REVIEW ON SYNTHESIS OF PILOCARPINE

A M.Sc Dissertation report by

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REVIEW ON SYNTHESIS OF PILOCARPINE

A DISSERTATION REPORT

Submitted in partial fulfilment

Of

The Degree of M.Sc (Organic Chemistry)

By

Ms. Shruti U. Naik

To the

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Goa University

Goa 403206

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CERTIFICATE

This is to certify that the dissertation entitled ***"Review on synthesis of pilocarpine"*** is bonafide work carried out by Ms. Shruti Ulhas Naik under my supervision in partial fulfilment of the requirement for the award of degree of Master of Sciences in Chemistry at the School of Chemical Sciences, Goa University.

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STATEMENT

I hereby declare that the dissertation report titled ***"REVIEW ON THE SYNTHESIS OF PILOCARPINE"*** submitted to the School of Chemical Sciences,Goa University is based on the original work done by me under the guidance of Dr. Vinod K. Mandrekar,Assistant Professor of Organic Chemistry.The information provided in this report is authentic to the best of my knowledge and the same has not been submitted to any other institution or university for the award of degree or diploma.

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CONTENTS

INTRODUCTION

Alkaloids are the organic compounds found in fungus,bacteria and the plant kingdom.Majority of these compounds exhibits an alkaline character since they are composed of one or more nitrogen atoms in heterocyclic ring.Alkaloids are known for their pharmacological and toxicological activities.[1] Thousands of alkaloids have been isolated over the past one thousand fifty years and a number of high standard techniques can now be operated to evaluate the pharmacological properties of these substances. Thus new application of these alkaloids have been discovered , as in the case of Pilocarpine which has been isolated in 1875 and practiced for decades in the treatment of glaucoma and xerostomy.[2]Several alkaloids have been isolated from the South American shrub *pilocarpus jaborandi*,such as pilocarpine,isopilocarpine,pilocarpidine,isopilocarpidine,pilosine,isopilosine,epiisopilosine,13-7-noria,N,N-dimethyl-5-methoxy-tryptamine,epiisopiloturine,(11)-dehydro-pilocarpine,N,N-dimethyl tryptamine,plastidesmine(1H)-4-methoxy-2-quinolone and dictamine.[3]Jaborandi leaves are the only known source of pilocarpine,an imidazole alkaloid derived from histidine[4]

The alkaloid Pilocarpine in particular was first discovered by Hardy and Gerrard as well as Petit and Polonovski.Its structure was determined by Jowett and Zav'yalov and absolute configuration was determined by Hill and Barcza.[5]

In 1972,DeGraw obtained pilocarpine as the main product by synthesizing the cis-homopilopic acid via catalytic hydrogenation of metals.After that, the study on the production of pilocarpine by chemical synthesis has made progress and has been artificially synthesized.[6]

Properties of pilocarpine

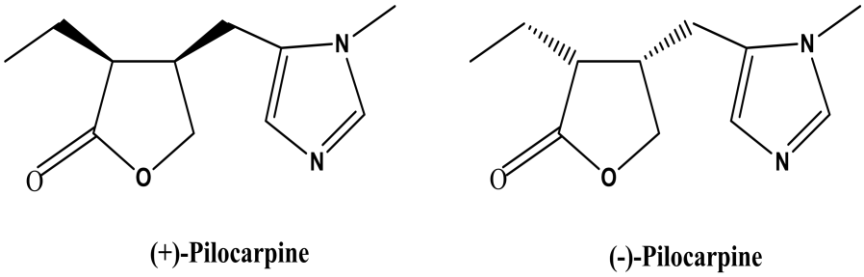


Figure: Structure of naturally occurring (+)-Pilocarpine and its

IUPAC name of pilocarpine is (3S,4R)-3-ethyl-4-[(3-methylimidazol-4-yl)methyl]oxalan-2-one.

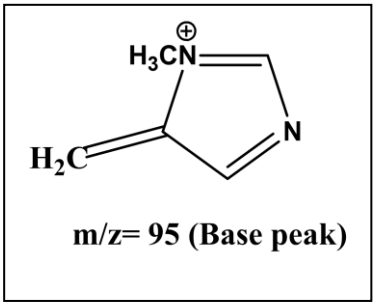
It is white crystalline powder with melting point 174-178°C, completely soluble in water,slightly soluble in ethanol,and insoluble in chloroform and diethyl ether.[7] The major binding cites of pilocarpine have been assumed to be the lactone ring oxygen and carbonyl oxygen atoms and the imidazole nitrogen atoms in the protonated form.[8] Pilocarpine is a cholinergic parasympathomimetic agent.It increases salivary, gastric and lacrimal secretion.[9] These include diaphoretic effects: stimulation of the parasympathetic system, miotic action: and particularly applications in ophthalmology.[10], [11]It is used for the treatment of glaucoma and xerostomia(salivary gland dysfunction).[10]The imidazole alkaloid (+)-pilocarpine and analogs have been the subject of study due to their therapeutic potential for the symptomatic treatment of Alzheimer's disease.[12], [13]

Pilocarpine is used as miotic agent for controlling the elevated intraocular pressure associated with glaucoma.But, the drug presents significant delievery problems resulting in a short duration of action and undesirable side effects such as myopia and miosis.Since these delievery problems are dependent upon physiochemical properties of the drug, it seems likely that the delievery characteristics of pilocarpine can be improved by using the prodrug approach.[14], [15]

Spectral data of pilocarpine

UV (EtOH) λmax 217 (end absorption).

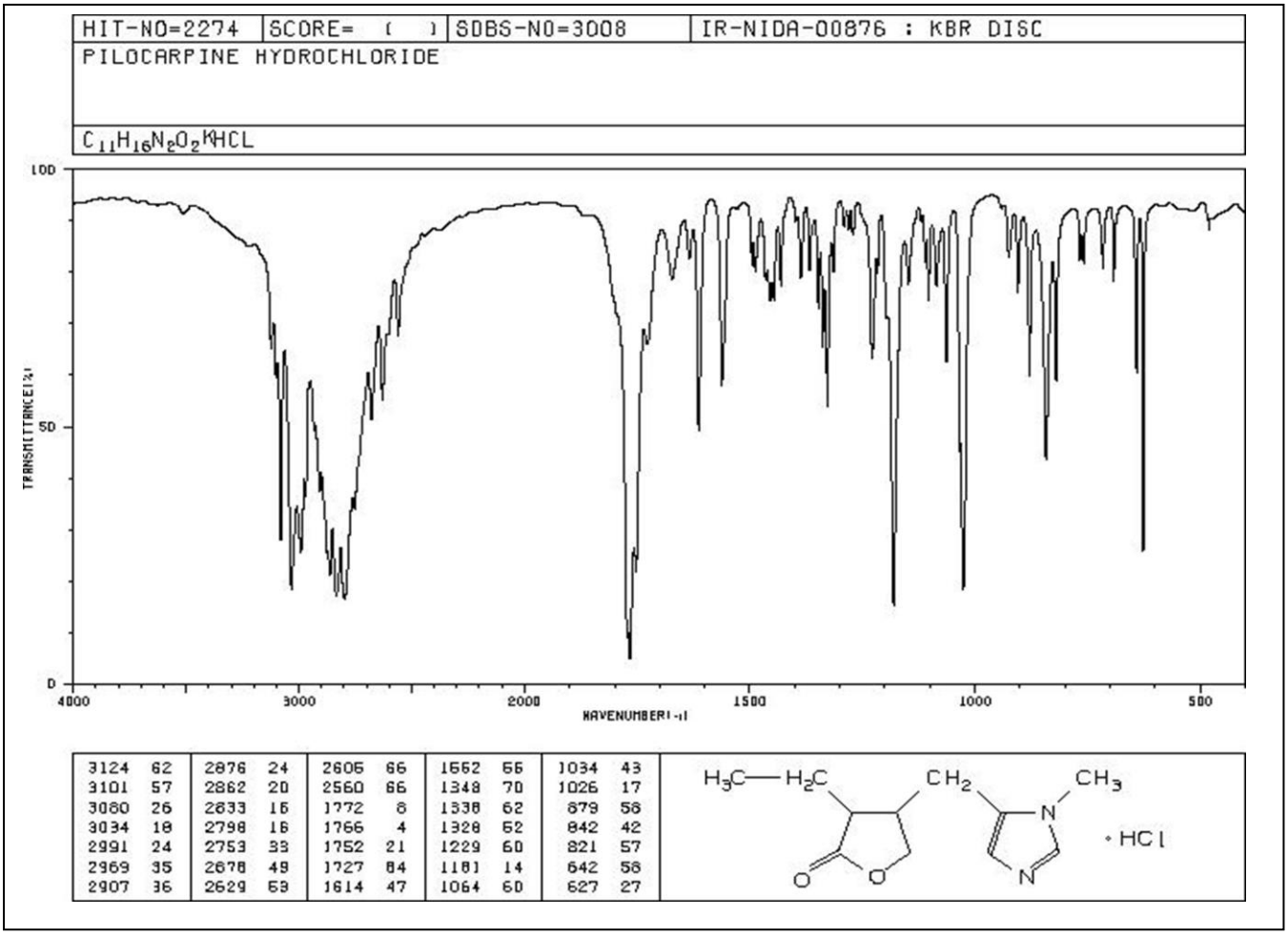
1H NMR (CDCl3) δ 1.12 (t, 3H, J 7.2 Hz), 1.58 (m, 1H), 1.92 (m, 1H), 2.42 (dd, 1H, J 15.7, IR (CHCl3) νmax 2,995, 1,770 (butyrolactonic C¼O), 1,500, 1,180 cm?1. 11.5 Hz), 2.68 (m, 2H), 2.83 (m, 1H), 3.57 (s, 1H), 4.11 (dd, 1H, J 9.1, 2.5 Hz), 4.20 (ddd, 1H, J 9.1, 5.4, 1.8 Hz), 6.81 (br s, 1H), 7.43 (br s, 1H).



m/z= 95 (Base peak)

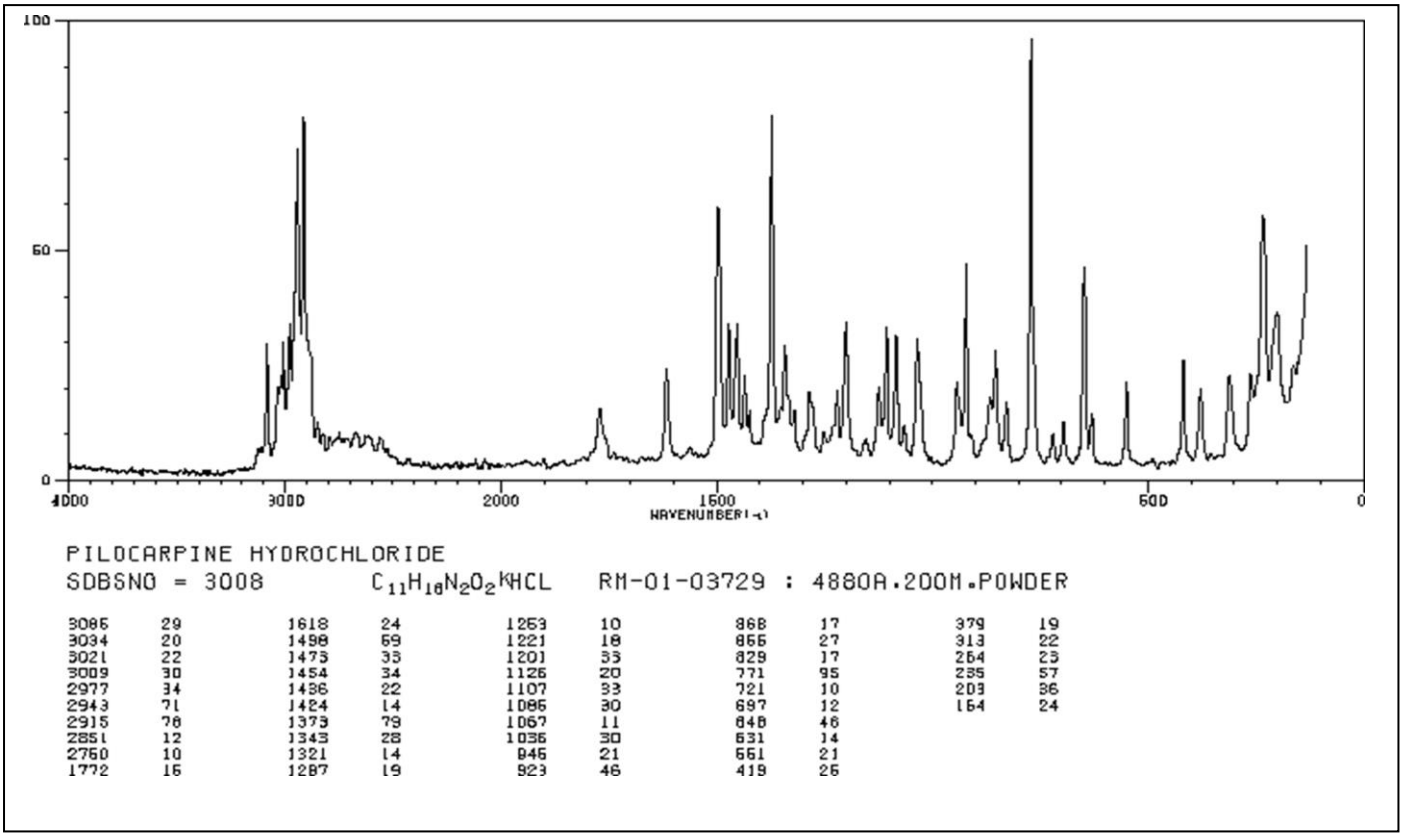
MS, m/z (relative intensity) 208 (M+, 8), 95 (M+_ 113) (100 %).[16]

IR spectra



3124	62	2876	24	2605	66	1562	66	1034	43
3101	57	2862	20	2560	66	1348	70	1026	17
3080	26	2833	16	1772	8	1338	62	879	56
3034	18	2798	16	1766	4	1328	62	842	42
2991	24	2753	33	1752	21	1229	60	821	57
2969	35	2678	49	1727	64	1181	14	642	56
2907	36	2629	59	1614	47	1064	60	627	27

Raman spectra



PILOCARPINE HYDROCHLORIDE

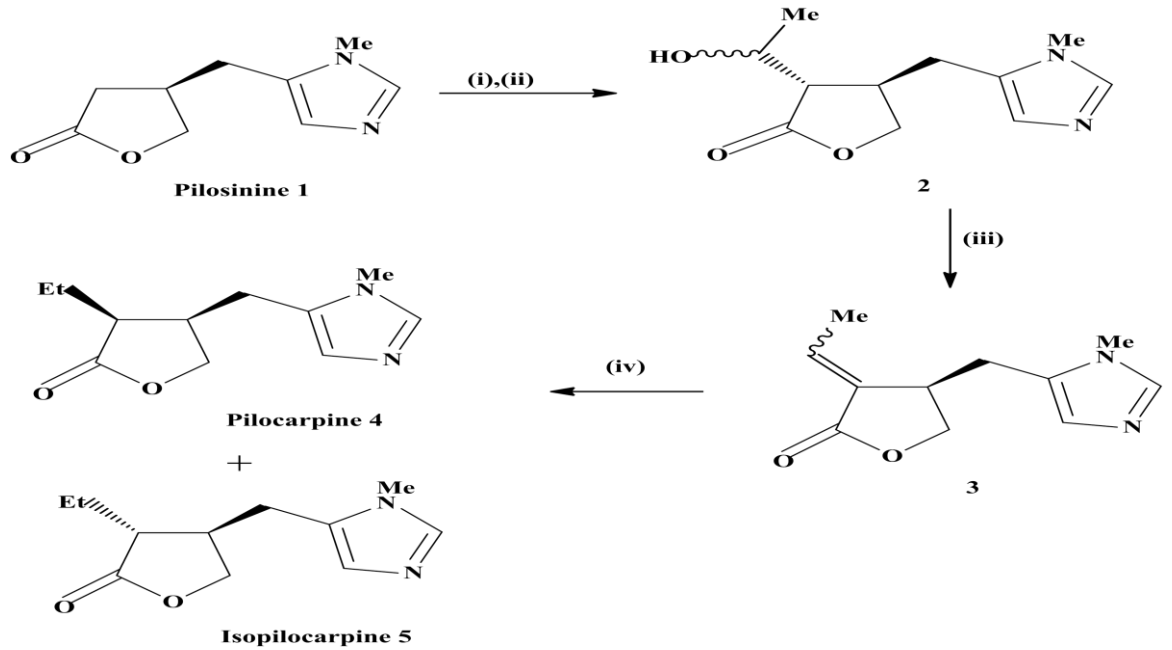
SDBSNO = 3008 $C_{11}H_{16}N_2O_2$KHCL RM-01-03729 : 488OA.200M.POWDER

3086	29	1618	24	1253	10	868	17	379	19
3034	20	1498	69	1221	18	855	27	313	22
3021	22	1473	35	1201	33	829	17	264	23
3009	30	1454	34	1126	20	771	95	235	57
2977	34	1436	22	1107	33	721	10	203	36
2943	71	1424	14	1086	30	697	12	164	24
2915	78	1373	79	1067	11	648	46		
2851	12	1343	28	1036	30	631	14		
2760	10	1321	14	945	21	561	21		
1772	15	1287	19	923	48	419	25		

LITERATURE REVIEW

Synthesis of racemic pilocarpine via pilosinine

Link and Bernauer proposed a strategy involving the elaboration of (+)-pilosinine **1** via acetylation and reduction to give α-hydroxyethyl lactone **2**.Subsequent elimination via the corresponding acetate and hydrogenation of the resulting α-ethylidene lactone **3** gave 93:7 mixture of pilocarpine **4** and isopilocarpine **5.(scheme 1)** [17]



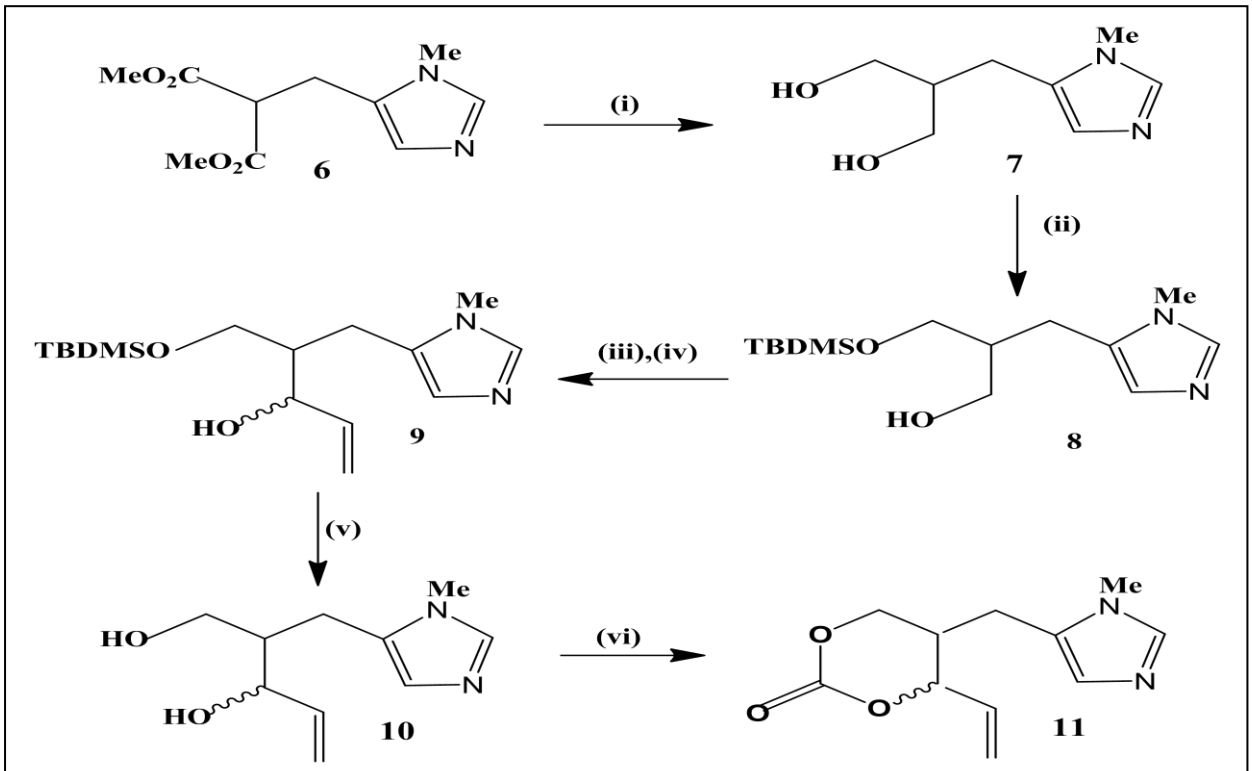
Scheme 1: Synthesis of pilocarpine and isopilocarpine from pilosinine

Reaction conditions (scheme 1):(i)-KO^tBu, EtOAc,^tBuOH,**(ii)** PtO_2,H_2(50 atm),MeOH, rt,**(iii)** Ac_2O, AcOH, 70–130°C,**(iv)** PtO_2,H_2(50 atm), MeOH, rt

Synthesis of pilocarpine using a precursor 1,3-dioxan-2-one

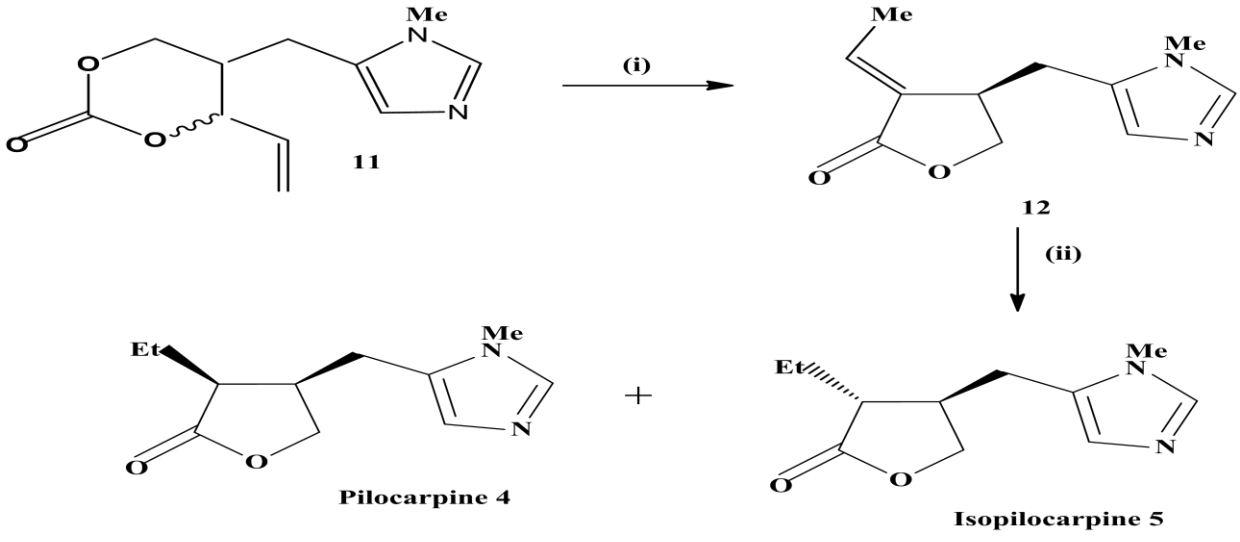
1,3-dioxan-2-one **11** was synthesised from diester **6** via initial reduction with $LiAlH_4$ to give diol **7**. Monosilylation of **7** was achieved via deprotonation with NaH followed by the addition of TBDMSCl to give monosliylated oxidation of the free hydroxyl group within **8** under Swern conditions gave the corresponding aldehyde which was found to be unstable and was therefore treated immediately with vinylmagnesium bromide to give **9** as a 50:50 mixture of diastereoisomers. Removal of the O-TBDMS protecting group was accomplished with HF in MeCN to give diol **10** which was used directly in the next step. Diol **10** was treated with 1,10-carbonyldi-imidazole (CDI) to give 1,3-dioxan-2-one

11, 90% yield as a 50:50 mixture of diaste-reoisomers.**(scheme 2)** Synthesized 1,3-Dioxan-2-one **11** was used as a precursor for the synthesis of pilocarpine and isopilocarpine.[17]**(Scheme 3)**



Scheme 2: Synthesis of 1,3-dioxane-2-one

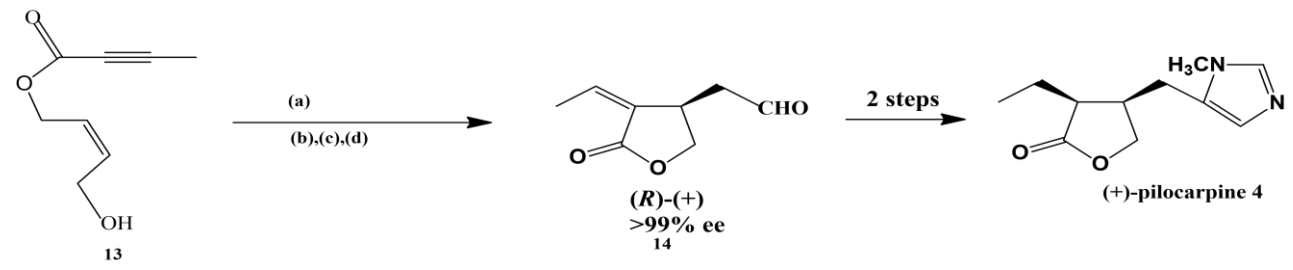
Reaction conditions (scheme 2) :(i)- $LiAlH_4$,THF,reflux,**(ii)-** NaH,DMF,rt then TBDMSCl,0°C to rt,**(iii)-** DMSO,$(COCl)_2$,CH_2Cl_2,-78°C to rt,**(iv)-** vinylmagnesium bromide,THF,rt,**(v)-** HF,MeCN,rt,**(vi)**- CDI,CH_2Cl_2,rt



Scheme 3: Synthesis of pilocarpine and isopilocarpine via 1,3-dioxane-2-one.

Rh catalysed asymmetric synthesis of (+)-pilocarpine

The first asymmetric Rh catalysed 1,6-enyne cycloisomerization reactions utilizing precursor catalysts containing chiral bidentate phosphanes and phosphinites was reported.This catalyst system was employed in an asymmetric synthesis of (+)-pilocarpine.[18]

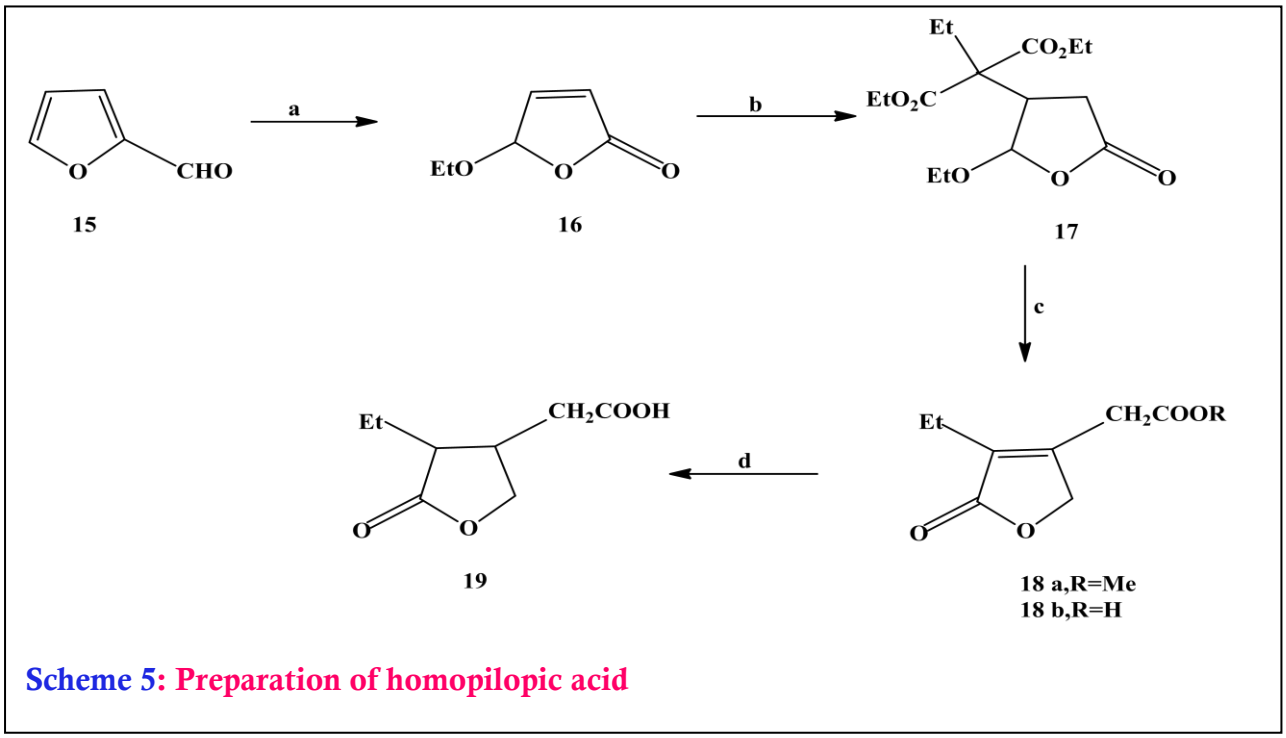


Scheme 4:Rh catalysed asymmetric synthesis of (+)-pilocarpine

Reaction conditions (scheme 4) (a)- [{Rh(cod)Cl}$_2$](5 mol %) **,(b)-**(*R*)-BINAP(11 mol %), **(c)-**$AgSbF_6$(20 mol%), **(d)-**$ClCH_2CH_2Cl$,RT,2-10 min

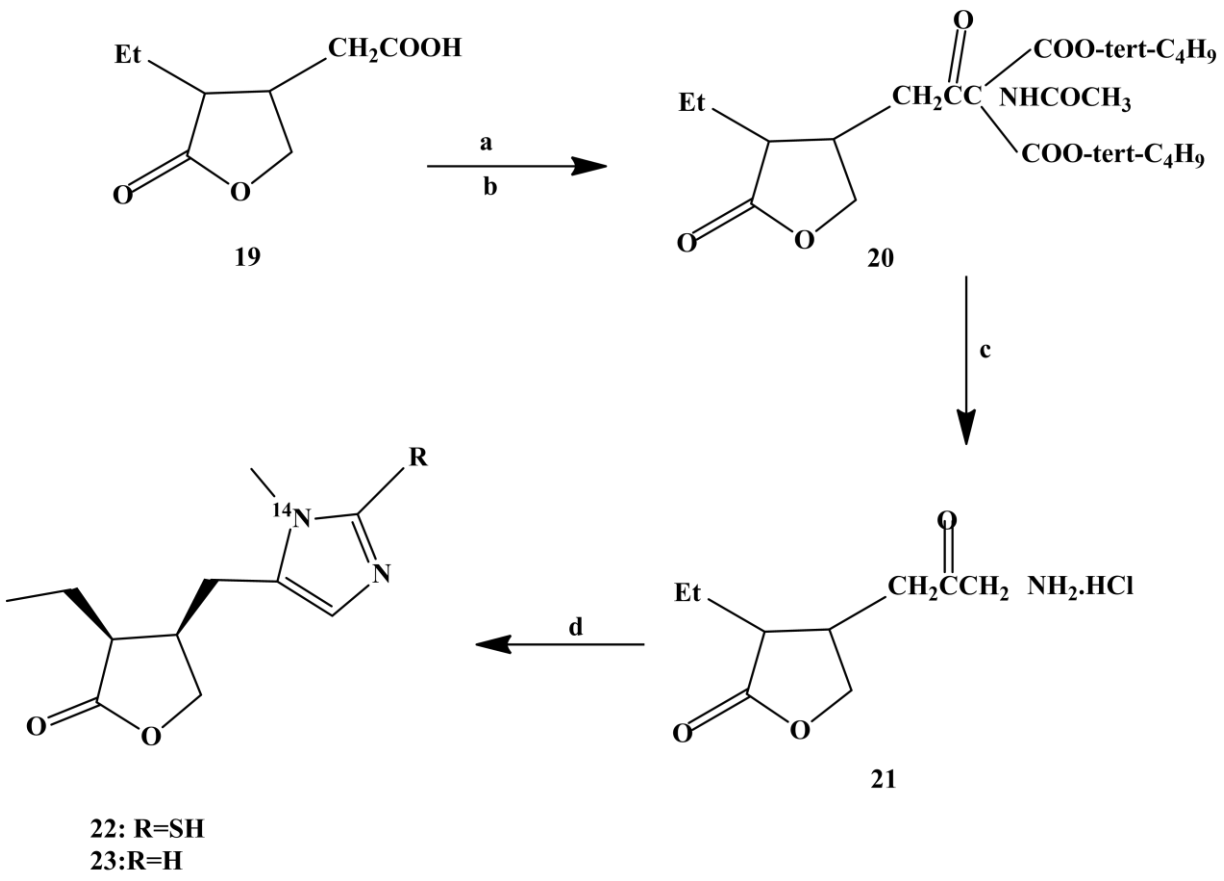
Synthesis of *d*-pilocarpine-N^{14}-CH_3

In one the of the researches,Synthesis of *d*-Pilocarpine was carried out via *dl*-homopilopic acid.The main precursor homopilopic acid was prepared by air oxidation of an ethanolic solution of furfural **15** in the presence of sunlight to give 2-oxo-5-ethoxy-2,5-dihydrofuran **16.**Michael condensation of **16** with diethylmalonate gave the lactone ester **17**.Treatment of **17** with hot HBr in AcOH gave an 88% of acid **18b** which was converted to methyl ester **18a.**[6] Optical resolution of the racemic acid was attained via salt formation with (+)-α-methylbenzylamine to provide the required *d*-homopilopic acid.This acid was transformed to the acid chloride,which was used for the acylation of di-tert-butylacetamidomalonate.Acid hydrolysis of the ester **20** caused cleavage of the tert-butyl groups and successive decarboxylation to afford the homopilopyl aminomethyl ketone **21** as the hydrochloride salt.Condensation of **21** with methyl isocyanate-$^{14}CH_3$(prepared from ^{14}C-methylamine hydrochloride) gave *d*-2-mercaptopilocarpine-*N*-$^{14}CH_3$ **22**,which was desulfurized with peroxide to yield *d*-pilocarpine-*N*-$^{14}CH_3$ **23**.[19]



Scheme 5: Preparation of homopilopic acid

Reaction conditions (scheme 5): (a)-Air oxidation,sunlight ,**(b)-**CH_2 $(CO_2Et)_2$,Michael condensation,**(c)-**Hot HBr in AcOH,**(d)-**α-methylbenzylamine.



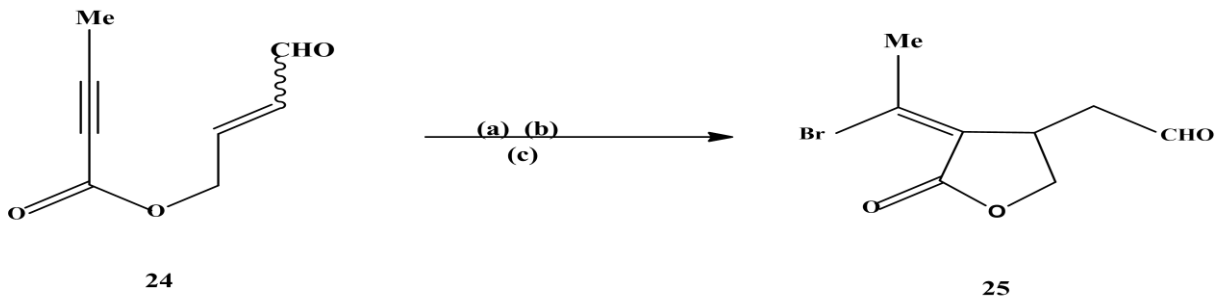
Scheme 6: Synthesis of *d*-pilocarpine-*N*-$^{14}CH_3$

Reaction conditions (scheme 6): (a)-$SOCl_2$,**(b)-**di-tert-butylacetamidomalonate,**(c)-** H^+/H_2O,Heat,**(d)-**methyl isocyanate-$^{14}CH_3$.

Application of Intramolecular alkyne-α,β-unsaturated carbonyl coupling in the formal synthesis of (+)-pilocarpine

Some years back, intramolecular alkyne-α,β-unsaturated carbonyl coupling and its application in the formal synthesis of (+)-pilocarpine was reported in one of the research paper.The starting material **1** for the precursor of (+)-pilocarpine synthesis was prepared by selective manifestation of 2-butene -1,4-diol.In the presence of $Pd/(OAc)_2$,tetra butyl ammonium bromide, and acetic acid.The reaction was carried out in a number of solvents such as MeCN,EtOH and CH_2Cl_2 to obtain cylization product **2**.The reaction was smooth and rapid with best yield. The reaction mechanism was close enough to the intermolecular alkyne α,β-unsaturated carbonyl coupling reaction: first, halopalladation of **24** gives vinylpalladium intermediate, which undergoes intramolecular carbon-carbon double bond introduction followed by protonolysis of the newly formed C-Pd bond to produce **25** and regenerate the Pd(II) catalytic species. Development of the facile method for the preparation of polyfunctionalized aldehydic γ-lactone created a way for its application in the synthesis of the natural product, (+)-pilocarpine.**(scheme 7)**

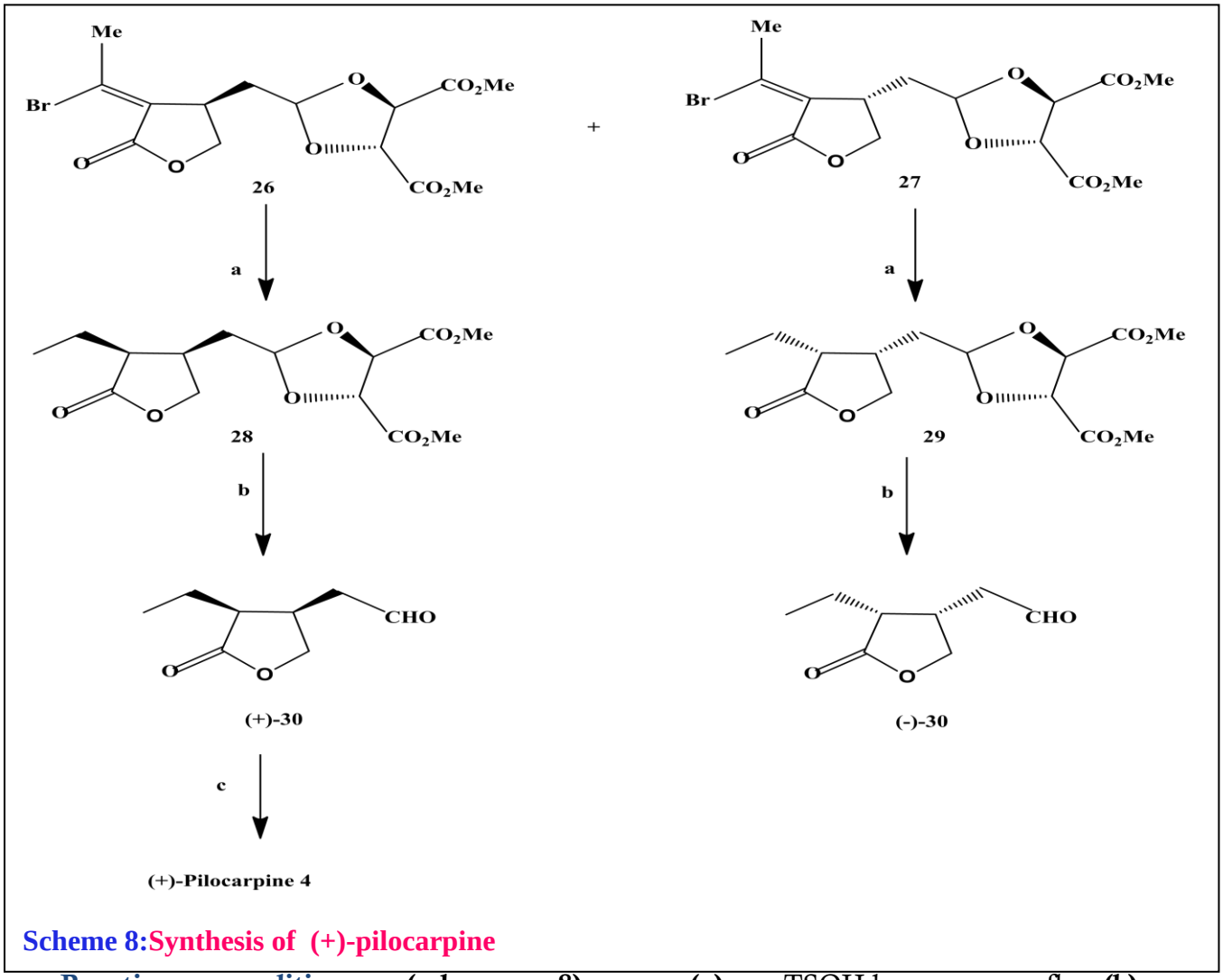
The pair of diastereomeric acetals **26** and **27** from **25** and dimethyl(+)-tartarate was prepared to get optically active homopilopic acldehyde **30**.The acetals **26** and **27** were easily separated by column chromatography on silica gel.Then Pd/C catalyzed hydrogenation of **28** and **29** followed by hydrolysis in the presence of $HOAc/H_2O$ afforded enantiomeric **(+)-30** and **(-)-30**, respectively. In these two reactions, no trans-substituted lactone isomer was obtained. The high stereoselectivity is noteworthy because direct hydrogenation of **25** only gave a 2:3 mixture of **30** and its trans isomer.With **(+)-30** in hand, the aldehyde function can be transformed to imidazole ring to complete the synthesis of (+)-pilocarpine.**(scheme 8)**[20]



24 25

Scheme 7: Palladium catalyzed intramolecular alkyne-α,ß-unsaturated carbonyl coupling

Reaction conditions (scheme 7) (a)-$Pd(OAc)_2$, **(b)-**TBAB**, (c)-**HOAc,rt.



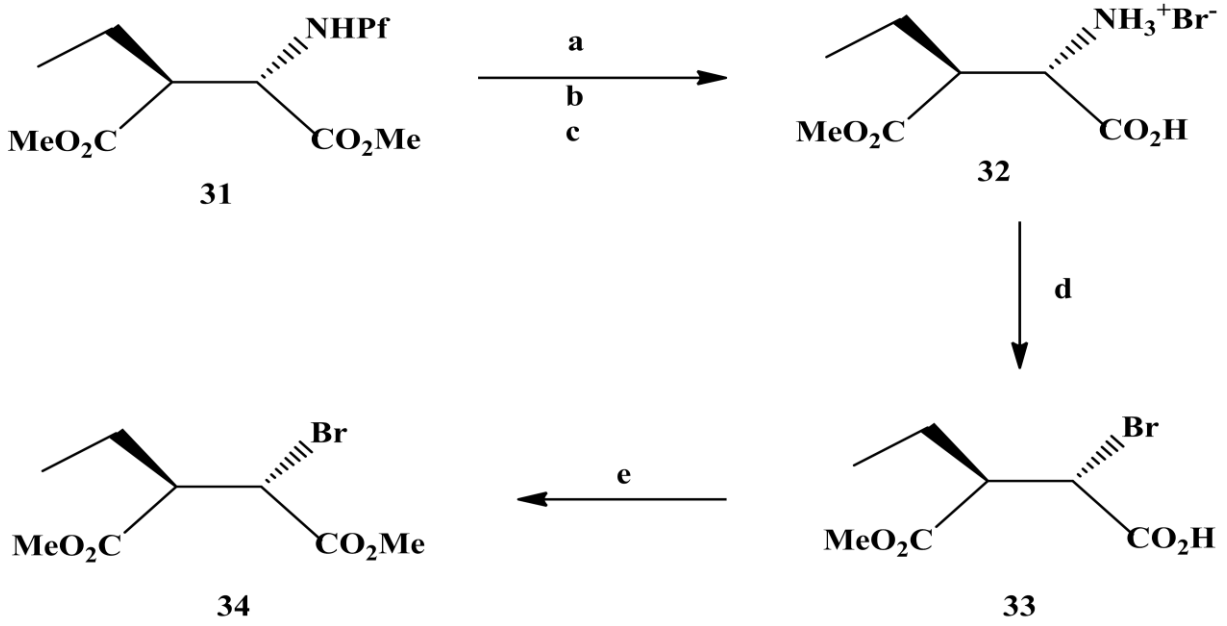
Scheme 8:Synthesis of (+)-pilocarpine

Reaction conditions (scheme 8) : (a)- TSOH,benzene reflux**,(b)-** Pd/C,NaOAc,EtOAc,H_2**,(C)**- HOAc,H_2O**,(d)-** CH_3NH_2, TosMic, DCM, benzene,NEt_3, 7 d, 23◦C

Synthesis of (+)-Pilocarpine from lactone.

Using one pot reaction sequence 3-*(S)*-ethylaspartate **(31)** was converted to bromo acid **(32).**The selective hydrolysis of the α-methyl ester with basic copper(II) carbonate under neutral conditions was the key feature of this sequence.First, using Pd/C in methanol in the presence of hydrobromic acid the removal of phenylfluorenyl nitrogen protecting group of **(31)** was achieved.It was then treated with a suspension of basic copper(II) carbonate in aqueous methanol, followed by destruction of the intermediate copper complex of the amino acid **(32)** with hydrogen sulphide.From this catalysed hydrolytic

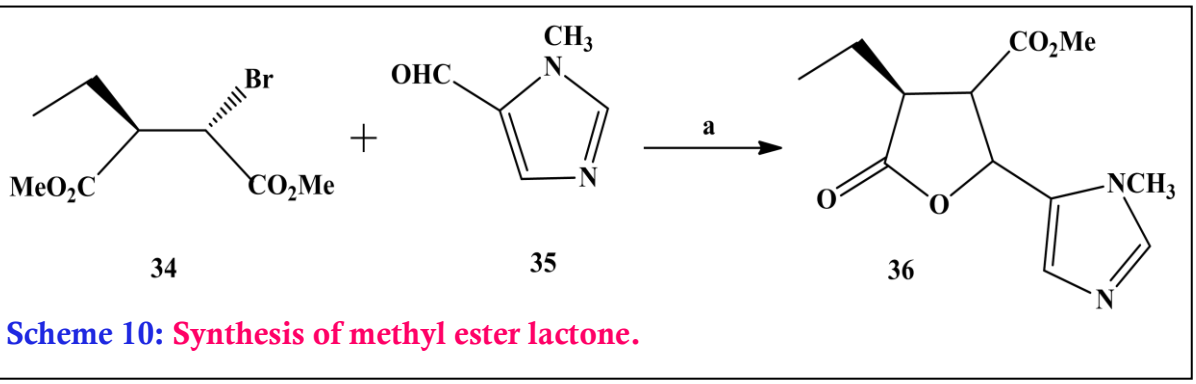
process amino acid **(32)** obtained as the hydrobromide salt.This was converted to bromo acid **(33)** via sodium nitrite and potassium bromide in hydrobromic acid.This sequence proceeded in excellent overall yield (93%).Further,dimethyl ester **(34)** was prepared using sulphuric acid in refluxing methanol in (91%) yield. [21]



Scheme 9: Conversion of aspartate to bromosuccinate dimethyl ester.

Reaction conditions (scheme 9) :**(a)-**H_2,HBr-HOAc,Pd/C **(b)-** $CuCO_3.Cu(OH)_2$,CH_3OH/H_2O **(c)-**H_2S **(d)-** $NaNO_2$,HBr/KBr **(e)-** H_2SO_4,MeOH.

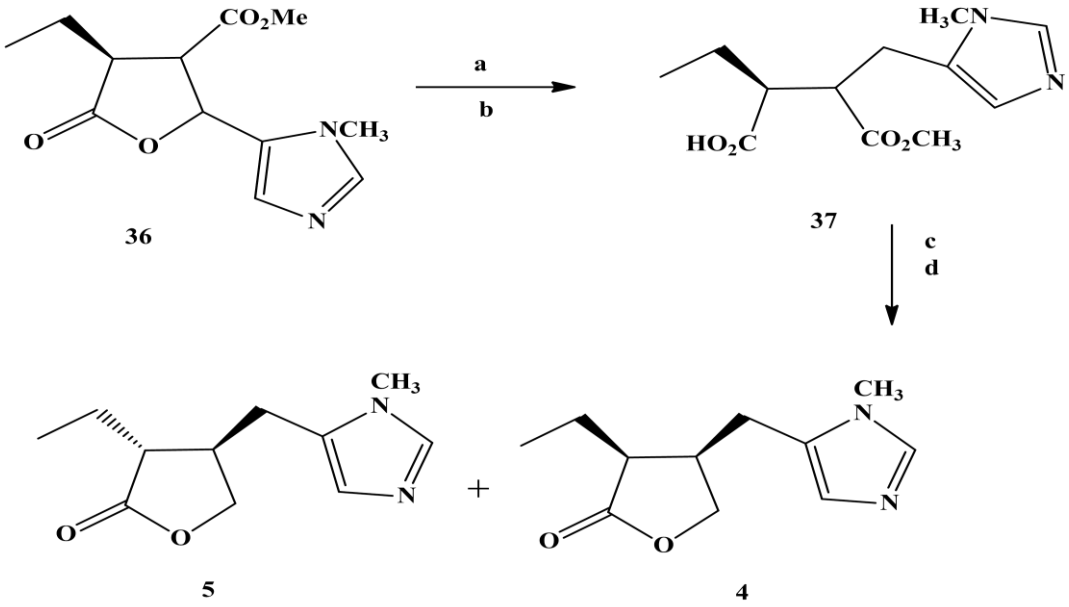
Lactone synthesis via reformatsky reaction was achieved by employing bromosuccinate dimethyl ester **(34)** and 1-methylimidazole-5-carboxaldehyde **(35)** with Zinc/silver couple and diethylaluminium chloride giving methyl ester lactone **(36)** in (94%) yield.



Scheme 10: Synthesis of methyl ester lactone.

Reaction conditions (scheme 10) :**(a)-**Zn(Ag),Et_2AlCl,THF/Hexane ,25°C,1.5 h.

Reaction of methyl ester lactone **(36)** with hydrogen at 60 psi in the presence of 5% palladium on charcoal in methanol for 60 h afforded monomethyl ester **(37).**This ester in the presence of $LiBH_4$ and acid afforded 91/9 mixture of (+)-pilocarpine **(4)** and isopilocarpine **(5)**.



Scheme 11: Synthesis of (+)-pilocarpine from lactone.

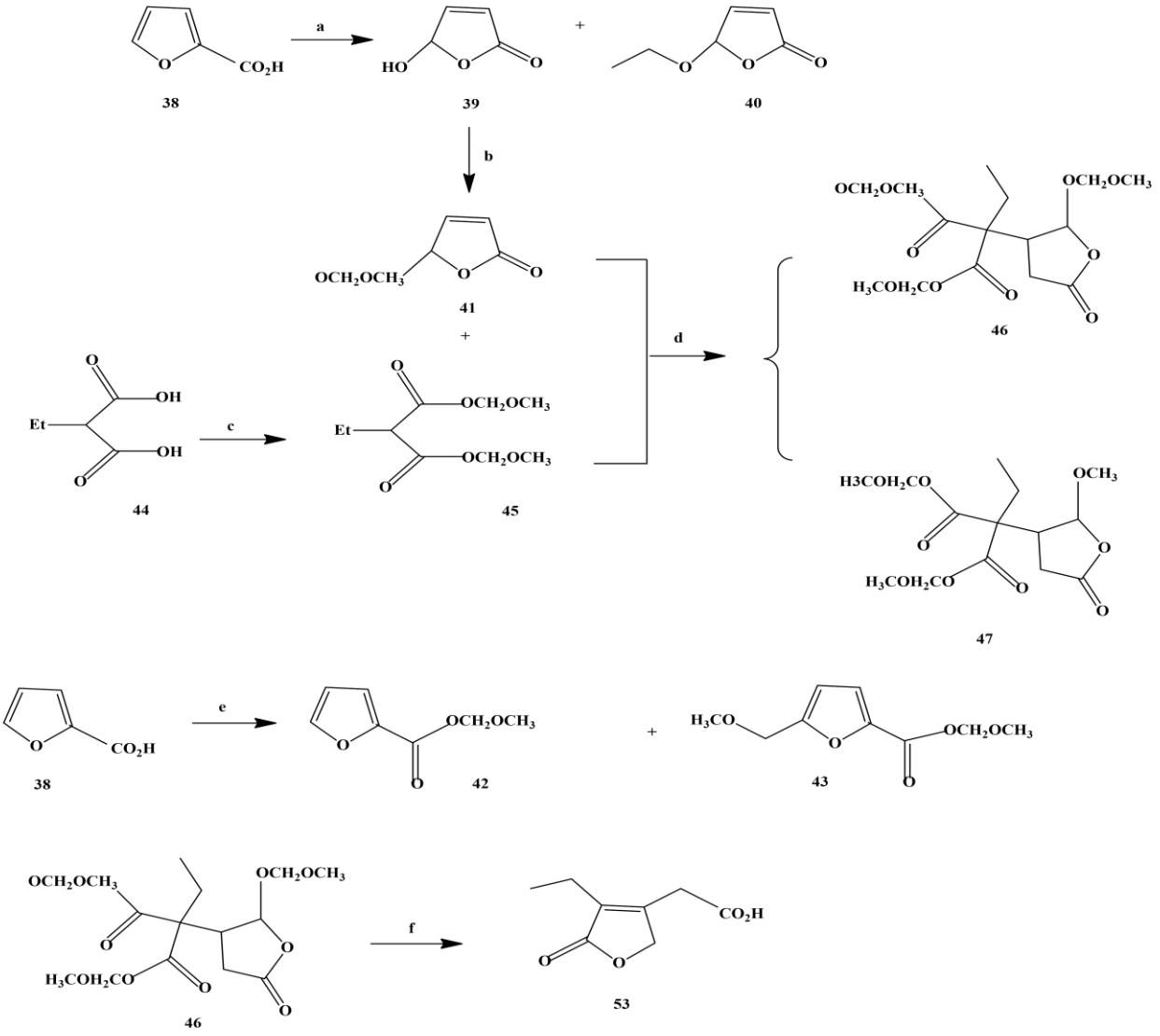
Reaction conditions (scheme 11) :**(a)-**H_2/Pd/C,CH_3OH **(b)-** $LiBH_4$ **(c)-**HCl

Synthesis of both pilocarpine enantiomers using dehydrohomopilopic acid

In one of the researches,synthesis of both pilocarpine enantiomers were carried out.The readily accessible furan-2-carboxylic acid was used as the starting material.This starting material **38** in the presence of oxygen and sensitizer(Bengal rosa) was photooxidised to yield **39** and **40** in which **40** was the by-product formed.In the next step ,acetal protecting group was introduced.The reaction of **39** with dimethoxymethane in presence of solid P_4O_{10} produced methoxymethylated **41**.Applying this same condition to **38**, **42** and **43** was formed.Ethyl-malonic acid **44** was converted into its bis(methoxymethyl ester)**8**.The Michael addition of **45** with **41** formed **46**, and **47** as a by-product.A stobbe condensation of **48** produced dehydrohomopilopic acid.

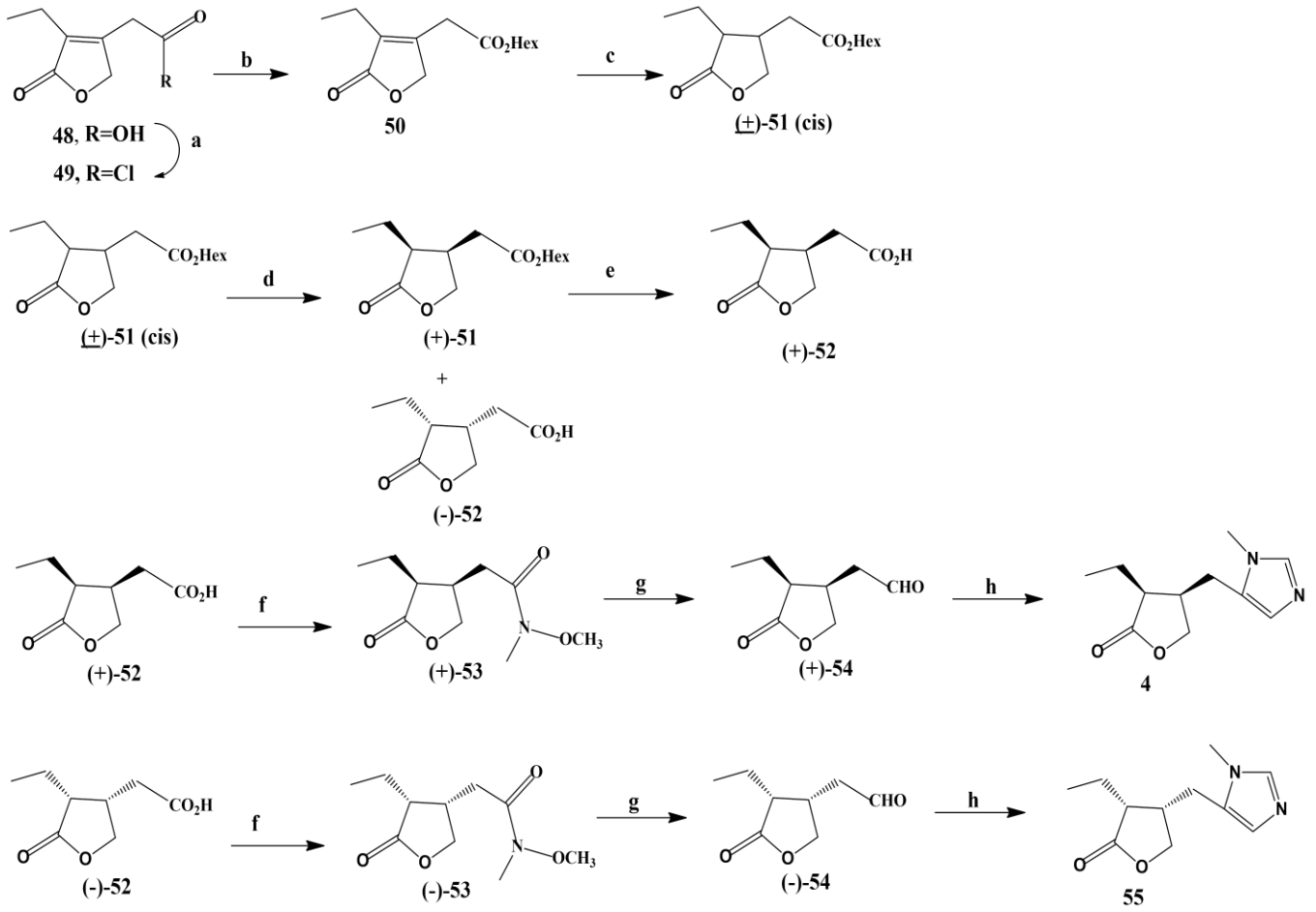
Dehydrohomopilopic acid was activated using thionyl chloride for the in situ generation of acid chloride, which is reacted with *n*-hexanol to produce ester **50**.Using Rh/Al_2O_3 hydrogenation of the ester **50** was carried out to produce cis-configurated **(±)-51.**This ester was hydrolysed by suitable hydrolytic enzyme lipase PS of Amano under Ph-stat conditions at pH=7 to yield **(+)-51** and **(-)-52**.Enzymatic hydrolysis of **(+)-51** using pig

liver esterase(PLE) at pH=7 afforded **(+)-52** with enantiomeric excess of ee > 99.Thus both the enantiomers of homopilopic acid **52** was produced in pure enantiomeric form.For the synthesis of both the enantiomers of pilocarpine, N-methylmorpholine,isobutyl chloroformate andN,O-dimethylhydroxylamine hydrochloride were reacted with **52** to yield Weinreb acetamides **(-)-53** and **(+)-53**.These Weinreb acetamides after treating with *p*-tosylmethylisocyanide in the presence of triethylamine produced (-)-pilocarpine **(55)** and (+)-pilocarpine **(4)**.[5]



Scheme 12: Preparation of dehydrohomopilopic acid for the synthesis of pilocarpine.

Reaction conditions (scheme 12):**(a)-** hγ, Bengal rosa, 8 h, 20◦C, 76% (of 3) and 5% (of 4),**(b)-** $CH_2(OCH_3)2$, P_4O_{10}, DCM,20◦C, 5 h, 98%.**(c)-** $CH_2(OCH_3)2$, P_4O_{10}, DCM, 20◦C, 5 h, 99%,**(d)-** THF, Na, 25◦C, 15 h, 72%,**(e)-** $CH_2(OCH_3)2$, P_4O_{10} ,DCM, 20◦C, 5 h, 77% and 19%,**(f)-** HBr, reflux, 2 d, 83%



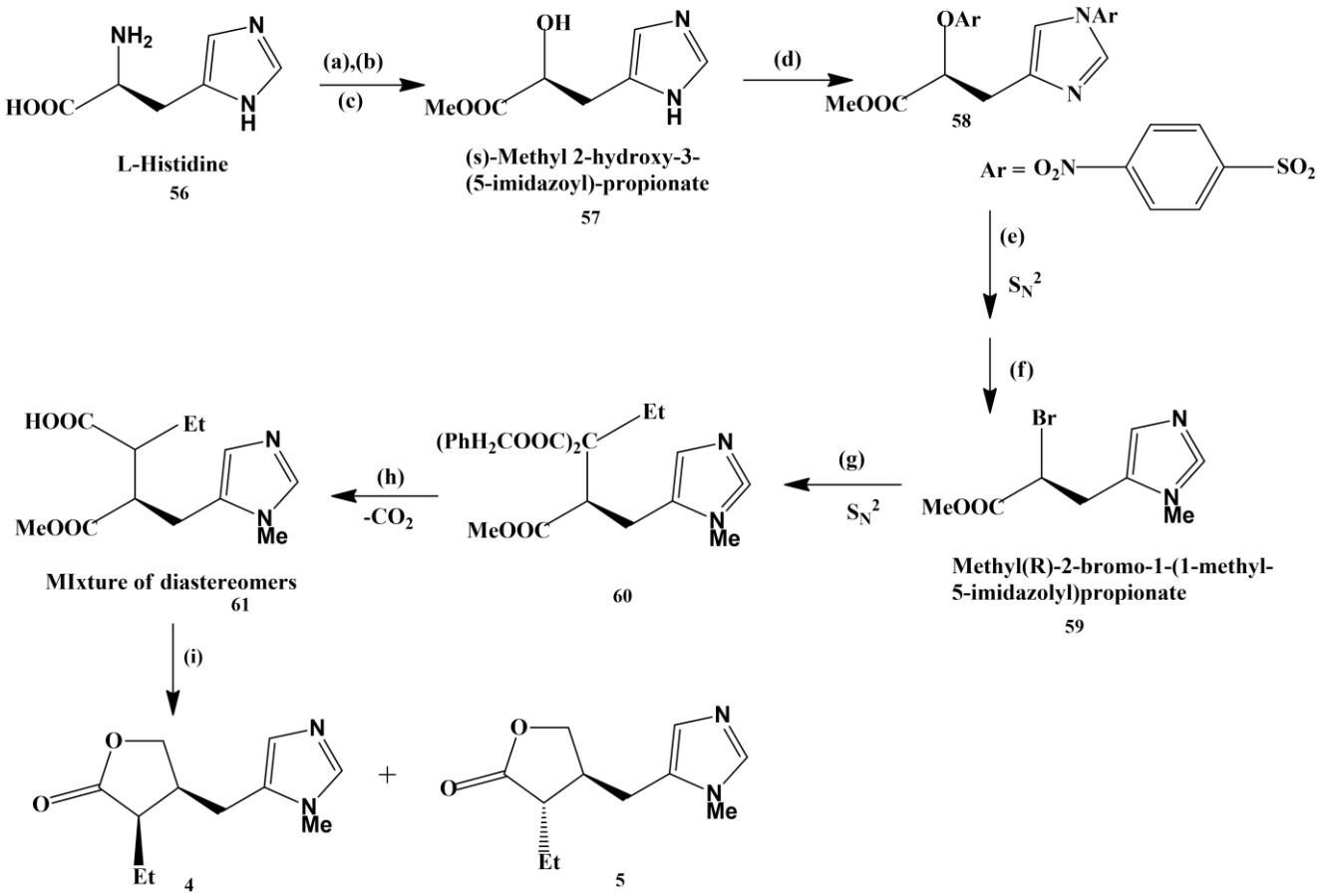
Scheme 13: Synthesis of (+)-pilocarpine and (-)-pilocarpine

Reaction conditions (scheme 13) :(a)-$SOCl_2$, reflux, 3 h**,(b)-** Hex-OH, reflux, 16 h, 98%,(c)- Rh/Al_2O_3 , H_2(1 at),THF, 5 d**,(d)-** Lipase PS, pH = 7.0, 2 d, 22°C, 48%**,(e)-** PLE, pH = 7.0, 22°C , 2 d, 96%,**(f)-** N-methylmorpholine,iBu-chloroformate,N,O-dimethylhydroxylamine hydrochloride, (23◦C, 1 d, 84%,**(g)-** $LiAlH_4$, Et_2O, 23◦C, 30 min, 95%,**(h)-** CH_3NH_2, TosMic, DCM, benzene,NEt_3, 7 d, 23◦C, 59%

Stereoselective synthesis of (+)-pilocarpine and (+)-isopilocarpine from L-histidine

This synthesis was reported by by Noordam et al.First step starts with diazotization of amino group to yield hydroxyl compound.Then conversion of hydroxyl compound to bromo derivative via 4-nitobenzenesulphonyl derivative accompanying walden

inversion.After methylation,it suffers another inversion when alkylated with dibenzyl ethyl malonate.Hydrogenolysis results in debenzylation of the compound.Decarboxylation followed by reduction using $LiBH_4$ yields the hydroxy acid.This hydroxyl acid undergoes lactonization to yield 1:1 mixture of (+)-pilocarpine and (+)-isopilocarpine in 85% yield.[16]



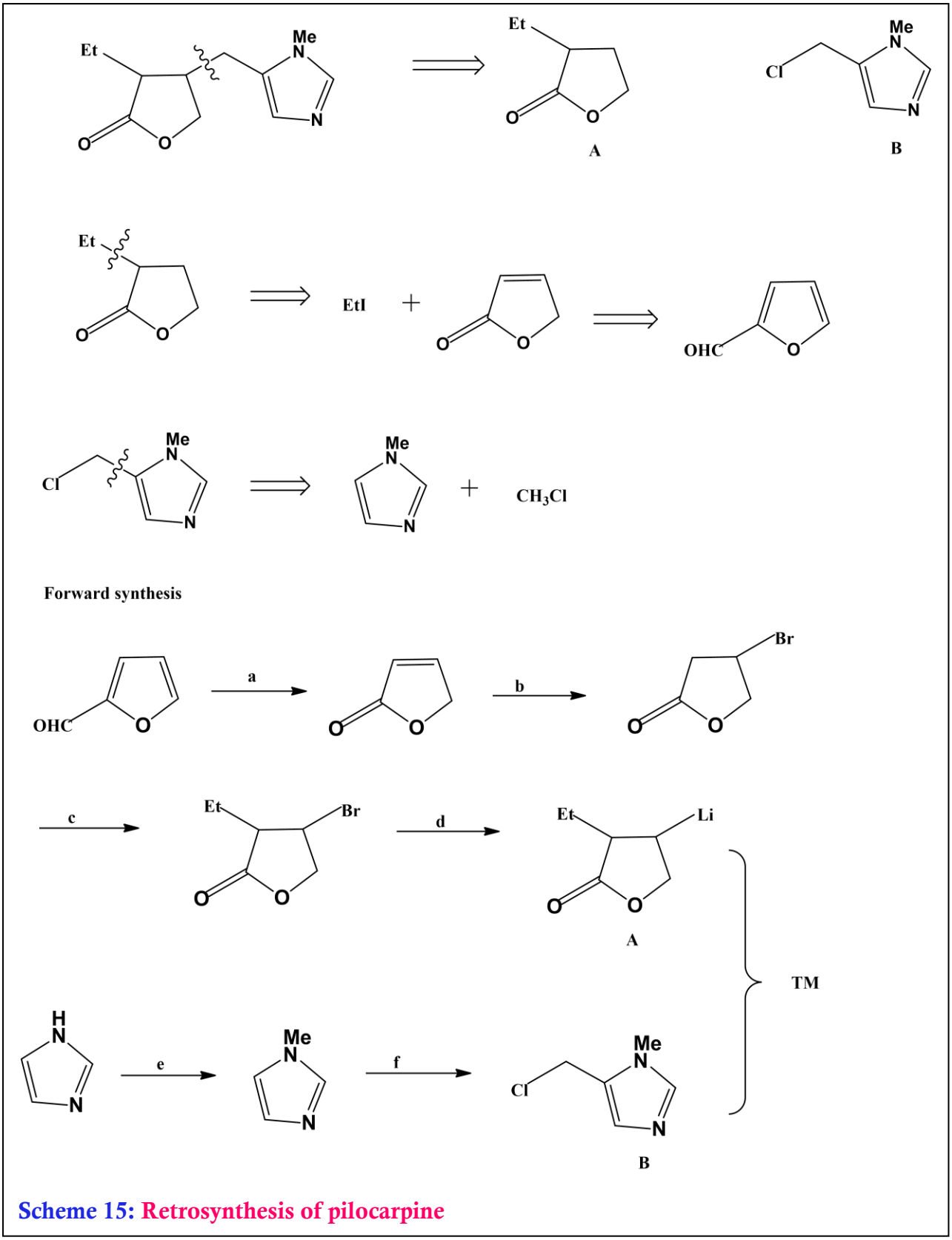
Scheme 14: Synthesis of (+)-pilocarpine and (+)-isopilocarpine using L-Histidine

Reaction conditions (scheme 14) :(a) AgNO2, aq. orthophosphoric acid (diazotization),**(b)** H2S (removal of Ag**),(c)** dry HCl, anhydrous MeOH (esterification),**(d)** *p*-Nitrobenzenesulfonyl chloride

(OH esterification and regioselective N 3 protection, N1 is not

protected probably due to steric hindrance by bulky C5 substitue*nt*),**(e)** LiBr, 2-butanone (Walden inversion), **(f)** Trimethyloxonium fluoborate (Me 3OBF4), MeNO2 (N1 -methylation),**(g)** Dibenzyl ethyl malonate, NaH, DMF CH(Et)(COOCH2Ph)2, (*S*N2 inv.),**(h)** AcOH, then 140 0C (hydrogenolysis, *CO2*), **(i)** LiBH4 , 2-propanol, then acidify (COOMe CH 2OH, then lactonization

RETROSYNTHETIC APPROACH OF PILOCARPINE



Scheme 15: Retrosynthesis of pilocarpine

Reaction conditions (scheme 15) :(a)-H_2O_2,HCOOH,**(b)-**HBr,**(c)-**LDA,EtI,**(d)-**n-BuLi,-78°C,**(e)-**n-BuLi,MeI,**(f)-**HCHO,HCl

CONCLUSION

It was found that esterification, hydrogenation and enzymatic hydrolysis followed by the reduction of weinreb amides and the single-step attachment of a 1-methyl-imidazole residue allowed for the convenient synthesis of both enantiomers of pilocarpine in good overall yields. Under Rh catalysis ,the starting material for the (+)-pilocarpine synthesis was accessible in high yields(90-98%) and enatioselectivities >99% ee.An efficient stereoselective cyclization method by palladium-catalyzed intramolecular enyne coupling of 4'-oxo-2'-alkenyl-2-alkynoates was developed which features in step wise synthesis of homopilopic acid.

A high yielding synthesis of racemic pilocarpine from starting material pilosinine was achieved.This synthetic strategy is practical,high yielding and potentially applicable to other members of the jaborandi family and their derivatives.

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