Chapter 1. Synthetic Studies towards D- (+)-Biotin

Section 1

Introduction to biotin
1.1.1 Introduction

D-(+)-Biotin (vitamin H) is a water-soluble vitamin (Fig. 1). Isolation of 1 has been accomplished independently from three natural sources. Kogl et al.\textsuperscript{1} have firstly characterized it as a growth factor for yeast in 1936 in its methyl ester form, which was originally referred to as “bios IIb” and later as “biotin.” The same compound was called “coenzyme R” by Nilsson \textit{et al}.\textsuperscript{2} and West \textit{et al}.\textsuperscript{3} in 1939, who isolated it from a root nodule bacteria, \textit{Rhizobium trifolii}. Boas has noticed that uptake of excess amount of egg white in rats results in severe disorders, leading to death.\textsuperscript{4} Because of dramatICAL improvement of the dysfunction by dietary 1, the compound was named “protective factor X.” Gyorgy \textit{et al}.\textsuperscript{5} have finally assured the identity of the compound both with biotin and coenzyme R, and dubbed it “vitamin H.”

\begin{center}
\textbf{Figure 1.} Structure of biotin (1)
\end{center}

The compound 1 is a cofactor of carboxylation enzymes and plays crucial roles in the metabolism of fatty acids, sugars, and \(\alpha\)-amino acids. In addition to the increasing application to feed additives, recent reports have revealed that 1 enhances insulin secretion in animals, suggesting it for a promising therapeutic candidate for an anti-diabetic drug. The remarkably strong affinity of 1 with avidin and streptavidin has been extensively applied for such technologies as photoaffinity labeling.

1.1.1.1 Structure determination

Chemically biotin is (\(+\)-\textit{cis}-hexahydro-2-oxo-1\textit{H}-thieno [3,4-\textit{d}]-imidazole-4-oxo-1\textit{H}-thieno [3,4-\textit{d}]-imidazole-4-valeric acid. The empirical formula for biotin \(\text{C}_{10}\text{H}_{16}\text{N}_{2}\text{O}_{3}\text{S}\) was established in 1941 and the full structure in 1942 by du Vigneaud.\textsuperscript{6,7} The structure was confirmed by the first total synthesis of biotin in Merck Laboratories by Harris and co-workers in 1943.\textsuperscript{8} X-ray crystallographic analyses of \textit{bis} 4-bromoanilide of \textit{N}-l-carboxybiotin in 1966 (Fig. 2)\textsuperscript{9} and biotin itself in 1985,\textsuperscript{10} have substantiated the structure of 1, involving the absolute configuration.
Figure 2. Structure of bis 4-bromoanilide of N-1-carboxybiotin

According to these data, ureido ring is planar while the thiophane ring has an envelope conformation (Fig. 3). The valeric acid side chain is not fully extended but twisted and there is a strong interaction between C-6 and N-3', a feature of importance in determining the biochemical reactivity of biotin.

Figure 3. Crystal structure of biotin (1)

Biotin has three contiguous chiral carbon atoms and therefore, four diastereomeric racemic forms are possible, of which only (+)-biotin I is biologically active, while, epi, allo and epi-allo-biotin I, II, and III respectively and their enantiomers are biologically inactive. Of the four diastereomeric racemic forms, only D-(+)-biotin occurs in nature whereas other isomers are of synthetic origin.

1.1.1.2 Biosynthesis

A number of fungi and bacteria synthesize biotin from pimelic acid by a metabolic pathway, whose last step involves the conversion of dethiobiocin to biotin. This pathway has been thoroughly investigated. All the intermediates from pimelic acid to dethiobiocin are formed by classical biochemical reactions. Recently Marquet and co-workers solved the elucidation of the mechanism for the transformation of dethiobiocin to biotin. Evidence has been presented that the
biosynthesis of biotin in *Aspergillus niger* and *E. Coli* proceeds by the introduction of sulfur at C-1 and C-4 of dethiobiotin without apparent involvement of C-2 and C-3.\textsuperscript{15,16} A more recent study clearly demonstrates that sulfur is introduced at C-4 of dethiobiotin with loss of the 4 pro S hydrogen atom. Since the configuration of biotin at C-3 is S, it follows that sulfur is introduced with retention of configuration at C-4, the prochiral center of dethiobiotin.

### 1.1.1.3 Deficiency

Onset of biotin deficiency has first been recognized by Boas as early as in 1924, who demonstrated excess feeding of raw egg white to rats resulted in fatal loss of body weight as well as skin and hair abnormalities (*vide supra*).\textsuperscript{4} Strong binding of biotin to avidin, present in the egg white, inhibits uptake of biotin through gastrointestinal tracts.

The biotin deficiency has been well investigated on poultries because of the economic significance. It is characterized by a series of clinical manifestations, such as locomotor\textsuperscript{17} and skin lesion,\textsuperscript{18} and rough and broken feathers. Hatchability\textsuperscript{19} may be affected by the deficiency and the amount of biotin in eggs is known to decline in adult birds.\textsuperscript{20} Broiler chickens of 3–4 weeks sometimes die suddenly after a few hours of recumbency. The disease is called “fatty liver and kidney syndrome (FLKS)’’\textsuperscript{21} and characterized as considerable systemic lipids infiltration, especially in liver and kidney. Pigs, another important livestock, suffer from severe lameness\textsuperscript{22} and inability in the reproductive performance upon biotin deficiency. The dangerous effects of chickens and pigs are cured by supplementation of biotin as a feed additive.\textsuperscript{23-25}

Biotin-dependent multiple carboxylase defects in man are known to occur because of mutation of holocarboxylase\textsuperscript{26} or biotinidase, which are much less common than in poultries.\textsuperscript{27} Biotin deficiency may trigger teratogenesis. For instance, fetuses from dams given a biotin-deficient diet throughout gestation show some characteristics of intrauterine growth retardation including abnormal liver weight and a higher brain/liver ratio.\textsuperscript{28}

### 1.1.1.4 Uses

Therapeutic efficacy of biotin, which provides a novel way to tackle diabetes, has been evaluated in patients with non-insulin-dependent diabetes mellitus. Biotin has been suggested to enhance glucose-induced insulin secretion in isolated perfused
pancreas islets of rats. When the islets were stimulated with glucose and biotin, the ATP/ADP ratio and glucose oxidation, assessed by carbon dioxide production, elevated to ca. 160% and 200%, respectively, of those observed in the islets treated with glucose alone. The data suggest that biotin enhances the degradation of glucose in the islets and, accordingly, accelerates the production of ATP, which results in the surge of the glucose-induced insulin secretion. Because of enhancement of the insulin secretion by biotin shortly following the stimulation, biotin is likely to induce the activities not by mediating via gene expression events but by direct participation in the process of glucose-induced insulin secretion.

It is used in pharmaceuticals for the preparation of ointments, tonics, etc. It is also used in poultry for rapid growth of chicks and healthy hatching of eggs. In recent years a utilization of strong biotin avidin complex has emerged in biochemistry as an important and versatile method for isolation, localization, immunoassay and drug delivery. It has been recently recognized that biotin finds use in cosmetic and it is administered orally for brittle nails and hair loss.

**Avidin-biotin system in immunochemistry:**

Biotin is known to display high affinity to avidin and its structural homolog, streptavidin. The binding ability is among the highest so far known for ligand–protein interactions. Avidin and streptavidin are isolated from *Streptomyces avidinii* and from hen egg white, respectively. Avidin is a tetramer containing four identical subunits of molecular weight 15,000. Each subunit contains a high affinity binding site for biotin with a dissociation constant of approximately 10^{-15} M. The binding is undisturbed by extremes of pH buffer salts or even chaotropic agents, such as guanidine hydrochloride (up to 3 M). The strength of the avidin-biotin interaction has provided the researcher with a unique tool for use in immunoassays, receptor studies, immunocytochemical staining and protein isolation.

The avidin-biotin system is particularly well suited for use as a bridging or sandwich system in association with antibody-antigen interactions. The biotin molecule can easily be activated and coupled to either antigens or antibodies, usually with complete retention of activity. Subsequently avidin can be conjugated with enzymes, fluorochromes, ferritin or colloidal markers and used as high affinity secondary reagents, which can greatly increase the sensitivity of an assay. In addition, since only one conjugate preparation is required for many different assays, the biotin-avidin system can be very attractive for use in immunological procedures.
**Biotin derivatives in use:**

The following are some of the biotin derivatives in use.

*Biotin derivatives as gelators of organic solvents:*\(^{36}\)

\[ \text{IV} \]

\[ n = 15, 11, 10, 7, 5, 2 \]

The recovery of spilled solvents, disposal of used cooking oil and novel drug delivery systems have been suggested as possible applications for gelling compound. Several of these compounds are capable of forming stable gels with a variety of organic solvents.

*Biotin derivatives as anti HIV protease inhibitors:*\(^{37}\)

\[ \text{V} \]

Several bis-N-alkylated (+)-biotin derivatives were synthesized and evaluated for activities against HIV-1 protease. The most potent inhibitor V, has \( K_i \) of 0.50 mM and antiviral \( IC_{90} \) of 7 mM. The (+)-biotin analogues in general have good translations from enzymic \( K_i \) to antiviral cell assay \( IC_{90} \). Also, other derivatives of biotin like \( N \)-hydroxysuccinimidobiotin, sulfosuccinimidobiotin, \( N \)-iodoacetyl-N-biotinylhexylenediamine, biotinhydrazide, immo-bilized biotin, biotincellulose of biotin are commonly used in different applications.

Biotin possesses a deceptively simple-looking structure. Its skeleton consists of a biheterocyclic core, to which is attached a carboxybutyl side chain. The heterocyclic system comprises a cyclic urea and a tetrahydrothiophene ring (which will subsequently be called thiophane). It further possesses three contiguous stereocenters on the thiophane ring in the all-cis configuration. Because of the fundamental and commercial importance, biotin has, ever since it was discovered, attracted the attention of both academic and industrial synthetic chemists.
A continuous endeavor over a period of more than 60 years has now resulted in more than 50 original contributions on the total synthesis of biotin. Many of earlier syntheses known were lengthy involving a number of steps, without any stereochemical control. Then there was a drought of published information for 20 years when no significant progress in biotin synthesis was made. However, the recent recognition of the importance of biotin in poultry, biochemistry and pharmaceutical formulations, revived the interest in this molecule, and this is evident by a boom in a number of international patents (around 60) between 1970-2010. The above figure excludes the applications of biotin in biochemistry and related subjects.

1.1.2 Synthesis of biotin: A literature survey

Asymmetric synthesis has acquired tremendous importance, especially in the pharmaceutical industry, since it is frequently the case that only a particular optically active isomer is therapeutically active. There is thus a continuing need for new methods of carrying out asymmetric syntheses and specific catalysts having a high degree of asymmetric induction for particular stereocenters; the synthesis should lead to the desired isomer in high optical purity and in high chemical yield. Since review on synthesis of all categories up to 2005 has been covered by Amar Gopal,\textsuperscript{38a} Priti Soni,\textsuperscript{38b} Ramakrishna,\textsuperscript{38c} from this group, as well as is reviewed by De Clercq in 1997\textsuperscript{39} and by Seki in 2006,\textsuperscript{40} syntheses of optically pure biotin reported after 2005 and representative syntheses of each strategy have been described in this present section. Schemes constitute the vehicle of the synthetic chemist. They are conceived so that the chemist can grasp the important stages in each shown sequence. Relevant experimental conditions are listed, including yields when they have been clearly reported in the original literature. The following stereochemical designations are used in the schemes: an unprefixed arabic numeral is used for achiral molecules and for chiral molecules which possess the correct enantiomeric configuration for eventual conversion into (+)-biotin; the opposite enantiomeric configuration is indicated by prefix \textit{ent} and racemic mixtures by the prefix \textit{rac}. Throughout the section/thesis, the atom numbering along the thiophane nucleus shown below will be used:
Because of the biological activity of (+)-biotin (1) found in only the enantiomer shown in Figure 1, chiral synthesis leading to the single enantiomer is required. The compound 1 is featured by sulphur containing bicyclic ureido skeleton that supports 4-carboxybutyl chain at C-2. The first total synthesis of 1 has been accomplished by Harris et al.\textsuperscript{8} in 1943. The synthesis involves 12 steps sequence starting from L-cystine. However, construction of the asymmetric centers is totally nonstereoselective, resulting in racemic mixtures of three diastereomers, rac-biotin, rac-allo biotin, and rac-epiallo biotin. The racemic biotin was finally resolved into 1 through optical resolution using L-arginine as the resolving agent. Although the method provided 1 in relatively less number of steps, \textit{i.e.} 12 steps from L-cystine, it has drawbacks of unsatisfactory yields and poor stereoselectivities as well as need for optical resolution at the final step. To figure out the complicating issues, considerable efforts have been devoted both in industries and academia. From a practical point of view, the synthetic schemes of 1 are classified as the following three approaches that utilize proficiency:

(1) Resolution

(2) Desymmetrization

(3) Asymmetric induction.

1.1.2.1 Resolution-

\textbf{Goldberg and Sternbach’s approach: Late stage resolution} (Pat. 2,489232, Nov. 22, 1949; Chem. Abstr. \textbf{1951}, 45, 184.)

In 1946 Goldberg and Sternbach\textsuperscript{41-43} described the first economical synthesis of (+)-biotin starting from cheaply available fumaric acid (see Scheme 1).

Fumaric acid 2 is converted into the cyclic anhydride 4 \textit{via} a four step sequence involving bromination of fumaric acid to yield \textit{meso}-dibromo succinic acid, double substitution of the latter with benzyl amine, formation of the cyclic ureide 3 with phosgene, followed by formation of anhydride 4 upon treatment of 3 with acetic anhydride. At this stage \textit{cis} relation of the vicinal amino groups at C-3 and C-4 centers is fixed.
Scheme 1: Reagents and conditions: a) Br₂, water; b) PhCH₂NH₂, EtOH; c) COCl₂, KOH; d) Ac₂O; e) Zn, Ac₂O, HOAc; f) H₂S, HCl; g) KSH, EtOH; h) Zn, HOAc; i) ClMg(CH₂)₃OCH₃; j) HOAc; k) H₂, cat.; l) HBr; m) Silver d-camphorsulfonate, followed by fractional crystallization; n) NaCH(COOEt)₂; o) 48% HBr.

In the second stage, the thiophane nucleus is formed by conversion of meso-4 into thiolactone 6. This involves reduction of anhydride 4 with zinc in acetic acid, treatment of the resultant acetoxy lactone 5 with hydrogen sulfide, and its further reduction with zinc to yield thiolactone 6 in racemic form. In the third stage, part of the carboxybutyl chain of biotin is introduced via Grignard reaction with subsequent dehydration to from the exocyclic olefin 7 with undefined doublebond stereochemistry. Catalytic hydrogenation of the latter yields 8 with the desired all cis relative configuration, at centers C-2, C-3 and C-4. In the fourth stage, ether 8 is converted into the thiophanium salt 9 by treatment with hydrobromic acid (HBr).

At this point, resolution is effected by conversion of bromide 9 into the diastereomeric sulfonate salts 10 which are readily separated in excellent yield by simple fractional crystallization. In the final stage of the synthesis, the side chain is established by
reaction of diastereomer (-)-10 with sodium diethyl malonate. In this important step, selective attack is observed at the least hindered primary center of the trimethylene thiophanium moiety. Finally, heating with conc. hydrobromic acid effected hydrolysis, subsequent decarboxylation and debenzylation all in one operation, to furnish biotin.

Several intermediates in the above scheme, and in particular, thiolactone 6 has been obtained later by other groups thus constituting new formal synthesis of (+)-biotin.

The use of benzyl groups as protective groups in the imidazolidothiophane and related intermediates has been commonly utilized in almost all later syntheses.


In 1970 Gerecke, Zimmerman and Aschwanden from Hoffmann-La Roche (Basel and Paris) reported on a further important development of the original Goldberg-Sternbach scheme that allows for the efficient production of the key thiolactone 6 in the required enantiomeric form starting from meso-acid 3.\textsuperscript{44} Crucial to this new development was the finding that lactone 13 could be converted in very high yield into the corresponding thiolactone 6 by treatment with potassium thioacetate in dimethylformamide at 150 °C (Scheme 2).

\[
\text{13} \xrightarrow{a} \text{6}
\]

**Scheme 2: Reagents and conditions:** a) CH$_3$COSK, DMF, 150 °C.

Hence, any synthesis of rac- or (+)-13 would constitute a new formal synthesis of rac- or (+)-biotin, respectively. The sequences that were originally used to convert the meso-compounds 3 into (+) - 13 are shown in Scheme 3.
Scheme 3: Reagents and conditions: a) Ac$_2$O, Zn/HOAc; b) NaOH, dioxane; c) R*-OH [(−)-menthol or (−)-borneol or (−)-4,4-dimethyl-3-hydroxydihydro-2(3H)-furanone], p-TsOH, PhCH$_3$; d) H$_2$SO$_4$, dioxane; e) NaBH$_4$, EtOH; f) CrO$_3$/H$_2$SO$_4$, dioxane.

The method involves conversion of 3 into hydroxylactone 11 (undefined stereo-chemistry) via acetoxy lactone 5 (Scheme 3). Treatment of 11 with an optically active alcohol leads to a diastereomeric mixture composed of 12a and 12b (both as epimeric mixtures). Selectivity in obtaining cis- or trans epimers could not be realized. Depending on the optically active alcohol used, i.e. (−)-menthol, (−)-borneol, or (−)-4,4-dimethyl-3-hydroxydihydro-2(3H)-furanone, the obtained diastereomers 12 crystallize in a different order and only two of the four possible diastereomers could be obtained in pure form. The required lactone (+)-13 is further obtained from the a-series via acid hydrolysis followed by sodium borohydride reduction, while the unwanted stereoisomers of the b-series were recycled through acid hydrolysis followed by chromic oxidation to meso-3.

Field’s approach: Early stage resolution (J. Am. Chem. Soc. 1978, 100, 7424)

The approach of Field and co-workers at Hoffmann-La Roche (1978) presents several interesting aspects (Scheme 4). It involves the synthesis of the bicyclic dihydrothiophene derivative 20 in homochiral form, followed by catalytic hydrogenation. The enantioselectivity in the sequence is the result of an early
resolution of the acid 16. In synthesis, the first step involves a conjugate addition of thioglycolic acid 14, to the unsaturated nitro ester 15. A salt of the (S)-enantiomer of 16 is obtained by treatment with (+)-R-methylbenzylamine (MBA). This salt is converted into the more stable dicyclohexylamine salt, which is converted to the phenol ester in 95% yield. When this ester is treated with (-)-R-methylbenzylamine in ethyl acetate, 18 is obtained as (-)-R-MBA salt. The construction of the imidazolone ring of 20 further involves a six-step sequence. After hydrogenation of the nitro group, the resulting amino ketone is treated with aqueous potassium cyanate and after acidification, alcohol 19 is obtained. Dehydration is effected in acetic acid. Finally, esterification and acetylation yields 20.

**Scheme 4: Reagents and conditions:**

- a) (+)-R-Methylbenzylamine ((+)-R-MBA), EtOAc; 30% yield, >97% ee after recrystallization;
- b) Dicyclohexylamine;
- c) PhOH, SOCl₂, pyridine (cat.);
- d) (+)-R-Methylbenzylamine, EtOAc;
- e) Pd/C (10%), H₂O; f) NaOH; g) HOAc, stripping at 55 °C;
- h) MeOH, H⁺;
- i) Ac₂O, 110 °C;
- j) H₂ (550 psi), 5% Pd/C (10% loading), Ac₂O, 85 °C;
- k) NaOH, CH₃OH.
Senuma’s approach: Auxillary based resolution (Chem. Pharm. Bull. 1990, 38, 882)

Senuma and co-workers reported an alternative method for the industrial resolution of hydroxy lactone 11 in 1990 (Scheme 5).\textsuperscript{46} It involves the direct resolution of the hydroxy lactone rac-11 (trans-epimer) with optically active amines. Thus the reaction of rac-11 with cinchonidine readily gave the cinchonidine salt of 21b in 45% yield with an optical purity evaluated at more than 98%. Upon acidification, the salt readily underwent cyclization to give a 42% overall yield of 11. Evaporation of the mother liquor of the salt afforded after acidification ent-11 in 36% yield. The undesired enantiomer is readily converted to meso-diacid 3 by facile oxidation with sodium chlorite. To find a more practical and inexpensive resolving agent applicable for industrial use, the authors also examined the optical resolution of rac-11 with various \(N\)-alkyl-\(D\)-glucamines.

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\begin{align*}
\text{Scheme 5: Reagents and conditions:} & \quad \text{a) Cinchonidine: 45\% of precipitated salt or } \text{N-}n\text{-butyl-}D\text{-glucosamine derivative: 46\% of precipitated salt; b) } HCl; \text{ c) } NaClO_2, 87\%.
\end{align*}
\]

Bihovsky’s approach: \(\alpha\)-Chlorination, diastereomer separation (Tetrahedron 1990, 46, 7667)

Bihovsky and Bodepudi\textsuperscript{47} succeeded in resolving 25 as shown in Scheme 6. The resolution was accomplished by separation of the diastereomeric alkoxy derivative 24a and 24b that were obtained by reaction of rac-23 with optically active secondary alcohols. The most efficient alcohol was (S)-(+)\-mandelic acid, since the
diastereomers could be readily separated by crystallization. Acid hydrolysis of 24b led to (+)-25 and hence to (+)-6, via oxidation or to 23 by treatment with HCl.

![Scheme 6: Reagents and conditions:](image)

**Scheme 6: Reagents and conditions:** a) NCS; b) R*-OH = (S)-(+) Mandelic acid, 75%; diastereomer separation by crystallization; CCl₄, reflux, 33% isolated yield with R* = CH(Ph)COOH; c) H₂SO₄, dioxane; d) HCl, CHCl₃; e) Et₃SiH, CF₃COOH.


Successful enzyme catalyzed kinetic resolutions were reported by Yamano et al. (Scheme 7). A variety of commercially available enzymes and microorganisms were investigated in order to effect the enantioselective hydrolysis of the ester 26, which was obtained by conventional acylations of rac-25. In a second approach, the same group found that direct resolution of alcohol 25 was accomplished via acylation with the lipoprotein from *Pseudomonas aeruginosa* TE 3285 in toluene. Curiously, addition of molecular sieves (MS) 4 Å to the reaction mixture improved the reactivity, while at the same time as addition of a small amount of water was found to be beneficial for the reaction.
Scheme 7: Reagents and conditions: a) Ac$_2$O, pyridine, 98%; b) Streptomyces rochei var. volubilis; 27% conversion; 92 and 94% ee after crystallization; c) LIP (P. aeruginosa TE3285; TOYOBO immobilized lipase), 0.3% H$_2$O, 4 Å molecular sieves (MS), PhCH$_3$, vinyl acetate; 56% conversion; 99 and 99.8% ee after crystallization of alcohol.

Volkmann’s approach: Late stage resolution (J. Am. Chem. Soc. 1983, 105, 5946)

Volkmann and co-workers of Pfizer Central Research disclosed a very short approach to 1 (Scheme 8). The approach is enantioselective through a resolution step at a late stage of the sequence. Central to the synthesis is the obtainment of thiazolidine 30 via a process which involves an ester enolate imine addition followed by an intramolecular amine/isothiocyanate condensation. The imine substrate 28 is obtained as the 3-thiazoline through reaction of brominated ethyl 7-oxoheptanoate 27 with sodium hydrogen sulfide, cyclohexanone, and ammonia. Crucial to the success of the addition of the lithium enolate of the isothiocyanato acetate ester 29 to thiazoline 28 is the prior activation of the imine through addition of an equivalent of boron trifluoride. In practice the diester 30 was obtained in 50% yield as the major product. Treatment of 30 with sodium borohydride resulted in the selective reduction of the α-amino ester to give alcohol 31. This was converted to a mixture of $d$-camphorsulfonates, which were separated by silica gel chromatography. Isomer 32 was converted upon acid treatment to 2-thiobiotin 33 in 83% yield. The reaction
conditions affected thiazolidine ring hydrolysis, thiophane ring formation, and ester hydrolysis. The eventual thiourea/urea transformation was realized by basic treatment with bromoethanol. In this procedure, the nucleophilic character of the thiourea sulfur atom was exploited in order to deliver, intramolecularly, the required oxygen atom via a labile alkoxyimimidazoline.

Scheme 8: Reagents and conditions:  
a) NaSH, cyclohexanone, NH$_3$, 90%;  
b) LDA, SCNCH$_2$COOEt, BF$_3$OEt$_2$, THF, -78 °C, 50%;  
c) NaBH$_4$, CH$_3$OH, THF, 0 °C, 90%;  
d) Et$_3$N, d-camphorsulfonyl chloride, CH$_2$Cl$_2$;  
e) CF$_3$COOH, H$_2$O, 45 to 100 °C, 83%;  
f) BrCH$_2$CH$_2$OH, N-methylpyrrolidinone, 110 °C, Na$_2$CO$_3$, 64%.

However, the classical Hoffmann La Roche synthesis with modifications is till date the commercially practiced technology. Although the method was initially impractical because of the use of optical resolution close to the final step, it was extended to an industrially viable approach by employing optically pure thiolactone 6 that was obtained by various methods involving optical resolution and Desymmetrization.
1.1.2.2 Desymmetrization-

Desymmetrization of meso-compounds is one of the most powerful transformations in asymmetric synthesis. Differentiation between two enantiotopic groups in such compounds leads to two or more stereocentres in only one step. Synthesis of 1, hitherto developed by Desymmetrization (Chart 1), includes five approaches,

i) Desymmetrization of meso-cyclic anhydride by enantioselective reduction

ii) Desymmetrization of meso-thiocarboxylic anhydride by enantioselective reduction

iii) Desymmetrization of meso-cyclic imide by enantioselective reduction

iv) Desymmetrization of meso-diester by enzymatic asymmetric hydrolysis

v) Desymmetrization of meso-cyclic anhydride by asymmetric alcoholysis

**Chart 1.** Earlier approaches for Desymmetrization.
i) Desymmetrization of meso-cyclic anhydride by enantioselective reduction-

If meso-cyclic anhydride/thiocarboxylic anhydride is treated with chiral reductant, it is possible to effect both desymmetrization and reduction to provide lactone/thiolactone in a single step.

**Matsuki approach** (*Tetrahedron Letters* 1993, 34, 1167)

![Scheme 9: Reagents and conditions:](image)

Scheme 9: Reagents and conditions: a) (R)-BINAL-H, -78°C to rt, THF, 76%.

In 1993 Matsuki and co-workers reported a highly enantioselective reduction of meso-1,2-dicarboxylic anhydride to yield optically active lactones using Noyori’s lithium aluminium hydride-ethanol-1,1’-bis-2-naphthol complex (BINAL-H). When applied to meso-4, the desired lactone 13 was directly obtained in 76% yield with 90% ee, which was enriched to 95% ee by recrystallization from benzene/cyclohexane (Scheme 9).

ii) Desymmetrization of meso thiocarboxylic anhydride by enantioselective reduction-

**Chen’s approach** (*Synthesis* 2000, 2004)

In 2000 Chen and co-workers reported an efficient and enantioselective synthesis of D-(-)-biotin using BINAL-H reduction of meso-thioanhydride 36 (Scheme 10). The synthesis started with cis-1,3-dibenzyl-2-imidazolidine-4,5-dicarboxylic acid 3. The key steps were the enantioselective reduction of meso-1,2-dicarboxylicthioanhydride 36 to prepare the (3S, 4R)-thiolactone 6, and the introduction of the side chain at C-2 in 6 via a modified Grignard reaction. This novel synthesis proceeded in six steps starting from 3 to afford 1 with 21% overall yield.
Scheme 10: Reagents and conditions: a) 1-Bromo-3-chloropropane, K$_2$CO$_3$, toluene, 80 °C, 94%; b) 47% HBr, NaBr, H$_2$SO$_4$, 50 °C, 86%; c) (CH$_2$OH)$_2$, TsOH, toluene, reflux, 92%; d) Mg, THF, rt, 83%; e) Ac$_2$O, 83% H$_3$PO$_4$ (cat.), reflux, 98%; f) Na$_2$S·9H$_2$O, THF, H$_2$O, rt, 49%; g) (R)-BINAL-H, THF, -78 °C to rt, 83%; h) 42, THF, reflux, then 30% H$_2$SO$_4$, 60 °C, 82%; i) I$_2$, KI, 10% NaOH, dioxane, 60 °C, 75%; j) 75% HCOOH, CH$_3$SO$_3$H, 10% Pd/C, reflux, 85%.

Figure 4. A possible mechanism for asymmetric reduction of thiocarboxylic anhydride 36.
The reaction mechanism of the asymmetric reduction of 36 with (R)-BINAL-H is worth noticing (Fig. 4). As was the case with the reduction of aromatic, acetylenic, and olefinic ketones by (R)-BINAL-H, the stereochemical outcome of the reduction is ascribed in large part to the electronic nature of the substituents rather than their steric factor. The reductant approaches to the convex side of thiocarboxylic anhydride 36. Si-face of the carbonyl group should be selectively reduced by means of a favorable n-π* interaction between lone pair electrons of oxygen atom in the reductant and anti-bonding orbital in the thioanhydride carbonyl group.

iii) Desymmetrization of meso-cyclic imide by enantioselective reduction

Stereoselective reduction of one of the carbonyls of meso-cyclic imide would lead to a product with three contiguous stereocentres. Speckamp et al.\textsuperscript{54} reported desymmetrization of meso-cyclic imide using CBS-catalyst (Scheme 11). In case of the cyclic meso-imides, the nitrogen moiety is the large substituent (R\textsubscript{L}), and the fused ring moiety is the small substituent (R\textsubscript{s}). Because the enantioselectivity of the reduction is higher if the difference in size between R\textsubscript{L} and R\textsubscript{s} is larger, a decrease of the size of R\textsubscript{s} should give a higher enantioselectivity (Fig. 5).

\textit{Scheme 11: Desymmetrization of meso-cyclic imide.}  
\textit{Fig.5. Possible mechanism}

\textbf{Shimizu’s approach (Tetrahedron Letters 1999, 40, 8873)}

In 1999 Shimizu and coworkers\textsuperscript{55} reported stereocontrolled reduction of meso-imides using oxazaborolidine (Scheme 12). The known meso-imide 34 was reduced using oxazaborolidine derived from L-threonine and borane-THF complex to give lactams 35 in high enantiomeric purity. This methodology was successfully applied to the synthesis of (+)-deoxybiotin in an enantio-controlled manner in good overall yield.
Scheme 12: Reagents and conditions: a) $\text{NaBH}_4$ (4.0 eq), THF-$H_2O$ (10:1); b) 2 N $H_2SO_4$-1,4-dioxane (8:1), 0 °C.


Catalytic asymmetric synthesis has been aimed at eliminating the use of stoichiometric amount of expensive chiral auxiliary. This was realized by the Chen group who developed catalytic version of the asymmetric synthesis of optically active lactone 13 through asymmetric reduction of imide 34 (Scheme 13).\(^{56}\) The meso-cyclic imide 34 was subjected to enantioselective reduction upon treatment with LiH and $\text{BF}_3 \cdot \text{Et}_2\text{O}$ in the presence of (1S,2S)-(+-)threo-1-(4-nitrophenyl)-2-amino-3-triphenylmethoxypropanol 46 under reflux in THF to give, after addition of sat. $\text{HCl/Et}_2\text{O}$ and filtration, 35 in 85% yield and 98% enantiomeric excess. Sterically demanding chiral environment given by 46 is responsible for inducing the high enantioselectivity.

Scheme 13: Reagents and conditions: a) 47, $\text{BH}_3 \cdot \text{SMe}_2$, THF, reflux; b) 80% NaH, $\text{BF}_3 \cdot \text{Et}_2\text{O}$, 48, THF, reflux.
In situ generated oxazaborolidine catalyst using a water-soluble small molecule amino alcohol as ligand 46 gives good results. However, on large-scale the recovery and purification of the catalyst are problematic. Immobilization of chiral oxazaborolidine catalysts would offer a solution to the problem. Polymer supported oxazaborolidine catalysts have wide uses in asymmetric organic synthesis owing to the ease of isolation of product from polymeric chiral catalyst, the convenient work-up procedure. Accordingly, the enantioselective reduction of meso-cyclic imide 34 catalyzed by a polymer-supported chiral oxazaborolidine derived from (S)-diphenylprolinol and polymer-bound sulfonyl chloride 47, or chiral polymer supported oxaborolidine 48 derived from polymer supported ligand was achieved (Scheme 13). The reduction using 80% NaH, BF\(_3\)·Et\(_2\)O, 48, THF, reflux was claimed to be advantageous over 47, BH\(_3\)·SMe\(_2\), THF, reflux in avoiding the use of BH\(_3\)-DMS and easy for large scale production which was replaced by NaH, and BF\(_3\)-Et\(_2\)O.

iv) Desymmetrization of meso-diester by enzymatic asymmetric hydrolysis-

**Iriuchijima’s approach** (Agric. Biol. Chem. 1982, 46, 1907)

The application of enzymatic resolution procedures to obtain the chiral lactone (+)-13 has been reported (Scheme 14). In 1982, Iriuchijima and co-workers described the asymmetric hydrolysis of the prochiral diester 49 with pig liver esterase (PLE).\(^{57}\) The meso-diester 49 has an interesting structure since it combines both a natural (S)-amino acid part and a unnatural (R)-amino acid part and enzymes are expected to preferentially hydrolyze the (S)-ester rather than the (R)-ester in 49. Hydrolysis of the di-n-propyl ester with PLE gave 50 in 85% yield. Further reduction with lithium borohydride yielded (+)-13 in 64% yield.

\[
\begin{align*}
\text{49} & \quad \overset{a}{\rightarrow} \quad \text{50} \\
\text{13} & \quad \overset{b}{\rightarrow} 
\end{align*}
\]

**Scheme 14: Reagents and conditions:** a) PLE, phosphate buffer; b) LiBH\(_4\) (75% ee; 87% ee after recrystallization).

![Scheme 15: Reagents and conditions:](image)

Scheme 15: Reagents and conditions: a) PLE, phosphate buffer; b) CH$_3$OCH$_2$Cl, N,N-diisopropylethylamine, CH$_2$Cl$_2$; c) LiAlH$_4$, ether/THF; d) Collins oxidation; e) HCl, THF/H$_2$O; f) Collins oxidation (93% ee).

The need to improve the enantioselectivity of the enzymic hydrolytic reaction prompted Sih in 1984 to use a different approach.$^{58}$ When the diacetate 51 was incubated with PLE, alcohol 52 was obtained (70% yield; 92% ee) indicating that the pro-R acetoxy group had been preferentially cleaved. Indeed, when 52 was subjected to a sequence involving Jones oxidation, basic hydrolysis, and lactonization, *ent*-13 was obtained. Eventually 52 was converted into the desired enantiomer 13 via the uneventful sequence shown in Scheme 15.


One of the serious drawbacks in enzymatic reaction comes from difficulty to separate the enzyme by filtration. Chen *et al.*$^{59}$ improved the workup to a great deal by the use of polymer supported pig liver esterase to achieve both high yield and high enantioselectivity (90% yield, 91% ee). The obtained half ester 55 was converted to lactone 13 by selective reduction of the ester group as described in Scheme 16.
Scheme 16. Reagents and conditions: a) CH$_3$OH, H$_2$SO$_4$, benzene, reflux, 6 h, 95%; b) Polymer-supported PLE, 0.1 M aqueous phosphate, 0.1 M aqueous NaOH, pH 8, 45 h, 30 °C, then 1 M aqueous HCl, 90%; c) LiEt$_3$BH, THF, 0 °C to rt, 6 h, then 1 M aqueous HCl, 45 °C, 1 h, 88%.

v) Desymmetrization of meso-cyclic anhydride by asymmetric alcoholsiy-

Deng’s approach (Synthesis 2001, 1737)

Scheme 17: Reagents and conditions: a) DHQD-PHN, MeOH, Et$_2$O, -40 °C; b) i) Cyanuric chloride, NMM, THF; ii) NaBH$_4$, H$_2$O; iii) aq. HCl.

A different method for the desymmetrization of meso-acid anhydride 4 has been achieved by applying a modern technology that utilizes catalytic asymmetric esterification wherein the use of a cinchona alkaloid derivative, DHQD-PHN (10 mol %) delivered an excellent ee (93% ee). Sodium borohydride used for the reduction of activated ester, which was in situ formed by the treatment with cyanuryl chloride, furnished the desired lactone 13 with the original asymmetric center virtually retained (Scheme 17).

In order to obtain a higher enantioselectivity, Chen’s group developed this strategy (Scheme 18) and the areas of improvements are: modification in the structure of cinchona catalyst and use of more rigid alcohol for alcoholysis. Firstly, they reported an inexpensive and easily available cinchona alkaloid-quinine-mediated desymmetrization of meso-cyclic anhydride 4 to prepare hemiester 38, a direct precursor to lactone 13. Using propargyl alcohol as nucleophile resulted hemiester 38a in excellent yield but moderate enantioselectivity and required 1.1 equivalent catalyst 1. Until quite recently, a remarkable breakthrough has been achieved by Connon et al. and Song et al., who independently observed the highly stereoselective methanolysis of cyclic anhydrides catalyzed by Cinchona-derived amine-thiourea bifunctional organocatalyst II (Fig. 6) at room temperature, with 10 mol% catalyst loading. Similar conditions were studied for the conversion of cyclic anhydride 4 into lactone 13. Due to the bulky size and the presence of multiple polar functionalities, a catalyst loading of 30 mol% was needed for desymmetrization of 4 with propargyl alcohol at room temperature, generating the hemiester 38b in 96% yield and 82% ee. Chen group also used Song’s protocol as being capable of facilitating the asymmetric methanolysis of various meso-cyclic anhydrides mediated by cinchona alkaloid-based sulfonamide III. However, under these conditions, the asymmetric desymmetrization of 4 using trans cinnamyl alcohol as the test nucleophile in MTBE with 0.5 equiv of Song’s catalyst III at ambient temperature affords the desired hemiester 38c with good enantioselectivity (92% ee).

Scheme 18: Reagents and conditions: a) Borohydride anion exchange resin (BER, 3.3 mmol BH4-/g), CaCl2, EtOH, r.t., 24 h; then 5% HCl, 55 °C, 0.5 h, 95% (over two steps).
Table 1

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Catalyst loading</th>
<th>Nucleophile (R)</th>
<th>Yield</th>
<th>ee</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1.1 equivalent</td>
<td>Propargyl alcohol</td>
<td>95</td>
<td>86</td>
</tr>
<tr>
<td>II</td>
<td>0.3 equivalent</td>
<td>Propargyl alcohol</td>
<td>96</td>
<td>82</td>
</tr>
<tr>
<td>III</td>
<td>0.5 equivalent</td>
<td>Trans Cinnamyl alcohol</td>
<td>98</td>
<td>92</td>
</tr>
<tr>
<td>IV</td>
<td>1.1 equivalent</td>
<td>MeOH</td>
<td>98</td>
<td>96</td>
</tr>
</tbody>
</table>

Figure 6. Structures of chiral amine organocatalysts

The efforts to develop asymmetric alcoholsysis were concluded by the Chen group with utilizing newly developed bifunctional cinchona alkaloid-derived squaramide IV. The catalyst was developed by the same group. The asymmetric desymmetrization of 4 upon treatment with 3 eq of methanol in the presence of 5 mol% catalyst IV in methyl tert-butyl ether (MTBE) at room temperature resulted in hemiester 38d in excellent yield with very low enantioselectivity (only 50% ee). It has been reported that the stoichiometric amount of organo-catalyst has found to constitute the key features for a highly efficient methanolytic desymmetrization of meso-cyclic anhydride in complex total synthesis due to the bulky size and the presence of multiple polar and basic functionalities of anhydrides. Then it was also
observed that anhydride 4 reacted very well leading to the corresponding product 38d in 98% yield with 96% ee when the catalyst loading increased from 5 to 110 mol%. The resulting chiral hemiester 38d was subjected to regioselective reduction with borohydride anion exchange resin (BER, 3.3 mmol BH4/g) in the presence of anhydrous CaCl₂ in EtOH for 18 h, subsequent acid-catalyzed lactonization with 5% HCl for 30 min at 55 °C furnished the desired lactone 13 in 95% overall yield with 98.5% enantiomeric excess.

Figure 7. The proposed transition-state model for the squaramide mediated enantioselective methanolysis reaction

To account for the observed stereochemical outcome of this reaction, a transition state model was proposed and is depicted in Figure 7. The NH groups of the squaramide IV moiety are expected to form hydrogen-bonding interaction with the carbonyl group to activate the electrophile (anhydride 4) and the quinuclidine group is believed to simultaneously act as a general base to activate the nucleophile (methanol).

1.1.2.3 Asymmetric induction-

Asymmetric induction⁶² in stereochemistry describes the preferential formation in a chemical reaction of one enantiomer or diastereomer over the other as a result of the influence of a chiral feature present in the substrate, reagent, catalyst or environment. Asymmetric induction is a key element in asymmetric synthesis. Asymmetric induction was introduced by Emil Fischer based on his work on carbohydrates. Several types of induction exist.

a) Internal asymmetric induction
b) Relayed asymmetric induction
c) External asymmetric induction
Internal asymmetric induction- Internal asymmetric induction makes use of a chiral center bound to the reactive center through a covalent bond and remains so during the reaction. The starting material is often derived from chiral pool synthesis. Chiral pool method for the synthesis of chiral compounds is efficient, when the carbon skeleton and functional groups as well as stereogenic centers of readily accessible natural product, that is chiral pool, are best suited for the synthesis of the target molecule.

Approaches involving L-cysteine/L-cystine as chirons:  
L-Cysteine/L-cystine, carrying amino and thiol groups and chiral center with S-configuration, may serve as a proper starting material for (+)-biotin (1). Intrigued by the structural features of L-cysteine/L-cystine, some synthetic approaches using L-cysteine/L-cystine as the starting material have recently been developed. The synthetic approaches are classified as three categories according to the place of the bond to be assembled to the bicyclic skeleton of 1 (Fig. 8): (1) C-3-N-3’ junction (De Clercq approach); (2) C-3-C-2 junction (Corey, Speckamp, and Chavan approaches); and (3) C-2-S-1 junction (Poetsch and Fujisawa approaches), are being discussed below in chronological order.

Poetsch’s approach (Chimia 1987, 41, 148)  
Poetsch et al. have reported an extremely concise synthesis of (+)-biotin (1) from L-cysteine (Scheme 19). L-Cysteine was first treated with benzaldehyde to simultaneously protect amino and thiol groups as a benzylidene acetal. The resulting thiazolidine derivative 57 was converted to bicyclic hydantoin 58 by the treatment with benzyl isocyanate and hydrochloric acid. Simple reduction of 58 with sodium borohydride furnished hydroxy derivative 59 that, upon activation by acetylation followed by treatment with trimethylsilyl cyanide (TMSCN) in the presence of titanium (IV) tetrachloride (TiCl₄), afforded cyano deriveative 60 in high yield.
Carboxylbutyl chain was efficiently installed through the treatment of 60 with a di-Grignard reagent followed by quenching with carbon dioxide. Reductive cleavage of carbon-sulfur bond with zinc dust, and subsequent cyclization and dehydration provided vinyl sulfide 62, a known precursor to 1. The use of less costly reagents and high overall yield as well as the minimum steps (9 steps) makes the process one of the best among the practical approaches to 1.

Scheme 1:

**Scheme 19: Reagents and conditions:** a) PhCHO; b) (i) BnNCO, (ii) HCl; c) NaBH₄, THF, H₂O; d) (i) Ac₂O (ii) TMSCN, TiCl₄; e) (i) BrMg(CH₂)₄MgBr, (ii) CO₂, (iii) HCl; (f) Zn, AcOH; (g) Piperidine, AcOH.

Corey’s Approach *(Tetrahedron Lett. 1988, 29, 57)*

Corey et al.*⁶⁴* have developed the synthesis of (+)-biotin (1) using radical cyclization of 67 as the key step (Scheme 20). 2,4-Dinitrophenyl sulfide 65 was first elaborated from L-cystine methyl ester hydrochloride 63. Alkyne moiety in 66 was introduced by nucleophilic substitution of 65 with cerium 2-(3-chloropropyl)acetylde. Amidoalkylation of diphenyl disulfide with phosphine-activated alcohol derivative, which was prepared by reduction of 66, afforded 67. Treatment of 67 with tricyclohexyl tin hydride in the presence of azobisisobutyronitrile (AIBN) provided the desired cyclized product 68, though in a moderate yield (40%). The resulting chloride 68 was then converted to 1 through substitution with sodium cyanide and hydrogenation, followed by hydrolysis and removal of the benzyl protective group.
Scheme 20: Reagents and conditions: a) (i) COCl₂, (ii) BnNH₂; (b) (i) Ph₃P, DME, (ii) HCl, (iii) 2,4-Dinitrobenzenesulfinyl chloride; (c) LiCC(CH₂)₃Cl, CeCl₃, (d) DIBAL-H; (e) (PhS)₂, n-Bu₃P; (f) (C₆H₁₁)₃SnH, AIBN; (g) NaCN; (h) H₂, Pd/C; (i) NaOH; (j) HBr.

De Clercq’s Approach (Tetrahedron Lett. 1993, 34, 4365)

De Clercq et al.⁶⁵ have described a conceptually elegant synthesis of (+)-biotin (1) (Scheme 21). Ene-azide 73, a substrate for ring formation, was obtained from L-cysteine. The Wittig reaction of aldehyde 70, which was readily derived from L-cysteine, installed 4-carboxybutyl chain. Treatment of the resulting olefin 71 with bromine followed by hydrogen bromide allowed three consecutive reactions: cleavage of benzylidene acetal, installation of a new tetrahydrothiophene ring, and removal of the protective group. Benzylation of 72 and subsequent formation of acyl azide and final elimination of bromide anion with 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) provided the required 73. Intramolecular [2+3] cycloaddition of 73 followed by elimination of nitrogen gas gave an intermediate 74 carrying the required bicyclic skeleton in good yield.
Scheme 21: Reagents and conditions: a) PhCHO, KOAc; b) ClCO₂Me, NaHCO₃; c) CH₂N₂; d) DIBAL-H; e) [Ph₃P(CH₂)₃CO₂H]Br, LDA; f) CH₂N₂; g) Br₂; h) HBr, AcOH; i) PhCHO, NaBH₃CN; j) COCl₂, DBU, NaN₃; k) DBU; l) Heat; m) H₂, Pd(OH)₂/C; n) HBr, reflux.

Fujisawa’s approach (J. Org. Chem. 1994, 59, 5865)

Fujisawa et al.⁶⁶ have developed the synthesis of (+)-biotin (1) using acid-catalyzed cyclization of yne thiol derivative 79 for the key step (Scheme 22). The compound 79 was synthesized from thiazolidine-4-carbaldehyde 75 that was readily derived from L-cysteine, through stereoselective addition of zinc salt of 2-butylnacetylide. The alcohol 76 obtained was stereoselectively converted to 78 by a sequence involving removal of Boc group, ureide formation, and cyclization via S₅² substitution. Simple treatment of 78 with p-toluenesulfonic acid led to cyclized product 80. Non-cyclized compound 79 was converted to 80 by the treatment with cesium hydroxide in a moderate yield (50%), owing to the uncontrolled regioselectivity of the cyclization. The compound 80 was transformed to 1 through hydrogenation and deprotection to 81, followed by subsequent microbial oxidation of the terminal methyl group. Practicability of the synthesis is, however lessened by the requirement of the bioprocess in the last step.
Scheme 22: Reagents and conditions: a) (i) 1-Hexyne, n-BuLi, (ii) ZnCl$_2$; b) (i) p-TsOH, MeOH, (ii) BnNCO, pyridine; c) KH, p-TsCl, THF, HMPA; d) TsOH, H$_2$O, MeOH; e) H$_2$, Pd/C; f) HBr; g) CsOH, H$_2$O, THF.


Speckamp and coworkers’ approach involves highly convergent synthetic scheme wherein 4-carboxybutyl chain was installed by alkylation of a thiol group with methyl 6-chloro-5-oxohexanoate (Scheme 23). Formation of the bicyclic skeleton of (+)-biotin (1) was carried out by intramolecular amidoalkylation of silyl enol ether, in situ generated from 84. The resulting mixture of diastereomers 85 and 86 was converted to an intermediate 87 through reduction with sodium borohydride followed by elimination of hydroxyl group. The compound 87 was led to 1 through hydrogenation and subsequent cleavage of benzyl groups.
Scheme 23: Reagents and conditions: a) DIBAL-H, THF, -70 °C, 1 h; b) MeO₂C(CH₂)₃C(O)CH₂Cl, Et₃N, 4 h; c) H₂SO₄/EtOH, methyl orange, pH=3.1, 0 °C, 2 h, 72%; d) 2.1 eq. of (TMS)CH₂CO₂Et, 0.03 eq. of TBAF, THF, -78 °C to 25 °C, 18 h, then 1.5 eq. of TMSOTf, DCM, -78 °C, 1 h, 78%; e) NaBH₄, MeOH, 25 °C; f) MeSO₂Cl, Et₃N, DCM; g) DBU, 60 °C, 2 h; h) KOH/MeOH, 2 h, 87%; i) H₂ (10 bar), 10% Pd/C, 2-propanol, 50 °C, 18 h; j) 48% HBr, 100 °C, 2 h, 85%.

 Independently, Chavan’s group has reported two syntheses of biotin on similar lines of intramolecular N acyliminium cyclisation shown in Scheme 24 and Scheme 25.


Conversion of thioaldehyde 90 to the corresponding silyl enol ether followed by trialkyl silyl triflate mediated cyclization in the presence of p-nitrobenzaldehyde as thiophenol scavenger lead to the thermodynamically more stable thiophane aldehyde 91 (Scheme 24). The synthesis of aldehyde 90 involves reduction of hydantoin ester 88 to yield the cyclic hemiacetal 89, which is further converted to 90 by treatment with thiophenol. The transformation of 91 into biotin involved first Wittig reaction
with the 4-carbon ylide, followed by deconjugation with base to yield the exocyclic olefin 92. Further catalytic hydrogenation led to dibenzyl biotin methyl ester 93, which on treatment with 48% HBr furnished D-(+)-biotin.

Scheme 24: Reagents and conditions: a) DIBAL-H, PhCH₃, 72%; b) p-TsOH, PhSH, 70%; c) ’BuMe₂SiCl, DBU, DCM; d) ’BuMe₂SiOTf (cat.), p-NO₂PhCHO, DCM, 87%; e) Ph₃P=CH-CH=CH-CO₂Me, DCM; f) DBU, DCM, 86%; g) H₂ (3 bar), Pd/C, MeOH, 92%; h) 48% HBr.

In the second approach (Scheme 25), the amidoalkylation was performed on hydroxy ureide 59 with 1,2-bis TMS 94 to furnish 95 in excellent yields. The ketone 95 was subjected to Baeyer-Villiger oxidation with tert-butylhydroperoxide in alkaline methanol to furnish the keto acid in 70% yield. This keto acid on esterification with diazomethane furnished the keto ester 96 in quantitative yield. The intermediate 96 being epimeric at C-7 with respect to (+)-biotin, was epimerized by reductive cleavage of carbon-sulfur bond with Zn/AcOH. Further cyclization of thiol thus obtained with the carbonyl function was performed in the presence of piperidine and acetic acid followed by dehydration to afford the olefin 97. Stereospecific
hydrogenation was carried out in the presence of 10% palladium on carbon to furnish \(N,N'\)-dibenzyl biotin methyl ester in quantitative yield. Removal of \(N\)-benzyl groups was achieved with aq. HBr (47%) at reflux temperature to afford (+)-biotin.

**Scheme 25: Reagents and conditions:**
- a) BF\(_3\).OEt\(_2\), DCM, 98%;
- b) 70% TBHP, KOH-MeOH, 15 min;
- c) \(\text{CH}_2\text{N}_2\), 10 min, 70%;
- d) Zn/AcOH, 80 °C, 5 h;
- e) AcOH/piperidine, 100 °C, 90 min, 70%;
- f) \(\text{H}_2/Pd\)-C, MeOH, 200 psi, 100%;
- g) 47% HBr, 5 h, 78%.

**Seki’s approach** (*Tetrahedron Letters* 2004, 45, 6579; *Chemistry- A European Journal* 2004, 10, 6102)

Seki *et al.* reported synthesis of biotin from L-cysteine utilizing Strecker reaction as key step.\(^{69}\) A syn-selective Strecker reaction of \(\alpha\)-amino aldehyde 99 to \(\beta\)-amino-\(\alpha\)-amino nitrile *syn* 100 was achieved to allow an access to thiolactone 6, a key intermediate for 1 (Scheme 26). Amino alcohol 98 was subjected to the Moffatt oxidation employing DCC in the presence of TFA and pyridine to effect a clean conversion to \(\alpha\)-amino aldehyde 99 (90% yield). The resulting solution of 99 in ethyl acetate was treated with sodium bisulfite (1.1 equiv) in water to provide the bisulfite adduct in excellent yields (99% conversion). The aqueous solution of bisulfite adduct was treated with benzylamine in a biphasic system involving CH\(_2\)Cl\(_2\) and water at 20 °C for 2 h. The resulting mixture containing an imine and a bisulfite adduct of the imine was treated with NaCN (1.2 equiv) at 8 °C and the whole was stirred at ambient temperature for 20 h to provide \(\alpha\)-amino nitrile 100 as a solution of CH\(_2\)Cl\(_2\) with high diastereoselectivity and in high yield (*syn/anti* = 11:1, 95% assay yield). The resulting CH\(_2\)Cl\(_2\) solution of 100 was directly subjected to amidation employing H\(_2\)O\(_2\), K\(_2\)CO\(_3\).
and DMSO. The reaction smoothly proceeded even in a mixed solvent of DMSO and CH$_2$Cl$_2$ to afford the corresponding amide 101 in quantitative yield. Syn 101 was allowed to undergo the ring transformation from 2-thiazolidinone to 2-imidazolidinone through $S,N$-carbonyl migration and subsequent hydrolysis to give thiol carboxylic acid 102 in 95% yield. The compound 102 obtained was cyclised and isomerized to thiolactone 6 in 93% yield.

**Scheme 26: Reagents and conditions:**

- **a)** DCC, TFA, pyridine, DMSO, EtOAc, 50 °C, 3 h;
- **b)** i) NaHSO$_3$, EtOAc, H$_2$O, 20 °C, 18 h; ii) BnNH$_2$ (1.7 equiv), CH$_2$Cl$_2$, 20 °C, 2 h, iii) NaCN (1.2 equiv), 8–20 °C 20 h;
- **c)** i) H$_2$O$_2$, K$_2$CO$_3$, DMSO/CH$_2$Cl$_2$, 20 °C, 2.5 h, ii) H$_2$O, filtration, iii) For the filtrate: HCl;
- **d)** i) DMF, 90 °C, 1 h, ii) HCl;
- **e)** DMF, TFA, pyridine, CHCl$_3$, 5 °C-reflux, 5 h;
- **f)** 120 °C, 5 h DMF.

In contrast, the anti isomer anti 101 was directly converted to 6 in 91% yield with heating at a higher temperature (120 °C) through the $S,N$-carbonyl migration followed by spontaneous cyclization.

**Approaches involving sugars as chirons:**

Syntheses of biotin utilizing monosaccharides as chiral synths are described below briefly. The monosaccharides that have been used as chiral starting materials for...
further multistep conversion to (+)-biotin are further shown in a way that immediately allows analysis of the sequences that will be necessary for their conversion to biotin. Crucial in the design of all syntheses in this area is the obtention of the thiophane nucleus via double S\textsubscript{N}2 displacement of a dimesylate obtained from a diol. All syntheses additionally have in common that the biotin carboxyalkyl side chain is introduced via Wittig condensation using 3-(methoxycarbonyl)-2-propenylidene triphenylphosphorane followed by catalytic hydrogenation. In all cases, the amino groups were introduced via sequences involving S\textsubscript{N}2 substitution of a leaving group by azide followed by reduction of the azide.

**Ohru’s approach** *(Tetrahedron Lett. 1975, 2765)*

![Scheme 27: Reagents and conditions](image)

**Scheme 27: Reagents and conditions:** a) PhCOCl, C\textsubscript{5}H\textsubscript{5}N; b) HOAc, H\textsubscript{2}O; c) NaIO\textsubscript{4}, CH\textsubscript{3}COCH\textsubscript{3}/H\textsubscript{2}O; d) Ph\textsubscript{3}P=CHCH=CHCOOCH\textsubscript{3}, CH\textsubscript{2}Cl\textsubscript{2}; e) H\textsubscript{2}, Pd/C, CH\textsubscript{3}OH; f) NaOCH\textsubscript{3}, CH\textsubscript{3}OH; g) NaBH\textsubscript{4}; h) CH\textsubscript{3}SO\textsubscript{2}Cl; i) Na\textsubscript{2}S, HMPA, 100 °C; j) 90% HCOOH, 20 °C; k) CH\textsubscript{3}SO\textsubscript{2}Cl; l) NaN\textsubscript{3}, HMPA, 80 °C; m) PtO\textsubscript{2}, MeOH/Ac\textsubscript{2}O; n) Ba(OH)\textsubscript{2}, H\textsubscript{2}O, 140 °C; o) COCl\textsubscript{2}.
In 1975, Ohrui et al.\(^\text{70}\) reported synthesis of biotin from mannofuranose as chiron (Scheme 27). In their approach the di-O-isopropylideneprotected \(R\)-D-mannofuranose 103 is converted to diol 106. The sequence involved formation of the benzoate of the anomeric alcohol, selective hydrolysis of the 5,6-isopropylidene group, and oxidative cleavage of the vicinal diol to yield aldehyde 104. Subsequent Wittig treatment and catalytic hydrogenation afforded 105. Base treatment of the latter generated the hemiacetal that was reduced to diol 106. After thiophane formation and hydrolysis the obtained diol 107 is converted to the diamine 108 via inversion. Final obtention of (+)-biotin occurs after saponification and phosgene treatment.

**Ravindranathan’s approach** (*Carbohydr. Res. 1984, 134, 332*)

A modified synthesis of (+)-biotin from D-glucose was reported by Ravindranathan and co-workers in 1984.\(^\text{71}\) The synthesis which is outlined in Scheme 28 leads to the Ohrui intermediate 105. The starting substance is D-glucurono-6,3-lactone 109 which is first reduced to L-gulono-1,4-lactone 110. The remaining steps are essentially similar to the Ohrui sequence (Scheme 27).

**Scheme 28: Reagents and conditions**: a) \(H_2\), Raney-Ni; b) \((\text{CH}_3)_2\text{C(OCH}_3)_2\), DMF, \(p\text{-TsOH}\); c) \(\text{NaBH}_4\), \(\text{CH}_3\text{OH}, 0 \degree\text{C}\); d) \(\text{PhCOCl}, \text{C}_5\text{H}_5\text{N}\); e) \(\text{CH}_3\text{OH}, \text{HCl}\); f) \(\text{NaIO}_4\), acetone/\(\text{H}_2\text{O}, 0 \degree\text{C}\); g) \(\text{Ph}_3\text{P=CHCH=CHCOOCH}_3, \text{CH}_2\text{Cl}_2\); h) \(H_2, \text{Pd/C}\).
Chavan’s approach *(Tetrahedron Lett. 2004, 45, 7307)*

Chavan’s group developed a sequence to the thiophone aldehyde 91 (Scheme 24) starting from D-glucosamine, by taking advantage of the correct absolute configuration of the amino group in D-glucosamine that will eventually appear at C-4 in (+)-biotin (Scheme 29). By treating glucosamine hydrochloride with BnNCO in the presence of sodium bicarbonate followed by heating in the presence of catalytic amount of pyridine, furnished the cis furanoid bicycle 114.

![Chemical diagram](image)

**Scheme 29: Reagents and conditions:**

- a) (i) BnNCO, aq. NaHCO₃; (ii) Pyridine (cat.), H₂O; b) p-TSA (cat.), acetone, rt; c) NaH, BnBr, DMF; d) p-TSA (cat.), THF–H₂O (9:1), reflux; e) NaIO₄, acetone–H₂O (9:1), rt; f) Ac₂O, Et₃N, DMAP (cat.), DCM; g) TMSCN, BF₃·Et₂O, DCM, -78 °C to rt; h) NaBH₄, MeOH, 0 °C to rt; i) TMSCl, MeOH, 40 °C; j) (i) NaIO₄, acetone–water (9:1), rt; (ii) Ethylene glycol, p-TSA, C₆H₆, reflux; k) Pd–CaCO₃, MeOH, rt; l) DBU (cat.), toluene, reflux; m) NaBH₄, EtOH, reflux; n) (i) MsCl, Et₃N, DMAP (cat.), 0 °C to rt; (ii) Na₂S, DMF, 100 °C; o) 6 N HCl, CH₃COOH, rt.
The diol 114 was protected as the acetonide and N-urea protected with benzyl bromide to furnish the protected bicyclic intermediate 115. Demasking of acetonide resulted in diol, which was cleaved to the aldehyde which was subsequently treated with acetic anhydride to furnish the exocyclic enol acetate 116. Intermolecular carbon–carbon bond formation via an acyliminium ion was effected by treating the enol acetate 116 with TMSCN and BF₃·Et₂O to furnish the cyano substituted intermediate 117. Compound 117 on reduction with NaBH₄ and further treatment with TMSCl in methanol resulted in methyl ester 118. The diol 118 was cleaved to aldehyde and subsequently protected as the dioxalane derivative 119. By following the sequence, selective O-debenzylation, epimerization and reduction dioxalane derivative 119 was converted into diol 120. The thiophane nucleus was synthesised via double Sₘ₂ displacement of a dimesylate obtained from the diol 120. Treatment with acid furnished thiophane aldehyde 91. The remaining steps are essentially similar to the previous approach (Scheme 24).

Approach involving L-aspartic acid as chiron:

Seki’s approach (Synthesis 2002, 3, 361)

In 2002 Seki et al. reported the synthesis of biotin starting from L-aspartic acid as chiral synthon (Scheme 30). The aldol reaction of an N-Cbz-3-amino-4-butanolide 122, derived from L-aspartic acid, with formaldehyde gave the trans-disubstituted 3-amino-4-butanolide 123 stereoselectively. Protection of the hydroxyl group of 123, amidation and oxidation provided the substituted L-asparagine derivative 126. The Hofmann rearrangement of 126 with sodium hypochlorite in the presence of sodium hydroxide and subsequent hydrogenation gave the bicyclic lactone 128, which upon dibenzylation and thionation, gave the thiolactone 6, a key intermediate for the synthesis of (+)-biotin.
Scheme 30: Reagents and conditions: a) i) Ac₂O; ii) NaBH₄, THF; iii) HCl; b) i) LDA, THF; ii) HCHO, −78 °C, trans/cis = 12:1; c) BOMCl, i-Pr₂NEt, THF, quant; d) NH₄OH, MeOH; e) Jones’ reagent, acetone; f) NaOCl, NaOH, H₂O; g) H₂, Pd(OH)₂/C, MeOH; h) BnBr, NaH, DMF; i) AcSK, DMF.

In **relayed asymmetric induction** the chiral information is introduced in a separate step and removed again in a separate chemical reaction. Special synths are called chiral auxiliaries.


Another interesting asymmetric approach has been developed by chemists at Lonza that centers around the hydrogenation of furoimidazole derivative 132 (Scheme 31). The synthesis of this intermediate 132 involves a straightforward four-step sequence starting from tetronic acid 129. Treatment of the latter with the diazonium salt derived from aniline leads to diazo compound 130 which is converted into 132 via reaction with a primary amine such as (S)-1-phenylethyl amine to afford 131 followed by reduction and subsequent imidazolone ring formation with ethyl chloroformate. It is interesting to note that both 132 and **ent-132** can lead to the
diastereomer with the desired (3S, 4R)-configuration depending on the hydrogenation conditions:

1. Rhodium on alumina in DMF for 132 (54% yield of crystalline 133).
2. Palladium on carbon in acetic acid for ent-132 (54% yield).\textsuperscript{74c}

\[
\begin{array}{c}
\text{HO} \quad \text{a} \quad \text{HO} \quad \text{b} \quad \text{c,d}
\end{array}
\]

\[
\begin{array}{c}
\text{129} \quad \text{130} \quad \text{131}
\end{array}
\]

\[
\begin{array}{c}
\text{Ph} \quad \text{H} \quad \text{Ph} \quad \text{H} \quad \text{Ph}
\end{array}
\]

\[
\begin{array}{c}
\text{132} \quad \text{133} \quad \text{134}
\end{array}
\]

\textit{Scheme 31: Reagents and conditions:} a) PhNH\textsubscript{2}, NaNO\textsubscript{2}, HCl; b) (R)-PhCH(NH\textsubscript{2})CH\textsubscript{3}, B(OEt)\textsubscript{3}, PhCH\textsubscript{3}, 80 °C; c) H\textsubscript{2}, Pt/C, EtOAc, 40 bar; d) ClCOOEt, Et\textsubscript{3}N, CH\textsubscript{3}CN, reflux; e) H\textsubscript{2}, Rh/Al\textsubscript{2}O\textsubscript{3}, DMF, 40 bar; f) NaH, DME, PhCH\textsubscript{2}Br; g) CH\textsubscript{3}COSK, CH\textsubscript{3}CON(CH\textsubscript{3})\textsubscript{2}, 150 °C.

In external asymmetric induction chiral information is introduced in the transition state through a catalyst of chiral ligand. This method of asymmetric synthesis is economically most desirable.


The reduction of achiral 135 in the presence of a rhodium complex and a chiral ferrocenylyphosphine ligand 136 into 137 (95% yield; 90% ee), constitutes a sole example where the chirality is introduced by a catalytic pathway (Scheme 32).
Scheme 32: Reagents and conditions: a) Rh(0) = [Rh(norbornadiene)Cl]₂, PhCH₃, 70 °C, H₂, 50 bar.

Up to date only one approach is reported by this strategy.
1.1.3 References

3. West, P.; Wilson, P. Science 1939, 89, 607.


Chapter 1. Synthetic Studies towards D-(+)-Biotin

Section 2

A novel and enantioselective synthesis of D-(+)-Biotin via Sharpless asymmetric dihydroxylation strategy
1.2.1 Summary

The present section deals with the enantioselective synthesis of D-(+)-biotin. The key steps in the sequence are the Sharpless asymmetric dihydroxylation of a (E)-ethyl 3-(2-chlorocyclohex-1-en-1-yl)acrylate derivative to establish the stereocenters of D-(+)-biotin, establishment of the carboxyalkyl side chain by unmasking of cyclohexene by ozonolysis and enzymatic hydrolysis of a thioacetate.

1.2.2 Introduction

Over the past decades, many efforts have been made on the development of an efficient process for the total synthesis of D-(+)-biotin (1) and a number of new synthetic approaches involving different strategies for the control of three adjacent chiral centers are reported in the literature.\(^1\) It may be emphasized that very few syntheses of D-(+)-biotin involving intermolecular asymmetric induction are reported in the literature.\(^{1a}\) Many synthetic approaches, such as diastereoisomeric or enzymatic resolutions,\(^2\) chiral pool methods involving carbohydrates,\(^3\) cysteine\(^4\) and L-aspartic acid\(^5\) have been described. This research group has been actively involved in the stereoselective synthesis of D-(+)-biotin via chiral pool methods and has reported D-(+)-biotin syntheses starting from L-cysteine\(^6\) and glucosamine.\(^7\) Herein, contributions towards developing an asymmetric total synthesis of 1 starting from commercially available achiral starting material \textit{viz.} cyclohexaneone, is described.

1.2.3 Present work

The envisaged retrosynthetic strategy for biotin (1) is delineated in Scheme 1. A linear synthetic strategy was invoked wherein olefin 2 was conceived as the direct precursor to 1. Olefin 2 would then in turn be accessed from thioacetate 3 upon hydrolysis and intramolecular cyclization. The carboxyalkyl side chain of biotin (1) could be unmasked by ozonolysis of cyclohexene 5. The cyclohexene 5 could be readily obtained from azidoamine 6 by reduction and urea formation. The vicinal diamine group in 6 could be introduced via sequences involving opening of cyclic sulfite which could be prepared from diol 7 and \textit{S_N}2 substitution of a leaving group by azide. Further analysis indicated that the diol compound 7 could be synthesized from the unsaturated ester compound 8 by using Sharpless asymmetric dihydroxylation.
Chapter 1. Section 2

(SAD). The unsaturated ester 8 could be obtained from easily available, inexpensive starting material 9 via Vilsmeier-Haack reaction and Wittig reaction (Scheme 1).

![Scheme 1. Retrosynthetic analysis for biotin 1.](image)

### 1.2.4 Results and discussion

Accordingly, the synthesis of biotin (1) began from cyclohexanone (9) as a commercially available starting material. Following a literature procedure, cyclohexanone (9) was subjected to Vilsmeier-Haack reaction to furnish aldehyde 10, which was homologated using Wittig reaction to afford unsaturated ester 8 in 76% yield (Scheme 2). The IR spectrum of compound 8 showed strong bands at 1713 and 1622 cm\(^{-1}\) indicating the presence of ester and olefin functionalities. The \(^1\)H NMR spectrum of compound 8 showed peaks at \(\delta\) 5.85 and 7.91 as doublets corresponding to the protons of double bond of unsaturated ester compound 8. The coupling constant of the two protons of the double bond was 15.9 Hz which revealed that the double bond had trans geometry. The \(^{13}\)C NMR spectrum showed peak at \(\delta\) 166.7
corresponding to the carbonyl carbon of ester functional group. Its DEPT NMR spectrum showed presence of five CH$_2$ groups, four for cyclohexene and one for ethyl ester. Finally, the structure of compound 8 was confirmed by HRMS analysis (Scheme 2).

Scheme 2. Reagents and conditions: a) POCl$_3$, DMF, DCM, 0 °C-rt, 4 h, 95%; b) Ph$_3$PCHCO$_2$Et, DCM, rt, 4 h, 76%.

This prochiral unsaturated ester 8 was deemed to be a suitable substrate for the installation of the chiral centers. The compound 8 was subjected to Sharpless asymmetric dihydroxylation (SAD)$^9$ conditions with (DHQD)$_2$PHAL as the chiral catalyst to yield chiral diol 7 in 84% yield with ≥99% ee.$^{10}$ The IR spectrum of compound 7 showed strong bands at 3446 and 1736 cm$^{-1}$ for free hydroxyl and ester functional groups respectively. In $^1$H NMR spectrum of compound 7, doublets at $\delta$ 5.85 and 7.91 were absent indicating the conversion of conjugated double bond into diol. $^{13}$C NMR and DEPT NMR spectra of compound 7 showed the peaks at $\delta$ 72.1 and 73.2 for corresponding methine carbons [C(OH)CH(OH)]. Finally the structure of compound 7 was confirmed by HRMS analysis. The enantiomeric excess of diol 7 was determined by chiral HPLC analysis (Scheme 3).

To install the azido functionality, diol 7 was converted into cyclic sulfite/sulfate. Direct conversion of sulfite/sulfate to the corresponding diazide proved unsuccessful. Hence it was decided to convert sulfite to diamine in a stepwise fashion. This was done by regioselective opening of this cyclic sulfite using NaN$_3$ in DMF to get the $\beta$-azido ester 11 in 75% yield (over two steps).$^{11}$ The IR spectrum of azido ester 11 showed strong bands at 2104 and 1736 cm$^{-1}$ indicating the presence of azide and ester functionalities respectively. Presence of broad band at 3448 cm$^{-1}$ indicated the presence of hydroxyl group. Peak at $\delta$ 5.01 in its $^1$H-NMR spectrum appeared as a doublet accounting for one proton adjacent to hydroxyl group. The proton adjacent to azide merged with the methylene protons of ester which appeared as multiplet at $\delta$ 4.19-4.36 integrating for three protons. Its $^{13}$C-NMR spectrum
showed characteristic peak at $\delta$ 172.4 which was assigned to carbonyl carbon. Its DEPT spectrum showed presence two CH carbons that appeared at $\delta$ 65.1 and 71.6, while five CH$_2$ carbons appeared at chemical shifts in accordance with the structure of 11. Finally the structure of compound 11 was confirmed by HRMS analysis.

**Scheme 3.** Reagents and conditions: a) (DHQD)$_2$PHAL, OsO$_4$, $K_3[Fe(CN)_{6}]$, $K_2CO_3$, $^1$BuOH:H$_2$O (1:1), 2 days, 84%, $\geq$99% ee; b) i) SOCl$_2$, Et$_3$N, DCM, 0 °C, 15 min; ii) NaN$_3$, DMF, rt, 4 h, 75% (over two steps); c) i) Ph$_3$P, Et$_2$O, rt, 2 h; ii) b) CbzCl, $K_2CO_3$, DCM, 0 °C, 80% (over two steps).

Azide was reduced under Staudinger reaction condition$^{12}$ followed by protection using CbzCl to furnish the carbamate 12. The IR spectrum of 12 displayed a strong absorption band at 1690 cm$^{-1}$ indicating the presence of carbamate functionality while absence of absorption at 2104 cm$^{-1}$ indicated azide reduction. $^1$H NMR spectrum of compound 12 showed the presence of a new multiplet which appeared at $\delta$ 7.27-7.40 integrating for five protons which was attributed to Cbz group while multiplet appeared at $\delta$ 5.25-5.29 corresponding to proton of NH-CO-Bn. $^{13}$C NMR spectrum showed the signals that appeared at $\delta$ 67.0 and 155.4 corresponding to carbons of Cbz group (NH-CO-CH$_2$-Ph and NH-CO-CH$_2$-Ph respectively). Additionally, DEPT experiment also showed the presence of six CH$_2$ and five CH carbons supporting the formation of 12. Finally, the structure of compound 12 was confirmed by HRMS analysis (Scheme 3).

The second amine was installed by converting hydroxyl group in 12 to its mesylate using mesyl chloride and triethylamine, which was displaced by azide using sodium azide in DMF to give azidoamine 6 in 82% yield (over two steps). The IR
The IR spectrum of compound 5 showed strong bands at 1818, 1726, 1750 and 1694 cm\(^{-1}\) for carbonyl groups of ethyl carbamate, benzyl carbamate, ethyl ester and cyclic urea. The \(^1\)H NMR spectrum of compound 5 showed multiplet at \(\delta\) 1.25-1.38 for six protons corresponding to ethyl ester (\(\text{CH}_3\text{CH}_2\text{OCO-}\)) and ethyl carbamate (\(\text{CH}_3\text{CH}_2\text{OCO-N}\)). \(^{13}\)C NMR spectrum showed peaks at \(\delta\) 147.4, 150.3, 151.0 and 168.6 for the carbonyl groups of carbamates, ester and urea. Finally, the structure of compound 5 was confirmed by HRMS analysis.

After having compound 5 in hand, ester was reduced using NaBH\(_4\) in methanol at 0 °C, to furnish hydroxyl compound 13. Interestingly, here proximal carbamate was also selectively hydrolyzed during reduction. The IR spectrum of compound 13 showed the absence of bands at 1818 and 1750 cm\(^{-1}\) for acyclic ethyl carbamate and ester indicating the hydrolysis during reduction of 5. The \(^1\)H NMR spectrum of compound 13 showed the absence of double doublet at \(\delta\) 6.28 and a multiplet at \(\delta\) 1.25-1.38 for six protons corresponding to acyclic ethyl carbamate and ester indicating the hydrolysis during reduction of 5. \(^{13}\)C NMR and DEPT NMR spectra were also in good agreement with the structure of compound 13. Finally, the structure of compound 13 was confirmed by HRMS analysis (Scheme 5).
The protection of primary hydroxyl group in 13 with tert-butylchlorodimethylsilane in the presence of imidazole in dichloromethane gave TBS ether 14 in 93% yield. The IR spectrum of compound 14 showed the absence of broad band at 3370 cm\(^{-1}\) for hydroxyl group indicating the protection of hydroxyl group. Its \(^1\)H-NMR spectrum showed peaks as singlets at \(\delta\) 0.02 and 0.83 for six and nine protons respectively clearly showing the presence of TBS group. Peaks at \(\delta\) -5.5, -5.4 and 25.7 in its \(^{13}\)C-NMR spectrum corresponded to TBS group. Finally, the structure of compound 14 was confirmed by HRMS analysis.

![Scheme 5](image)

**Scheme 5.** Reagents and conditions: a) NaBH\(_4\), MeOH, 0 °C, 4 h, 79%, 30 min; b) TBSCI, imidazole, DMAP, DCM, 0 °C-rt, 24 h, 93%; c) NaH, BnBr, THF, 0 °C, 3 h, 95%.

On treatment with sodium hydride and benzyl bromide, 14 was converted to its bisbenzyl imidazolidone 15 in 95% yield. An important and interesting feature of this transformation is the formation of bis N-benzyl derivative which involves deprotection of carbamate followed by alkylation in one pot. The IR spectrum of 15 displayed the strong absorption band at 1698 cm\(^{-1}\) indicating the presence of cyclic urea functionality while absence of absorption at 1775 cm\(^{-1}\) indicated carbamate hydrolysis. The \(^1\)H NMR spectrum showed four upfield signals at \(\delta\) 4.00, 4.08, 4.52 and 4.93 for four protons and multiplet at \(\delta\) 7.24-7.32 for ten protons indicating that the presence of two benzyl groups. This was further confirmed by its \(^{13}\)C NMR and DEPT NMR spectra, which showed a \(-\text{CH}_2-\) carbon singlets at \(\delta\) 46.2 and 46.9 for the benzylic carbon. Finally, the structure of compound 15 was confirmed by HRMS analysis (Scheme 5).
On performing ozonolysis reaction in MeOH:DCM (1:5) solvent system, imidazolidone 15 was converted into ketoester 4 in 92% yield. The IR spectrum of ketoester 4 showed strong absorption bands at 1733, 1706 and 1690 cm\(^{-1}\) indicating the presence of ester, ketone and cyclic urea functionalities respectively. The \(^1\)H NMR spectrum of compound 4 showed a singlet at \(\delta\) 3.66 for three protons indicating the presence of methyl ester. The \(^{13}\)C NMR spectrum showed peaks at \(\delta\) 173.4 and 207.4 corresponding to ester and ketone functional groups respectively. Finally, the structure of compound 4 was confirmed by HRMS analysis.

Scheme 6. Reagents and conditions: a) \(O_3\), MeOH:DCM (1:5), NaHCO\(_3\), -78 °C, 30 min, 92%; b) CSA, MeOH, rt, 30 h, 90%; c) i) TsCl, Et\(_3\)N, DMAP, DCM, 0 °C-rt, 24 h, 85%; ii) KSAc, DMF:THF, 80 °C, 2 h, 83 %.

Deprotection of TBS ether of 4 was carried out using camphorsulfonic acid in MeOH to afford the corresponding alcohol 16 in 90% yield. The IR spectrum of 16 showed broad absorption band at 3409 cm\(^{-1}\) indicating the presence of hydroxyl group. The \(^1\)H NMR spectrum of compound 4 showed absence of peaks at \(\delta\) 0.02 and 0.83 for six and nine protons respectively clearly showing the absence of TBS group. This was further confirmed by its \(^{13}\)C NMR and DEPT NMR spectra. Finally, the structure of compound 4 was confirmed by HRMS analysis.

After diamine installation only thing remaining was to construct tetrahydrothiophene ring. Introduction of sulfur was done by converting the primary hydroxyl group into a good leaving group. Accordingly, the hydroxyl compound 16
was converted to its tosylate derivative and it was in turn displaced by potassium thioacetate in DMF:THF (2:3) to furnish the thioacetate 3 in 83% yield (Scheme 6). The IR spectrum of thioacetate 3 showed strong absorption band at 1710 cm\(^{-1}\) indicating the presence of acetate functionality. The \(^1\)H NMR spectrum of compound 3 showed singlet at \(\delta 2.24\) for three protons indicating the presence of thioacetate (CH\(_2\)CO-S-) group. \(^1\)C NMR spectrum showed peaks at \(\delta 159.0, 173.4, 194.2\) and 206.6 for the corresponding carbonyl group of urea, ester, thiocetate and ketone. Finally, structure of compound 3 was confirmed by HRMS analysis (Scheme 6).

All the structural constituents for biotin were present and in place in compound 3, the only thing remaining to complete synthesis was acetate deprotection of 3 and the construction of thiophane ring. Efforts towards this seemingly simple deprotection of acetate 3 to thiol 17 failed under a variety of reaction conditions (Table 1).

![Diagram](image)

**Table 1.** Attempts towards acetate deprotection

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pyrrolidine, Et(_3)N, MeOH</td>
<td>Dimerization</td>
</tr>
<tr>
<td>2</td>
<td>K(_2)CO(_3), MeOH</td>
<td>Dimerization</td>
</tr>
<tr>
<td>3</td>
<td>Acetyl chloride, MeOH</td>
<td>SM recovered(^a)</td>
</tr>
<tr>
<td>4</td>
<td>DBU, toluene, RT</td>
<td>SM recovered(^a)</td>
</tr>
<tr>
<td>5</td>
<td>DBU, toluene, 80 °C</td>
<td>Complex reaction mixture</td>
</tr>
<tr>
<td>6</td>
<td>TiCl(_4), Zn, DCM</td>
<td>SM recovered(^a)</td>
</tr>
<tr>
<td>7(^b)</td>
<td>Lipase <em>(Candida cylindrica)</em></td>
<td>SM recovered(^a)</td>
</tr>
<tr>
<td>8(^b)</td>
<td>Lipase <em>(Candida rugosa)</em></td>
<td>17 in 80%</td>
</tr>
</tbody>
</table>

\(^a\)SM: starting material; \(^b\)in phosphate buffer (pH 6.8).

Finally the hydrolysis under enzymatic condition using lipase *(Candida rugosa)* gave the thiol 17 in 80% yield. The IR spectrum of thiol 17 showed absence of absorption band at 1710 cm\(^{-1}\) indicating the absence of acetate functionality. The \(^1\)H NMR spectrum of compound 17 showed absence of singlet at \(\delta 2.24\) for three protons.
indicating the absence of thioacetate, whereas it showed a double doublet at δ 1.11 for one proton characteristic of thiol (-CH₂-SH). \(^{13}\)C NMR spectrum showed absence of peaks at δ 194.2 indicating hydrolysis of 3. Finally, structure of compound 3 was confirmed by HRMS analysis.

Correction of stereochemistry at C-3 of 17 was achieved by epimerization with catalytic amount of DBU in refluxing toluene, which on concomitant cyclisation and elimination using \(p\)TSA resulted in the formation of olefin 2 in 82% yield over two steps (Scheme 7). The \(^1\)H NMR spectrum of compound 2 revealed a characteristic triplet at δ 5.54 integrating for one proton which was assigned to the olefinic proton. Finally, HRMS analysis confirmed the molecular formula (calculated for \(C_{25}H_{28}O_3N_2NaS\) 459.1713, found 459.1707). The olefin 2 thus obtained was identical with an authentic sample, with respect to IR, NMR, mass spectra, and specific rotation,\(^6\) prepared by a different route. Since the conversion of olefin 2 to (+)-biotin (1) has been reported by this group\(^6,7\) and others,\(^1\) this constitutes a formal synthesis of (+)-biotin.

**1.2.5 Conclusion**

In conclusion, an asymmetric synthesis of D-(+)-biotin has been achieved employing Sharpless asymmetric dihydroxylation as the single source of chirality. The enantioselectivity is introduced by intermolecular asymmetric induction, which is the first report of its kind utilized in the synthesis of biotin. The carboxyalkyl side chain of D-(+)-biotin is introduced by unmasking of cyclohexene by ozonolysis, which is also a novel strategy in synthesis.
1.2.6 Experimental

**(E)-Ethyl 3-(2-chlorocyclohex-1-en-1-yl)acrylate (8):** A mixture of anhydrous DCM (80 mL) and anhydrous DMF (15.7 mL, 204.08 mmol) was cooled to 0 ºC using ice, to that was added POCl₃ (15.1 mL, 163.26 mmol) dropwise and stirred further for 2 h at room temperature. Cyclohexanone 9 (10.0 g, 102.04 mmol) in DCM (20 mL) was added dropwise at 0 ºC and further stirred for 4 h at room temperature. Reaction mixture was quenched first by using ice followed by careful addition of sat. NaHCO₃ solution. Reaction mixture was allowed to separate in a separating funnel. The organic layer was separated and the aqueous layer was again extracted twice using DCM (50 mL). The collected organics were dried over anhydrous Na₂SO₄, filtered and concentrated in vacuo to furnish chloroaldehyde 10 (14.0 g, 95%) as a crude product.

The crude aldehyde (14 g, 97.22 mmol) was dissolved in DCM (150 mL) and to that was added Ph₃PCHCO₂Et (50.7 g, 145.83 mmol). The reaction mixture was stirred further for 4 h. The solvent was removed under reduced pressure and the crude residue was directly adsorbed on simple silica gel and was purified using flash chromatography (SiO₂, 04:96 EtOAc: Pet. ether) to furnish the α,β-unsaturated ester 8 as a colorless liquid.

*R*: 0.6 (Pet. ether-ethyl acetate, 9:1).

**Yield:** 25.7 g, 76%.

**MF:** C₁₁H₁₅ClO₂, **MW:** 214.68.

**IR (CHCl₃, cm⁻¹):** v max 2938, 1713, 1622, 1292, 1175.

**¹H NMR (200 MHz, CDCl₃+CCl₄):** δ 1.30 (t, J=7.1 Hz, 3 H), 1.62 - 1.76 (m, 4 H), 2.26 (brs, 2 H), 2.51 (brs, 2 H), 4.21 (q, J=7.1 Hz, 2 H), 5.85 (d, J=15.9 Hz, 1 H), 7.91 (d, J=15.9 Hz, 1 H).

**¹³C NMR (100 MHz, CDCl₃+CCl₄):** δ 14.2, 21.7, 23.3, 26.1, 35.1, 60.1, 118.1, 128.5, 139.0, 141.1, 166.7.

**HRMS ESI [M+H]⁺ calcd for C₁₁H₁₆O₂Cl 215.0833, found 215.0832.**
(2S,3R)-Ethyl 3-(2-chlorocyclohex-1-en-1-yl)-2,3-dihydroxypropanoate (7):

To a mixture of K$_2$Fe(CN)$_6$ (13.8 g, 42.03 mmol), K$_2$CO$_3$ (5.8 g, 42.03 mmol) and (DHQD)$_2$PHAL (0.109 g, 0.14 mmol) in $^1$BuOH-H$_2$O (1:1, 120 mL) cooled at 0 °C was added osmium tetroxide (1 mL of 0.1 M solution in toluene, 0.4 mol %) followed by methane sulfonamide (1.32 g, 14.01 mmol). After stirring for 5 min at 0 °C, the olefin 8 (3 g, 14.01 mmol) was added in one portion. The reaction mixture was stirred at 0 °C for 2 days and then quenched with solid sodium sulfite (6 g). The stirring was continued for an additional 45 min and then the solution was extracted with EtOAc (3 × 30 mL). The combined organic layers were dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (SiO$_2$, 3:7 EtOAc:Pet. ether) to provide the diol 7 as a white solid.

**R$_f$:** 0.3 (Pet. ether-ethyl acetate, 8:2).

**Yield:** 2.92 g, 84%.

**MF:** C$_{11}$H$_{17}$ClO$_4$, **MW:** 248.70.

**Melting Point:** 65-67 °C.

$[^{[\alpha]}]^{25}_{D} = +5.2$ (c 1.92, CHCl$_3$).

**IR** (CHCl$_3$, cm$^{-1}$): $v_{\text{max}}$ 3446 (broad), 2937, 1736, 1105.

$^1$H NMR (500 MHz, CDCl$_3$+CCl$_4$): $\delta$ 1.31 (t, $J=7.1$ Hz, 3 H), 1.59 - 1.79 (m, 4 H), 2.12 - 2.43 (m, 4 H), 2.82 (brs, 1 H), 3.29 (brs, 1 H), 4.20 - 4.34 (m, 3 H), 4.95 (d, $J=4.2$ Hz, 1 H).

$^{13}$C NMR (125 MHz, CDCl$_3$+CCl$_4$): $\delta$ 14.2, 22.0, 23.7, 25.5, 34.1, 62.1, 72.1, 73.2, 128.5, 132.6, 172.8.

**HRMS** (ESI) [M+Na]$^+$ calcld for C$_{11}$H$_{17}$O$_4$ClNa 271.0708, found 271.0706.

(2S,3S)-Ethyl 3-azido-3-(2-chlorocyclohex-1-en-1-yl)-2-hydroxypropanoate (11):

To a cooled (0 °C) solution of diol 7 (2.5 g, 10.08 mmol) in DCM (25 mL) was added Et$_3$N (2.82 mL, 20.16 mmol) followed by thionyl chloride (1.02 mL, 12.09 mmol) in a dropwise manner. The resulting reaction mixture was stirred for two hours at rt and concentrated under reduced pressure to obtain the sulfite as a thick oil. The crude sulfite was dissolved in DMF (30 mL) and to this solution...
was added sodium azide (1.3 g, 20 mmol) and stirred overnight. The reaction mixture was quenched using water (150 mL) and was extracted in ethyl acetate (2 × 20 mL). The combined organics were washed with water, dried over anhydrous Na₂SO₄, filtered and concentrated in vacuo carefully. The crude product was purified on flash chromatography (SiO₂, 2:8 EtOAc: Pet. ether) to afford azidoalcohol 11 as a gummy liquid.

**RF:** 0.5 (Pet. ether-ethyl acetate, 8:2).

**Yield:** 2.05 g, 75%.

**MF:** C₁₁H₁₆ClN₃O₃, **MW:** 273.71.

[α]₂⁵<sub>D</sub> = -40.9 (c 1.32, CHCl₃).

**IR (CHCl₃, cm⁻¹):** νmax 3448 (broad), 2938, 2104, 1736, 1603, 1267.

**¹H NMR (200 MHz, CDCl₃ + CCl₄):** δ 1.34 (t, J=7.1 Hz, 3 H), 1.54 - 1.88 (m, 4 H), 2.19 (brs, 2 H), 2.40 (brs, 2 H), 2.92 (brs, 1 H), 4.19 - 4.36 (m, 3 H), 5.01 (d, J=5.9 Hz, 1 H).

**¹³C NMR (100 MHz, CDCl₃ + CCl₄):** δ 14.1, 22.1, 23.6, 26.2, 34.2, 62.3, 65.1, 71.6, 128.6, 132.6, 172.4.

**HRMS (ESI) [M+Na]⁺ calcd for C₁₁H₁₆O₃ClN₃Na 296.0772, found 296.0770.**

(2S,3S)-Ethyl 3-(((benzyloxy)carbonyl)amino)-3-(2-chlorocyclohex-1-en-1-yl)-2-hydroxy propanoate (12): To a solution of azidoalcohol 11 (2 g, 7.32 mmol) in diethyl ether (20 mL) was added PPh₃ (4.57 g, 18.31 mmol) and stirred until the evolution of nitrogen gas ceased (2 h). After completion of reaction, the solvent was evaporated under reduced pressure to obtain the amine as thick oil. The crude amine was dissolved in DCM (20 mL) and to this solution was added K₂CO₃ (2.51 g, 18.2 mmol) followed by CbzCl (1.49 mL, 8.74 mmol) and stirred overnight (12 h). The reaction mixture was filtered and filtrate was treated with water (30 mL) and was extracted with DCM (2 × 20 mL). The combined organics were washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated in vacuo. The crude product was purified on flash chromatography using silica gel (2:8 EtOAc: Pet. ether) to afford carbamate 12 as a gummy liquid.

**RF:** 0.4 (Pet. ether-ethyl acetate, 7:3).

**Yield:** 2.2 g, 85%.
MF: C$_{19}$H$_{23}$ClNO$_5$, MW: 381.85.

[α]$^D_{25}$ = -17.94 (c 1.59, CHCl$_3$).

IR (CHCl$_3$, cm$^{-1}$): v$_{max}$ 3393 (broad), 2932, 1732, 1690, 1511, 1218.

$^1$H NMR (500 MHz, CDCl$_3$+CCl$_4$): δ 1.30 (t, J=7.2 Hz, 3 H), 1.57 - 1.69 (m, 4 H), 2.10 (brs, 2 H), 2.22 - 2.45 (m, 2 H), 2.96 (brs, 1 H), 4.07 - 4.19 (m, 1 H), 4.20 - 4.31 (m, 1 H), 4.39 (d, J=4.3 Hz, 1 H), 5.05 - 5.14 (m, 2 H), 5.25 - 5.29 (m, 1 H), 5.55 (d, J=8.2 Hz, 1 H), 7.27 - 7.40 (m, 5 H).

$^{13}$C NMR (125 MHz, CDCl$_3$+CCl$_4$): δ 14.0, 22.2, 23.6, 27.0, 34.3, 54.2, 62.3, 67.0, 72.4, 128.2, 128.3 (2C), 128.5 (2C), 129.4, 131.0, 136.4, 155.4, 172.5.

HRMS (ESI) [M+H]$^+$ calcd for C$_{19}$H$_{25}$O$_5$NCl 382.1416, found 382.1408.

(2R,3S)-Ethyl 2-azido-3-(((benzyloxy)carbonyl)amino)-3-(2-chlorocyclohex-1-en-1-yl) propanoate (6): To a stirred solution of carbamate 12 (2 g, 5.27 mmol) in dry DCM (20 mL) was added Et$_3$N (2.21 mL, 15.81 mmol) at 0 °C, followed by dropwise addition of mesyl chloride (0.64 mL, 7.9 mmol) and the reaction mixture was stirred for 4 h under nitrogen atmosphere. The reaction mixture was diluted with DCM (20 mL) and washed with saturated solution of sodium bicarbonate (20 mL) and water (20 mL). The organic layer was separated, dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under reduced pressure to afford O-mesyl compound.

To a solution of crude O-mesyl compound in anhydrous DMF (25 mL) was added sodium azide (0.68 g, 10.44 mmol) and the reaction mixture was stirred at 50 °C for 12 h under nitrogen atmosphere. After completion of the reaction (monitored by TLC), the reaction mixture was cooled to room temperature, diluted with water (75 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were washed with brine, dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO$_2$, 7:93 EtOAc: Pet. ether) to afford azide 6 as a gummy liquid.

$R_f$: 0.5 (Pet. ether-ethyl acetate, 9:1).

Yield: 2.12 g, 82%.

MF: C$_{19}$H$_{23}$ClN$_4$O$_4$, MW: 406.86.
[\alpha]_{D}^{25} = +11.56 (c 1.73, CHCl₃).

IR (CHCl₃, cm⁻¹): vmax 2930, 2114, 1742, 1505, 1216.

¹H NMR (400 MHz, CDCl₃+CCl₄): δ 1.26 (t, J=7.0 Hz, 3 H), 1.60 - 1.73 (m, 4 H), 1.98 - 2.19 (m, 2 H), 2.39 (brs, 2 H), 4.13 - 4.34 (m, 2 H), 4.48 (brs, 1 H), 4.99 - 5.15 (m, 2 H), 5.21 - 5.38 (m, 2 H), 7.27 - 7.44 (m, 5 H).

¹³C NMR (100 MHz, CDCl₃+CCl₄): δ 14.1, 22.1, 23.4, 27.0, 34.0, 53.4, 62.3, 64.8, 67.1, 128.1, 128.2 (2C), 128.5 (2C), 130.0, 130.6, 136.3, 155.2, 167.7.


(4R,5S)-1-Benzyl 3,4-diethyl 5-(2-chlorocyclohex-1-en-1-yl)-2-oximiazolidine-1,3,4-tricarboxylate (5): To a solution of azide 6 (2.1 g, 5.17 mmol) in diethyl ether (20 mL) was added PPh₃ (3.39 g, 12.92 mmol) and stirred until evolution of nitrogen gas ceased (2.5 h). After completion of reaction the solvent was evaporated under reduced pressure to obtain the amine as thick oil. The crude amine was dissolved in DCM (25 mL) and to this solution was added Et₃N (5.74 mL, 40.96 mmol) followed by ethyl chloroformate (3.88 mL, 40.96 mmol) at 0 °C and stirred (12 h). The reaction mixture was quenched with water (30 mL) and was extracted in DCM (2 × 20 mL). The combined organics were washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated in vacuo. The crude product was purified by flash chromatography (SiO₂, 8:2 EtOAc: Pet. ether) to afford urea 5 as a gummy liquid.

Rᶠ: 0.4 (Pet. ether-ethyl acetate, 7:3).

Yield: 2.1 g, 86%.

MF: C₂₃H₂₇ClN₂O₇, MW: 478.92.

[α]_{D}^{25} = -8.12 (c 2.6, CHCl₃).

IR (CHCl₃, cm⁻¹): vmax 2929, 1818, 1750, 1726, 1694, 1437, 1250.

¹H NMR (200 MHz, CDCl₃+CCl₄): δ 1.25 - 1.38 (m, 6 H), 1.53 - 1.71 (m, 4 H), 1.89 (brs, 2 H), 2.18 - 2.45 (m, 2 H), 4.18 - 4.41 (m, 5 H), 5.15 (d, J=12.2 Hz, 1 H), 5.29 (d, J=2.9 Hz, 1 H), 5.38 (d, J=12.2 Hz, 1 H), 7.27 - 7.44 (m, 5 H).
$^{13}$C NMR (100 MHz, CDCl$_3$+CCl$_4$): $\delta$ 14.07, 14.14, 21.6, 23.2, 24.1, 33.9, 55.4, 58.0, 62.4, 63.7, 68.4, 128.3 (2C), 128.47, 128.53 (2C), 129.5, 131.7, 134.9, 147.4, 150.3, 151.0, 168.6.

HRMS (ESI) [M+Na]$^+$ calcd for C$_{23}$H$_{27}$ClN$_2$O$_7$Na 501.1399, found 501.1400.

(4R,5S)-Benzyl 5-(2-chlorocyclohex-1-en-1-yl)-4-(hydroxymethyl)-2-oxoimidazolidine 1-carboxylate (13): To a solution of urea 5 (1.8 g, 3.76 mmol) in methanol (15 mL) was added NaBH$_4$ (0.57 g, 15.04 mmol) at 0 $^\circ$C portionwise. The reaction mixture was allowed to stir for 4 h at room temperature. After completion of reaction, the reaction mixture was concentrated under reduced pressure. The aq. solution of NH$_4$Cl was added to the semisolid mass and allowed to stir for 30 min and the reaction mixture was extracted with ethyl acetate (2 $\times$ 20 mL). The combined organic layer was dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The crude product was purified on flash chromatography (SiO$_2$, 9:1 EtOAc: Pet. ether) to afford alcohol 13 as a white solid.

$R_f$: 0.3 (Ethyl acetate) long tail.

Yield: 0.9 g, 79%.

MF: C$_{18}$H$_{21}$ClN$_2$O$_4$, MW: 364.11.

Melting point: 165-167 $^\circ$C.

$\lbrack \alpha\rbrack_{D}^{25} = -65.54$ (c 2.0, CHCl$_3$).

IR (CHCl$_3$, cm$^{-1}$): v max 3370 (broad), 2931, 2860, 1773, 1389, 1337, 1117.

$^1$H NMR (400 MHz, CDCl$_3$+CCl$_4$): $\delta$ 1.35 - 1.55 (m, 3 H), 1.61 - 2.01 (m, 4 H), 2.13 - 2.37 (m, 3 H), 3.33 (brs, 1 H), 3.54 - 3.65 (m, 1 H), 3.73 - 3.76 (m, 1 H), 4.99 (d, $J$=12.1 Hz, 1 H), 5.19 (d, $J$=3.5 Hz, 1 H), 5.33 (d, $J$=12.1 Hz, 1 H), 7.26 - 7.35 (m, 5 H).

$^{13}$C NMR (100 MHz, CDCl$_3$+CCl$_4$): $\delta$ 21.8, 23.4, 24.4, 33.9, 56.7, 57.9, 64.1, 67.5, 128.3 (3C), 128.4 (2C), 128.8, 131.6, 135.4, 151.1, 156.5.

HRMS (ESI) [M+Na]$^+$ calcd for C$_{18}$H$_{23}$O$_4$N$_2$ClNa 387.1082, found 387.1080.

(4R,5S)-Benzyl 4-(((tert-butyldimethylsilyl)oxy)methyl)-5-(2-chlorocyclohex-1-en-1-yl)-2-oxoimidazolidine-1-carboxylate (14): To a solution of alcohol 13 (800 mg, 2.65 mmol) in anhydrous
DCM (10 mL) was added imidazole (360 mg, 5.3 mmol) followed by addition of TBSCI (600 mg, 3.97 mmol) and DMAP (cat.) at 0 °C under nitrogen atmosphere. The reaction was allowed to stir at room temperature for 24 h. After completion of reaction (monitored by TLC), the reaction mixture was diluted with water (15 mL) and extracted with DCM (3 × 15 mL). The combined organic layer was dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (SiO$_2$, 1:1 EtOAc: Pet. ether) to afford TBS ether 14 as a colorless liquid.

$\text{Rf: } 0.5$ (Pet. ether-ethyl acetate, 1:1).

$\text{Yield: } 1.17 \text{ g, 93\%.}$

$\text{MF: } C_{24}H_{35}ClN_2O_4Si, \text{ MW: } 479.08.$

$[\alpha]^{25}_D = -47.5 (c 4.0, \text{ CHCl}_3).$

$\text{IR (CHCl}_3, \text{ cm}^{-1}): \nu_{\text{max}} 2923, 2857, 1775, 1390, 1333, 1108.$

$^1\text{H NMR (400 MHz, CDCl}_3+\text{CCl}_4): } \delta 0.02 (s, 6 \text{ H}), 0.83 (s, 9 \text{ H}), 1.48 - 1.63 (m, 4 \text{ H}), 1.85 - 2.03 (m, 2 \text{ H}), 2.18 - 2.26 (m, 2 \text{ H}), 3.28 - 3.31 (m, 1 \text{ H}), 3.63 (qd, J=10.4, 4.4 \text{ Hz}, 2 \text{ H}), 5.06 (d, J=12.3 \text{ Hz}, 1 \text{ H}), 5.17 (d, J=2.5 \text{ Hz}, 1 \text{ H}), 5.32 (d, J=12.3 \text{ Hz}, 1 \text{ H}), 6.70 (s, 1 \text{ H}), 7.25 - 7.38 (m, 5 \text{ H}).$

$^{13}\text{C NMR (100 MHz, CDCl}_3+\text{CCl}_4): } \delta -5.5, -5.4, 18.1, 21.7, 23.4, 24.4, 25.7 (3\text{ C}), 33.8, 56.3, 57.8, 65.2, 67.4, 128.1, 128.27 (2\text{ C}), 128.34 (2\text{ C}), 128.8, 131.8, 135.6, 150.9, 155.9.$

$\text{HRMS (ESI)} [\text{M+Na}^+] \text{ calcld for } C_{24}H_{35}O_4N_2ClNaSi 501.1947, \text{ found 501.1947.}$

$(4R,5S)-1,3$-Dibenzyl-4-(((tert-butyldimethylsilyl)oxy)methyl)-5-(2-chlorocyclohex-1-en-1-yl)imidazolidin-2-one (15): To the suspension of 60% NaH (137 mg, 5.72 mmol) (washed with dry petroleum ether 2-3 times) in dry THF (10 mL) was added TBS ether 14 (1.1 g, 2.29 mmol) in anhydrous THF (5 mL) at 0 °C and stirred for 10 min. Then benzyl bromide (0.72 mL, 5.72 mmol) was added drop wise and reaction mixture was stirred for 3 h at room temperature. On completion of the reaction, it was quenched by the addition of saturated ammonium chloride solution, extracted with ethyl acetate (2 × 15 mL) and washed with water followed by brine. The combined organic layers were dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (SiO$_2$, 1:1 EtOAc: Pet. ether) to afford TBS ether 14 as a colorless liquid.
flash chromatography (SiO$_2$, 1:9 EtOAc: Pet. ether) to afford benzyl protected cyclic urea 15 as a white solid.

$R_f$: 0.6 (Pet. ether-ethyl acetate, 8:2).

Yield: 1.14 g, 95%.

MF: C$_{30}$H$_{41}$ClN$_2$O$_2$Si, MW: 525.19.

Melting Point: 87-89 °C.

[$\alpha$]$^D_{25}$ = -33.1 ($c$ 2.9, CHCl$_3$).

IR (CHCl$_3$, cm$^{-1}$): $\nu_{\text{max}}$ 2930, 2857, 1698, 1448, 1252, 1119.

$^1$H NMR (200 MHz, CDCl$_3$+CCl$_4$): $\delta$ -0.04 (s, 3 H), -0.02 (s, 3 H), 0.83 (s, 9 H), 1.08 - 1.65 (m, 6 H), 2.18 - 2.24 (m, 2 H), 3.09 (dt, $J$=6.1, 3.9 Hz, 1 H), 3.40 - 3.67 (m, 2 H), 4.00 (d, $J$=12.3 Hz, 1 H), 4.08 (d, $J$=12.3 Hz, 1 H), 4.52 (d, $J$=15.0 Hz, 1 H), 4.56 - 4.59 (m, 1 H), 4.93 (d, $J$=15.0 Hz, 1 H), 7.24 - 7.32 (m, 10 H).

$^{13}$C NMR (125 MHz, CDCl$_3$+CCl$_4$): $\delta$ -6.5, -5.5, 18.3, 21.7 (2C), 23.6, 25.9 (3C), 34.3, 46.2, 46.9, 56.6, 57.8, 62.6, 127.3, 127.4, 128.3 (2C), 128.4 (2C), 128.5 (2C), 128.8 (2C), 130.2, 131.1, 137.4, 137.4, 160.3.

HRMS (ESI) [M+Na]$^+$ calecd for C$_{30}$H$_{41}$O$_2$ClNaSi 547.2518, found 547.2523.

**Methyl 6-((4R,5R)-1,3-dibenzyl-5-(((tert-butyldimethylsilyl)oxy)methyl)-2-oxoimidazolidin-4-yl)-6-oxohexanoate (4):** To a cooled solution of benzyl protected cyclic urea 15 (1.1 g, 2.10 mmol) and sodium hydrogen carbonate (352 mg, 4.20 mmol) in dichloromethane (20 mL) and methanol (4 mL) at -78 °C, ozone was bubbled. Ozone introduction was stopped when solution turned blue (35 min) and dimethyl sulfide (1 mL, excess) was added at the same temperature. The solution was allowed to warm to room temperature. The solvent was evaporated under reduced pressure, washed with water (15 mL) and extracted with ethyl acetate (2 × 20 mL). The combined organic layer was dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (SiO$_2$, 2:8 EtOAc: Pet. ether) to afford methyl ester 4 as white solid.

$R_f$: 0.5 (Pet. ether-ethyl acetate, 7:3).

Yield: 1.07 g, 92%.

MF: C$_{31}$H$_{44}$N$_2$O$_3$Si, MW: 552.77.
Melting Point: 62-64 °C.

\[[\alpha]_{D}^{25}\] = -12.18 (c 2.0, CHCl₃).

IR (CHCl₃, cm⁻¹): v max 2924, 1733, 1706, 1690, 1463, 1252.

¹H NMR (400 MHz, CDCl₃+CCl₄): δ -0.06 (s, 3 H), -0.05 (s, 3 H), 0.81 (s, 9 H), 1.32 - 1.39 (m, 4 H), 1.98 - 2.08 (m, 1 H), 2.11 - 2.23 (m, 3 H), 3.16 (q, J=4.3 Hz, 1 H), 3.39 - 3.55 (m, 2 H), 3.66 (s, 3 H), 3.70 (d, J=4.3 Hz, 1 H), 4.04 (d, J=14.8 Hz, 2 H), 4.89 (d, J=15.3 Hz, 1 H), 4.82 (d, J=14.8 Hz, 1 H), 7.19 - 7.33 (m, 10 H).

¹³C NMR (100 MHz, CDCl₃+CCl₄): δ -5.6, -5.5, 18.3, 22.5, 24.2, 25.9 (3 C), 33.7, 37.4, 46.3, 47.2, 51.5, 56.4, 62.7, 63.5, 127.69, 127.75, 128.1 (2 C), 128.74 (2 C), 128.77 (2 C), 128.8 (2 C), 136.5, 137.0, 159.6, 173.4, 207.4.

HRMS (ESI) [M+Na⁺] calcd for C₃₁H₄₄O₅N₂NaSi 575.2912, found 575.2915.

Methyl 6-((4R,5R)-1,3-dibenzyl-5-(hydroxymethyl)-2-oximidazolidin-4-yl)-6-oxohexanoate (16): To a solution of ester 4 (1 g, 1.81 mmol) in MeOH (10 mL) was added camphorsulfonic acid (42 mg, 0.18 mmol) at 0 °C. The reaction was allowed to stir at room temperature for 30 h. After completion of reaction (monitored by TLC), the reaction mixture concentrated under reduced pressure. The aq. solution of sodium hydrogen carbonate was added to the semisolid mass and extracted with ethyl acetate (2 × 20 mL). The combined organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (SiO₂, 6:4 EtOAc: Pet. ether) to afford alcohol 16 as a white solid.

R_f: 0.3 (Pet. ether-ethyl acetate, 6:4).

Yield: 713 mg, 90%.

MF: C₂₅H₃₀N₂O₅, MW: 438.51.

Melting Point: 84-86 °C.

\[[\alpha]_{D}^{25}\] = -11.12 (c 2.0, CHCl₃).

IR (CHCl₃, cm⁻¹): v max 3409 (broad), 2928, 1726, 1682, 1451, 1238.

¹H NMR (500 MHz, CDCl₃+CCl₄): δ 1.36 (brs, 4 H), 2.10 - 2.25 (m, 4 H), 2.76 (brs, 1 H), 3.20 (brs, 1 H), 3.49 (d, J=11.6 Hz, 1 H), 3.62 (brs, 1 H), 3.67 (s, 3 H), 3.86 (s, 1 H), 4.12 - 4.15 (m, 2 H), 4.75 - 4.89 (m, 2 H), 7.19 - 7.40 (m, 10 H).
\[ \text{\^{13}C NMR (125 MHz, CDCl}_3+\text{CCl}_4): \delta 22.4, 24.1, 33.6, 37.7, 46.2, 47.4, 51.5, 56.7, 61.5, 63.5, 127.78, 127.8, 128.0 (2 C), 128.5 (2 C), 128.75 (2 C), 128.83 (2 C), 136.2, 136.8, 160.0, 173.5, 207.6.} \]

HRMS (ESI) [M+Na]^+ calcld for \( \text{C}_{25}\text{H}_{30}\text{O}_5\text{N}_2\text{Na} \) 461.2047, found 461.2045.

Methyl 6-((4R,5R)-5-((acetylthio)methyl)-1,3-dibenzyl-2-oximidazolidin-4-yl)-6-oxohexanoate (3): To a stirred solution of alcohol 16 (630 mg, 1.44 mmol) in dry DCM (10 ml) was added Et\( _3 \)N (0.3 mL, 2.16 mmol) at 0 °C, followed by addition of tosyl chloride (328 mg, 1.73 mmol) and DMAP (cat.). The reaction mixture was stirred for 24 h under nitrogen atmosphere. After completion of the reaction (monitored by TLC), the reaction mixture was washed with saturated solution of sodium bicarbonate (10 mL), water (10 mL) and extracted with DCM (2 × 20 mL). The combined organic layer was dried over anhydrous Na\( _2 \)SO\( _4 \), filtered and concentrated under reduced pressure to afford \( O \)-tosyl compound, which was used as such for next transformation.

To a solution of crude \( O \)-tosyl compound in anhydrous DMF (8 mL) and anhydrous THF (12 mL) was added sodium thioacetate (208 mg, 1.83 mmol) and the reaction mixture was stirred at 80 °C for 2 h under nitrogen atmosphere. After completion of the reaction (monitored by TLC), the reaction mixture was cooled to room temperature, diluted with water (50 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were washed with brine, dried over anhydrous Na\( _2 \)SO\( _4 \), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO\( _2 \), 2:8 EtOAc: Pet. ether) to afford thioacetate 3 as a yellow liquid.

\( R_f \): 0.4 (Pet. ether-ethyl acetate, 7:3).

Yield: 502 mg, 83%.

MF: \( \text{C}_{27}\text{H}_{32}\text{N}_2\text{O}_5\text{S} \), MW: 496.61.

\([\alpha]^{25}_D = +40.0 \) (c 1.0, CHCl\( _3 \)).

IR (CHCl\( _3 \), cm\(^{-1} \)): \( \text{vmax} 2923, 2851, 1728, 1710, 1695, 1451, 1215. \)

\( ^1\text{H NMR (500 MHz, CDCl}_3+\text{CCl}_4): \delta 1.31 - 1.42 (m, 4 H), 1.99 - 2.09 (m, 1 H), 2.10 - 2.21 (m, 3 H), 2.24 (s, 3 H), 2.85 (dd, \( J=14.3, 6.3 \) Hz, 1 H), 3.15 (dd, \( J=14.3, 2.7 \) Hz,
Methyl 6-((4R,5R)-1,3-dibenzyl-5-(mercaptomethyl)-2-oxoimidazolidin-4-yl)-6-oxohexanoate (17): A suspension of 3 (300 mg, 2.5 mM) in phosphate buffer (pH 6.8, 50 mL) was purged with a stream of nitrogen for 5 min, lipase (150 mg) from Candida rugosa (706 units/mg) added, and the contents were stirred vigorously. After 2 h, the reaction mixture was extracted with DCM (3 × 20 mL). The organic extracts were washed with brine, dried over anhydrous Na₂SO₄, and then evaporated to give thiol 17. The purification of thiol was effected by flash chromatography (SiO₂, 2:8 EtOAc: Pet. ether) to afford thiol 17 as a yellow liquid.

**Rf:** 0.4 (Pet. ether-ethyl acetate, 7:3).

**Yield:** 219 mg, 80%.

**MF:** C₂₅H₃₀N₂O₄S, MW: 454.58.

**[a]²⁵_D:** + 17.5 (c 2.6, CHCl₃).

**IR (CHCl₃, cm⁻¹):** vmax 3016, 2923, 2851, 1725, 1695, 1451, 1215.

**¹H NMR (200 MHz, CDCl₃+CCl₄):** δ 1.11 (dd, J=9.2, 7.8 Hz, 1 H), 1.27 - 1.44 (m, 4 H), 2.01 - 2.28 (m, 4 H), 2.47 - 2.71 (m, 2 H), 3.21 - 3.38 (m, 1 H), 3.65 (s, 3 H), 3.77 (d, J=4.5 Hz, 1 H), 4.02 (d, J=15.2 Hz, 1 H), 4.08 (d, J=14.8 Hz, 1 H), 4.85 (d, J=14.8 Hz, 1 H), 4.89 (d, J=15.2 Hz, 1 H), 7.19 - 7.36 (m, 10 H).

**¹³C NMR (50 MHz, CDCl₃+CCl₄):** δ 22.3, 24.0, 26.8, 33.5, 37.7, 45.7, 47.3, 51.4, 56.2, 64.5, 127.7 (2 C), 127.9 (2 C), 128.0, 128.4, 128.6 (2 C), 128.7 (2 C), 135.9, 136.3, 159.4, 173.4, 207.4.

(Z)-Methyl 5-((3aS,6aR)-1,3-dibenzyl-2-oxotetrahydro-1H-thieno[3,4-d]imidazol-4(2H)-ylidene)pentanoate (2): Thiol 17 (200 mg, 0.44 mmol) was dissolved in 5 mL toluene and DBU (0.03 mmol) was added drop wise at room temperature under nitrogen atmosphere with continuous stirring. After complete addition, the reaction mixture was heated at 100 °C for 3 h. After the reaction was completed, the toluene was removed under reduced pressure, diluted with ethyl acetate and washed with water (5 mL) and the organic layer was separated and washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure and further it was subjected to elimination.

The crude cyclised hydroxyl compound (200 mg, 0.44 mmol) was dissolved in 5 mL toluene, pTSA (0.025 mmol) was added at room temperature and the reaction mixture was stirred continuously under nitrogen atmosphere. Progress of the reaction was monitored by TLC, which indicated that no unreacted starting material remained after 4 h. Solvent was removed under reduced pressure and ethyl acetate was added. The reaction mixture was neutralized with sodium bicarbonate solution and washed with water (5 mL). The separated organic layer was washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to furnish a residue. The purification of the residue was effected by flash chromatography using silica gel (SiO₂, 2:8 EtOAc: Pet. ether) to afford olefin 2 as a yellow liquid.

*Rf*: 0.4 (Pet. ether-ethyl acetate, 7:3).

**Yield**: 157 mg, 82%.

**MF**: C₂₅H₂₈N₂O₃S, **MW**: 436.56.

\[\alpha\]^2⁵₂^D = + 194 (c 1, CHCl₃).

**IR (CHCl₃, cm⁻¹)**: v max 3032, 2928, 1743, 1634, 1440.

**¹H NMR (500 MHz, CDCl₃+CCl₄)**: δ 1.65 - 1.75 (m, 2 H), 2.03 - 2.11 (m, 2 H), 2.27 (t, J=7.5 Hz, 2 H), 2.93 - 2.97 (m, 2 H), 3.66 (s, 3 H), 4.01 (d, J=15.5 Hz, 1 H), 4.10 (ddd, J=9.0, 7.5, 4.0 Hz, 1 H), 4.20 (d, J=15.5 Hz, 1H), 4.73 (d, J=10.1 Hz, 1 H), 4.80 (d, J=14.5 Hz, 1 H), 4.95 (d, J=15.6 Hz, 1 H), 5.41 (t, J=7.0 Hz, 1 H), 7.29 - 7.37 (m, 10 H).

1.2.7 NMR spectra

**1H NMR spectrum of compound 8 (CDCl₃ + CCl₄, 200 MHz)**

**13C NMR spectrum of compound 8 (CDCl₃ + CCl₄, 100 MHz)**
Chapter 1. Section 2

DEPT spectrum of compound 8 (CDCl$_3$ + CCl$_4$, 100 MHz)

$^1$H NMR spectrum of compound 7 (CDCl$_3$ + CCl$_4$, 500 MHz)
Chapter 1. Section 2

\[ ^{13} \text{C NMR spectrum of compound 7 (CDCl}_3 + \text{CCl}_4, 125 \text{ MHz)} \]

\[
\begin{array}{l}
\text{Chemical Shift (ppm)} \\
172.80 \\
132.64 \\
128.47 \\
96.17 \\
77.00 \\
73.17 \\
72.08 \\
62.13 \\
34.09 \\
25.50 \\
23.67 \\
21.99 \\
14.17 \\
\end{array}
\]

\[ \text{DEPT spectrum of compound 7 (CDCl}_3 + \text{CCl}_4, 125 \text{ MHz)} \]

\[
\begin{array}{l}
\text{Chemical Shift (ppm)} \\
73.16 \\
72.07 \\
62.13 \\
34.08 \\
25.49 \\
23.66 \\
21.99 \\
14.17 \\
\end{array}
\]
**HPLC chromatogram of racemic diol 7**

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<th>C Area</th>
<th>Area %</th>
</tr>
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<td>53.848</td>
</tr>
<tr>
<td>9.342</td>
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</table>

**Totals** 21093300 100.000

Project Leader: Dr. S. P. Chavan
Column: Chiracel OJ-H (250 x 4.6mm)
Mobile Phase: IPA:PET ETHER (7.5:92.5)
Wavelength: 220 nm
Flow Rate: 1 ml/min
Conc.: 2 mg/1.0 ml
Inj vol.: 5 ul.

---

**HPLC chromatogram of chiral diol 7**

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<th>Retention Time</th>
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<tr>
<td>9.625</td>
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</table>

**Totals** 7695001 100.000

Project Leader: Dr. S. P. Chavan
Column: Chiracel OJ-H (250 x 4.6mm)
Mobile Phase: IPA:PET ETHER (7.5:92.5)
Wavelength: 220 nm
Flow Rate: 1 ml/min
Conc.: 2 mg/1.0 ml
Inj vol.: 5 ul.
**Chapter 1. Section 2..............**

**$^1$H NMR spectrum of compound 11 (CDCl$_3$ + CCl$_4$, 200 MHz)**

![H NMR spectrum of compound 11](image)

**$^{13}$C NMR spectrum of compound 11 (CDCl$_3$ + CCl$_4$, 100 MHz)**

![C NMR spectrum of compound 11](image)
Chapter 1. Section 2. 

DEPT spectrum of compound 11 (CDCl₃ + CCl₄, 100 MHz)

1H NMR spectrum of compound 12 (CDCl₃ + CCl₄, 500 MHz)
$^{13}$C NMR spectrum of compound 12 (CDCl$_3$ + CCl$_4$, 125 MHz)

DEPT spectrum of compound 12 (CDCl$_3$ + CCl$_4$, 125 MHz)
1H NMR spectrum of compound 6 (CDCl₃ + CCl₄, 400 MHz)

13C NMR spectrum of compound 6 (CDCl₃ + CCl₄, 100 MHz)
DEPT spectrum of compound 6 (CDCl$_3$ + CCl$_4$, 100 MHz)

$^1$H NMR spectrum of compound 5 (CDCl$_3$ + CCl$_4$, 200 MHz)
Chapter 1. Section 2

$^{13}$C NMR spectrum of compound 5 (CDCl$_3$ + CCl$_4$, 100 MHz)

DEPT spectrum of compound 5 (CDCl$_3$ + CCl$_4$, 100 MHz)
**\(^1\)H NMR spectrum of compound 13 (CDCl\(_3\) + CCl\(_4\), 400 MHz)**

*Diagram showing the \(^1\)H NMR spectrum with chemical shifts marked.*

**\(^13\)C NMR spectrum of compound 13 (CDCl\(_3\) + CCl\(_4\), 100 MHz)**

*Diagram showing the \(^13\)C NMR spectrum with chemical shifts marked.*
DEPT spectrum of compound 13 (CDCl$_3$ + CCl$_4$, 100 MHz)

1H NMR spectrum of compound 14 (CDCl$_3$ + CCl$_4$, 400 MHz)
13C NMR spectrum of compound 14 (CDCl₃ + CCl₄, 100 MHz)

DEPT spectrum of compound 14 (CDCl₃ + CCl₄, 100 MHz)
**Chapter 1. Section 2**

$^1$H NMR spectrum of compound 15 (CDCl$_3$ + CCl$_4$, 200 MHz)

$^13$C NMR spectrum of compound 15 (CDCl$_3$ + CCl$_4$, 125 MHz)
Chapter 1. Section 2

DEPT spectrum of compound 15 (CDCl$_3$ + CCl$_4$, 125 MHz)

$^1$H NMR spectrum of compound 4 (CDCl$_3$ + CCl$_4$, 400 MHz)
$^{13}$C NMR spectrum of compound 4 (CDCl$_3$ + CCl$_4$, 100 MHz)

DEPT spectrum of compound 4 (CDCl$_3$ + CCl$_4$, 100 MHz)
$^1$H NMR spectrum of compound 16 (CDCl$_3$ + CCl$_4$, 500 MHz)

$^{13}$C NMR spectrum of compound 16 (CDCl$_3$ + CCl$_4$, 125 MHz)
Chapter 1. Section 2

DEPT spectrum of compound 16 (CDCl$_3$ + CCl$_4$, 125 MHz)

$^1$H NMR spectrum of compound 3 (CDCl$_3$ + CCl$_4$, 500 MHz)
**13C NMR spectrum of compound 3 (CDCl$_3$ + CCl$_4$, 125 MHz)**

- **Chemical Shift (ppm):**
  - 206.56
  - 194.19
  - 173.41
  - 159.05
  - 136.54
  - 136.20
  - 128.81
  - 128.72
  - 128.32
  - 127.84
  - 96.16
  - 77.25
  - 77.00
  - 76.74
  - 64.83
  - 54.23
  - 51.49
  - 47.04
  - 45.86
  - 37.76
  - 33.61
  - 31.44
  - 30.54
  - 24.10
  - 22.43

**DEPT spectrum of compound 3 (CDCl$_3$ + CCl$_4$, 125 MHz)**

- **Chemical Shift (ppm):**
  - 128.81
  - 128.32
  - 127.82
  - 64.82
  - 54.22
  - 51.50
  - 47.05
  - 45.86
  - 37.77
  - 33.61
  - 31.44
  - 30.55
  - 24.11
  - 22.43
Chapter 1. Section 2

\(^1\)H NMR spectrum of compound 17 (CDCl\(_3\) + CCl\(_4\), 200 MHz)

\[^{13}\)C NMR spectrum of compound 17 (CDCl\(_3\) + CCl\(_4\), 50 MHz)
Chapter 1. Section 2

DEPT spectrum of compound 17 (CDCl₃ + CCl₄, 50 MHz)

H NMR spectrum of compound 2 (CDCl₃ + CCl₄, 200 MHz)
1.2.8 References


10. Enantiomeric excess (% ee) was determined by Chiral HPLC analysis (Chiralcel OJ-H (250×4.6 mm), mobile phase: isopropanol: pet. ether = 7.5: 92.5, wave length = 220 nm, flow rate = 1 ml/min).


Chapter 1. Synthetic Studies towards D-(+)-Biotin

Section 3

Formal Synthesis of Biotin via Epoxidation
1.3.1 Summary

The present section deals with the formal synthesis of biotin. New expedient and short synthesis of biotin has been achieved from the common starting material *viz* (E)-ethyl 3-(2-chlorocyclohex-1-en-1-yl)acrylate derivative.

1.3.2 Introduction

As described in section I of this chapter the synthesis of biotin is a good synthetic challenge in both industry and academia. Biotin is a large-scale commercial product, hence it was decided to shorten the sequence of reaction and also avoid the use of hazardous chemical like sodium azide to introduce amine functionality. In keeping with the interest in the expedient construction of the ureido ring, it was settled to apply epoxidation and its opening with amine for the efficient synthesis of biotin.

![Figure 1. Structure of biotin (1)](image)

Epoxides are versatile and important intermediates in organic chemistry. The strain of three membered heterocyclic ring makes them accessible to a large variety of reagents. The regio-chemistry in epoxide-opening reactions of 2,3-epoxy alcohols depend on the steric and electronic factors in the substrates and on reaction conditions. Nucleophilic substitution under neutral and basic conditions occurs preferentially from the less substituted side in an $S_N2$ manner, where the configuration of the attacked carbon is inverted. Nucleophilic attack under acidic conditions occurs at the more substituted side in an $S_N2$ manner. With sterically unbiased epoxy alcohols or their O-protected derivatives, epoxide opening with nucleophiles occurs preferentially at C-3 (Figure 2).

![Figure 2](image)
1.3.3 Present work

Owing to their ring strain, epoxides are very prone to nucleophilic ring opening reactions with various nucleophiles. Taking account of this, retrosynthesis for intermediate 2 is as evidenced in Scheme 1. Conversion of 2 to biotin (1) was already discussed in previous section of this chapter. The intermediate 2 could be accessed from diamine 3 by urea formation. The diamine could be obtained from hydroxyl compound 4 through S_N2 displacement by nucleophile. The hydroxyl compound 4 in turn could be derived from epoxide 6 through standard synthetic transformations.

![Scheme 1. Retrosynthetic analysis for biotin 1](image)

1.3.4 Results and discussion

According to retrosynthetic analysis, the cabamate 5 was synthesized from epoxy alcohol 6 and which in turn synthesized from (E)-ethyl 3-(2-chlorocyclohex-1-en-1-yl)acrylate (7). The synthesis started from the same unsaturated ester 7 (For preparation refer chapter 1, section 2). Unsaturated ester 7 was reduced by using DIBAL-H to furnish allylic alcohol 8, followed by epoxidation with m-CPBA and NaH_2PO_4 to get epoxy alcohol 6. The crude epoxide 6 was treated by benzyl isocynate and pyridine in DCM to provide epoxy carbamate 5. The subsequent intramolecular ring opening of 5 required extensive experimentation. Ultimately, the use of sodium hexamethyldisilazide in THF provided the desired oxazolidinone 4 in 63% yield (over four steps). The IR spectrum of compound 4 showed strong bands.
at 3370 and 1749 cm\(^{-1}\) indicating the presence of hydroxyl and carbamate functionalities. \(^1\)H NMR spectrum of compound 4 showed set of multiplets appeared at \(\delta 1.52 - 1.82\) and 2.29 - 2.48 integrating for five protons and three protons which were attributed to cyclohexene ring protons while doublets appeared at \(\delta 4.27\) and 4.74 corresponding to methylene protons of -CO-NR-CH\(_2\)-Ph group. \(^{13}\)C NMR spectrum showed the signals that appeared at \(\delta 46.4\) and 159.2 corresponding to carbon of (-CO-NR-CH\(_2\)-Ph and -CO-NR-CH\(_2\)-Ph) carbamate functionality. Additionally DEPT experiment also showed the presence of six CH\(_2\) and seven CH supporting the formation of 4. Finally, peak at \(m/z\) 343.95 (M+Na\(^+\)) in its mass spectrum confirmed the formation of 4 (Scheme 2).

![Scheme 2](image)

**Scheme 2.** Reagents and conditions: a) DIBAL-H, DCM, -20 °C, 2 h; b) m-CPBA, Na\(\text{H}_2\)PO\(_4\), DCM, 0 °C, 45 min; c) BnNCO, Py, DCM, rt, 12 h; d) NaHMDS, THF, 0 °C, 30 min, 63% (over four steps).

The hydroxyl group of 4 was activated as the methanesulfonate ester. Displacement with potassium phthalimide was attempted but it failed. Hence it was displaced by sodium azide as nitrogen containing nucleophile to provide azido oxazolidinone 9 in 80% yield over two steps\(^6\). The IR spectrum of azido oxazolidinone 9 showed strong bands at 2103 and 1755 cm\(^{-1}\) indicating the presence of azide and carbamate functionalities respectively. Peak at \(\delta 5.06\) in its \(^1\)H-NMR spectrum appeared as a doublet accounting for one proton adjacent to azide group. Its \(^{13}\)C-NMR spectrum showed characteristic peak at \(\delta 158.6\) which was assigned to carbonyl carbon of cabamate functionality. In its DEPT spectrum CH and CH\(_2\) carbons appeared at chemical shifts in accordance with the structure of 9. Further, peak at \(m/z\) 347.41 (M+H\(^+\)) in its mass spectrum confirmed the formation of 9.
Azide was reduced under Staudinger reaction condition and amine was protected with (Boc)$_2$O anhydride in THF: H$_2$O system (1:1), gave 3 in 89% yield over two steps. The IR spectrum of 3 displayed a strong absorption bands at 1809 and 1755 cm$^{-1}$ indicating the presence of carbamate functionalities while absence of absorption at 2103 cm$^{-1}$ indicated azide reduction. $^1$H NMR spectrum of compound 3 showed the presence of a new singlet which appeared at $\delta$ 1.47 integrating for nine protons which was attributed to Boc group. $^{13}$C NMR spectrum showed the signals that appeared at $\delta$ 28.4, 80.4 and 155.3 corresponding to carbons of Boc group [NH-CO-O-C(CH$_3$)$_2$, NH-CO-O-C(CH$_3$)$_2$ and NH-CO-O-C(CH$_3$)$_2$ respectively]. Additionally, DEPT experiment also showed the presence of six CH$_2$ and six CH carbons supporting the formation of 3. Further, peak at m/z 443.12 (M+Na)$^+$ in its mass spectrum confirmed the formation of 3.

![Scheme 3](image)

*Scheme 3. Reagents and conditions: a) i) MsCl, Et$_3$N, DCM, 0 °C, 30 min, ii) NaN$_3$, DMF, RT, overnight, 80% (over two steps); b) i) Ph$_3$P, THF, RT, 2 h, ii) (Boc)$_2$O, THF:H$_2$O (1:1), 89% (over two steps) c) NaH, THF, 80 °C, 6 h. 92%.*

On treatment with NaH, 3 was converted into uera 10 with free primary hydroxyl in 92 % yield. The IR spectrum of compound 10 showed the absence of bands at 1809 and 1755 cm$^{-1}$ for acyclic tert-butyl carbamate and cyclic carbamate indicating the hydrolysis during reaction, also showed broad band at 3337 cm$^{-1}$ indicating the presence of hydroxyl group. The $^1$H NMR spectrum of compound 10 showed the absence of singlet at $\delta$ 1.47 integrating for nine protons attributed to Boc group indicating the hydrolysis during reaction. $^{13}$C NMR and DEPT NMR spectra
were also in good agreement with the structure of compound 10. Further, peak at m/z 343.04 (M+Na)^+ in its mass spectrum confirmed the formation of 10 (Scheme 3).

The primary hydroxyl group of 10 was protected as silyl ether by using TBSCl, imidazole and DMAP in DCM gave 11 in 93% yield. The IR spectrum of compound 11 showed the absence of broad band at 3337 cm\(^{-1}\) for hydroxyl group indicating the protection of hydroxyl group. Its \(^1\)H-NMR spectrum showed peaks as singlets at \(\delta\) 0.00 and 0.84 for six and nine protons respectively clearly showing the presence of TBS group. Further, peak at m/z 436.21 (M+H)^+ in its mass spectrum confirmed the formation of 10 (Scheme 4).

\[
\text{Scheme 4. Reagents and conditions: a) TBSCl, imidazole, DMAP, DCM, 24 h, 93%; b) NaH, BnBr, THF, 0°C, 3 h, 95%}.\]

On treatment with sodium hydride, benzyl bromide 11 was converted to dibenzyl protected imidazolidone 2 in 95% yield. The IR spectrum of 2 displayed the strong absorption band at 1698 cm\(^{-1}\) indicating the presence of cyclic urea functionality. The \(^1\)H NMR spectrum showed four upfield signals at \(\delta\) 4.00, 4.08, 4.52 and 4.93 for four protons and multiplet at \(\delta\) 7.24-7.32 for ten protons indicating that the presence of two benzyl groups. This was further confirmed by its \(^{13}\)C NMR and DEPT NMR spectra, which showed a -CH\(_2\)- carbon singlets at \(\delta\) 46.2 and 46.9 for the benzylic carbon. Finally, the structure of compound 2 was confirmed by HRMS analysis (Scheme 4). Fallowing this route, it was able to access biotin in 22 steps and in 7.6% overall yield but this was not an azide free synthesis.

As Biotin has promising biological activity, was an attempt made to reduce number of steps and avoid use of sodium azide to make it a practical method. Keeping these things in mind, synthesis was modified. As depicted in Scheme 5, the synthesis began with the same starting material unsaturated ester 7. Reduction of ester and epoxidation was done by the same procedure as described in Scheme 2. The primary hydroxyl group of epoxy alcohol 6 was protected as its silyl ether by using TBSCl,
imidazole and DMAP in DCM to furnish 12 in 93% yield. The IR spectrum of compound 12 showed the presence of band at 1255 cm\(^{-1}\) for C-O-C ether group. Its \(^1\)H-NMR spectrum showed peaks as singlets at \(\delta\) 0.08, 0.09 and 0.91 for three, three and nine protons respectively clearly showing the presence of TBS group. Peaks at \(\delta\) - 5.3, -5.2 and 26.0 in its \(^{13}\)C-NMR spectrum corresponded to TBS group. Further, peak at \(m/z\) 303.77 (M+H)+ in its mass spectrum confirmed the formation of 12 (Scheme 4).

![Image of the scheme showing reagents and conditions](image)

**Scheme 5: Reagents and conditions:** a) TBSCl, imidazole, DMAP, DCM, 24 h, 93%; b) BnNH\(_2\), ZrCl\(_4\), 30 min, RT, 85%; c) BnNCO, DCM, RT, 12 h, 95%; d) KH, TsCl, HMPA, THF, 0°C, 1 h, 81%.

Epoxide 12 on ring opening aminolysis by using zirconium chloride with benzyl amine, afforded aminol 13 in 50% yield.\(^7\) The IR spectrum of 13 showed broad band at 3341 cm\(^{-1}\) indicating the presence of hydroxyl group. \(^1\)H NMR spectrum of compound 13 showed the presence of a new multiplet which appeared at \(\delta\) 7.12 - 7.38 integrating for five protons which was attributed to benzyl protons. \(^{13}\)C NMR spectrum showed the signals that appeared at \(\delta\) 46.9, 127.3, 128.3 and 128.8 corresponding to carbons of Bn group (N-CH\(_2\)-Ph). Additionally, DEPT experiment also showed the presence of six CH\(_2\) and eight CH carbons supporting the formation of 13. Further, peak at \(m/z\) 411.09 (M+H)+ in its mass spectrum confirmed the formation of 13.

Aminol 13 on treatment with benzyl isocyanate gave acyclic urea 14 in 95% yield. The IR spectrum of compound 14 showed strong bands at 3337 and 1686 cm\(^{-1}\) indicating the presence of hydroxyl and urea functionalities. \(^1\)H NMR spectrum of
compound **14** showed the presence of a two multiplets which appeared at $\delta$ 7.13 - 7.24 and 7.25 - 7.37 integrating for total ten protons which was attributed for two Bn protons. $^{13}$C NMR spectrum showed the signals that appeared at $\delta$ 45.0 and 50.8 corresponding to carbons of Bn group (N-CH$_2$-Ph). Additionally, DEPT experiment also support the formation of **14**. Further, peak at $m/z$ 565.11 (M+ Na)$^+$ in its mass spectrum confirmed the formation of **14**.

Selective cyclization to the imidazolidone **2** was accomplished under carefully controlled conditions by combining 5 equivalent of potassium hydride and 1.2 equivalent of $p$-toluenesulfonyl chloride in the presence of 30 equivalent of hexamethylphosphoramide in THF which resulted in imidazolidone **2** in 40% yield (Scheme 5).

The imidazolidone **2** thus obtained was identical with an authentic sample, with respect to IR, NMR and mass spectra prepared by a different route. Since the conversion of imidazolidone **2** to biotin (**1**) has been reported in previous section, this constitutes a formal synthesis of biotin.

### 3.3.5 Conclusion

The crucial intermediate for biotin has been accessed from two different strategies to reduce number of steps and avoid use of hazardous sodium azide.
3.2.6 Experimental

3-Benzyl-4-(2-chlorocyclohex-1-en-1-yl)(hydroxy)methyl)oxazolidin-2-one (4):

To a stirred solution of α,β-unsaturated ester 7 (3.0 gm, 14.01 mmol) in dry CH$_2$Cl$_2$ (30 mL) was added DIBAL-H (28.03 mL, 28.03 mmol, 1 M solution in toluene) at -20 °C slowly over period of 15 min and stirred for another 2 h. TLC showed complete conversion of ester to allylic alcohol 8. Reaction was quenched by careful addition of pre-cooled MeOH (3 mL) and allowed to warm to 0 °C. Roche’s salt (saturated solution of sodium potassium tartrate, 30 mL) was added and stirred for 0.5 h after which organic layer was separated and aqueous layer was washed with CH$_2$Cl$_2$ (3 × 20 mL). Combined organic layers were washed with brine, dried over anhydrous Na$_2$SO$_4$, filtered and concentrated in vacuo and used as such for next reaction.

To a cold (0 °C), magnetically stirred solution of allylic alcohol 8 (2.3 g, 12.36 mmol) in distilled DCM (20 mL), NaH$_2$PO$_4$ (2.2 g, 18.54 mmol) was added followed by 60% m-CPBA (4.25 g, 14.83 mmol) added portion wise and stirred for 45 min at same temperature. The reaction was quenched with solid NaHCO$_3$ and stirred for further 15 min. The reaction mixture was extracted with DCM (3 × 10 mL) and the combined organic layer was washed with brine (10 mL) and dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The crude reaction mixture was used as such in the next reaction without further purification.

To a solution of epoxy alcohol 6 (2.0 g, 10.63 mmol) in anhydrous DCM (20 mL) was added pyridine (1.65 mL, 21.26 mmol) followed by addition of BnNCO (1.69 mL, 12.76 mmol) at room temperature under nitrogen atmosphere. The reaction was allowed to stir at same temperature for 12 h. After completion of reaction (monitored by TLC), the reaction mixture was diluted with water (15 mL) and extracted with DCM (3 × 15 mL). The combined organic layer was dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under reduced pressure and used as such in the next reaction without further purification.

To a cold (0 °C), magnetically stirred solution of epoxy carbamate 5 (1.00 g, 3.11 mmol) in dry THF (10 mL), NaHMDS (1.95 mL, 3.73 mmol) was added dropwise and stirred for 30 min. The reaction was quenched by addition of aq. NH$_4$Cl, the
aqueous phase was extracted with ethyl acetate (3 × 5 mL). The combined organic layer was washed with brine (20 mL) and dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure and the crude residue was purified using flash chromatography (SiO₂, 3:7 EtOAc: Pet. ether) to furnish 4 as a white solid.

**Rₛ:** 0.4 (Pet. ether-ethyl acetate, 7:3).

**Yield:** 0.88 g, 63% (over four steps).

**MF:** C₁₇H₂₀ClNO₃, **MW:** 321.80.

**MP:** 110-112 ºC.

**IR (CHCl₃, cm⁻¹):** v max 3370 (broad), 2928, 1749, 1416.

**¹H NMR (400 MHz, CDCl₃+CCl₄):** δ 1.52 - 1.82 (m, 5 H), 2.29 - 2.48 (m, 3 H), 3.30 (brs, 1 H), 3.83 (ddd, J=8.8, 6.2, 2.7 Hz, 1 H), 4.18 (t, J=8.8 Hz, 1 H), 4.27 (d, J=15.1 Hz, 1 H), 4.44 (dd, J=8.8, 6.2 Hz, 1 H), 4.74 (d, J=15.1 Hz, 1 H), 5.01 (s, 1 H), 7.26 - 7.40 (m, 5 H).

**¹³C NMR (125 MHz, CDCl₃+CCl₄):** δ 22.0, 23.6, 25.8, 34.0, 46.4, 58.0, 62.8, 67.4, 128.0, 128.3 (2 C), 128.9 (2 C), 129.0, 131.8, 136.3, 159.2.

**MS (ESI):** m/z = 343.95 (M + Na)⁺.

**4-Azido(2-chlorocyclohex-1-en-1-yl)methyl)-3-benzyloxazolidin-2-one (9):** To a stirred solution of carbamate 4 (0.7 g, 2.18 mmol) in dry DCM (10 mL) was added Et₃N (0.9 mL, 6.54 mmol) at 0 ºC, followed by dropwise addition of mesyl chloride (0.35 mL, 4.36 mmol) and the reaction mixture was stirred for 30 min under nitrogen atmosphere. The reaction mixture was diluted with DCM (10 mL) and washed with saturated solution of sodium bicarbonate (20 mL) and water (20 mL). The organic layer was separated, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to afford O-mesyl compound.

To a solution of crude O-mesyl compound in anhydrous DMF (10 mL) was added sodium azide (0.71 g, 10.9 mmol) and the reaction mixture was stirred at room temperature for 12 h under nitrogen atmosphere. After completion of the reaction (monitored by TLC), the reaction mixture was diluted with water (75 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced
The residue was purified by flash chromatography (SiO$_2$, 1:9 EtOAc: Pet. ether) to afford azide 6 as a gummy liquid.

$R_f$: 0.5 (Pet. ether-ethyl acetate, 8:2).

**Yield:** 0.6 g, 80%.

**MF:** $C_{17}H_{19}ClN_4O_2$, **MW:** 346.12.

**IR (CHCl$_3$, cm$^{-1}$):** $\nu_{max}$ 3032, 2859, 2103, 1755, 1496.

**$^1$H NMR (400 MHz, CDCl$_3$):** $\delta$ 1.60 - 1.70 (m, 5 H), 2.20 - 2.55 (m, 3 H), 3.58 - 3.65 (m, 1 H), 3.90 - 3.97 (m, 1 H), 4.07 - 4.15 (m, 1 H), 4.49 (d, $J = 15.1$ Hz, 1 H), 4.89 (d, $J = 15.1$ Hz, 1 H), 5.06 (d, $J = 9.3$ Hz, 1 H), 7.32 - 7.38 (m, 5 H).

**$^{13}$C NMR (100 MHz, CDCl$_3$):** $\delta$ 21.8, 23.4, 25.5, 34.0, 47.7, 54.6, 64.1, 66.5, 126.8, 127.9, 128.4 (2 C), 128.8 (2 C), 135.3, 136.3, 158.6.

**MS (ESI):** $m/z = 347.41$ (M+H)$^+$.  

**tert-Butyl-3-benzyl-2-oxooxazolidin-4-yl)(2-chlorocyclohex-1-en-1-yl)methyl carbamate (3):** To a solution of azide 9 (0.6 g, 1.73 mmol) in diethyl ether (20 mL) was added PPh$_3$ (1.14 g, 4.33 mmol) and stirred until the evolution of nitrogen gas ceased (2 h). After completion of reaction, the solvent was evaporated under reduced pressure to obtain the amine as thick oil. The crude amine was dissolved in THF:H$_2$O (1:1, 20 mL) and to this solution was added (Boc)$_2$O (0.56 mL, 2.57 mmol) and stirred overnight (12 h). The reaction mixture was extracted with EtOAc (2 × 20 mL). The combined organics were washed with brine, dried over anhydrous Na$_2$SO$_4$, filtered and concentrated in vacuo. The crude product was purified on flash chromatography using silica gel (3:7 EtOAc: Pet. ether 2:8 EtOAc: Pet. ether) to afford carbamate 9 as a solid.

$R_f$: 0.5 (Pet. ether-ethyl acetate, 1:1).

**Yield:** 0.64 g, 89%.

**MF:** $C_{22}H_{29}ClN_2O_4$, **MW:** 420.93.

**MP:** 102-104 ºC

**IR (CHCl$_3$, cm$^{-1}$):** $\nu_{max}$ 3421, 2983,1809, 1755, 1478.

**$^1$H NMR (500 MHz, CDCl$_3$+CCL$_4$):** $\delta$ 1.47 (s, 9 H), 1.57 - 1.83 (m, 4 H), 2.05 - 2.13 (m, 2 H), 2.42 (brs, 2 H), 3.82 - 3.88 (m, 1 H), 4.00 - 4.26 (m, 3 H), 4.82 (brs, 1 H),
4.99 (d, J = 14.9 Hz, 1 H), 5.05 - 5.23 (m, 1 H), 7.17 - 7.26 (m, 2 H), 7.28 - 7.40 (m, 3 H).

$^{13}$C NMR (125 MHz, CDCl$_3$+CCl$_4$): δ 22.2, 23.3, 27.8, 28.4 (3 C), 34.2, 47.9, 54.9, 55.0, 65.4, 80.4, 128.2 (3 C), 128.9, 129.0 (2 C), 135.7, 155.3, 159.4.

MS (ESI): m/z = 443.12 (M+Na$^+$).

1-Benzyl-4-(2-chlorocyclohex-1-en-1-yl)-5-(hydroxymethyl)imidazolidin-2-one (10): To a suspension of NaH (0.1 g, 4.28 mmol) in dry DMF (10 mL) at 0 °C was added a solution of 3 (0.6 g, 1.42 mmol) in DMF (2 mL) and refluxed for 6 h. The reaction mixture was cooled and quenched by addition of water (30 mL) and was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine, dried over anhydrous Na$_2$SO$_4$, filtered and solvent was removed in vacuo. The crude compound was purified by flash column chromatography (SiO$_2$, 7:3 EtOAc: Pet. ether) to afford 10 as solid.

Yield: 0.42 g, 92%.

MF: C$_{17}$H$_{21}$ClN$_2$O$_2$, MW: 320.82.

MP: 86-88 ºC.

IR (CHCl$_3$, cm$^{-1}$): v$_{max}$ 3337 (broad), 2925, 2824, 1676, 1537.

$^1$H NMR (400 MHz, CDCl$_3$): δ 1.46 - 1.71 (m, 5 H), 1.94 (brs, 1 H), 2.11 - 2.22 (m, 1 H), 2.28 - 2.42 (m, 2 H), 3.18 - 3.34 (m, 1 H), 3.60 (d, J=11.0 Hz, 1 H), 3.77 (d, J=10.0 Hz, 1 H), 4.26 (d, J=15.2 Hz, 1 H), 4.59 (brs, 1 H), 4.71 (d, J=15.2 Hz, 1 H), 4.97 (d, J=6.6 Hz, 1 H), 7.29 - 7.40 (m, 5 H).

$^{13}$C NMR (100 MHz, CDCl$_3$): δ 21.9, 23.6, 25.0, 34.2, 45.8, 52.3, 60.9, 61.3, 127.8, 128.2 (2 C), 128.8 (2 C), 130.1, 131.5, 137.2, 161.7.

MS (ESI): m/z = 343.04 (M+Na$^+$).

1-Benzyl-5-(((tert-butyldimethylsilyl)oxy)methyl)-4-(2-chlorocyclohex-1-en-1-yl)imidazolidin-2-one (11): To a solution of alcohol 10 (0.4 g, 1.25 mmol) in anhydrous DCM (10 mL) was added imidazole (0.26 g, 3.75 mmol) followed by addition of TBSCl (0.38 g, 2.50 mmol) and DMAP (cat.) at 0 °C under nitrogen atmosphere. The reaction was allowed to stir at room
temperature for 24 h. After completion of reaction (monitored by TLC), the reaction mixture was diluted with water (15 mL) and extracted with DCM (3 × 15 mL). The combined organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (SiO₂, 3:7 EtOAc: Pet. ether) to afford TBS ether 11 as a colorless liquid.

**Rf**: 0.5 (Pet. ether-ethyl acetate, 3:2).

**Yield**: 0.5 g, 93%.

**MF**: C₂₃H₃₅ClN₂O₂Si, **MW**: 435.08.

**IR (CHCl₃, cm⁻¹)**: \( \nu_{\text{max}} \) 2925, 2824, 1674, 1514, 1438.

**¹H NMR (200 MHz, CDCl₃+CCl₄)**: δ 0.00 (s, 6 H), 0.84 (s, 10 H), 1.43 - 1.72 (m, 5 H), 1.96 - 2.34 (m, 3 H), 3.09 - 3.27 (m, 1 H), 3.46 - 3.73 (m, 2 H), 4.00 (d, J=15.0 Hz, 1 H), 4.42 (s, 1 H), 4.72 (dd, J=5.7, 1.9 Hz, 1 H), 4.82 (d, J=15.0 Hz, 1 H), 7.09 - 7.37 (m, 5 H).

**MS (ESI)**: m/z = 436.21 (M+H)⁺.

1,3-Dibenzyl-4-(((tert-butyldimethylsilyl)oxy)methyl)-5-(2-chlorocyclohex-1-en-1-yl)imidazolidin-2-one (2): To the suspension of 60% NaH (68 mg, 1.74 mmol) (washed with dry petroleum ether 2-3 times) in dry THF (10 mL) was added TBS ether 11 (0.5 g, 1.16 mmol) in anhydrous THF (5 mL) at 0 °C and stirred for 10 min. Then benzyl bromide (0.17 mL, 1.39 mmol) was added drop wise and reaction mixture was stirred for 3 h at room temperature. On completion of the reaction, it was quenched by the addition of saturated ammonium chloride solution, extracted with ethyl acetate (2 × 10 mL) and washed with water followed by brine. The combined organic layers were dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (SiO₂, 1:9 EtOAc: Pet. ether) to afford benzyl protected cyclic urea 2 as a white solid.

**Rf**: 0.6 (Pet. ether-ethyl acetate, 8:2).

**Yield**: 0.57 g, 95%.

**MF**: C₃₀H₄₄ClN₂O₂Si, **MW**: 525.19.

**Melting Point**: 87-89 °C.

**IR (CHCl₃, cm⁻¹)**: \( \nu_{\text{max}} \) 2930, 2857, 1698, 1448, 1252, 1119.
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\[^1\text{H NMR (200 MHz, CDCl}_3+\text{CCl}_4):\] \(\delta\) -0.04 (s, 3 H), -0.02 (s, 3 H), 0.83 (s, 9 H), 1.08 - 1.65 (m, 6 H), 2.18 - 2.24 (m, 2 H), 3.09 (dt, \(J=6.1, 3.9\) Hz, 1 H), 3.40 - 3.67 (m, 2 H), 4.00 (d, \(J=12.3\) Hz, 1 H), 4.08 (d, \(J=12.3\) Hz, 1 H), 4.52 (d, \(J=15.0\) Hz, 1 H), 4.56 - 4.59 (m, 1 H), 4.93 (d, \(J=15.0\) Hz, 1 H), 7.24 - 7.32 (m, 10 H).

\[^{13}\text{C NMR (125 MHz, CDCl}_3+\text{CCl}_4):\] \(\delta\) -5.6, -5.5, 18.3, 21.7 (2C), 23.6, 25.9 (3C), 34.3, 46.2, 46.9, 56.6, 57.8, 62.6, 127.3, 127.4, 128.3 (2C), 128.4 (2C), 128.5 (2C), 128.8 (2C), 130.2, 131.1, 137.4, 137.4, 160.3.

**HRMS (ESI) [M+Na]^+** calcd for \(\text{C}_{30}\text{H}_{41}\text{O}_2\text{N}_2\text{ClNaSi}\) 547.2518, found 547.2523.

**tert-Butyl-3-(2-chlorocyclohex-1-en-1-yl)oxiran-2-yl)methoxy)dimethylsilane**

![Chemical structure](image)

(12): To a solution of epoxy alcohol 6 (800 mg, 4.25 mmol) in anhydrous DCM (10 mL) was added imidazole (434 mg, 6.38 mmol) followed by addition of TBSCI (770 mg, 5.10 mmol) and DMAP (cat.) at 0 °C under nitrogen atmosphere. The reaction was allowed to stir at room temperature for 24 h. After completion of reaction (monitored by TLC), the reaction mixture was diluted with water (15 mL) and extracted with DCM (3 × 15 mL). The combined organic layer was dried over anhydrous \(\text{Na}_2\text{SO}_4\), filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (SiO\(_2\), 0.4:96 EtOAc: Pet. ether) to afford TBS ether 12 as a colorless liquid.

**RF**: 0.5 (Pet. ether-ethyl acetate, 19:1).

**Yield**: 1.22 g, 93%.

**MF**: \(\text{C}_{15}\text{H}_{27}\text{ClO}_2\text{Si}\), **MW**: 302.91.

**IR (CHCl\(_3\), cm\(^{-1}\))**: \(\nu_{\text{max}}\) 2932, 2883, 1531, 1454, 1255.

\[^1\text{H NMR (200 MHz, CDCl}_3+\text{CCl}_4):\] \(\delta\) 0.08 (s, 3 H), 0.09 (s, 3 H) 0.91 (s, 9 H), 1.57 - 1.85 (m, 5 H), 1.94 - 2.16 (m, 1 H), 2.29 - 2.45 (m, 2 H), 3.03 - 3.10 (m, 1 H), 3.59 - 3.75 (m, 1 H), 3.81 - 4.04 (m, 2 H).

\[^{13}\text{C NMR (100 MHz, CDCl}_3+\text{CCl}_4):\] \(\delta\) -5.3, -5.2, 18.4, 21.7, 23.7, 24.2, 26.0 (3 C), 34.2, 54.4, 57.1, 63.4, 77.0, 128.8, 131.9.

**MS (ESI):** \(m/z = 303.77 (\text{M+H})^+\).

1-(Benzylamino)-3-((tert-butyl dimethylsilyl)oxy)-1-(2-chlorocyclohex-1-en-1-yl)propan-2-ol (13): \(\text{ZrCl}_4\) (42 mg, 5 mol%) was added to a magnetically stirred mixture of \(12\) (1.1 g, 3.64 mmol) and
benzyl amine (0.48 mL, 4.37 mmol) at room temperature under nitrogen. After completion of the reaction (30 min, TLC), the reaction mixture was diluted with Et₂O (15 mL) and the precipitated catalyst was separated by decantation of the supernatant ethereal solution. The catalyst was washed with Et₂O (10 mL) and the combined ethereal solutions were dried (Na₂SO₄) and concentrated in vacuo and the crude residue was directly adsorbed on simple silica gel and was purified using flash chromatography (SiO₂, 1:19 EtOAc: Pet. ether) to furnish the aminol 13 as a colorless liquid.

R<sub>f</sub>: 0.6 (Pet. ether-ethyl acetate, 9:1).

Yield: 1.26 g, 85%.

MF: C<sub>22</sub>H<sub>36</sub>ClNO<sub>2</sub>Si, MW: 410.07.

IR (CHCl<sub>3</sub>, cm<sup>-1</sup>): νmax 3341 (broad), 2930, 2857, 1683, 1531, 1454.

<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>+CCl<sub>4</sub>): δ 0.00 (m, 6 H), 0.81 (m, 9 H), 1.44 - 1.77 (m, 4 H), 2.25 - 2.94 (m, 4 H), 3.48 - 3.82 (m, 5 H), 4.08 (d, J=6.1 Hz, 1 H), 7.12 - 7.38 (m, 5 H).

<sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>+CCl<sub>4</sub>): δ -5.6, -5.5, 18.3, 21.7, 23.6, 25.9 (3 C), 34.3, 46.2, 46.9, 56.6, 57.8, 62.6, 127.3, 128.3 (2 C), 128.8 (2 C), 130.2, 131.1, 137.4.

MS (ESI): m/z = 411.09 (M+H)<sup>+</sup>.

1,3-Dibenzyl-1-3-((tert-butyldimethylsilyl)oxy)-1-(2-chlorocyclohex-1-en-1-yl)-2-hydroxypropylurea (14): To a solution of aminol 13 (1.2 g, 2.93 mmol) in anhydrous DCM (10 mL) was BnNCO (0.47 mL, 3.52 mmol) at room temperature under nitrogen atmosphere. The reaction was allowed to stir at room temperature for 12 h. After completion of reaction (monitored by TLC), the reaction mixture was diluted with water (15 mL) and extracted with DCM (3 × 10 mL). The combined organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure and was purified using flash chromatography (SiO₂, 1:9 EtOAc: Pet. ether) to furnish the urea 4 as a colorless liquid.

R<sub>f</sub>: 0.5 (Pet. ether-ethyl acetate, 4:1).

Yield: 1.51 g, 95%.

MF: C<sub>30</sub>H<sub>43</sub>ClN<sub>2</sub>O<sub>3</sub>Si, MW: 542.21.

IR (CHCl<sub>3</sub>, cm<sup>-1</sup>): νmax 3337, 2925, 2854, 1686, 1537, 1496.
1H NMR (400 MHz, CDCl3): δ 0.03 (s, 3 H), 0.06 (s, 3 H), 0.87 (s, 9 H), 1.55 - 1.78 (m, 4 H), 2.18 - 2.62 (m, 4 H), 3.56 - 3.74 (m, 2 H), 3.94 (brs, 1 H), 4.24 (d, J=4.6 Hz, 1 H), 4.29 - 4.39 (m, 1 H), 4.44 - 4.54 (m, 3 H), 4.96 (d, J=4.6 Hz, 1 H), 5.10 (brs, 1 H), 7.17 (m, 2 H), 7.25 - 7.37 (m, 8 H).

13C NMR (100 MHz, CDCl3): δ -5.42, -5.39, 18.2, 22.3, 22.7, 23.6, 25.9 (3 C), 27.8, 34.5, 45.0, 50.8, 61.9, 63.8, 72.6, 126.4 (2 C), 127.1, 127.3 (2 C), 127.3, 128.5 (2 C), 128.8 (2 C), 131.3, 132.1, 139.3, 158.9.

MS (ESI): m/z = 565.11 (M+Na)

1,3-Dibenzyl-4-(((tert-butyldimethylsilyl)oxy)methyl)-5-(2-chlorocyclohex-1-en-1-yl)imidazolidin-2-one (2): Under an argon atmosphere potassium hydride (35%, 210 mg, 1.84 mmol) was washed with n-hexane (10 mL × 2), and to it was added hexamethyldisilazane (1.8 mL, 11.1 mmol) and solutions of 14 (200 mg, 0.37 mmol) in THF (10 mL) and p-TsCl (84 mg, 0.44 mmol) in THF (5 mL) successively at 0 °C. After being stirred at room temperature for 2 h, the reaction mixture was quenched by sat. NH4Cl. The entire mixture was extracted with ethyl acetate, and the combined extracts were dried (Na2SO4) and concentrated to leave an oil which was purified by flash chromatography (SiO2, 1:9 EtOAc: Pet. ether) to afford benzyl protected cyclic urea 2 as a white solid.

Rf: 0.6 (Pet. ether-ethyl acetate, 8:2).

Yield: 157 mg, 81%.

MF: C30H41ClN2O2Si, MW: 525.19.

Melting Point: 87-89 °C.

IR (CHCl3, cm⁻¹): v max 2930, 2857, 1698, 1448, 1252, 1119.

1H NMR (200 MHz, CDCl3+CCl4): δ -0.04 (s, 3 H), -0.02 (s, 3 H), 0.83 (s, 9 H), 1.08 - 1.65 (m, 6 H), 2.18 - 2.24 (m, 2 H), 3.09 (dt, J=6.1, 3.9 Hz, 1 H), 3.40 - 3.67 (m, 2 H), 4.00 (d, J=12.3 Hz, 1 H), 4.08 (d, J=12.3 Hz, 1 H), 4.52 (d, J=15.0 Hz, 1 H), 4.56 - 4.59 (m, 1 H), 4.93 (d, J=15.0 Hz, 1 H), 7.24 - 7.32 (m, 10 H).

13C NMR (125 MHz, CDCl3+CCl4): δ -5.6, -5.5, 18.3, 21.7 (2C), 23.6, 25.9 (3C), 34.3, 46.2, 46.9, 56.6, 57.8, 62.6, 127.3, 127.4, 128.3 (2C), 128.4 (2C), 128.5 (2C), 128.8 (2C), 130.2, 131.1, 137.4, 137.4, 160.3.

HRMS (ESI) [M+Na]⁺ calcd for C30H41O2N2ClNaSi 547.2518, found 547.2523.
3.3.7 Spectra

**$^1$H NMR spectrum of compound 4 (CDCl$_3$, 400 MHz)**

**$^{13}$C NMR spectrum of compound 4 (CDCl$_3$+CCl$_4$, 125 MHz)**
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DEPT spectrum of compound 4 (CDCl₃+CCl₄, 125 MHz)

1H NMR spectrum of compound 9 (CDCl₃, 400 MHz)
$^{13}$C NMR spectrum of compound 9 (CDCl$_3$, 100 MHz)

[Chemical shifts and peaks are shown for $^{13}$C NMR spectrum]

DEPT spectrum of compound 9 (CDCl$_3$, 100 MHz)

[Chemical shifts and peaks are shown for DEPT spectrum]
\( ^1 \text{H NMR spectrum of compound 3 (CDCl}_3+\text{CCl}_4, 500 \text{ MHz) } \)

\begin{center}
\includegraphics[width=\textwidth]{1H_NMR.png}
\end{center}

\( ^{13} \text{C NMR spectrum of compound 3 (CDCl}_3+\text{CCl}_4, 125 \text{ MHz) } \)

\begin{center}
\includegraphics[width=\textwidth]{13C_NMR.png}
\end{center}
DEPT spectrum of compound 3 (CDCl₃+CCl₄, 125 MHz)

1H NMR spectrum of compound 10 (CDCl₃, 400 MHz)
$^{13}$C NMR spectrum of compound 10 (CDCl$_3$, 100 MHz)

DEPT spectrum of compound 10 (CDCl$_3$, 100 MHz)
\(^1\text{H NMR spectrum of compound 11 (CDCl}_3+\text{CCl}_4, \text{200 MHz)}\)

\(^1\text{H NMR spectrum of compound 12 (CDCl}_3, \text{200 MHz)}\)
$^{13}$C NMR spectrum of compound 12 (CDCl$_3$+CCl$_4$, 100 MHz)

DEPT spectrum of compound 12 (CDCl$_3$+CCl$_4$, 100 MHz)
$^1$H NMR spectrum of compound 13 (CDCl$_3$, 200 MHz)

$^{13}$C NMR spectrum of compound 13 (CDCl$_3$+CCl$_4$, 125 MHz)
DEPT spectrum of compound 13 (CDCl₃+CCl₄, 125 MHz)

1 H NMR spectrum of compound 14 (CDCl₃, 400 MHz)
$^{13}$C NMR spectrum of compound 14 (CDCl$_3$, 100 MHz)

DEPT spectrum of compound 14 (CDCl$_3$, 100 MHz)
3.3.8 References