ADVANCES IN THE CHEMISTRY OF BIOTIN

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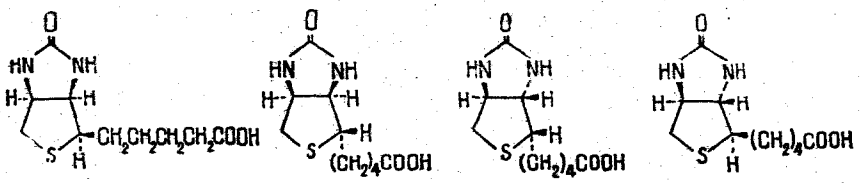
The review gives methods of synthesizing the biocatalyst biotin. The existing methods of obtaining biotin and its stereoisomers from dihaloalkanes, dicarboxylic acid esters, sugars, thiophene derivatives, etc., are discussed. The basic possibilities of the formation of biotin are considered.

One of the latest publications on the chemistry of the natural bioregulator (+)-biotin (vitamin H) belongs to 1973 [1] while the last 6-7 years have been marked by original investigations on the creation of more than 10 new syntheses of biotin based on various strategies. The advances in the field of the synthesis of biotin and its stereoisomers is the subject of the present review which, for the sake of exhaustive completeness, also includes earlier syntheses of biotin.

Biotin is an exogenous essential nutritional factor. It is one of the biocatalysts of reversible metabolic reactions of carbon dioxide transport in micro- and macroorganisms: carboxylation, trans-carboxylation, and the monodecarboxylation of polybasic organic acids. It performs its function in the form of $N_{(1)}$-carboxybiotin — the coenzyme of a number of coenzyme-A-dependent carboxylases.

Biotin, (4)-c-4-(δ-carboxybutyl)-2-oxohexahydro-(r-6a,c-3a)-thieno[3,4-d]imidazole is a colorless substance with mp 230-232°C (decomp.) and $[\alpha]^{22}$ +92° (c 0.3; 0.1 N NaOH). The structural formula of biotin was established in 1942 by Melville, du Vigneaud, et al. [2] and was confirmed through its x-ray structural analysis by Traub [3]; the absolute configuration of biotin was established from the results of x-radiographic investigations [4], and then the conformation of the side chain was refined [5, 6].

Biotin has three chiral carbon atoms and four stereoisomeric forms are known for it, of which only (+)-biotin itself (1) is biologically active; epi-, allo-, and epiallobiotins (2, 3, and 4, respectively), and also the diastereomeric (-)-biotin are biologically inactive.



1 2 3 4

The bicyclic skeleton of the biotin molecule consists of a planar imidazolidone ring cis-linked to a tetrahydrothiophene ring (φ 120°C) which has the envelope conformation. The δ-carboxybutyl side chain is present in position 4 in the cis configuration to the imidazolidone ring and in the "twist" conformation [5, 6].

The synthetic methods of obtaining (+)- and (±)-biotin and its stereoisomers can be grouped according to the methods of constructing the molecule and other general characteristics. The following syntheses have been created (the numbers of the schemes are given in parentheses): stereospecific (1, 5, 6, 10, 12-15); stereoselective (7, 16-18); from natural compounds — D-glucose, L-cysteine, D-mannose (1, 14, 15) and from L-cystine (11, 12); via an imidazoline or imidazolidine ring (1, 2, 16, 17); via a thiophene ring (2-5); via a dihydrothiophene ring (6, 7, 11, 18); via a thiophane ring (8-15); via a tetrahydrothienoimidazoline bicyclic system (6, 17); using phosgene for the formation of the imidazolidone ring (7-14); and the synthesis of stereoisomers (9-12).

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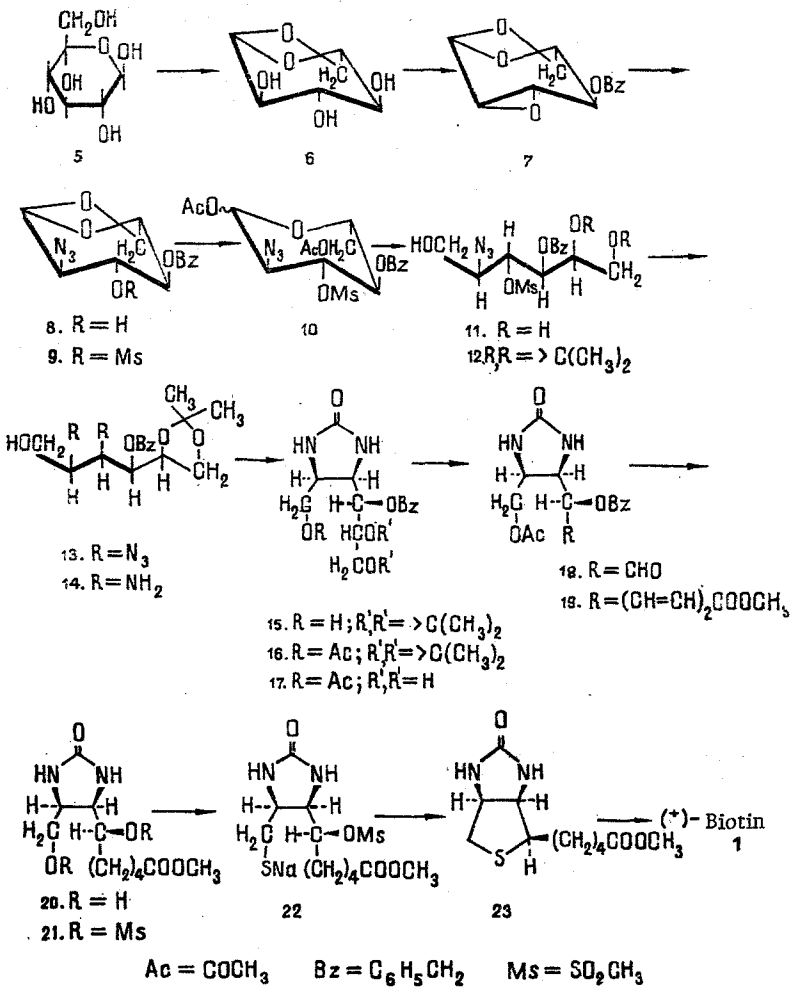
Stereospecific Synthesis of (+)-Biotin from D-Glucose

The synthesis by Ogawa et al. [7] (Scheme 1) is based on the transfer of the dextrorotatory optical activity of D-(+)-glucose to the optically active molecule of natural biotin through a series of stereospecific transformations, the introduction into the hexatomic carbohydrate molecule of two amino groups, the shortening of the carbon chain, introduction of the carboxybutyl substituent, the preparation of a substituted imidazolidone ring, and the building of the tetrahydrothiophene ring on to it in the last stage of the synthesis. To some extent, this chemical synthesis imitates the biological transformation of sugars by which some species of bacteria can use D-glucose in the biosynthesis of biotin [8]; in the last stages of biosyntheses there is possibly a similar biochemical scheme of the production of biotin from pimelic acid and cysteine [9, 10], or from dethiobiotin [11, 12].



Scheme 1

In the stereochemical transformation of β-D-glucopyranose (5) into the key intermediate compound (11) with the groupings necessary for the further transformations, the conformationally rigid [13] 1,6-anhydro-β-D-glucose, levoglucosan (6), is used, and from this benzyl 1,6:2,3-dianhydro-β-D-mannopyranoside (4) is obtained in four stages [14]. The diaxial opening of the epoxide of compound (7) with the aid of sodium azide and ammonium chloride in a mixture of 2-methoxyethanol and water (1:4) at 120°C gives benzyl 2-azido-2-deoxy-1,6-anhydro-β-D-glycopyranoside (8); yield 85% on compound (7). The hydrogen of the hydroxyl at $C_{(3)}$ is replaced by a methanesulfonate group by the usual method giving the mesyl-substituted monoazide (9). In order to achieve the smooth S_N2 replacement [15] of the mesyl group by an azide anion the steric environment of the mesyl group at $C_{(3)}$ must be changed [the attacking azide ion has a 1,2-interaction with the axial substituents at $C_{(2)}$ and $C_{(4)}$ in compound (9)]. For this purpose, as was shown [7], the flexible acyclic structure (12) is more suitable than the rigid 1,6-anhydro structure. Thus, the acetolysis of the mesyl derivative (9) in a boron trifluoride etherate mixture leads to the formation of an anomeric mixture of diacetates (10;

α:β = 8:3), which on solvolysis in 1% hydrogen chloride in methanol followed by reduction with sodium tetrahydroborate in ethanol in the presence of boric acids gives the triol (11) [yield 45% on compound (9)]. A second azide group is introduced into this compound — first, by the action of 2,2-dimethoxypropane in DMFA, themonoisopropylidene derivative (12) is formed and then, by the action of lithium azide in DMFA at 80°C, with reversal of the configuration, the diazide (13) [52% on compound (11)]. As the result of its selective hydrogenation [16] with Lindlar catalyst (palladium partially poisoned with mercury) in ethanol the diamine (14) is obtained in quantitative yield and then reaction with phosgene in carbon tetrachloride in a mixture with an aqueous solution of sodium carbonate gives the substituted imidazolidone (15) [87% on compound (13)].

The subsequent selective conversion of the dioxolane part of the substituted imidazolidone (15) into the side chain of the key intermediate compound (20) [40% on compound (15)] is achieved in six stages [7]. As the result of acetylation with acetic anhydride in pyridine of compound (15) the monoacetate (16) and, after deisopropylidenization in 80% acetic acid at 70°C, the diol (17) are obtained. The oxidation of the latter with $NaIO_4$ in 50% ethanol leads to the aldehyde (18) which is converted by the Wittig reaction with (3-methoxycarbonylprop-2-en-1-ylidene)triphenylphosphorane [17, 18] in dichloromethane into the ester (19), and then, by hydrogenation of the multiple bonds with 10% Pd/C in methanol followed by deacetylation with the aid of sodium methanolate in methanol, 4-(1'-hydroxy-5'-methoxycarbonylpentyl)-5-hydroxymethylimidazolid-2-one (20) is obtained [6.3% on compound (6)]. This diol ester (20) is finally converted into (+)-biotin by closing the tetrahydrothiophene ring with inversion at $C_{(4)}$; this is achieved by methanesulfonation with 15 equivalents of mesyl chloride in a mixture of pyridine and dichloroethane at −10°C to give compound (21) which, without isolation, is converted by reaction with an excess of sodium sulfide in DMFA at 100°C into compound (22) from which the methyl ester of biotin (23) and then (+)-biotin (1) [30% on compound (20)] are obtained. The overall yield of (+)-biotin is 1.9% on the 1,6-anhydro-β-D-glucose, levoglucosan (6).

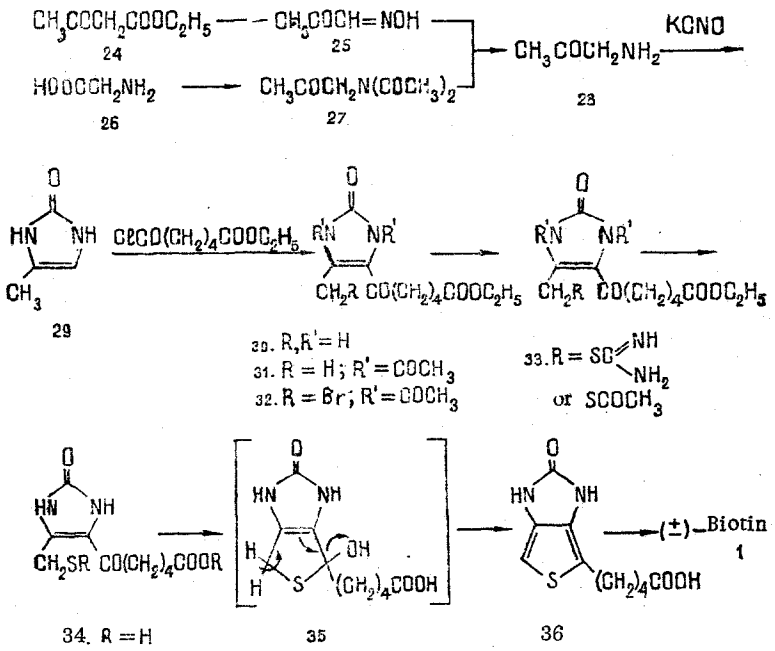
A readily available starting material for obtaining the levoglucosan [19, 20] is cellulose — its thermal decomposition under reduced pressure gives not less than 40% of levoglucosan [20].

According to another variant, the production of the intermediate 4-(1'-hydroxy-5'-methoxycarbonylpentyl)-5-hydroxymethylimidazolid-2-one (20) from D-glucose is effected by a multistage synthesis via 2-amino-2-deoxy-D-glucose (with a yield of 3.9% on this compound) [21].

Synthesis of (±)-Biotin from Acetoacetic Ester or Glycine via an Imidazolinone Derivative and Tetradehydrobiotin

This method of synthesizing biotin (Scheme 2) was developed by several groups of authors over 30 years — from 1945 to 1975. It consists in the initial formation of the imidazolinone ring with a methyl group in position 4 followed by the introduction of a 5-ethoxycarbonyl-1-oxopentyl group into the ring and then the creation on the imidazolinone ring of a thiophene ring conjugated with it which leads to 3a,4,6,6a-tetradehydrobiotin, which is reduced by ionic hydrogenation to (±)-biotin.

In 1948, Duschinsky and Dolan [22] synthesized 1,3-diacetyl-4-amidinothiomethyl-5-(5-ethoxycarbonyl-1-oxopentyl)imidazolin-2-one hydrobromide (33, R = $-SC(=NH)NH_2$). The action on acetoacetic ester (24) of sodium nitrite and mineral acid gave the ketoxime (25), which was hydrogenated over a palladium catalyst to the amino ketone (28) [89% on the (24)]. Without isolation, this was brought into reaction with potassium isocyanate in an aqueous medium in the presence of hydrochloric acid, which led to 4-methylimidazoline-2-one (29) (yield 73% on compound 28 or 65% on compound 24) [23]. The amino ketone (28) (31.5% on the 26) was also obtained from glycine (26) [24] and acetic anhydride by the Dakin-West reaction via compound (27). Compound (29) was alkylated by the Friedel-Crafts method with the chloride of monomethyl adipate in nitrobenzene in thepresence of aluminum chloride, giving 4-(5-ethoxycarbonyl-1-oxopentyl)-5-methylimidazolin-2-one (30) (62%) [33]. Its amino group was protected by acetylation, giving compound (31) (87%), and this was brominated in the methyl group by bromosuccinimide in carbon tetrachloride in the presence of potassium iodide to the 4-bromomethyl derivative (32) (92%) which was then converted by the action of thiourea in dioxane into the imidazolinone (33, R = $-SC(=NH)NH_2$) [68% on the compound (32) and 21% on the compound (24)] [22]. However, the authors were unable to cyclize compound (33) and convert it into tetradehydrobiotin (36).



Scheme 2

The bromine in compound (32) can be replaced by an acetylthio group by reaction with potassium mercaptoacetate, forming 1,3-diacetyl-4-acetylthiomethyl-5-(5-ethoxycarbonyl-1-oxopentyl)imidazolin-2-one (33, R = $-SCOCH_3$) (60%) [26, 27].

Twenty years later, in 1968, this synthetic route was repeated by Isaka et al. [28], and then compound (33) was subjected to hydrolysis under the action of 10% caustic soda and the resulting compound (34) was cyclized in hydrochloric acid at 70°C. A substance with mp 248-249°C was isolated to which the authors assigned an incorrect structure, namely 4-(4-carboxybutylidine)-3,4-dehydrothieno[3,4-d]imidazolin-2-one [28], while it was actually 3a,4,6,6a-tetradehydrobiotin (36) (56% on compound 33 and 12% on 24).

The correct structure of (36) was established in 1973 by Zav'yalov et al. [29]. On the basis of a comparison of PMR, IR, and UV spectra and also the absence of a depression of the melting point of a mixture with the known substance, they showed that the cyclization of compound (34) forms 3a,4,6,6a-tetradehydrobiotin (36). Esters of this compound were obtained [30].

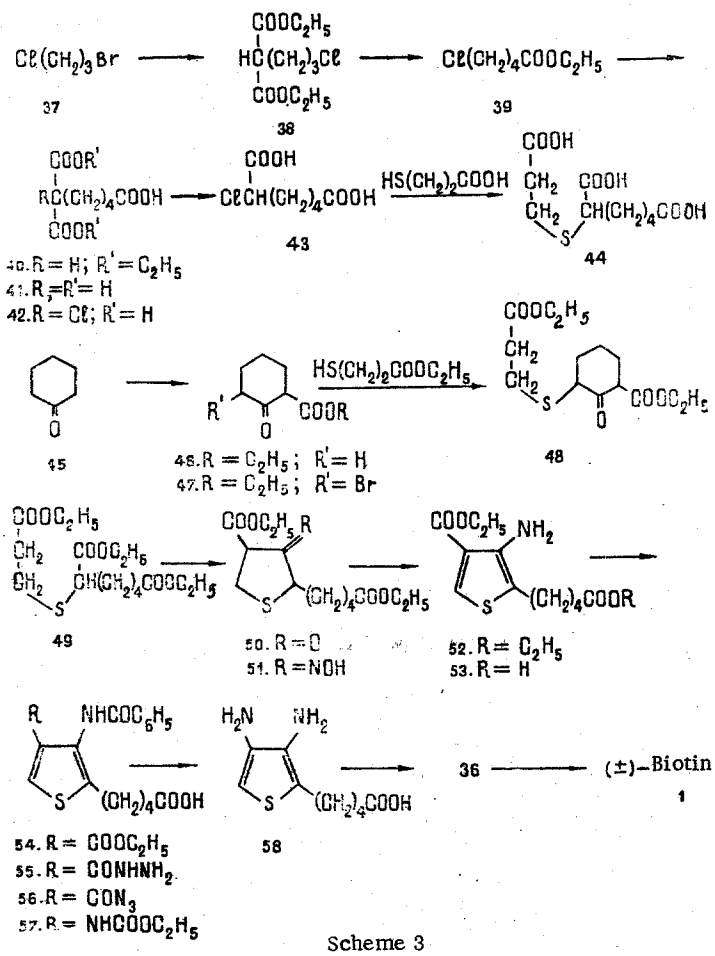
In 1974, Taguchi et al. [27] proposed for the cyclization of compound (34) to tetradehydrobiotin (36) a mechanism consisting in the initial formation of the hydroxy acid (35) followed by dehydration accompanied by double-bond migration and the creation of a conjugated thieno ring [the authors did not describe the experiment but gave the overall yield of compound (36) — 18% on the compound (24)].

In 1975 Zav'yalov et al. [31] effected the reduction of 3a,4,6,6a-tetradehydrobiotin (36) by ionic hydrogenation with triethylsilane in trifluoroacetic acid to (±)-biotin (1) (10%). Thus, the overall yield of (±)-biotin amounted to 1.2% on acetoacetic ester (24) and 0.46% on glycine (26).

Synthesis of (±)-Biotin from 1-Bromo-3-chloropropane via Chloropimelic Acid, Thiophene Derivatives, and Tetradehydrobiotin

In Cheney and Piening's synthesis [32, 33] (Scheme 3), α-chloropropionic acid was condensed with β-mercaptopropionic acid with the synthesis of a substituted 3-oxothiophane, the oxime of the latter was rearranged into 3-aminothiophene, and this was subjected to a number of conversions to give the key bicyclic thienoimidazole.

The α-chloropimelic acid (43) was obtained from 1-bromo-3-chloropropane (37) first by condensation with malonic ester to compound (38) (70%) [34] followed by decarboxylation to 5-chlorovaleric acid (39) (58%) [35, 36] which was again subjected to reaction with malonic ester to give compound (40) (72.5%); the ester groups were hydrolyzed to give the tricarboxyl-



Scheme 3

ic acid (41) (94%) which was chlorinated to form compound (42) (99%), and one of the carboxy groups was eliminated with the production of the chlorodicarboxylic acid (43) (~100%). α-Chloropimelic acid (43) was treated with β-mercaptopropionic acid in aqueous solution in the presence of caustic soda, and the resulting sulfidic triacid (44) was esterified to compound (49) [73.5% on the compound (43) and 20% on the (37)] [32].

In another variant [37] the starting material was cyclohexanone (45) which was converted via its ethoxycarbonyl derivative (46) into 2-bromo-6-ethoxycarbonylcyclohexanone (47); this was condensed with β-mercaptopropionic acid in the presence of sodium ethanolate and the ring of the compound (48) obtained was opened with caustic soda and, after esterification, compound (49) was obtained [34% on the compound (45)].

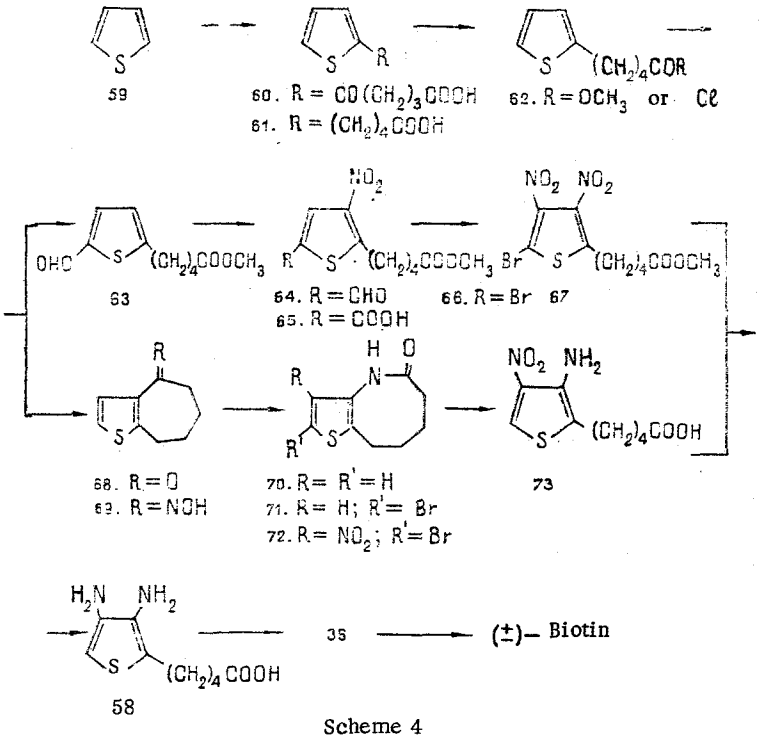
Diethyl α-(β-ethoxycarbonylethylthio)pimelate (49) was subjected to Dieckmann cyclization in benzene in the presence of sodium ethanolate to give 4-ethoxycarbonyl-2-(4-ethoxycarbonylbutyl)-3-oxothiophane (50) (89%), which was then converted into the oxime (51) (96%). For the synthesis of 4-(4-carboxybutyl)dihydro-1H-thieno[3,4-d]imidazole (36) use was made of the rearrangement of 3-hydroxyiminothiophane into 3-aminothiophene under the action of dry hydrogen chloride in ether [33]. By this reaction, the 4-ethoxycarbonyl-2-(4-ethoxycarbonylbutyl)-3-hydroxyiminothiophane (51) yielded 3-amino-4-ethoxycarbonyl-2-(4-ethoxycarbonylbutyl)-thiophene (52) (60.5%), from which the ethoxy group of the side chain at $C_{(4)}$ was eliminated by selective hydrolysis, leading to the acid (53) (82%). Then the ethoxycarbonyl group in position 4 of this acid was converted into the amino group of compound (58) via compound (54) (83%), the hydrazide (55) (91%), the azide (56) (93%), and the Curtius reaction to form the urethane (57) (95%). The 3,4-diamino-2-(4-carboxybutyl)thiophene (58) obtained by the hydrolysis of this urethane was treated with phosgene, which led to the formation of 3a,4,6,6a-tetradehydrobiotin (36) [58% on the compound (57), 4.3% on the (37), and 5.6% on the (45)] [32]. The reduction of this compound with hydrogen in the presence of MoS_3/Al gave a mixture of biotin and its isomers [38], from which it was freed by recrystallization from aliphatic alcohols. In this way, (±)-biotin (1) was obtained with a yield of 3-5% on the "aromatic biotin" (36), 0.13-0.21% on the initial 1-bromo-3-chloropropane (37), or 0.17-0.28% on cyclohexanone (45).

If one bears in mind the fact that recently [39] the hydrogenation of 3a,4,6,6a-tetradehydrobiotin (36) under a pressure of 135 atm in anhydrous ethanol with a threefold amount of 5% Pd/C catalyst at 70°C has given a 14.7% yield, the overall yield of (±)-biotin in the method under consideration may reach 0.6% on 1-bromo-3-chloropropane (37) or 0.82% on cyclohexanone (45).

Synthesis of (±)-Biotin from Thiophene via Tetradehydrobiotin

Two variants of the synthesis of 3a,4,6,6a-tetradehydrobiotin ("aromatic biotin") from thiophene are known – those of Nishimura and Imoto [40] and those of Gol'dfarb, Fabrichnyi et al. [41, 42] (Scheme 4). In both variants, nitro derivatives of thiophene are used; by successive reactions, thiophene is provided with the substituents that are necessary for the transformation of its molecule into "aromatic biotin," which can be converted into biotin by hydrogenation using the method of Vasilevskis and Caldwell just described [39].

Preparation of 3a,4,6,6a-Tetrahydrobiotin; Variant 1 [40]. First, a 4-carboxyl-1-oxobutylgroup is introduced into position 2 of thiophene (59) by the action of the monochloride of glutaric acid in the presence of stannic chloride. In the compound obtained (60) (87%) the keto group is reduced by hydrazine in the presence of caustic soda, leading to the formation of 2-(4-carboxybutyl)thiophene (61) (88%) the methyl ester of which (62, R = OCH_3) is then formed (85%).



Scheme 4

The introduction of a nitro group into position 3 of the thiophene molecule is possible only if position 2 is substituted by an electron-donating group, such as an alkyl group, and there is an electron-accepting group such as formyl or acetyl in position 5 [43]. Consequently, the ester (62) is formylated, and the resulting compound (63) (79%) is nitrated in position 3 giving the nitro derivative (64) (91%). For the introduction of a second nitro group into position 4, the formyl group in position 5 must be replaced by an electron-donating substituent such as bromine. For this purpose, the formyl group is oxidized with chromic acid to a carboxy group (compound 65) (76%) and this is converted via the silver salt into bromide by the Hansdiecker reaction giving the bromomononitro derivative (66) (53%). This compound is nitrated with a mixture of nitric and sulfuric acids to 5-bromo-2-(4-methoxycarbonylbutyl)-3,4-dinitrothiophene (67) (88%), which is then reduced with tin in concentrated hydrochloric acid to give the diamine (58) (91%). Since this compound is not stable in the air it is not isolated but is treated directly with phosgene in the presence of caustic soda to give 4-(4-carboxybutyl)-2-oxo-2,3-dihydro-1H-thieno[3,4-d]imidazole, 3a,4,6,6a-tetradehydrobiotin (36) [70% on the compound (58) and 11.8% on the initial compound (59)].

Preparation of 3a,4,6,6a-Tetradehydrobiotin; Variant 2 [41, 42]. Via compound (60), thiophene (50) is converted into 2-(4-carboxybutyl)thiophene (61) [76.5% on the (59)] in the same way as described above. By the action of thionyl chloride, compound (61) is converted into the acid chloride (62, R = Cl) (83%), and this is subjected to intramolecular cyclization by the Friedel-Crafts reaction in the presence of stannic chloride to form the bicyclic compound (68) (85%) [44], from which the oxime (69) is then obtained (90%). This cyclic oxime is subjected to Beckmann rearrangement in the presence of benzenesulfonyl chloride to form the lactam (70) (83%). In this way a potential amino group appears in position 3 [42]. In order to introduce a second amino group into position 4 via a nitro group, there must be an electron-donating substituent such as bromine in position 5. The lactam (70) is brominated with elementary bromine in acetic acid and the resulting compound (71) (85%) is nitrated with a mixture of potassium nitrate and sulfuric acid to the nitrolactambromo (72) (51%) from which the bromine is eliminated by boiling with copper powder in propionic acid, and the lactam ring is opened by hydrolysis with hydrochloric acid giving the nitro amine (73) in the form of the hydrochloride. The nitro group of compound (73) is reduced with tin in hydrochloric acid, and the diamine (58) is treated with phosgene in the presence of a base to give 3a,4,6,6a-tetradehydrobiotin (36) [34% on the compound (72) and 5.8% on the initial thiophene (59)] [41].

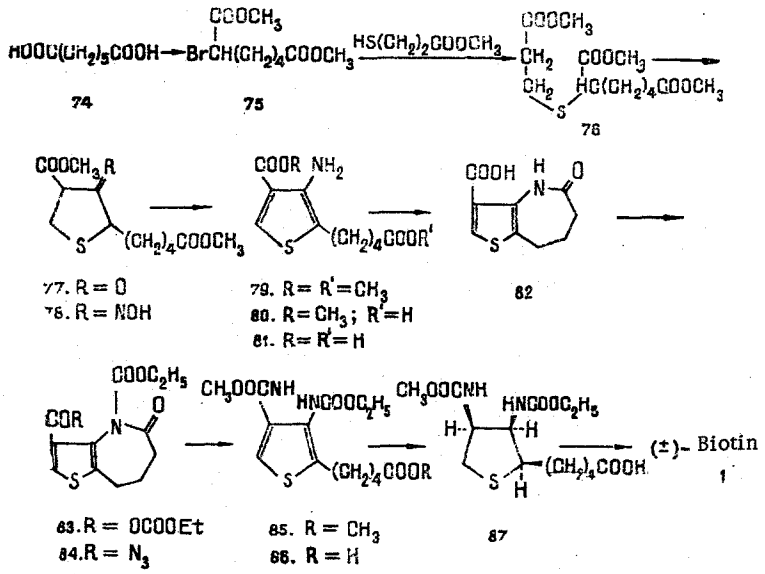
The authors concerned did not continue the proposed variants of the synthesis of 3a,4,6,-6a-tetradehydrobiotin as far as biotin itself. It is known that the reduction of thiophenes, including compound (36), presents great difficulties. However, Vasilevskis and Caldwell [39] have achieved some success — by the catalytic hydrogenation of 3a,4,6,6a-tetradehydrobiotin (36) in anhydrous ethanol with a threefold amount of the catalyst 5% Pd/C at 135 atm and 70°C they obtained (±)-biotin (1), contaminated with the initial compound and a desulfuration product, in a yield of 14.7%. If these facts are taken into account, the overall yield of (±)-biotin present in an unresolvable mixture with the initial compound (36) and a desulfuration product, calculated on thiophene (59), may amount to 1.7% by variant 1 and 0.85% by variant 2.

Stereospecific Synthesis of (±)-Biotin from Pimelic Acid via a 2,3,4-Trisubstituted Thiophene

The synthesis due to Confalone et al. [45, 46] (Scheme 5) makes use of the closure of an eight-membered lactam ring on the thiophene ring, the Curtius rearrangement in order to introduce an amino group into position 4, the substrate-specific hydrogenation of the 2,3,4-trisubstituted thiophene to the corresponding thiophane with the cis position of the substituents, and the ring-closure of a diurethane to form an imidazolidone ring.

The reaction of pimelic acid (74) with thionyl chloride and then with bromine in methanol forms dimethyl α-bromopimelate (75), which is treated with methyl β-mercaptopropionate giving dimethyl α-(β-methoxycarbonylethylthio)pimelate (76). Then the intramolecular condensation of this sulfide in the presence of sodium methanolate in benzene gives 4-methoxycarbonyl-2-(4-methoxycarbonylbutyl)-3-oxothiophane (77) (yield 14% on compound 74) [47]. The oxothiophane (77) is converted with hydroxylamine in pyridine into its oxime (78) (∿100%) which is subjected in ether in the presence of hydrogen chloride to an intramolecular rearrangement as a result of which an unsaturated (thiophene) ring is formed from the thiophane ring with the conversion of the hydroxyimino group into an amino group and the simultaneous splitting out of a molecule of water — a mixture of the amino diester (79) and the amino monoester (80) in a ratio of 6:1 is obtained (97%).

This mixture is hydrolyzed to the amino acid (81) (95%) which is boiled in xylene to bring about the cyclization of the aliphatic side chain to form an eight-membered carboxylactam on the thiophene ring — compounds (82) (87%). The action of chloroformyl ester on the carboxylactam (82) in the presence of triethylamine in acetone with cooling forms the intermediate mixed anhydride (83) which, without isolation, is treated with sodium azide to give the lactam of 4-azidocarbonyl-3-ethoxycarbonylamino-2-(4-carboxybutyl)thiophene (84) (∿100%). The Curtius rearrangement of the azide (84) with simultaneous methanolysis [48] and the simultaneous opening of the lactam ring is effected by boiling in methanol, the product being the diurethane methyl ester (85) (80%), the hydrolysis of which in caustic soda solution leads to the formation of 2-(4-carboxybutyl)-3-ethoxycarbonylamino-4-methoxycarbonylaminothiophene (86) (99%). By catalytic hydrogenation with 10% Pd/C at 50°C and 120 atm for 10 h in acetic acid, the thiophene diurethane (86) is converted into the thiophane mixed diurethane (87) (95%) with the complete cis configuration of the substituents. When this is treated with barium hydroxide at the boil in water, intramolecular cyclization to an imidazolidone ring with the formation of (±)-biotin (1) takes place (68%). The overall yield of biotin on the oxothiophane (77) is 37%, and on the pimelic acid (74) 5.2%.



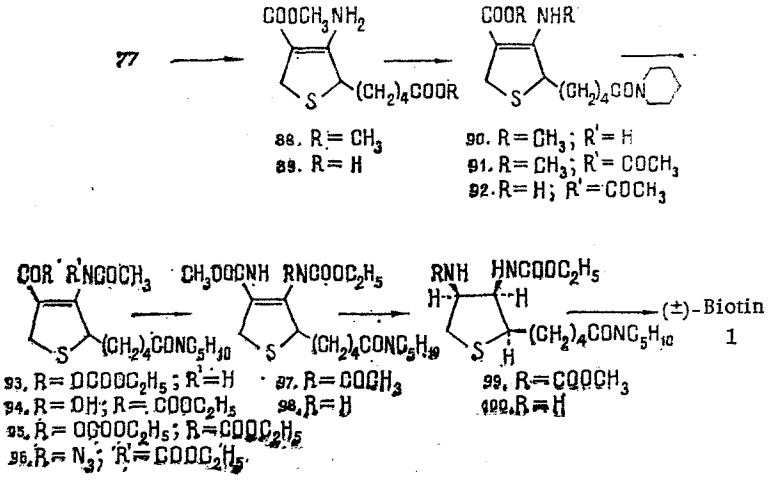
Scheme 5

In this synthesis, particularly effective is the use of a compound with two urethane groupings, since they are the precursors of the imidazolidone moiety of biotin and the closure of this ring does not require special reagents (phosgene) and the performance of supplementary reactions.

(±)-Biotin is obtained in lower yield if compound (82) is subjected to esterification and is converted by reaction with hydrazine into the azide and then the Curtius rearrangement is carried out and the diurethane methyl ester (85) is obtained from the resulting diaminothiophene lactam.

Stereospecific Synthesis of (±)-Biotin from Pimelic Acid via 2,5-Dihydrothiophene Derivatives

In the synthesis from pimelic acid due to Confalone et al. [49] (Scheme 6), the key compound is 4-methoxycarbonyl-3-oxothiophane with the aliphatic side chain characteristic for the biotin molecule; by a specific rearrangement, the oxo group is replaced by an enamine group and the carboxy group at $C_{(4)}$ is converted by a series of steps using the Curtius reaction into a urethane group and the imidazolidone ring of biotin is formed as the result of intramolecular cyclization of the urethane groupings.



Scheme 6

In this synthesis, 4-methoxycarbonyl-2-(4-methoxycarbonylbutyl)-3-oxothiophane (77) is obtained with a yield of 14% from pimelic acid (74) by a three-stage synthesis via compounds (75) and (76) (for a description, see above) [47]. The oxothiophane (77) is caused to react with ammonium formate at the boil in ethanol, whereupon a rearrangement of the exocyclic double

bond into an endocyclic unsaturated bond takes place with the introduction of an amino group into position 3 and the splitting out of water, which leads to the formation of 3-amino-4-methoxycarbonyl-2-(4-methoxycarbonylbutyl)-2,5-dihydrothiophene (88). The aliphatic ester group in this compound is subjected to selective hydrolysis under the action of sodium methanolate, and in the monoacid (89) obtained it is again protected, but now by an amide group — under the action of chloroformic ester in tetrahydrofuran in the presence of triethylamine followed by treatment with piperidine the piperidide (90) is formed. The relatively unreactive amino group of compound (90) is acylated under the action of acetic anhydride in the presence of perchloric acid, giving the acetyl derivative (91) [92% on the (77)] and then, after hydrolysis, 3-acetylamino-4-carboxy-2-(4-piperidinocarbonylbutyl)-2,5-dihydrothiophene (92) (94%).

In order to introduce an amino group into position 4 of this compound use is made of the Curtius reaction, for which compound (92) is first treated with two equivalents of chloroformic ester, which leads to the formation of the amido mixed anhydride (95) through the intermediate compounds (93) and (94); the subsequent addition of sodium azide gives the acyl azide (96) in the form of an oil. This undergoes rearrangement with simultaneous methanolysis to the urethane (97) on boiling in methanol [48] with a yield of 85% on the (92). By mild alkaline hydrolysis, compound (97) is selectively deacetylated to the tetrahydrofuran, giving the crystalline diurethane (98) (92%). Its stereospecific catalytic hydrogenation with 10% Pd/C (proportion of catalyst 6:1) in acetic acid at 50°C and 120 atm for 10 h gives the substituted thiophane (99) with the full cis configuration of the substituents in a yield of 91%. Alkaline hydrolysis of the compound leads to the formation of (±)-biotin (1) with a yield of 67%, apparently via the intermediate amine (100%) which cyclizes at the $C_{(3)}$ urethane group. The overall yield of (±)-biotin from the ketone (77) is ∿37% and from the pimelic acid (74) 5.2%.

Stereoselective Synthesis of (±)-Biotin from the Ester of Adipic Acid Semialdehyde via Derivatives of the Thienofuroxane Ring

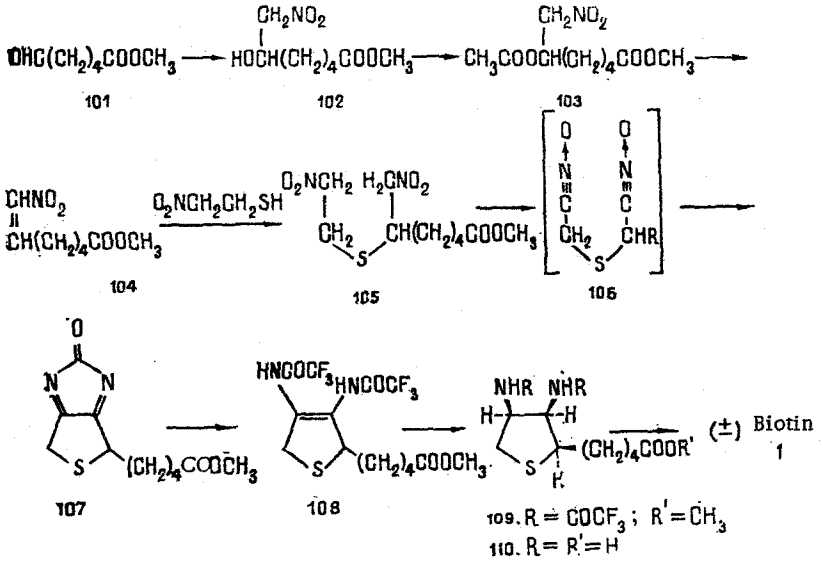
Marx et al. [50] have proposed a synthesis (Scheme 7) which passes through dinitro compounds containing the aliphatic side chain of biotin with the subsequent formation of a thienofuroxane bicyclic system and its further conversion into biotin via a diaminothiophane.

The condensation of the ester of adipic acid semialdehyde (101) [51] with nitromethane in methanol in the presence of caustic soda leads to the nitro alcohol (102) [52], which is converted via the nitro acetate (103) by the action of acetic anhydride and sulfuric acid into methyl 7-nitrohept-6-enoate (104) [38% on the (101)] [53]. 2-Nitrothioethanol is added to this nitroolefin (104) in ether, giving the di(nitroethyl) sulfidic ester (105) with a yield of 80% [50]. The 2-nitrothioethanol is obtained from 2-nitroethyl acetate by the action of trisodium phosphorothioate [54] in water followed by acid hydrolysis of the intermediate phosphorothioate. A chloroform solution of the dinitrosulfidic ester (105) is slowly added to a mixture of phosphorus oxychloride and triethylamine in chloroform giving the substituted 4H,6H-thieno-[3,4-d]furoxane (107) in the form of a mixture of isomers (81%); the reaction takes place through the intermediate formation of the dinitrile oxide (106). This structure of the compound (107) determines the stereochemistry of the functional groups to be introduced into the thiophane ring.

When the compound (107) is treated with the Zn/Ag couple [55] in the presence of trifluoroacetic anhydride in dimethoxyethane, a peculiar reduction takes place with the opening of the furoxane ring and the formation of an acylated enediamine — the 2,5-dihydrothiophene derivative (108) (40%) — together with a small amount of thiophene derivative as impurity. The catalytic hydrogenation of compound (108) to the diacyldiaminothiophanecarboxylic ester (109) with the total cis configuration of the substituents takes place with a 20% Pd/C catalyst under a pressure of 4 atm at 20°C for 24 h. After hydrolysis and deacetylation in methanol in the presence of potassium carbonate, the resulting diamine (110) is treated with phosgene in benzene, giving crystalline (±)-biotin (1) in a yield of 77% on the (108). The overall yield of biotin on the initial ester of adipic acid semialdehyde is 4.1%. The resolution of the racemic biotin into its optical antipodes with L-(+)-arginine gives natural (+)-biotin.

Synthesis of (±)-Biotin from 1,4-Dibromobutane via a 3-Oxothiophane Derivative

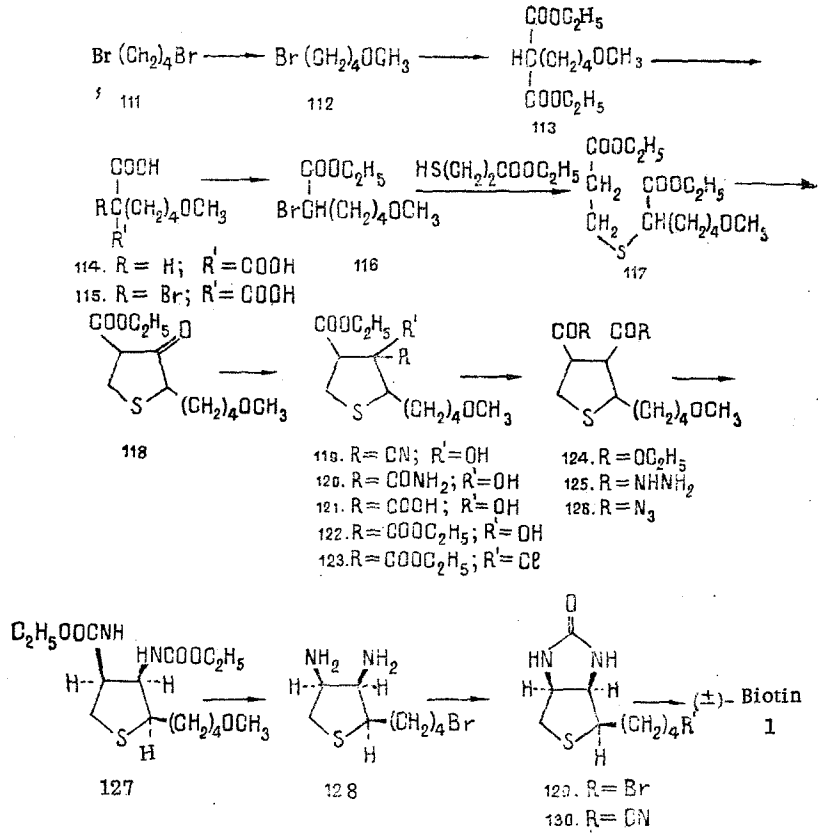
This synthesis was developed by Grüssner et al. [56, 57] (Scheme 8); it is nonstereospecific and is accompanied by the formation of a considerable amount of isomeric compounds



Scheme 7

and byproducts [58]; the final construction of the aliphatic chain of the biotin molecule is carried out on the preformed hexahydrothienoimidazole molecule with the complete cis configuration of the substituents.

The starting material in this synthesis is 1,4-dibromobutane (111), in which one bromine atom is replaced by a methoxy group, and the monobromo compound (112) is brought into reaction with malonic ester; subsequent hydrolysis and the decarboxylation of compound (113) to (114) and its bromination to compound (115) leads to ethyl 2-bromo-6-methoxycaproate (116). This compound is condensed with ethyl β-mercaptopropionate to form the sulfide (117) which gives the oxothiophane (118) by ring closure [59].

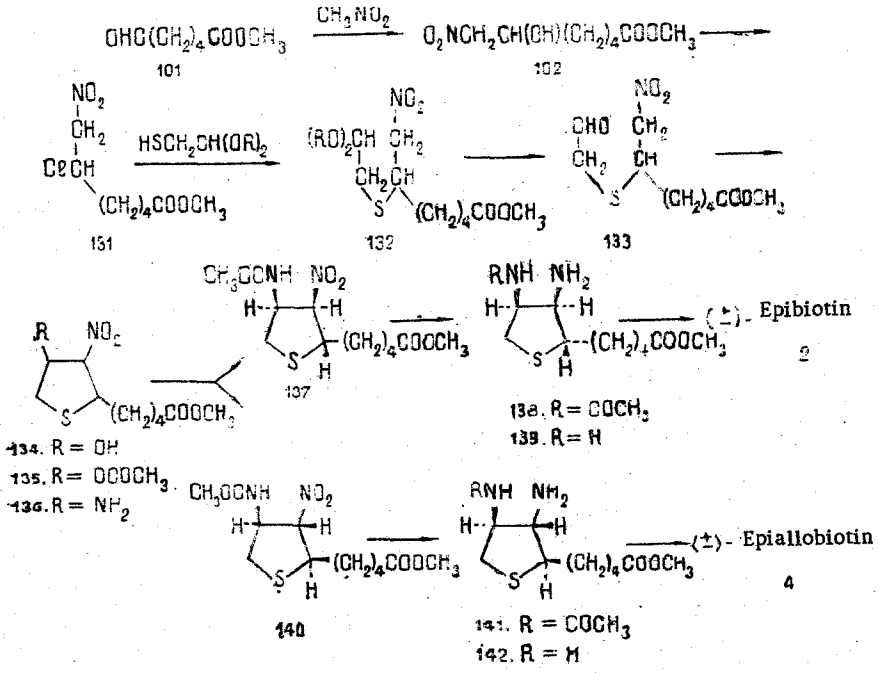


Scheme 8

A second carboxy group is introduced at $C_{(3)}$ into the 2-(4-methoxybutyl)-4-methoxycarbonyl-3-oxothiophane (118) by the cyanohydrin synthesis and subsequent transformations via compounds (119-123), and then from the diester (124) and hydrazine hydrate the noncrystalline dihydrazide (125) is obtained and this is converted via the diazide (126), using the Curtius reaction, into the diurethane (127). The action of 48% hydrobromic acid on this compound eliminates the protective groups and simultaneously replaces the methoxy side chain by bromine. The resulting diamine (128) with the compete cis configuration of the substituents is treated with phosgene, which forms the imidazolidone ring, and then the intermediate compound (129) is converted via the biotin nitrile (130) in low yield into (±)-biotin (1).

Synthesis of (±)-Epibiotin and (±)-Epiallobiotin from the Ester of Adipic Acid Semialdehyde via 3-Nitrothiophane Derivatives

This is one of the first syntheses of stereoisomers of biotin and was developed by Grob and Sprecher [52] (Scheme 9).

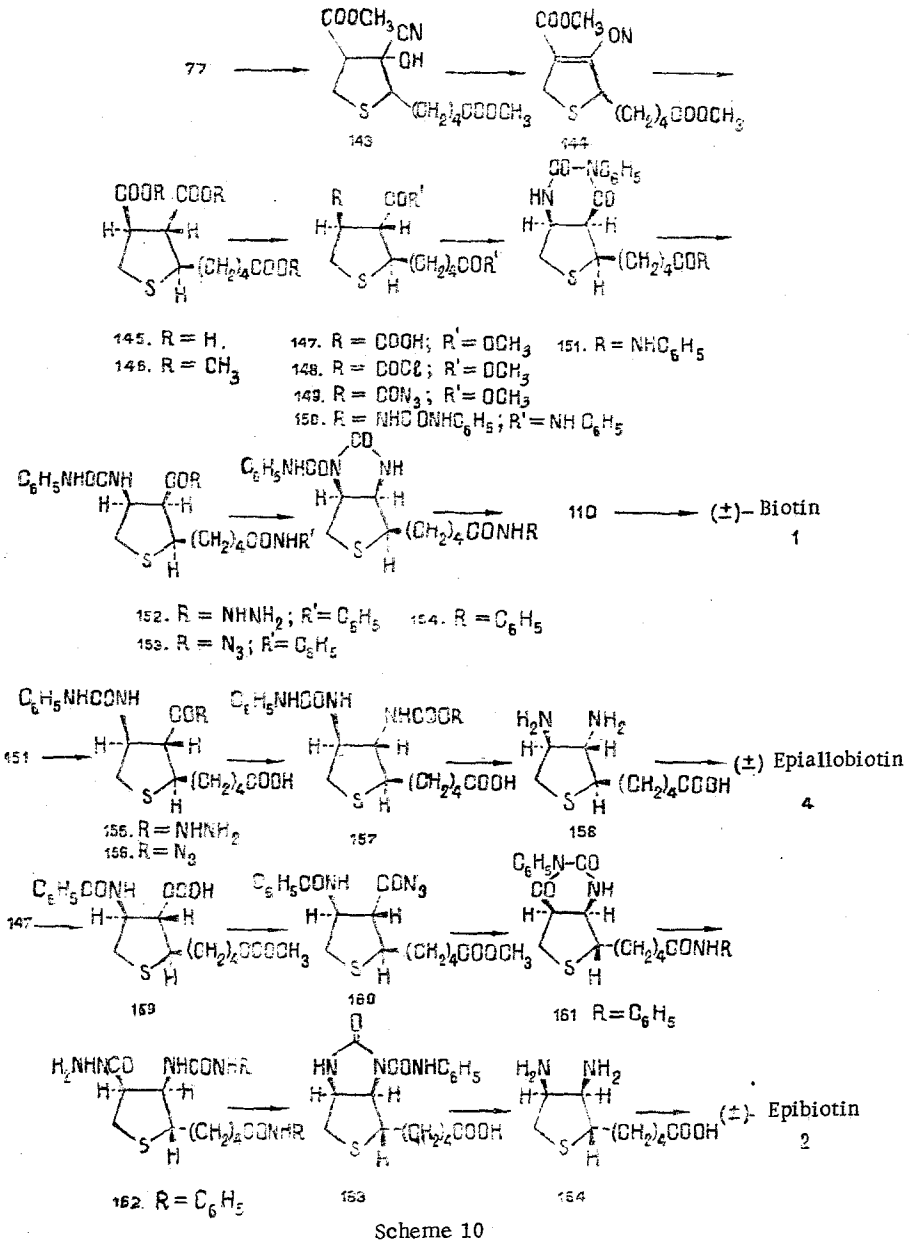


Scheme 9

As the starting material in this synthesis they use the ester of adipic semialdehyde (101), from which by a series of transformations -- the formation of the nitro derivative (102), the chloro derivative (131), the sulfidic ester (132), and the sulfidic aldehyde (133) — a mixture of isomeric esters of 2-(4-carboxybutyl)-3-nitro-4-hydroxythiophane (134), isolated in the form of two crystalline nitrobenzoates, is obtained. From these stereoisomeric hydroxynitrothiophanes (134) are obtained successively: the acetoxy derivative (135); by the action of ammonia in dioxane solution the nitro amines (136); and then the stereoisomeric nitroacetamides (137) and (140); by reduction with amalgamated aluminum the monoacetyl diamines (138) and (141), and then the epidiamine (139) and the epiallodiamine (142). The closure of the imidazolidone rings of these two compounds under the action of phosgene leads to (±)-epibiotin (2) and (±)-epiallobiotin (4).

Stereospecific Synthesis of (±)-Biotin, (±)-Epibiotin, and (±)-Epiallobiotin from Pimelic Acid via a Substituted trans-3,4-Dicarboxythiophane

This synthesis of biotin and its stereoisomers was developed by Baker et al. [47, 60-62] (Scheme 10). It consists in the initial formation of a key substituted thiophane — 4-methoxycarbonyl-2-(4-methoxycarbonylbutyl)-3-oxothiophane — from pimelic acid with the subsequent transformation of the substituents into 3,4-diamino groups with the required stereoisomerism which is achieved by reversing the configuration of substituents in the formation of new rings and their opening. As a result, the imidazolidone ring is created on the diaminothiophane.



Scheme 10

First from pimelic acid (74) 4-methoxycarbonyl-2-(4-methoxycarbonylbutyl)-3-oxothiophane (77) is obtained in a yield of 14% on compound (74) via a series of intermediate compounds (75) and (76) (for description, see above). The oxothiophane (77) is converted by means of the cyanohydrin synthesis, dehydration, hydrolysis of the nitrile group to a carboxy group, and reduction via compounds (143) and (144) into 3,4-dicarboxy-2-(4-carboxybutyl)thiophane in the form of several stereoisomers of which only the trans-3,3-dicarboxylic acid (145) is used in the subsequent synthesis [47, 60]. The $C_{(4)}$ carboxy group of this compound is converted into a phenylureido group, with the formation of compound (150) [61]. For this purpose the dicarboxylic acid (145) is converted into the triester (146) which is subjected to selective hydrolysis with one equivalent of alkali. The free carboxy group of the monoacid (147) formed is converted into the chloride (148) and then into the azide (149), from which the isocyanate is obtained, and this is condensed with aniline to form compound (150). The cyclic anilide (151) is obtained from this by the action of acetic anhydride under the catalytic action of sodium acetate, with reversal of the trans configuration at $C_{(3)}$ to the cis configuration.

The action of an equimolecular amount of hydrazine hydrate on the anilide (151) at 100°C for 10 min leads to the opening of the cyclic anilide and the formation of the cis-hydrazide (152), from which, via the azide (153) followed by Curtius rearrangement, compound (154) is obtained. Its hydrolysis and the subsequent reaction of the r-4,c-3-diamino-c-2-(δ-carboxybutyl)thiophane (110) with phosgene gives (±)-biotin (1) with an overall yield of 1.7% on

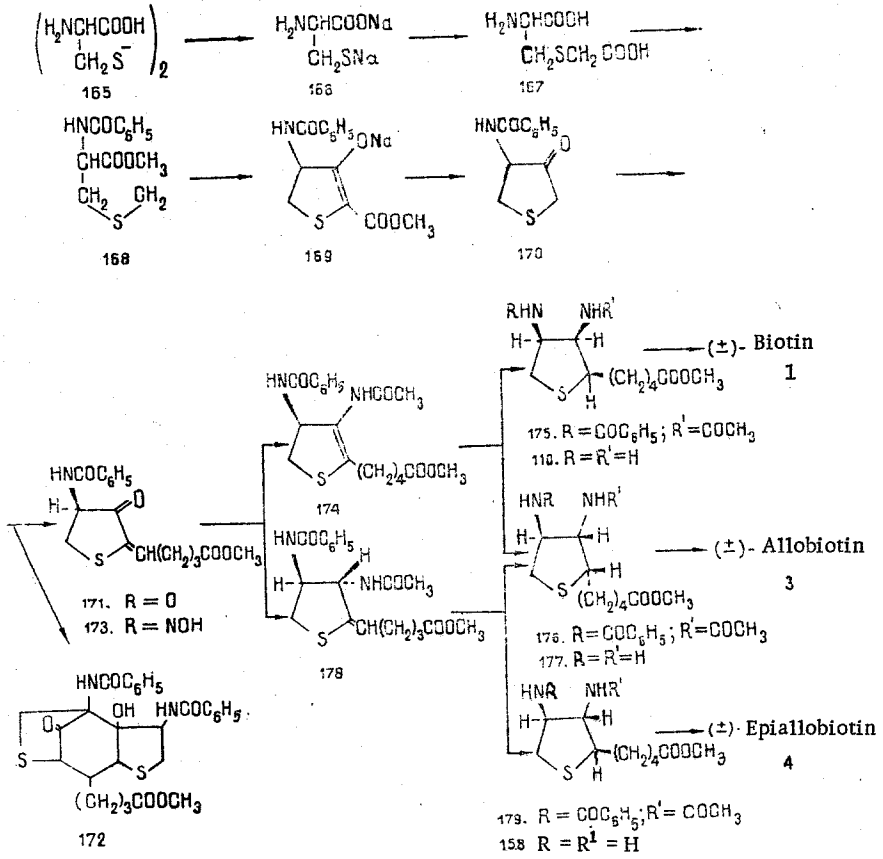
the pimelic acid (74) [61]. If the amide group of the cyclic anilide is hydrolyzed and the product is treated with an excess of hydrazine hydrate in aqueous solution at 100°C for 2 h, the trans-hydrazide (155) is formed by virtue of the fact that the substituent at $C_{(3)}$ undergoes reversal of the cis configuration to the trans configuration (the same cis-trans inversion of a carboxyl takes place in the presence of caustic soda or sodium methanolate); a series of subsequent conversions via compounds (156), (157), and r-4,t-3-diamino-c-2-(4-carboxybutyl)thiophane (158) gives (±)-epiallobiotin (4) [6].

The preparation of epibiotin starts from the intermediate diester (147) and then, via the 4-carbanilido-3-carboxythiophane (159), in which the carboxy group at $C_{(3)}$ is converted into the azide (160) and by the Curtius reaction into the intermediate isocyanate, the cyclic cis-ureide (161) is obtained, its formation being connected with reversal of the configuration of the carboxy group at $C_{(4)}$. Then, as the result of a number of successive transformations via compounds (162), (163), and r-4,c-3-diamino-t-2-(4-carboxybutyl)thiophane (167), (±)-epibiotin (2) is obtained [62].

Synthesis of (±)-Biotin, (±)-Allobiotin, and (±)-Epiallobiotin from L-Cystine via Thiophane Derivatives

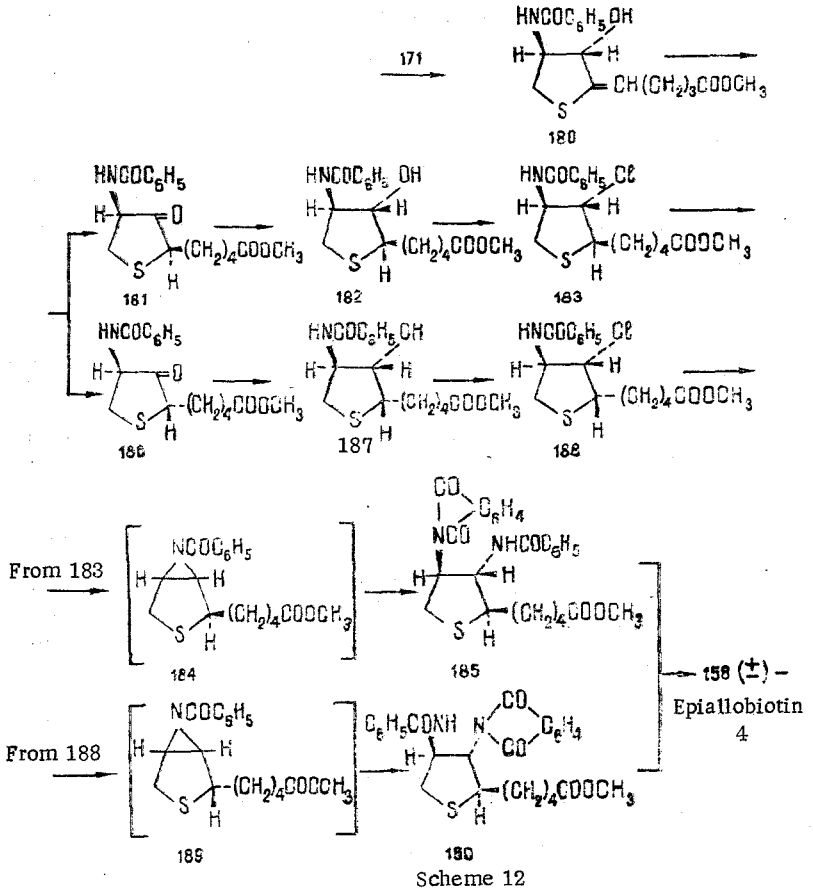
In 1944-1945, Harris, Folkers, et al. [63, 64] performed the first total synthesis of (±)-biotin (Scheme 11) and thereby definitively confirmed its structure. Since the synthesis has no steric directivity, a number of intermediate substituted thiophanes are obtained in the form of mixtures of isomers with diffrent cis-trans configurations of the substituents. The separation of the isomers is carried out on the basis of their different solubilities. In the last stage of the synthesis the imidazolidone ring is constructed on the thiophane ring. The synthesis gives three stereoisomeric forms of biotin.

L-Cystine (165) is reduced with sodium in liquid ammonia to L-cysteine (sodium derivative, 166) which is condensed with chloroacetic acid to form S-carboxymethylcysteine (167) [82.2% on the (165)]. Protective groups are introduced into the amino and carboxy groups by benzoylation and esterification, and the dimethyl ester of N-benzoyl-S-carboxymethylcys-



Scheme 11

teine (168) is obtained with a yield of 48.6% [63]. By Dieckmann intramolecular condensation, compound (168) is converted into the enolic form (sodium derivative) β-keto ester (169) (89%), which by decarboxylation in a mixture of acetic and hydrochloric acids forms 4-benzoylamino-3-oxothiophane (170) with a yield of 76.5%. Then the aliphatic side chain characteristic of the biotin molecule is introduced into position 2 of this compound by condensation with the methyl ester of glutaric acid semialdehyde [53, 65, 66], leading to the production of 4-benzoylamino-2-(4-methoxycarbonylbutylidene)-3-oxothiophane (171) (53%) with an overall yield of 14.4% on the initial L-cystine [63]. As was found, this reaction takes place ambiguously and through a Diels-Alder reaction the product of the diene condensation of the enolic form of 4-benzoylamino-3-oxothiophane (170, dienophile) with the enolic form of compound (171) (diene) —3,4-dibenzoylamino-3a-hydroxy-7-methoxycarbonylpropyl-5-oxoperhydro-6,4-epithiobenzo[b]thiophene (172) — is formed simultaneously [67].



Scheme 12

As a result of a number of nonstereospecific reactions, compound (171) gives three diastereoisomeric forms: (±)-biotin (1), (±)-allobiotin (3), and (±)-epiallobiotin (4). First, compound (171) is converted into the oxime (173) (59%) which is reduced with zinc in a mixture of acetic anhydride and acetic acid to a mixture of two isomeric compounds (174) (34%) and (178), which, after their separation and catalytic hydrogenation over a Pd catalyst, form three stereoisomeric compounds (175) (68%), (176), and (179). After the separation and treatment of each of these compounds with an aqueous solution of barium hydroxide the stereoisomeric diaminothiophanecarboxylic acids (110), (177), and (158) are obtained which, by treatment with phosgene, give racemic biotin (1) in a yield of 57% on the (175), racemic allobiotin (3), and racemic epiallobiotin (4) [63, 64]. The yield of (±)-biotin (1) is 7.8% on the 4-benzoylamino-2-(4-methoxycarbonylbutylidene)-3-oxothiophane (171), or 1.1% on the initial L-cystine (165).

Stereospecific Synthesis of (±)-Epiallobiotin from L-Cystine via 3-Hydroxythiophane Derivatives

The synthesis due to Mikhno et al. [68-71] (Scheme 12) is based on an intermediate compound obtained (in accordance with Scheme 11) from L-cystine and consists in the transformation of the substituent at $C_{(3)}$ with the use of stereodirected reduction of the cyclic keto

group to a hydroxy group, and its replacement by chlorine and then by an amino group by a Gabriel reaction taking place in peculiar fashion with subsequent formation of the imidazolidone ring on the thiophane molecule.

L-Cystine (165) is first converted via compounds (166-170, Scheme 12) into 4-benzoylamino-2-(4-methoxycarbonylbutyldiene)-3-oxothiophane (171) [63], in which the oxo group is reduced to a hydroxy group by sodium tetrahydroborate in methanol — the reaction takes place in stereodirected fashion leading to r-4-benzoylamino-2-(4-methoxycarbonylbutylidene)-t-3-oxothiophane (180) [68]. Then this compound is subjected to a prototropic rearrangement under the action of hydrogen chloride in methanol, which leads to the formation of equal amounts of two isomers at position 2 of the thiophene ring — r-4-benzoylamino-c-2-(4-methoxycarbonylbutyl)-3-oxothiophane (181) and r-4-benzoylamino-t-2-(4-methoxycarbonylbutyl)-3-oxothiophane (186) [69].

The oxothiophanes (181) and (186) are stereodirectedly reduced with sodium tetrahydroborate to the corresponding hydroxythiophanes (182) and (187), from which, by the action of thionyl chloride, the 3-chloro derivatives with the retention of the configurations of the initial compounds — r-4-benzoylamino-t-3-chloro-c-2-(4-methoxycarbonylbutyl)thiophane (183) and r-4-benzoylamino-t-3-chloro-t-2-(4-methoxycarbonylbutyl)thiophane (188) — are obtained [70]. By the Gabriel reaction, the chlorine in these compounds is replaced by an amino group [71]. The reaction takes place in a peculiar manner. Initially, the trans-3,4-aminohalo-substituted thiophanes (183) and (188) undergo intramolecular cyclization with the formation of intermediate compounds apparently containing aziridine rings (184) and (189), and then a nucleophilic attack by the phthalimide on the $C_{(4)}$—N bond [in compound (184)] gives t-3-benzoylamino-c-2-(4-methoxycarbonylbutyl)-r-4-phthalimidothiophane (185) (an intermigration, as it were, of the substituents in positions 3 and 4 takes place, but the configurations of the substituents at $C_{(2)}$, $C_{(3)}$, and $C_{(4)}$ do not change), and attack at the $C_{(3)}$—N bond [in compound (189)] gives r-4-benzoylamino-c-2-(4-methoxycarbonylbutyl)-t-3-phthalimidothiophane (190) (reversal of the configurations of the substituents at $C_{(3)}$ and $C_{(4)}$ takes place). The phthalimide derivatives (185) and (190) are hydrolyzed with 48% hydrobromic acid, and the diaminothiophene (158) obtained is phosgenated in an aqueous medium in the presence of sodium carbonate, giving (±)-epiallobiotin (4) [71].

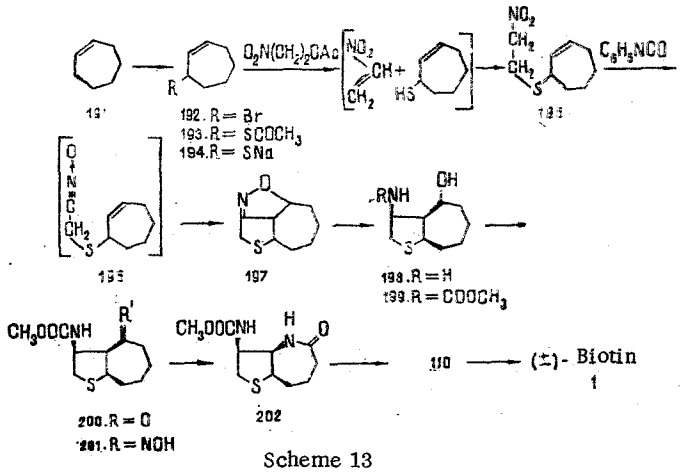
Stereospecific Synthesis of (±)-Biotin from Cycloheptene

In this synthesis, Confalone et al. [72] (Scheme 13) used the intramolecular [3+2]-cycloaddition of an olefinic nitrile oxide in a stereospecific synthesis of a key bicyclic amino alcohol — 3-amino-4-hydroxytetrahydrothienocycloheptane.

Cycloheptene (191) is subjected to allyl bromination with N-bromosuccinimide [73], giving 3-bromocycloheptene (192) with a yield of 60%, and this is converted by the action of mercaptoacetic acid in acetonitrile into 3-acetylthiocycloheptane (193) with a yield of 71%. The sodium mercaptide (194) is obtained with a yield of ∿100% from this in the presence of sodium ethanolate in ethanolic solution and then, by the action of one equivalent of nitroethyl acetate, 3-nitroethylthiocycloheptene (195) is obtained with a yield of 99%. Apparently, nitroethylene and a mercaptan are first obtained as intermediate compounds which then condense. The treatment of compound (195) with phenyl isocyanate in the presence of triethylamine as catalyst in benzene leads via the intermediate intramolecular [3+2]-cycloaddition of the nitrile oxide [compound (196)] to the stereospecific production of the tricyclic isoxazolotetrahydrothienocycloheptane (197) (80%). At this state of the synthesis two of the three asymmetric centers of biotin are created.

The third asymmetric carbon atom with the cis configuration of the substituent is created in compound (197) as the result of the action of lithium tetrahydroaluminate, whereupon the N—O bond is cleaved and the imino group is reduced, giving the bicyclic amino alcohol (198) with a yield of 92%. The subsequent course of the reaction consists in the oxidation of the hydroxy group at $C_{(4)}$ to a carbonyl function and the introduction of a nitrogen atom into the $C_{(3a)}$ position.

The amino group of the amino alcohol (198) is protected by reaction with methyl chloroformate, and the resulting compound (199) (94%) is converted by the action of acetic anhydride in dimethyl sulfoxide into the amino ketone (200) with a yield of 99%. The possibility of epimerization at $C_{(3a)}$ to the more stable trans configuration is hereby excluded. Then the reaction of compound (200) with hydroxylamine in a mixture of pyridine and ethanol gives the oxime in the stereoisomeric anti form (201) (90%) with traces of the unwanted syn form, and



Scheme 13

by the Beckmann rearrangement the oxime (201) forms a substituted thiophane with an eight-membered lactam ring (202) with a yield of 22%.

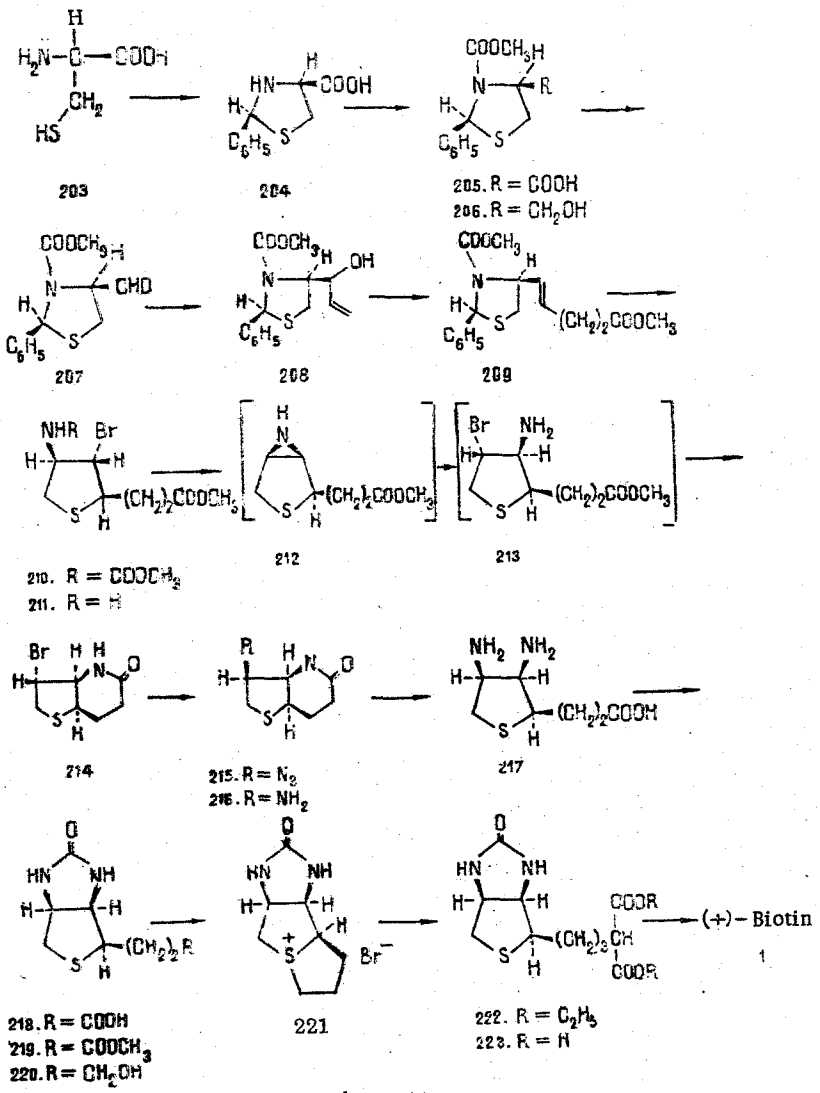
The completing stage of the synthesis consists in the prolonged boiling of compound (202) with barium hydroxide in aqueous solution followed by the reaction of the resulting r-2,c-3-diamino-c-2-(4-carboxybutyl)thiophane (110) with phosgene to give (±)-biotin (1) with a yield of 80% on compound (202). The overallyield of (±)-biotin from cycloheptene amounts to 4.3%.

Stereospecific Synthesis of (+)-Biotin from L-(+)-cysteine

This synthesis due to Confalone et al. [74] (Scheme 14) is based on the production from L-(+)-cysteine first of a thiazolidine derivative and on a new specific oxidative cyclization of this compound with rearrangement into a substituted thiophane. In the subsequent transformations, the amino group of cysteine, which must be present at $C_{(4)}$ of the thiophane structure, is transferred to position 3 as the result of a rearrangement of an aminobromothiophane. The synthesis is not accompanied by racemization and ensures 100% optical purity of the biotin structure characteristic for (+)-cysteine.

L-Cysteine (203) is first condensed with benzaldehyde in 95% ethanol to give 4-carboxy-2-phenylthiazolidine (204) (98%), in which the cyclic imino group is protected by a methoxycarbonyl grouping under the action of chloroformic ester. In the thiazolidine derivative formed (205) (97%), the carboxylic function is selectively reduced by diborane in tetrahydroduran, giving the alcohol (206) (99%), which is converted by oxidation with chromium trioxide in a mixture of pyridine and methylene chloride into the key aldehyde 4-formyl-3-methoxycarbonyl-2-phenylthiazolidine (207)(80%). Then this compound is condensed with vinylmagnesium chloride by the Grignard reaction in methylene chloride at −75°C to form the allyl alcohol (208) (87%), which was subjected to Claisen rearrangement at 85°C with trimethyl orthoacetate in benzene in the presence of propionic acid as catalyst to form the transolefin 3-methoxycarbonyl-c-4-(4-methoxycarbonylbut-1-enyl)-r-2-phenylthiazolidine (209) with a yield of 95%. This thiazolidine is subjected to stereospecific oxidative cyclization-rearrangement under the action of pyridine perbromide hydrobromide in methanol to the substituted thiophane t-3-bromo-r-4-(methoxycarbonylamino)-c-2-(β-methoxycarbonylethyl)thiophane (210) (47%). After selective elimination of the protective grouping under the action of hydrogen bromide in acetic acid, the aminobromothiophane (211) was obtained with a yield of 79% in the form of the hydrobromide.

The subsequent synthesis of biotin was associated with the necessity for the direct S_N2 replacement of the bromine atom at $C_{(3)}$ by an azide group with the reversal of the configuration, which was achieved by heating the aminobromothiophane in acetic acid in the presence of sodium acetate at the boil. This reaction apparently takes place through an intermediate aziridine ring [in compound (212)] with its subsequent opening and the formation of the intermediate 3-amino-4-bromo derivative (213), which leads to an optically active trans-bromolactam — the lactam of t-3-amino-r-4-bromo-t-2-(β-carboxyethyl)thiophane (214) (96%). The treatment of the trans-bromolactam (214) with lithium azide in dimethylformamide at 130°C brings about the inversion of the configuration at $C_{(4)}$ and the formation of the cis-azidolactam (215) (16%) and also the formation of the main product of this reaction — a 2,3-dihydrothiophene without a substituent at $C_{(4)}$.



Scheme 14

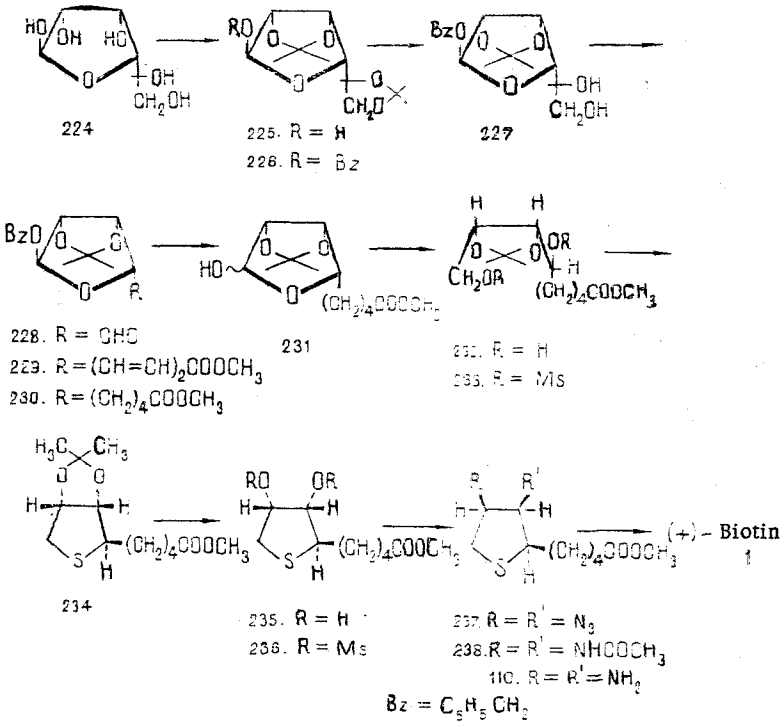
After the catalytic hydrogenation of compound (215) with 10% Pd/C in ethanol at 3 atm, the cis-aminolactam (216) is obtained with a yield of 86%. The hydrolysis of this compound with barium hydroxide to the diamino acid (217) with the complete cis configuration of the substituents, and subsequent treatment with an aqueous solution of phosgene in the presence of sodium bicarbonate, gives bisnorbiotin (218), isolated in the form of its methyl ester (219) [45% on the compound (216)]. The reduction of compound (219) with lithium tetrahydroborate in boiling tetrahydrofuran leads to bisnorbiotinol (220) (87%), which, on treatment with hydrobromic acid in acetic acid, is smoothly converted into the thiophanium bromide (221) in a yield of 80%. Its conversion into (+)-biotin by the addition of a two-carbon-atom fragment is carried out [75] by condensing the thiophanium bromide (221) with malonic ester, leading to the diester (222) (68%) which, after hydrolysis by barium hydroxide, is isolated in the form of the dicarboxylic acid (223). The decarboxylation of one carboxy function by boiling in water leads to (+)-biotin (1) in a yield of about 70% on the compound (222). The overall yield of (+)-biotin amounts to 3.6% on the initial L-(+)-cysteine.

Complete Stereospecific Synthesis of (+)-Biotin from D-Mannose via Thiophane Derivatives

In Ohrui and Emoto's [76] (Scheme 15), an optically active hexose is used to obtain natural optically active (+)-biotin.

From α-D-mannose (224) is obtained 2,3:5,6-di-O-isopropylidene-α-D-mannofuranose (225) [77], and then, by reaction with benzoyl chloride in pyridine, its 1-O-benzoate (226) (yield 97%), which is converted by selective hydrolysis with 70% acetic acid at 20°C into 1-O-benzoyl-2,3-O-isopropylidene-α-D-mannofuranose (227) (93%). In order to introduce the aliphatic

chain characteristic of the biotin molecule into position 4 of this compound, the 5,6-diol grouping is converted into an aldehyde group, which is achieved by shortening the carbon chain by periodate oxidation in aqueous acetone, and then the aldehyde (228) obtained is used in the Wittig reaction with an excess of 3-methoxycarbonylprop-2-en-1-ylidenetriphenylphosphorane [78] in dichloromethane, as a result of which methyl 1-O-benzoyl-2,3-O-isopropylidene-5,6,7,8-tetradeoxy-5,7-dieno-α-D-lyxo-nano-1,4-furanoate (229) is obtained with a yield of 90% on compound (227). After the hydrogenation of compound (229) on Pd/C catalyst in methanol, its derivative with a methoxycarbonylbutyl substituent (230) is obtained (97%).



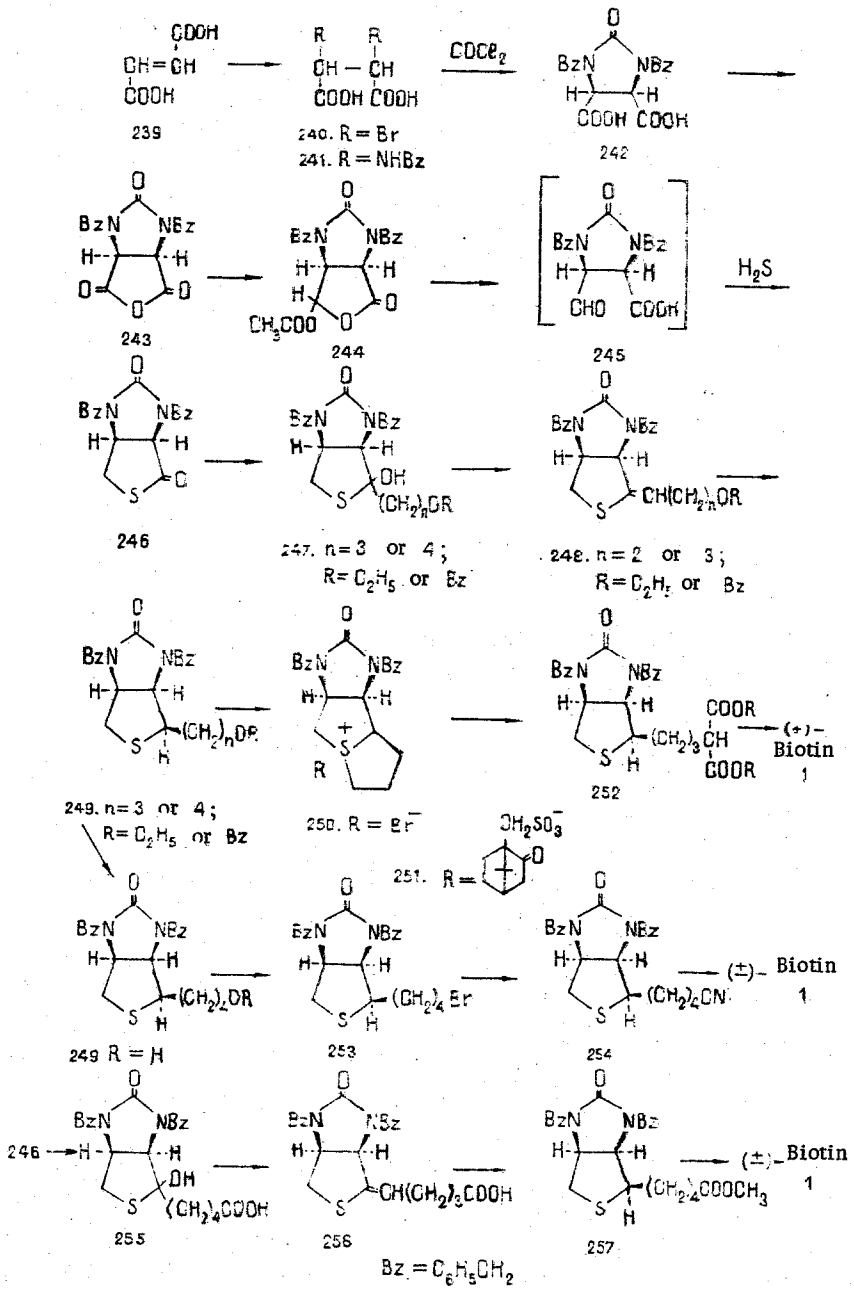
Scheme 15

The subsequent reactions are directed to the formation of the thiophane ring. The hydrolysis of compound (230) with sodium methanolate in methanol gives the hemiacetal (231) and, after reduction with sodium tetrahydroborate, methyl 7,8-O-isopropylidene-2,3,4,5-tetradioxo-L-lyxo-nonoate (232) [85% on the (230)]. Then this compound is treated with methanesulfonyl chloride to give the dimesylate (233) (96%), which with sodium sulfide in hexamethylphosphoramide at 100°C is converted into the thiophane derivative (234) with a yield of 75%.

The isopropylidene protection is eliminated in 90% formic acid at 20°C. This gives the diol (235) (92%), the hydroxy groups of which are converted into amino groups. For this purpose, compound (235) is subjected to mesylation to give the dimesylate (236) (95%), and after reaction with sodium azide in hexamethylphosphoramide at 80°C the diazide (237) is formed (78%). The catalytic reduction of the azido groups with a platinum catalyst in methanol and acetic anhydride at 20°C for 4 h leads to the diamine r-4,c-3-di(acetylamino)-c-2-(4-methoxycarbonylbutyl)thiophane (238) (58%). If reduction is carried out for 1 h, it is possible to obtain only a monoacetylaminomonoazide. (+)-Biotin (1) is obtained in a yield of ∿60% from compound (238) after hydrolysis with barium hydroxide in water at 140°C for 14 h via the intermediate r-4,c-3-diamino-c-2-(4-carboxybutyl)thiophane followed by its phosgenation [63]. The overall yield of (+)-biotin is about 8.0% on the initial D-mannose (224).

Stereoselective Synthesis of (+)- and (±)-Biotins from Fumaric Acid via cis-4,5-Dicarboxyimidazolid-2-one and Hexahydrothienoimidazole Derivatives

This synthesis of (+)-biotin was created by Goldberg and Sternbach [75, 79-81] (Scheme 16) as early as 1949. It consists in the stereospecific formation of a cis-substituted imidazolidone from fumaric acid, its conversion into tetrahydrofuro[3,4-d]imidazole with subsequent transformation into hexahydrothieno[3,4-d]imidazole, and the formation of the third asymmetric center with the aliphatic side chain by the cis-stereoselective reduction of an exocyclic double bond.



Scheme 16

The stereospecific addition of bromine to fumaric acid (239) leads to the formation of meso-dibromosuccinic acid (240) (80%) [82], in which the halogen is replaced by benzylamino groups with the formation of meso-α,β-di(benzylamino)succinic acid (241) (88%) [83] with no change in the configuration of the substituents, after which the compound is treated with phosgene to give 1,3-dibenzyl-cis-4,5-dicarboxy-2-oxotetrahydroimidazole (242) (61%) [79].

The configuration of the chiral $C_{(4)}$ and $C_{(5)}$ atoms in the imidazolidone ring are retained. In the further course of the synthesis, the thiophane ring is built up on the imidazolidone ring as base. Under the action of acetic anhydride, the anhydride 243 is obtained with a yield of 80% from the cis-dicarboxylic acid (242), and this is reduced with zinc in a mixture of acetic acid and acetic anhydride to the acyloxylacetone (244) (50%) [79]. This compound can be obtained from the cis-dicarboxylic acid (242) by catalytic reduction with hydrogen in the presence of palladium or Raney nickel followed by acetylation [80].

Then compound (244) is treated with hydrogen sulfide in the presence of hydrogen chloride in an alcoholic medium and is reduced with zinc in acetic acid via the aldehydo acid (245) to the key 1,3-dibenzyl-2,4-dioxohexahydrothieno[3,4-d]imidazole (246) [80]. This can also be obtained by the action of hydrogen sulfides (NaHS, KHS, LiHS) in an aqueous alcoholic medium

followed by reduction with zinc in hydrochloric acid [84]. The yield in this stage of the synthesis is low. However, the yield can be raised markedly [85] if the reaction of the compound (244) with hydrogen sulfide in the presence of hydrogen chloride is carried out in dioxane. The reaction takes place through the intermediate 1,3-dibenzyl-6-chloro-2,4-dioxohexahydrothieno[3,4-d]imidazole, which is reduced by zinc in acetic acid to compound (246) [75-80% on the compound (244) and 13% on the compound (239)]. Compound (246) can also be obtained by the reduction of the anhydride (243) with $NaBH_4$ or KBH_4 in dimethylformamide to an intermediate lactone followed by the replacement of the oxygen by sulfur under the action of hydrogen sulfides [86].

Then the aliphatic side chain is introduced into position 4 of compound (246) by condensation [75] with 3-ethoxypropylmagnesium bromide to give 1,3-dibenzyl-(3-ethoxypropyl)-4-hydroxyhexahydrothieno[3,4-d]imidazole (247, n = 3) (30-40%), which is dehydrated (87%) to form compound (248, n = 2) with an exocyclic double bond. This is hydrogenated over a Pd or Pd/C catalyst to compound (240, n = 3). The reduction of the double bond takes place stereoselectively with the formation mainly of compound (249, n = 3) having the cis configuration of the substituent at $C_{(4)}$; simultaneously a certain amount of isomer with the trans configuration is formed which subsequently leads to the production of epibiotin as a byproduct [88]. The authors do not give the ratio of the isomers.

Resolution into optical isomers is performed at the intermediate stages of the synthesis of biotin. For this purpose, compound (249, n = 3) is first treated with hydrogen bromide in acetic acid. The formation of a tricyclic salt — the bromide (250) — takes place [12% on compound (246)] [81], from which with d-camphorsulfonic acid, twodiastereomeric forms separable by crystallization are obtained. The enantiomer (251) formed from the (-)-thiophanium bromide (250) and d-camphorsulfonic acid corresponds to (+)-biotin. The second enantiomer cannot be recovered for re-use in the synthesis, since the three chiral atoms independent from one another must form epimers [89].

The enantiomer (251) is treated with sodiomalonic ester, and compound (252) is boiled in 48% hydrobromic acid, which leads to the hydrolysis of the ester groups, the decarboxylation of one of the carboxy groups of the side chain, and the elimination of the benzyl protective groups, giving (+)-biotin (1) [79] with a yield of about 2% on the initial fumaric acid (239).

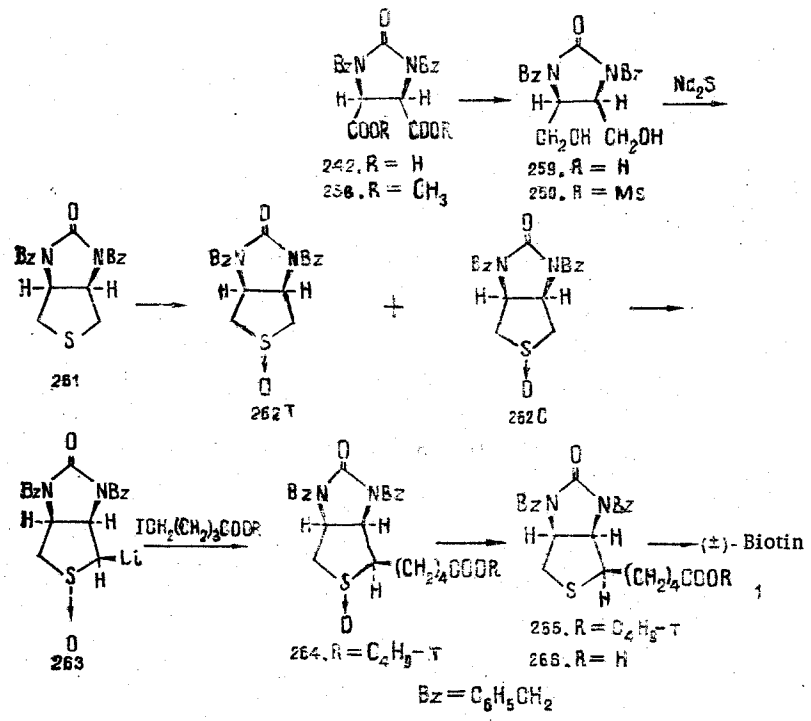
In subsequent investigationsto improve this scheme the yield of biotin was raised [88]. Thus, the dioxothienoimidazole (246) is condensed with 4-benzyloxybutylmagnesium bromide in benzene and the resulting hydroxy compound (247, n = 4) (74%) is dehydrated by heating in acetic acid to form 70% of compound (248, n = 3), which is hydrogenated in methanol in the presence of Pd/C to 1,3-dibenzyl-c-4-(4-benzyloxybutyl)-2-oxo-1H-(r-6a,c-3a)thieno[3,4-d]-imidazole (249, n = 4). The benzyl protection of the hydroxy group is eliminated by heating in 70% ethanol solution saturated with hydrogen chloride. This gives compound (249, R = H) which is brominated with phosphorus tribromide in carbon tetrachloride, the bromide (253) (68%) is heated with sodium cyanide in alcoholic solution to give the nitrole (254), and this is then heated in 48% hydrobromic acid to hydrolyze the nitrile group and to bring about debenzylation, giving (±)-biotin (1) with a yield of 28% on the compound (246) or 3.6% on the initial fumaric acid (239).

In another method [90], compound (246) is treated with tetramethylenedimagnesium dichloride with subsequent treatment by carbon dioxide and acidification to give (±)-N,N'-dibenzyl-4-hydroxybiotin (255), which is dehydrated by heating in acetic acid to compound (256). This is hydrogenated to the methyl ester of N,N'-dibenzylbiotin (257) on an acid-resistant catalyst — Ni on kieselguhr — in methanol at 160-180°C under a pressure of 100 atm. On reduction with a Pd/C catalyst, partial desulfuration of the molecule takes place. The ester (257) is hydrolyzed and debenzylated by heating in 48% hydrobromic acid to give (±)-biotin (1) with a yield of 49% on compound (255) and 4.7% on the initial fumaric acid (239).

Later, the resolution into optical isomers was carried at earlier stages of the synthesis of biotin and, namely, on the hydroxylactone [89], which is readily obtained by hydrolysis of the acetoxylactone (244) in an alkaline medium. The separation was carried out by the use of optically active alcohols [(-)-menthol, (-)-borneol, orothers] in the presence of strong acids, which leads to the formation of diastereomeric pseudoesters capable of separation by recrystallization. In a second variant the separation into optical isomers is carried out on monoesters of the dicarboxylic acids obtained from the anhydride (243). It has also been proposed [91] to carry out resolution into optical isomers on the dicarboxylic acid (242) with optically active amines.

Stereoselective Synthesis of (±)-Biotin from Fumaric via Hexahydrothienoimidazole Sulfoxide

This synthesis due to Lavielle, Marquet, et al. [92-94] (Scheme 17) is based on the creation from fumaric acid of hexahydrothieno[3,4-d]imidazole sulfoxide and the introduction into its molecule of the aliphatic side chain characteristic for biotin by stereoselective alkylation.



Scheme 17

Fumaric acid (239) is converted via meso-dibromosuccinic acid (240) and meso-α,β-di(benzylamino)succinic acid (241) (for description, see above) into 1,3-dibenzyl-cis-4,5-dicarbozyimidazolid-2-one (242) [yield 76% on the compound (241) and 59% on the (239)] [79]. The carboxy groups of compound (242) are methylated with diazomethane in ether and the resulting compound (258) (97%) is reduced with lithium tetrahydroaluminate in a mixture of tetrahydrofuran and ether to 1,3-dibenzyl-cis-4,5-di(hydroxymethyl)imidazolidone (252) (98%) which, under the action of mesyl chloride in dichloromethane in the presence of diethylamine at 0°C, gives the di(mesyloxymethyl) derivative (260) in a yield of 95%. The latter compound under the action of sodium sulfide in ethanol at the boil forms the key intermediate bicyclic compound — 1,3-dibenzyl-2-oxohexahydrothieno[3,4-d]imidazole (261) with a yield of 95%.

The subsequent synthesis of biotin consists in the stereodirected formation of the sulfoxide of the bicyclic compound (262C), its highly selective acylation in order to introduce the carboxybutyl side chain into the molecule, and the subsequent elimination of the S-oxide group and the protective groups. Oxidation of the bicyclic compound (261) with sodium periodate in aqueous methanol [95] forms the two sulfoxides (262C) and (262T) with the cis and trans configurations of the S-oxide group in relation to the configuration of the conjugated hydrogen atoms of positions 3a and 6a, respectively. The oxidation reaction takes place with almost quantitative yield, the isomers 262C/262T being formed in a ratio of 90:10. The configurations of the isomeric sulfoxides were established by PMR spectroscopy. For the subsequent synthesis of biotin, the cis isomer of the S-oxide of 1,3-dibenzyl-2-oxohexahydrothieno-[3,4-d]imidazole (262C) is used.

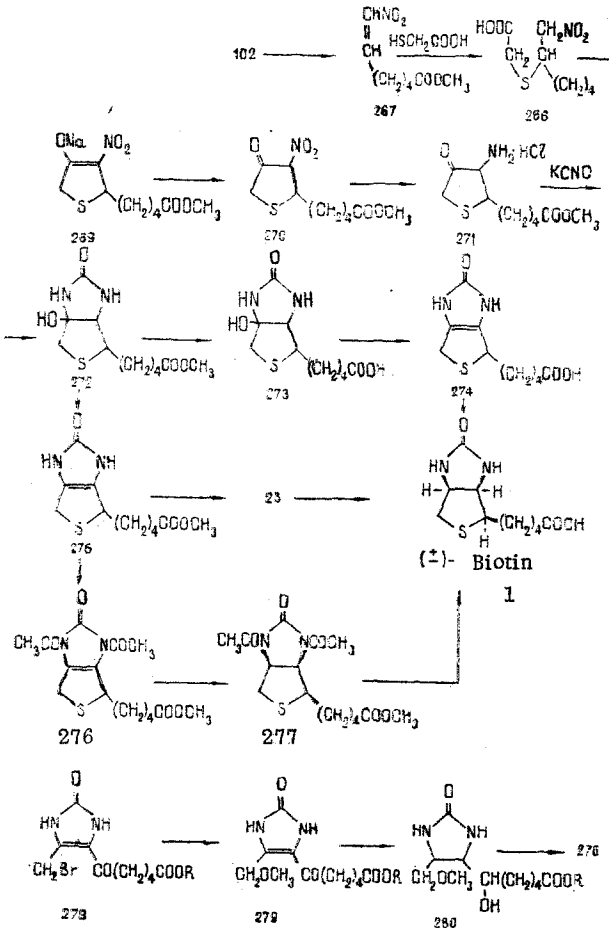
The stereoselectife alkylation of the sulfoxide (262C) takes place via the lithium derivative (263) in the trans-2 position to the S-oxide group and, consequently, leads to the introduction of the substituent into the linked imidazolidone ring of the biotin molecule in the cis position. Originally, the lithium derivative (263) was obtained by the action on the S-oxide of methyllithium in a mixture of hexamethylphosphorotriamide (HMPTA) and tetrahydrofuran at −30°C, and it was then treated with five equivalents of tert-butyl ω-iodovalerate at the same temperature. This forms only one isomer with the side chain in the trans position to

the S-oxide group, i.e., in the cis position to the linked imidazolidone ring, to give cis-1,3-dibenzyl-c-4-(4-tert-butoxycarbonylbutyl)-2-oxo-hexahydro(r-6a,c-3a)thieno[3,4-d]imidazole (264). The course of the alkylation reaction depends on the reagents and solvents used. Thus, with the use of CH_3Li and a mixture of HMPTA and THF the reaction product contains 262C/264T in a ratio of 20:80, with HMPTA and triglyme the ratio is 12:88, and with THF 58:42, and in the case of BuLi the yield falls to 30%. With an unsuitably selected solvent, the alkylation reaction is complicated by the fact that the intermediate carbocation may undergo β-elimination or protonation concurrently.

The S-oxide group of compound (264) is readily reduced by the action of titanium trichloride in a mixture of chloroform and methanol [96] or by triphenylphosphine in CCl_4 [97]. The tert-butyl ester group in the compound formed (265) (89%) is hydrolyzed by boiling in acetic acid, and the compound obtained (266) (96%) is debenzylated in 40% hydrobromic acid [79] to (±)-biotin (1) with a yield of 50% on the (266) or 3.1% on fumaric acid (239). In this biotin synthesis, the benzyl protective group can be replaced by an allyl group which is eliminated at the end of the synthesis under milder conditions than the benzyl group. The yields of some intermediate compounds are somewhat lower in the case of the allyl group.

Stereoselective Synthesis of (±)-Biotin from the Ester of Adipic Acid Semialdehyde via a Protected Ketothiophane and Dehydrobiotin

In this synthesis, due to Fild and Caldwell [98, 99] (Scheme 18), the construction of the biotin molecule is based on a compound containing its aliphatic side chain, the thiophane ring being formed first and the imidazolone ring being constructed upon it. Stereospecificity is achieved as the result of the reduction of 3α,6α-dehydrobiotin or its ester.



Scheme 18

The methyl ester of adipic acid semialdehyde (101) is condensed with nitromethane in DMFA to form methyl 6-hydroxy-7-nitroheptanoate (102) in a yield of 89% [52], and this is dehydrated in the presence of magnesium sulfate and piperidine to the derivative (267) which is then condensed with mercaptoacetic acid at 3°C to form methyl 6-carboxymethylthio-7-nitro-

heptanoate (268) [80% on the (101)]. In the form of its salt with dicyclohexylamine, this compound is cyclized under the action of dicyclohexylcarbodiimide to 4-hydroxy-2-(4-methoxycarbonylbutyl)-3-nitro-2,5-dihydrothiophene, which is isolated in the form of the sodium derivative (269) (45%). When this is treated with sulfuric acid in methanol, a prototropic rearrangement of the nitro enol (269) to the nitro ketone (270) takes place with a yield of 68%. The catalytic hydrogenation of the nitro group to an amino group with Pd/C leads to the hydrochloride of 3-amino-2-(4-methoxycarbonylbutyl)-4-oxothiophane (271) the reaction of which with potassium isocyante forms the bicyclic 6a-hydroxy-4-(4-methoxycarbonylbutyl)-2-oxohexahydrothieno[3,4-d]imidazole — the methyl ester of 6a-hydroxybiotin (272) [18.2% on the (270)].

Originally, 6a-hydroxybiotin (273) was obtained from this ester of 6a-hydroxybiotin (272) and was then dehydrated by acetic anhydride to 3a,6a-dehydrobiotin (274) (65%), and, finally, by reduction with sodium tetrahydroborate in aqueous methanol it was converted into (±)-biotin (1) with a yield of 4.3% on the initial methyl ester of adipic acid semialdehyde (101). According to another variant, the dehydration of the 6a-hydroxybiotin ester (272) with acetic anhydride gives the 3a,6a-dehydrobiotin ester (275) with a yield of 65%. This is reduced with triethylsilane in trifluoroacetic acid to the methyl ester of biotin (23) [21% on the (275) and 2.0% on the (101)], and hydrolysis gives (±)-biotin (1) [98, 99].

A modification of Vasilevskis and Caldwell's method of synthesizing biotin [39] in its last stages has permitted the yield of biotin to be considerably increased. The catalytic hydrogenation of dehydrobiotin or its methyl ester in acetic acid gives a low yield of biotin. The situation is different if the dehydrobiotin (274) or its methyl ester (275) is subjected to diacetylation with acetic anhydride to form compound (276) (80%) which, after isolation, is hydrogenated in a mixture of acetic acid and acetic anhydride with a 5% Pd/C catalyst at a pressure of 35 atm and a temperature of 85°C — the methyl ester of N,N'-diacetylbiotin (277) is obtained with a yield of 88%. After the removal of the protective groups by hydrolysis, this compound is converted into (±)-biotin (1) with an overall yield of 6.7% on the initial ester of adipic acid semialdehyde (101). A method has been proposed for obtaining the 3a,6a-dehydrobiotin ester (275) which is based [100] on the protected imidazoline (278); the synthesis of an analogous compound is shown in Scheme 2 via the methoxymethyl derivative (279) which is reduced by sodium tetrahydroborate to the hydroxy derivative (280). This compound is converted into (275) under the action of hydrogen sulfide and $TiCl_4$ in methylene chloride at −70°C.

According to Vasilevskis et al. [101], optically active (+)-biotin (1) is obtained by the method shown in Scheme 18 except that in one of the early stages of synthesis an optically active dextrorotatory salt with (+)-α-methylbenzylamine in ethyl acetate is obtained from methyl 6-carboxymethylthio-7-nitroheptanoate (268). The pure S-enantiomer is obtained from the salt and from this (+)-biotin is synthesized in high yield.

Many of the syntheses of biotin considered above lead to the formation of racemic (±)-biotin. Natural (+)-biotin is obtained from it by resolving the racemate into antipodes via a diastereomeric ester or salt with an optically active alcohol or base — (-)-hydroxyphenylacetic acid [102] or L-(+)-arginine [103].

In conclusion, it must be mentioned that the first nonstereospecific synthesis of biotin from L-cystine by Scheme 11 has not lost its value at the present time, since developments of a stereospecific variant of the synthesis based on intermediate compounds may give good results. The industrial synthesis of biotin is based on the patents of Goldberg and other workers by Scheme 16. The new syntheses of biotin by Schemes 15-18 and others may also be of interest for technical development.

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A STUDY OF THE PRODUCTS OF THE HYDROLYSIS OF THE XYLAN OF *Melilotus albus* BY ENDO-1,4-β-XYLANASE

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The enzymatic hydrolysis of a 4-O-methylglucuronoxylan of white sweetclover (*Melilotus albus*) by a highly purified homogeneous endo-1,4-β-xylanase from *Aspergillus niger* 14 has shown that the enzyme hydrolyzes 97% of the polysaccharide in 72 h. Acidic and neutral oligosaccharides were found in the hydrolysate after the action of the enzyme. An investigation of the hexauronic acid isolated has shown that the glucuronic acid is attached to the nonreducing end of the xylooligosaccharide, which demonstrates the specific action of the enzyme on the polysaccharide.

The action of an endo-1,4-β-xylanase with a molecular weight of 24,000-27,000 produced by the fungus *Aspergillus niger* 14 in surface cultivation on wheat bran has been studied [1]. The endo-1,4-β-xylanase was purified by precipitation from an extract of the culture with isopropanol, fractionation with ethanol, gel filtration through a column of Sephadex G-50, chromatography and rechromatography on a column of hydroxylapatite, and chromatography and rechromatography on CM-cellulose [2, 3]. The degree of purification was 5000.

The homogeneity of the enzyme used was established by electrophoresis in polyacrylamide gel and isoelectric focusing, by the use of an ultracentrifuge, and by gel filtration through a column of Sephadex G-200 [2].

The endo-1,4-β-xylanase did not cleave xylobiose and methyl D-xyloside and did not hydrolyze the (1→4) bonds between the β-D-glucopyranose residues in the molecules of cotton fiber, filter paper, and sodium carboxymethylcellulose and the 1→3 bonds in laminarin and did not hydrolyze the 1→4 and 1→6 bonds in starch molecules, andit did not contain as impurities pectolytic and proteolytic enzymes, β-1,4-mannanases and α-1,6-galactosidases.

In experiments in which it was incubated with the arabinoglucuronoxylan isolated from wheat straw and with [^{14}C]xylose the endo-1,4-β-xylanase from the fungus *Aspergillus niger* 14 exhibited no transglycosylase activity, and it hydrolyzed xylotriose and xylotetraose to xylobiose and xylose, hydrolyzed xylopentaose mainly to xylotetraose, and hydrolyzed xylohexaose to xylopentaose and xylotetraose. The rate of cleavage of the oligosaccharides rose with an increase in the degree of polymerization.

Under conditions of the continuous removal of the hydrolysis products, using dialysis bags of collagen film placed in a vessel containing 0.01 M acetate buffer at pH 4.2 with continuous stirring of the solution and the replacement of the buffer every 3 h, after 72 h almost complete (97.7%) cleavage by the endo-1,4-β-xylanase of the 4-O-methylglucurono-β-D-xylan isolated from the stems of the herb white sweetclover (*Melilotus albus*) took place.

The unhydrolyzed insoluble residue contained a polysaccharide with a molecular weight of 7400. Its degree of polymerization was 56, $[\alpha]_D^{20}$ −37°. It was constructed of β-D-xylose residues linked to one another by (1→4)-bonds together witha small amount of D-glucuronic and 4-O-methyl-D-glucuronic acids.

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