 5 of the imidazolidine ring, throughout the synthetic scheme, are established by the starting material, fumaric acid [110-17-8] 2. Fumaric acid is brominated to dibromosuccinic acid [608-36-6] 2 followed by reaction with benzylamine to give dibenzylaminosuccinic acid [55645-40-4] 2. Treatment with phosgene forms the imidazolone ring of cycloacid [51591-75-4] 2. Cycloacid is dehydrated with acetic anhydride to form cycloanhydride [56688-83-6] 2. Opening of cycloanhydride with cyclohexanol forms the racemic monoester 2. The mixture is resolved by salt formation with (+)-ephedrine [299-42-3] 2 in high yield. The desired *d*-ephedrine salt undergoes acid cleavage, followed by selective reduction of the ester functionality with lithium borohydride to form, after acidification, the *d*-lactone [56688-82-5] 2. The undesired *l*-ephedrine salt is recycled via the meso-cycloacid to cycloanhydride. The *d*-lactone, which possesses the desired configuration at two of the stereocenters, is converted to the *d*-thiolactone [56688-83-6] 2 using potassium thioacetate. The introduction of the C-5 side chain is accomplished by reacting the thiolactone with the Grignard reagent derived from 3-methoxypropyl chloride, followed by dehydration to give the thiophene [85611-62-7] 2. Stereoselective hydrogenation establishes the third stereocenter and affords the thiophane [33607-59-9] 2. The thiophane undergoes acid-catalyzed cyclization to form the key intermediate,

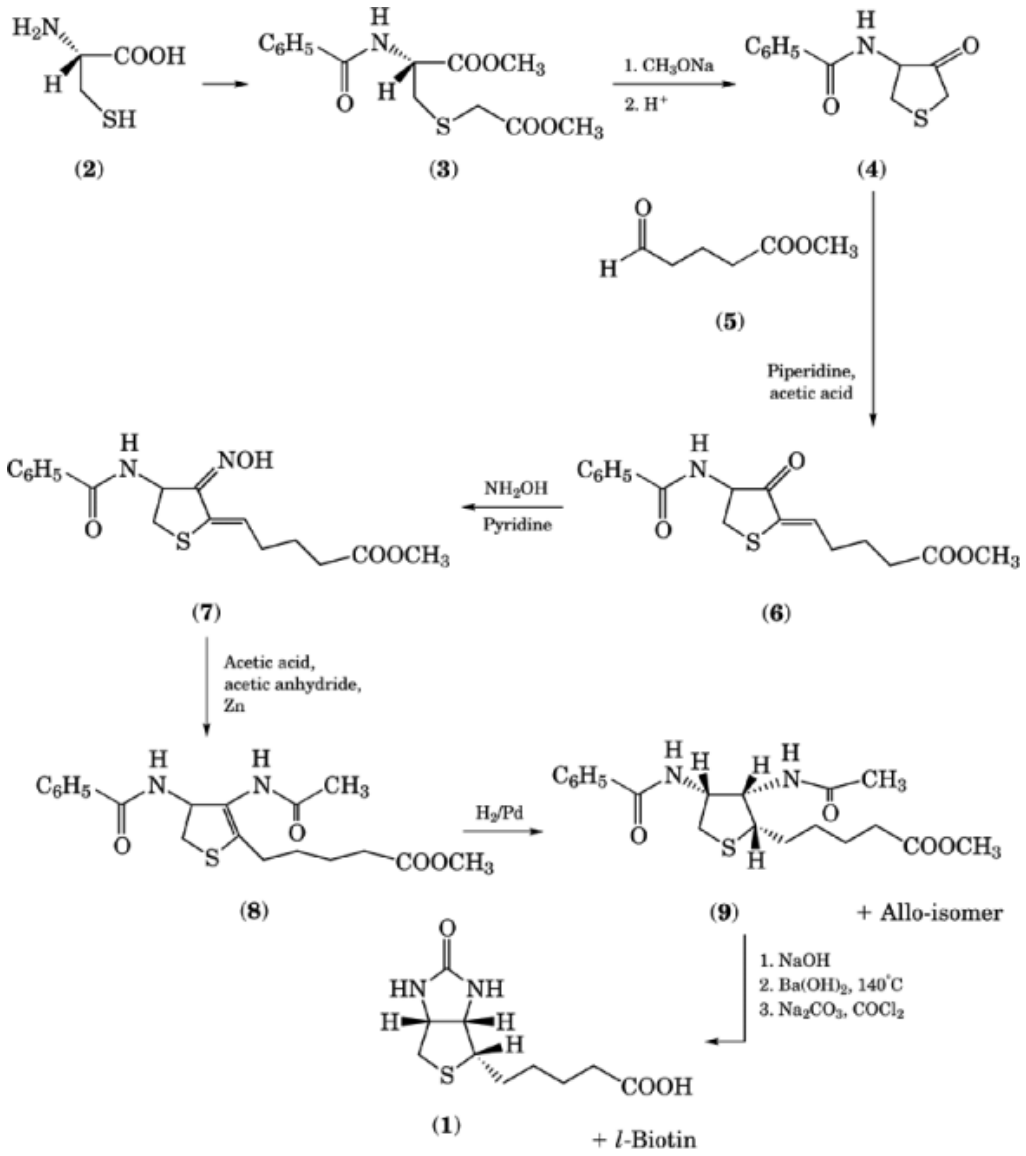


Fig. 1. Synthetic pathway for racemic biotin.

l-thiophanium bromide [60209-10-1] 2. The last two carbons of the biotin side chain are added by reaction of thiophanium bromide with sodium dimethylmalonate to form the diester [8554-84-3] 2. Hydrolysis of the ester groups, decarboxylation and debenzylation using strong acid forms homochiral, pure *d*-biotin. Although a number of significant modifications to the Sternbach synthesis have appeared over the last 45 years, it is still the basis of today's industrial preparations.

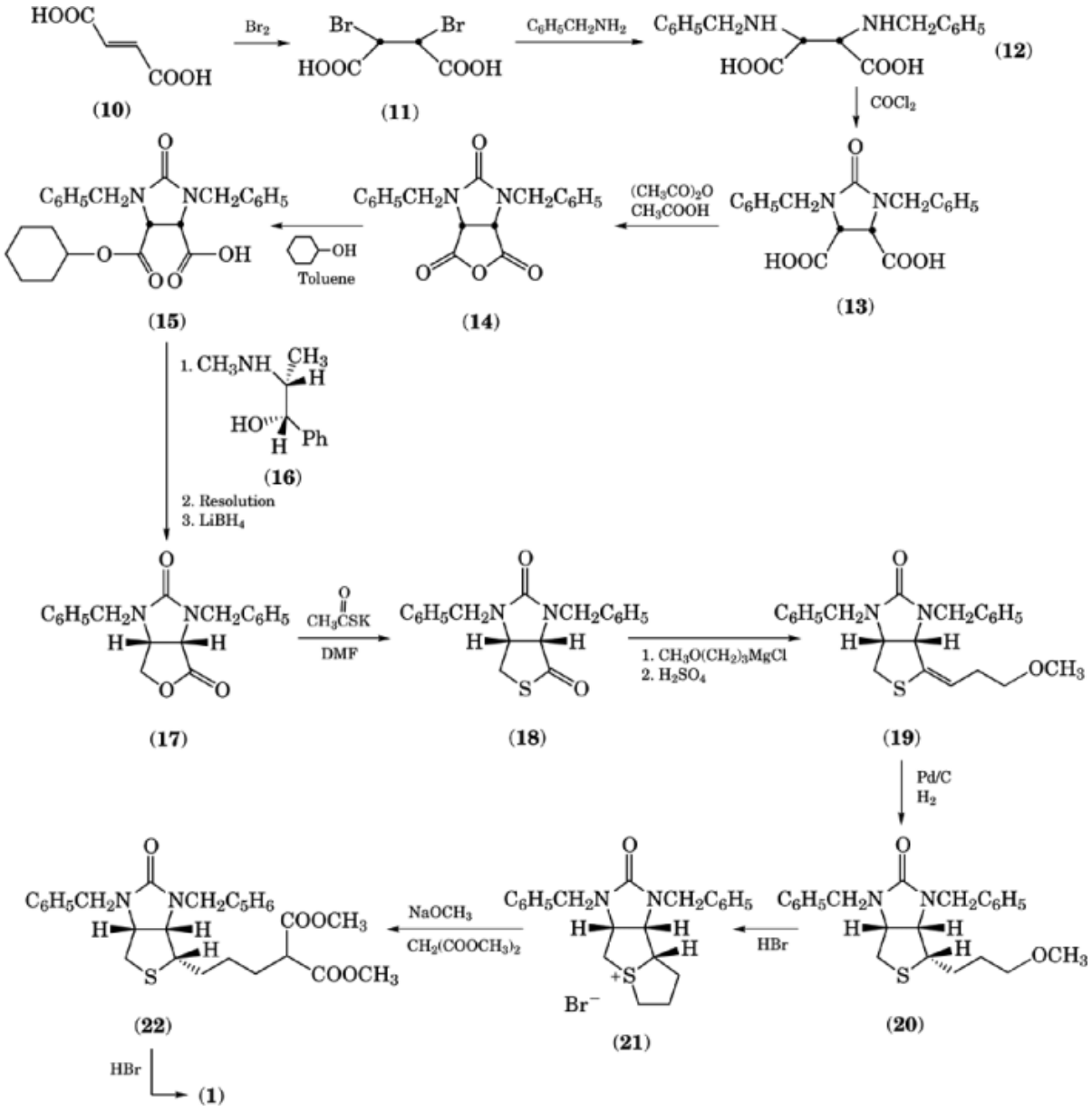


Fig. 2. Synthetic pathway for *d*-biotin (Sternbach synthesis).

6.3. Synthetic Drawbacks

The major drawbacks in the Sternbach-Goldberg synthesis are the resolution/recycling of the intermediate that leads to *d*-lactone and the multiple manipulations required to add the five-carbon side chain. This sequence is inefficient, bringing with it a net loss of methanol, hydrogen bromide, carbon dioxide, and water that were once part of the molecule. In the resolution of the intermediate that leads to *d*-lactone, 50% of the product is the undesirable isomer, although this material is converted to the cycloacid for recycling. This is inherently

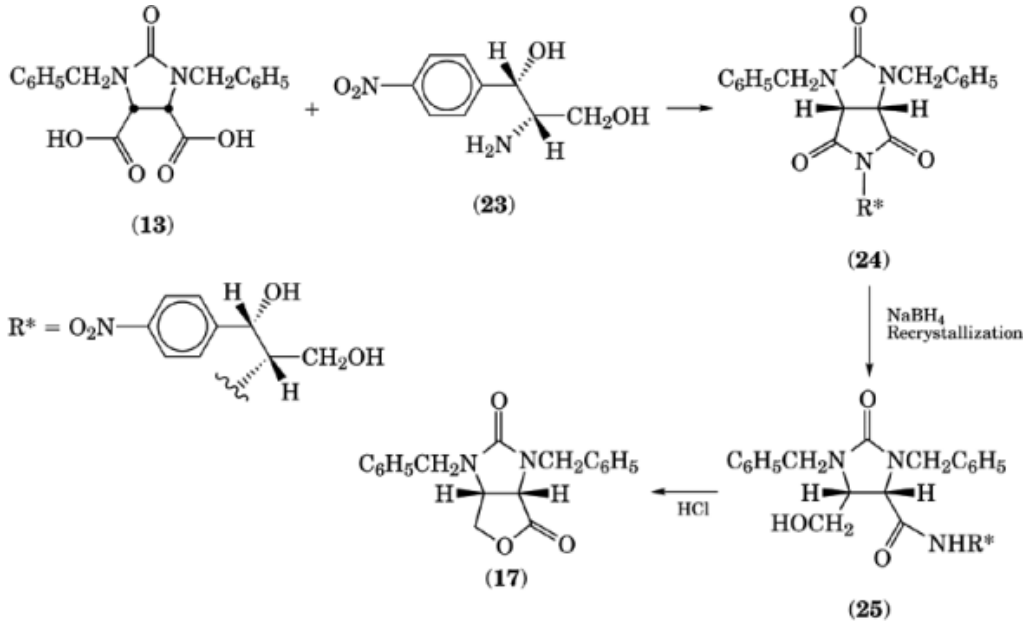


Fig. 3. Synthetic pathway for *d*-biotin (Sumitomo synthesis).

inefficient and limits single-run production capacity by at least 50%. Recycling the undesired isomer also requires additional labor.

6.4. Industrial Synthetic Improvements

One significant modification of the Sternbach process is the result of work by Sumitomo chemists in 1975, in which the optical resolution–reduction sequence is replaced with a more efficient asymmetric conversion of the *meso*-cycloacid 2 to the optically pure *d*-lactone 2 (Fig. 3) (25). The cycloacid is reacted with the optically active dihydroxyamine [2964-48-9] 3 to quantitatively yield the chiral imide [85317-83-5] 3. Diastereoselective reduction of the pro-*R*-carbonyl using sodium borohydride affords the optically pure hydroxyamide [85317-84-6] 3 after recrystallization. Acid hydrolysis of the amide then yields the desired *d*-lactone 3. A similar approach uses chiral alcohols to form diastereomic half-esters stereoselectivity. These are reduced and directly converted to *d*-lactone (26). In both approaches, the desired diastereomeric half-amide or half-ester is formed in excess, thus avoiding the costly resolution step required in the Sternbach synthesis.

Another modification of the Sternbach method involves the direct attachment of the C-5 side chain to thiolactone or a functionalized thiolactone intermediate. One such method, possibly utilized by Sumitomo and reported several times by Lonza (27–29), involves the treatment of the thiolactone 2 with the five-carbon Wittig reagent 4 to give the olefin 4. Hydrogenation of the thiophene 4 followed by acid hydrolysis completes the synthesis of *d*-biotin. Another method that directly attaches the five-carbon side chain is patented by Lonza (Fig. 4). This method involves a Wittig reaction of a phosphonium salt derived from the thiolactone 4 and a C-5 aldehyde. The thiolactone 3 is reduced to the thiophanol 3, which is converted to the Wittig salt 4 with Ph_3PH^+ BF^-_3. The ylide 4, in the presence of base, undergoes condensation with the aldehyde ester to form the thiophene 4, which in turn is catalytically hydrogenated to *d*-biotin.

A final variation of the Sternbach method was reported by Lonza (27–29, 31). This method (Fig. 5) not only involves direct attachment of the C-5 side chain to the lactone [118609-09-9] 5, but also introduces the

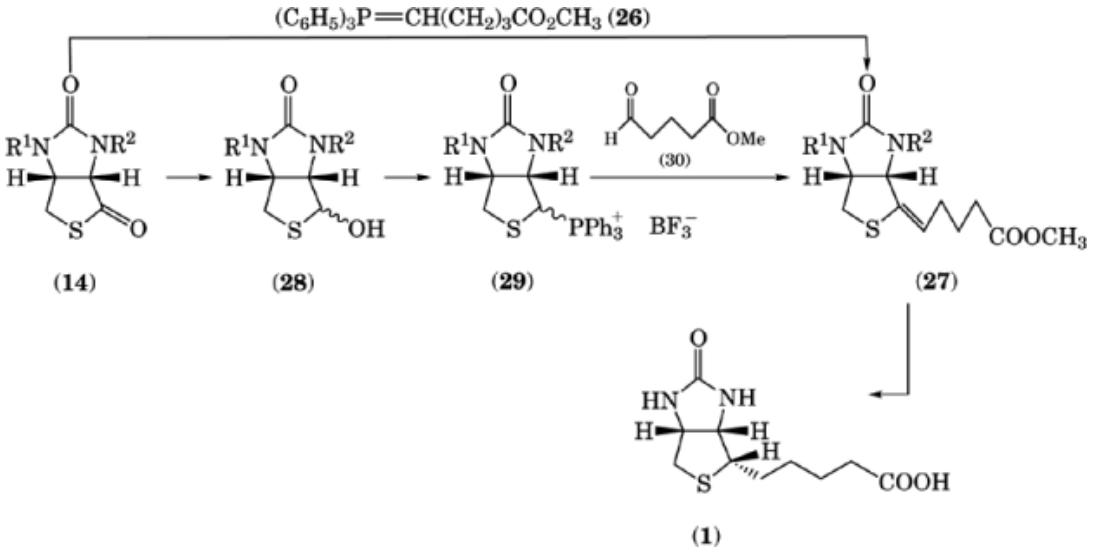


Fig. 4. Synthetic pathways for *d*-biotin (Lonza syntheses).

key stereocenters early in the synthetic pathway via a chiral mono-protected imidazole intermediate [116291-87-3] 5. Catalytic diastereoselective hydrogenation of the furoimidazole 5 affords the *d*-lactone 5 as the major product, in 54% yield. The undesired diastereomeric *l*-lactone 5 can be easily separated by chromatography or crystallization. In the step following separation, the *d*-lactone is converted to the thiolactone [118609-15-7] 5 using a thiocarboxylic acid salt. The thiolactone undergoes a Grignard or Wittig reaction to incorporate the C-5 side chain. The thiophene [118609-16-8] 5 is then catalytically hydrogenated. This process hydrogenates the carbon–carbon double bond and also removes the chiral protecting group on the nitrogen. This process takes advantage of a short synthetic pathway and the use of a relatively inexpensive starting material. A recent modification of this Lonza process uses a chiral-substituted ferrocenyldisphosphine with a rhodium catalyst to stereoselectively reduce the enamine 5 to the *d*-lactone 5 in 99% yield. This improvement eliminates the need to separate the undesired *l*-lactone by chromatography or crystallization and increases the yield of the desired lactone from 54 to 99%, making the process extremely efficient (32, 33).

6.5. Novel Synthetic Methods

More recently, several novel syntheses of *d*-biotin, starting from a variety of chiral starting materials, have been developed. Seven of these synthetic pathways start from L-cysteine (34–41), two from L-cystine (42, 43), two from D-arabinose (44, 45), and one from ribitol (46). Each of these methods has at least one of the following drawbacks: multiple steps with overall low yields; low yield steps associated with cyclization reactions; safety issues associated with reagents such as metal azides, methyl iodide, diazomethane, organoazide reagents, etc; environmental issues with reagents and by products such as triphenyl phosphine oxide and stannane salts; and costs of reagents such as 1,3-dicyclohexylcarbodiimide and trialkyltin hydride. Only one synthetic pathway starts from an achiral starting material, 2,5-dihydrothiophene-1,1-dioxide; this route requires a stereochemical resolution step (47–49). The drawbacks of this pathway are the commercially unavailable starting material and low conversions in several steps.

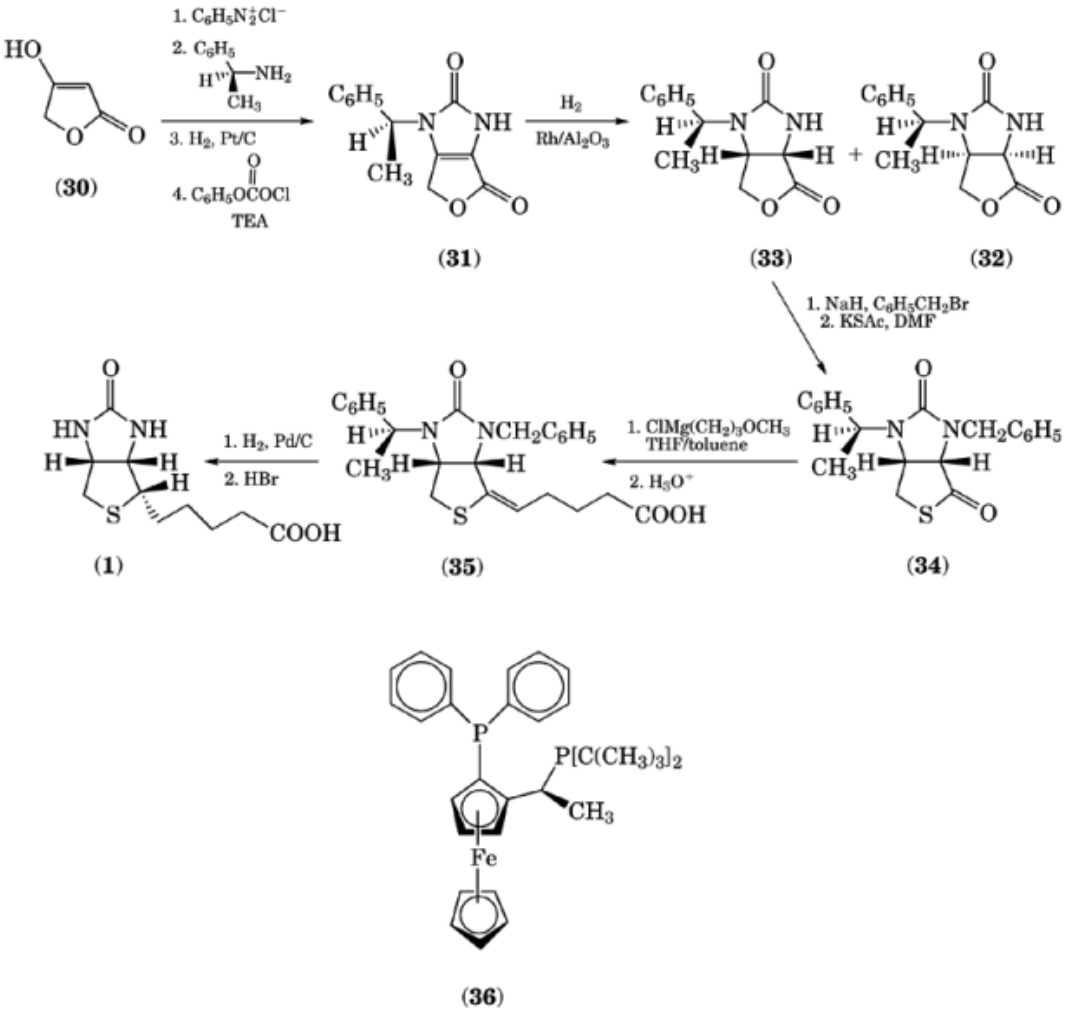


Fig. 5. Synthetic pathway for *d*-biotin (Lonza synthesis). An improved process uses the chiral ferrocenyldisphoshine **(36)** to introduce stereospecificity during the hydrogenation of lactone **(31)**.

7. Biosynthesis

Biotin is produced by a multistep pathway in a variety of fungi, bacteria, and plants (50–56). The established pathway (50, 56) in *E. coli* is shown in Figure 6. However, little is known about the initial steps that lead to pimelyl-CoA or of the mechanism of the transformation of desthiobiotin to biotin. Pimelic acid is believed to be the natural precursor of biotin for some microorganisms (51).

Evidence that pimelic acid is a biotin precursor has been found in *Bacillus* species by feeding pimelic acid and observing the concomitant increase in biotin titers. Also, if labeled pimelic acid is used, labeled biotin is formed. In the biosynthetic pathway, pimelic acid [111-16-0] 6 is converted into pimelyl-CoA 6 by the enzyme pimelyl-CoA-synthetase in the presence of ATP and Mg^{2+} at 32°C and pH 7–8 (57). On the other hand, *E. coli* does not seem to rely on pimelic acid as a starting material for biotin synthesis. *E. coli* seems to form pimelyl-CoA by a pathway similar to that of fatty acid and polyketide synthesis (58).

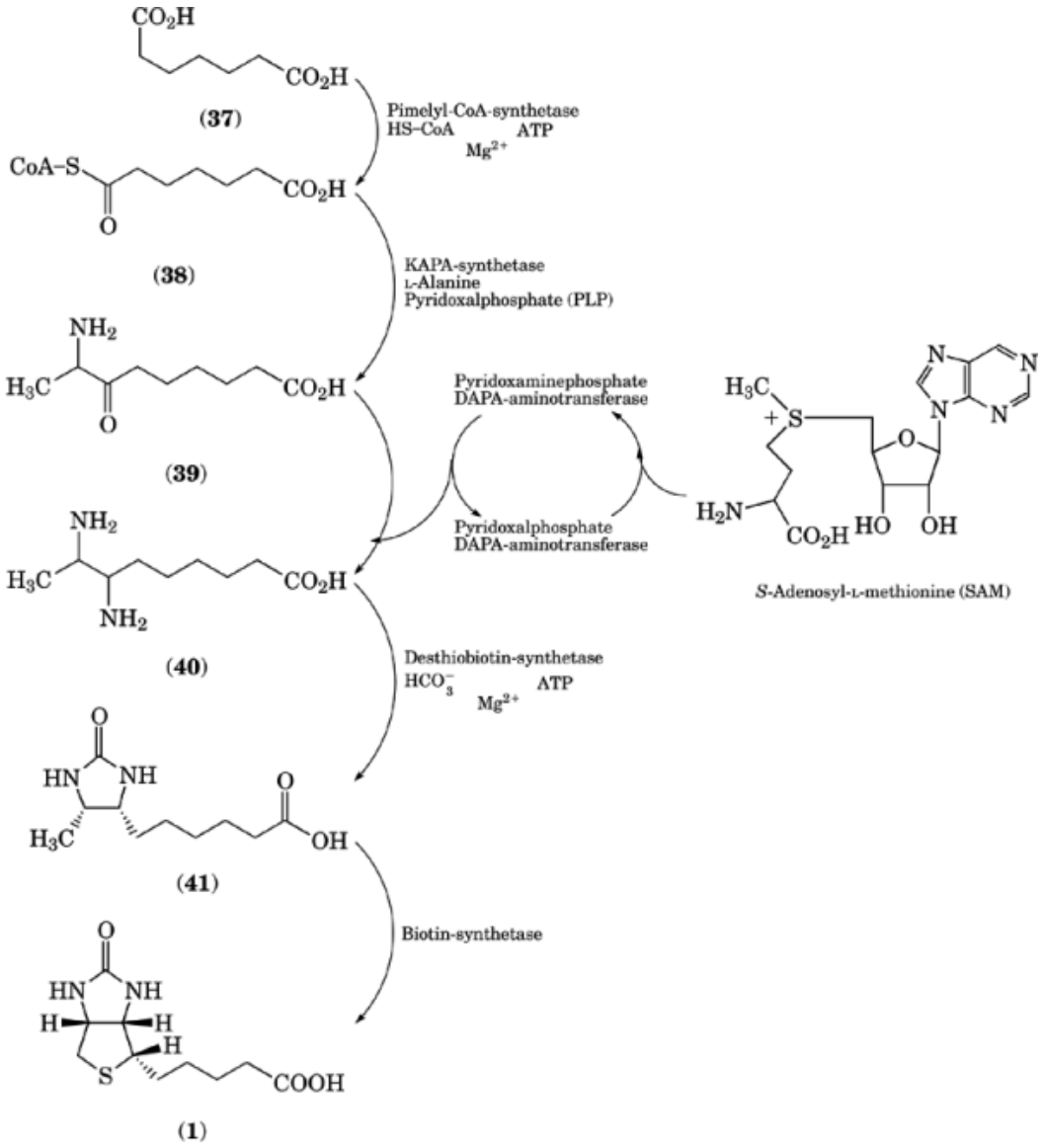


Fig. 6. Biosynthetic pathway of *d*-biotin.

l-CoA 6 is transformed into 7-keto-8-aminopelargonic acid [4707-58-8] (7-KAPA) 6 by reaction with L-alanine. The reaction requires pyridoxal-5′-phosphate (PLP) as a co-reactant and is catalyzed by the enzyme KAPA-synthetase (59). 7-Keto-8-amino-pelargonic acid 6 is converted into 7,8-diamino-pelargonic acid [157120-40-6] (DAPA) 6 by the enzyme DAPA-aminotransferase, which uses *S*-adenosyl-L-methionine (SAM) instead of a simple amino acid as the amino group donor (60, 61). 7,8-Diaminopelargonic acid 6 is converted by the enzyme DTB-synthetase to desthiobiotin [533-48-2] 6. The optimal biological production of desthiobiotin requires ATP, Mg^{2+} and bicarbonate at 50°C and pH 7–8 (62–66). Finally desthiobiotin 6 is converted to biotin, supposedly by the enzyme biotin synthetase, which is encoded by the Bio B gene (56).

Table 3. Organisms Used for Biotin Fermentation

Organism name	Titer, mg/L	References
Cornebacteria flavum		(70, 71)
Brevibacterium flavum	0.5[a]	(70, 72)
Serratia marcescens		(73–78)
	600	73
	500	74
	120	(75, 78)
	83	77
Escherichia coli	2	(79, 80)
Bacillus sphaericus		(81–85)
	70	81
	20	82
	365	83
Rhodotorula rubra		86
Sporobolomyces roseus		86
Yarrowia lipolytica		86
Candida shehatae		87
Rhizopus delemar	0.6	(88, 89)

[a]

The conversion of desthiobiotin to biotin occurs in growing as well as resting cells in which the internal source of sulfur is believed to be cysteine or methionine. The introduction of sulfur into desthiobiotin has been investigated by several groups and it is proposed that the sulfur is introduced at C-4 of desthiobiotin with retention of configuration (67–69). The biosynthesis of biotin has led several groups to explore the feasibility of synthesizing biotin on a commercial scale by microorganisms. To date, no commercial scale total synthesis of biotin by fermentation is known. However, several microorganisms and mutant strains have been evaluated (Table 3). One of the problems associated with biotin biosynthesis via fermentation is that total biotin production decreases with increasing biotin concentrations in the fermentation broth. In fact, biotin strongly inhibits all steps of the biosynthesis except the synthesis of pimelyl-CoA. This inhibition by biotin leads to the isolation of several biotin vitamers. A vitamer is a compound, structurally similar to a vitamin, that exhibits varying degrees of vitamin activity. The most important biotin vitamer is desthiobiotin. Inhibition of biotin biosynthesis is the subject of much research.

8. Analytical Methods

8.1. Biological Assay

Various analytical methods, including microbiological, biological, chemical, enzymatic and chromatographic assays, have been used to determine biotin levels in food, feed, and body fluids (79–81). The biological assay of biotin is conducted with rats or chicks made biotin-deficient by special diets containing either raw egg whites or avidin. The assay is based on growth response curves since test-animal weight gain is proportional to the logarithm of the biotin dose. A biological assay of biotin is advantageous because no pretreatment of the sample is necessary and the assay measures the amount of biotin available to the test animal. However, biological assays are time-consuming (four weeks to actually run the assay and six to seven weeks to prepare the biotin-deficient animals), costly to run, and require a large number of chicks or rats for accurate results.

8.2. Microbiological Assay

An alternative to the biological assay is the microbiological assay, which takes less time, costs less, requires less space, and has greater sensitivity than the biological assay. The typical microorganisms used for a microbiological assay of biotin are *Lactobacillus casei* ATCC 7469, *Neurospora crassa*, *Ochromonas danica*, *Saccharomyces cerevisiae* ATCC 7745, and *Lactobacillus plantarum* ATCC 8014. *Lactobacillus plantarum* ATCC 8014 is the test organism employed by most laboratories for biotin assay. Since biotin occurs in both the free and bound forms in nature and *L. plantarum* ATCC 8014 responds only to free biotin, all available biotin must be converted to free biotin prior to the microbiological assay. Bound biotin is usually converted to free biotin by acid hydrolysis with sulfuric acid or by digestion with papain. Hydrochloric acid should not be used in place of sulfuric acid for the acid hydrolysis because it may inactivate biotin. Lipids that stimulate the growth of *L. plantarum* interfere with the assay and have to be removed by filtration of the acid extracts or by preliminary ether extraction prior to the assay. A microbiological assay involves extraction, addition of graded levels of standard and sample to the assay tubes, addition of the medium, sterilization of the assay tubes, inoculation with *L. plantarum* and incubation. After the cell growth stops, the biotin content of the test material is determined by measuring the growth response of the organism (eg, colorimetrically, spectrophotometrically, or titrimetrically with sodium hydroxide) as compared to cell growth with known biotin concentration standards. Microbiological assay of biotin using *L. plantarum* is capable of detecting as little as 0.05 ng of biotin/mL of test solution.

8.3. Isotope Dilution Assay

An isotope dilution assay for biotin, based on the high affinity of avidin for the ureido group of biotin, compares the binding of radioactive biotin and nonradioactive biotin with avidin. This method is sensitive to a level of 1–10 ng biotin (82–84), and the radiotracers typically used are [^{14}C]biotin (83), [3H]biotin (84, 85) or an ^{125}I-labeled biotin derivative (86). A variation of this approach uses ^{125}I-labeled avidin (87) for the assay.

8.4. High Performance Liquid Chromatography Analysis

The analysis of biotin has also been achieved by high performance liquid chromatography (hplc). Biotin has been analyzed in B-complex tablets, vitamin premixes, and multivitamin–multimineral preparations by reverse-phase, high performance liquid chromatographic methods (hplc) using a C^{18} column and uv detection at either 230 nm or 200 nm (88–91). Although this method can detect biotin in vitamin premixes, it is not sensitive enough to determine typical biotin levels in food or feed. Another method, one having greater sensitivity, is a reverse-phase ion-interaction reagent hplc method, with a biotin detection limit of 4 μg. An hplc method with even greater sensitivity involves derivatizing biotin prior to chromatographic separation. This technique can be used for either uv-absorbing derivatives, such as the bromoacetophenone ester (92), or fluorescent derivatives, such as methyl methoxycoumarin ester (92) and anthryldiazomethane ester (93). The biotin detection limit for the bromoacetophenone ester derivative at uv 254 nm was 50 ng. The detection limit for the methyl methoxycoumarin ester at excitation wavelength 360 nm and emission wavelength 410 nm was 5 ng; whereas, the limit of the anthryldiazomethane ester at excitation wavelength at 365 nm and emission wavelength 412 nm was 0.1–10 ng.

8.5. Gas Chromatography Analysis

From a sensitivity standpoint, a comparable technique is a gas chromatographic (gc) technique using flame ionization detection. This method has been used to quantify the trimethylsilyl ester derivative of biotin in agricultural premixes and pharmaceutical injectable preparations at detection limits of approximately 0.3 μg (94, 95).

9. Specifications and Product Forms

According to the *Food Chemicals Codex* (96), the biologically active, food-grade form of biotin must have an assay of 97.5%, a melting point in the range of 229–232°C, and a specific rotation at 25°C in 0.1 *N* NaOH in the range of + 89–93°. It must also contain less than 3 ppm of arsenic and less than 10 ppm of heavy metals, eg, lead, mercury, and copper. Finally, it must be able to be quantitatively sieved through U.S. Standard Sieves No. 80 using a mechanical shaker. Biotin is listed as GRAS in the *Code of Federal Regulations* (97, 98) for use as a nutrient or dietary supplement (21 CFR 182.5159). The specifications for *d*-biotin for pharmaceutical applications are similar to those for food and are listed in the *United States Pharmacopeia* (99). No specifications for the optically inactive, *d*,*l*-biotin are given for either food or pharmaceutical applications.

10. Economic Aspects

The biotin market is divided between agricultural and human use, with ∼90% of biotin used in the animal health care market and ∼10% for the human nutritional market. The major producers of biotin are Hoffmann-La Roche, Lonza, E. Merck-Darmstadt, Rhône-Poulenc, Sumitomo Pharmaceutical, E. Sung, and Tanabe Seiyaku (100). Worldwide production of biotin in 1994 was approximately 60 metric tons. The list price for pure biotin in 1995 was ∼$7.00/g; whereas, the list price for technical feed-grade biotin was ∼$5.50/g. Biotin is used in various pharmaceutical, food, and special dietary products, including multivitamin preparations in liquid, tablet, capsule, or powder forms. One of the commercially available products of *d*-biotin is Britrit-1, which is a 1% biotin trituration used in food premixes.

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Related Articles

Vitamins, Survey; Ascorbic Acid; Folic Acid; Niacin, Nicotinamide, and Nicotinic Acid; Pantothenic Acid; Pyridoxine (B6); Riboflavin (B2); Thiamine (B1); Vitamin A; Vitamin B12