The thesis entitled **" Synthesis of peptide bioactives using unusual amino acids and new applications of polymethylhydrosiloxane (PMHS)**" is divided into three chapters.

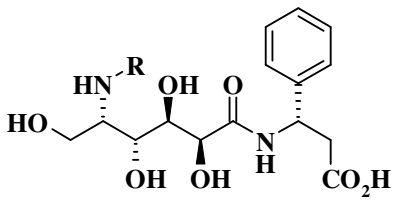
CHAPTER I: Chapter I deals with the synthesis of Pyloricidins: Synthesis of (2*S*,3*R*,4*R*,5*S*)-5-(*t*-butyloxycarbonyl)amino-2,3,4,6-tetrahydroxyhexanoyl-*β*-D-phenyl alanine methyl ester.

CHAPTER II: Chapter II deals with the stereoselective synthesis of peptides derived from norbornadiene: New surrogates for secondary structures on the peptide of *3-aminobicyclo[2.2.1]hept-5-ene-2-carboxylic acid* as monomer

CHAPTER III: This chapter deals with development of novel reduction procedures using polymethylhydrosiloxane (PMHS).

CHAPTER I: Deals with the synthesis of pyloricidins: Synthesis of (2*S*,3*R*,4*R*,5*S*)-5-(*t*-butyloxycarbonyl)amino-2,3,4,6-tetrahydroxyhexanoyl-*β*-D-phenylalanine methyl ester**.**

The pyloricidins **1**, isolated from *Bacillus sp*. HC-70 and *Bacillus sp.* HC-72, have shown exceptional anti-*Helicobacter pylori* antibiotic properties. *Helicobacter pylori*, a gram-negative bacterium, infection of this is the major causative factor of a number of gastric and duodenal pathologies. Several classes of compounds have been identified as anti-*H. Pylori* agents. Incomplete eradication of *H. pylori* has been achieved with some anti-microbial agents such as amoxicillin and clarithromycin due to their degradation by gastric acid. Efforts have been directed to develop or isolate a new series of compounds having antibiotic properties, which can totally eradicate these bacteria. Even though it is biologically important, recently this research gained prominence. (Fig 1).



1

a) Pyloricidin A :R= H-L-Val-L-Val-L-Leu

b) Pyloricidin A_1 :R= H-L-Val-L-Ilu-L-Leu

c) Pyloricidin A_2:R= H-L-Val-L-Leu-L-Leu

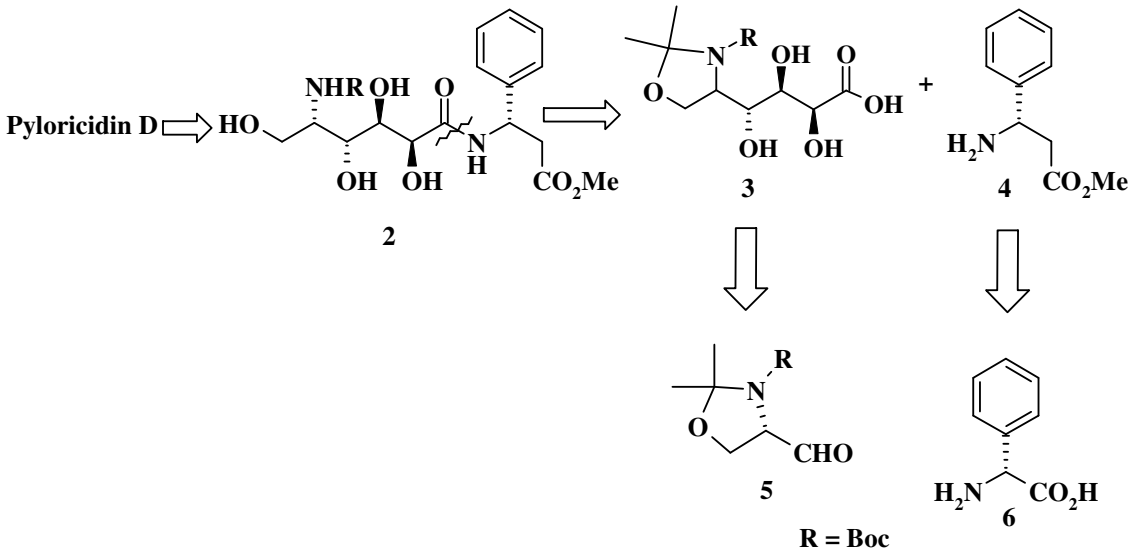
d) Pyloricidin B :R= H-L-Val-L-Leu

e) Pyloricidin C :R= H -Leu

f) Pyloricidin D :R= H-

Fig 1

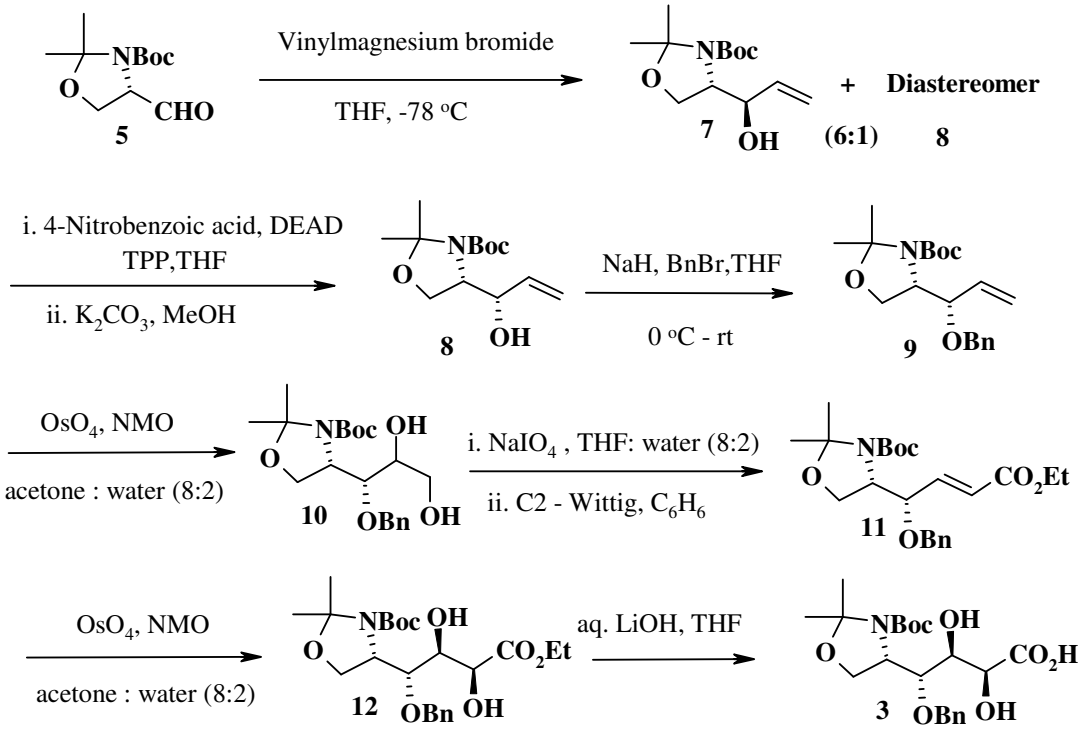
As a part of continued interest in developing simple and elegant strategies for the synthesis of bioactive natural and unnatural products, especially combining chiron approach with asymmetric synthesis, herein, we disclose the full findings on the N- and C-terminal protected (2*S*,3*R*,4*R*,5*S*)-5-amino-2,3,4,6-tetrahydroxyhexanoyl phenyl-alanine, which constitutes the key backbone of pyloricidins. This synthesis was taken up as part of designing new hybrid analogues of peptide bioactives. In the retrosynthetic analysis of pyloricidin D the connection of two fragments can be made with amide bond between two amino acids, which led to the key building blocks **3** and **4** respectively. The easily available Garner aldehyde **5** and L-phenylglycine **6** were effectively transformed to the target compound **2** involving very straightforward but high yielding reactions (Scheme 1).



Scheme 1

Synthesis of acid **3** started from readily available Garner aldehyde **5**. Grignard reaction on aldehyde **5** with vinyl magnesium bromide in tetrahydrofuran at –78 ^{o}C gave the allyl alcohols **8** and **7** with 1:6 *syn-anti* selectivity. The two diastereomers were readily separated by column chromatography. The ratio of the isomers was determined based on the weight of the products isolated. Inversion of hydroxy group in alcohol **7** under Mitsunobu conditions using triphenylphosphine/diethyl azodicarboxylate and 4-nitrobenzoic acid in THF at 0 ^{o}C to room temperature and followed by hydrolysis of nitro

benzoate with K_2CO_3 in MeOH produced the *syn* alcohol **8** in 30% yield for two steps. The spectral data (optical rotation and ^{1}H NMR) of compound **8** and minor diastereomer obtained during vinylation of Garner aldehyde were comparable confirming the inversion of hydroxy group. Allylic hydroxy group of compound **8** was protected as benzyl ether using NaH and benzyl bromide in THF. Dihydroxylation of olefin **9** using OsO_4 and NMO in acetone : H_2O (8:2) afforded the diol **10**. After periodate oxidation of diol **10**, the resultant aldehyde was treated with (carboethoxymethylene)- triphenylphosphorane in benzene to afford the unsaturated ester **11**.

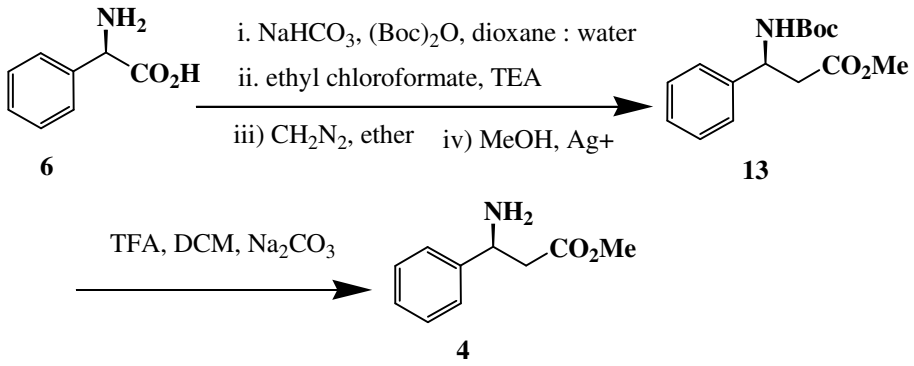


Scheme 2

Ester **11** was dihydroxylated using OsO_4 and NMO in acetone:water (8:2) to furnish the diol **12** with 13:1 diastereomeric ratio. This ratio was determined using hypersil OD reverse phase column and MeOH : water / 70: 30 as eluents at 254 nm. Hydrolysis of the diol **12** using aq. LiOH gave the dihydroxy acid **3** (Scheme 2).

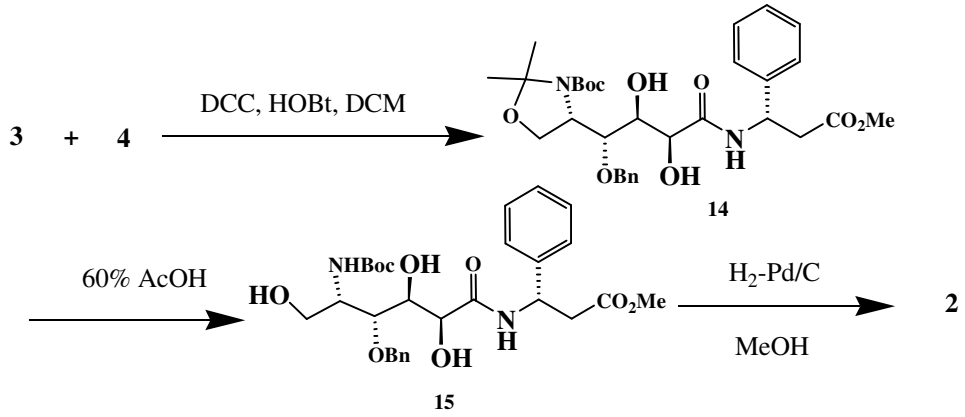
After completion of the synthesis of dihydroxy acid **3** next attempts were directed towards the synthesis of *β*-phenylalanine methyl ester **4,** which was prepared from phenylglycine **6**. Homologation of *N*-Boc protected phenyl glycine with ethyl

chloroformate, triethylamine, CH_2N_2 and silverbenzoate in MeOH followed by deprotection of Boc group with trifluoroaceticacid in CH_2Cl_2 and subsequent basification with Na_2CO_3 gave the β-phenylalanine methylester **4** (Scheme 3).



Scheme 3

Amide bond between acid **3** and amine **4** was achieved using dicyclohexyl carbodiimide, hydroxy benzotriazole in methylenechloride to give **14** in 60% yield. Deprotection of acetonide group under acidic conditions (80% AcOH) gave the triol **15**. Finally, debenzylation of the triol **15** yielded the target compound **2** (Scheme 4).



Scheme 4

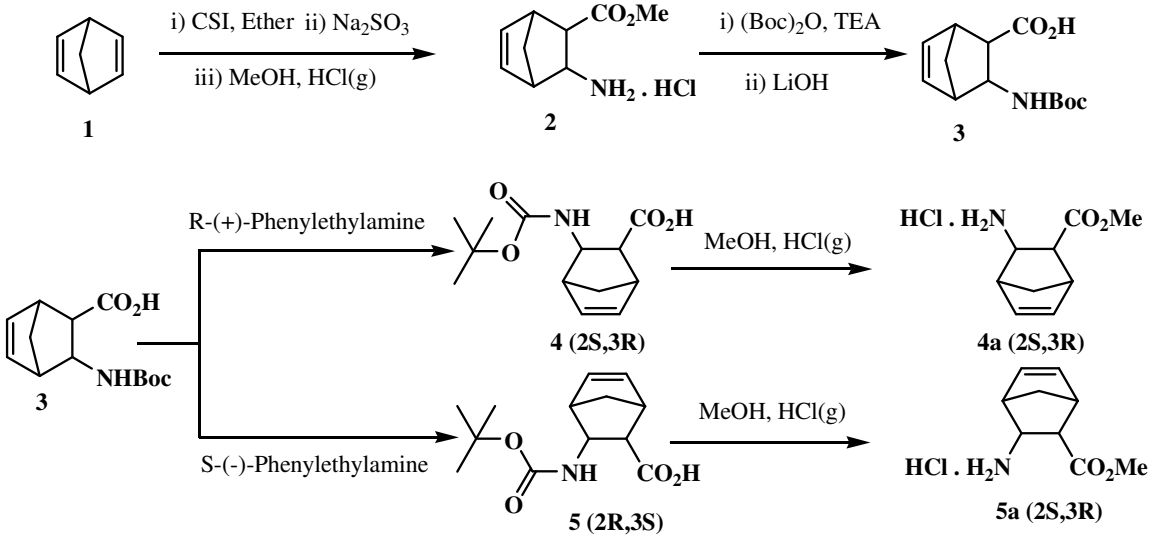
CHAPTER II: It deals with the stereo selective synthesis of peptides derived from norbornadiene: New surrogates for secondary structures on the peptide of *3-aminobicyclo[2.2.1]hept-5-ene-2-carboxylic acid* as monomer.

A template for the synthesis of parallel and antiparallel β-strands would be valuable in view of the role played by this secondary structure in the biological activity of proteins. It is well-known that proteases bind their substrates and inhibitors by generating β-strands or sheets, and the conformational requirement has been influential in the design of inhibitors of renin and of HIV-1 protease. Moreover, it has been reported that protein-DNA interactions can occur with the protein interface in a β-strand conformation. One of the important goals in peptide chemistry is to be able to design short peptides that can mimic some important aspect of protein structure or function. Recently our group has reported different helical structures with a new class of *cis*-furanoid β-sugar amino acid, which have been prepared from D-glucose. The present work focuses the synthesis of stereo-specific oligomers of bicyclic *exo-cis- β*-amino acids derived from norbornadiene and characterization of the secondary folding pattern is extensively studied by using NMR, MD and CD spectra.

Norbornadiene is a strained bicyclic compound used extensively in mechanistic organic chemistry and energy chemistry. However, this scaffold is not well utilized by bioorganic chemist. We have modified norbornadiene to an unusual amino acid (vide infra) and oligomerized to obtain homo hexamers and heteromers with other unusual amino acids. The secondary folding pattern is extensively studied by using modeling, NMR and CD spectra. The syntheses of enantiomerically pure **4** and **5** are based on the following sequence (Scheme 5): (i) preparation of the racemic amino acid **2** by dipolar cycloaddition of norborna 2,5- diene and chlorosulphonylisocyanate (ii) *N*-^{t}butyloxycarbonyl protected amino acid **3** (iii) separation of the enantiomers either by using optical resolution method using enatiomerically pure phenylethylamine as the chiral auxiliary or by induced crystallization (iv) separately esterification and *N*-Boc deprotection (v) oligomerization (Scheme 6).

2,2 Dipolar cycloaddition of norborna-2,5- diene and chlorosulphonylisocyanate gives tricyclic chlorosulphonyl derivative in ether at 0 ^{o}C. The chlorosulphonyl derivative was reduced with Na_2SO_3 and resulting azitidinone transformed into methyl ester of *cis*

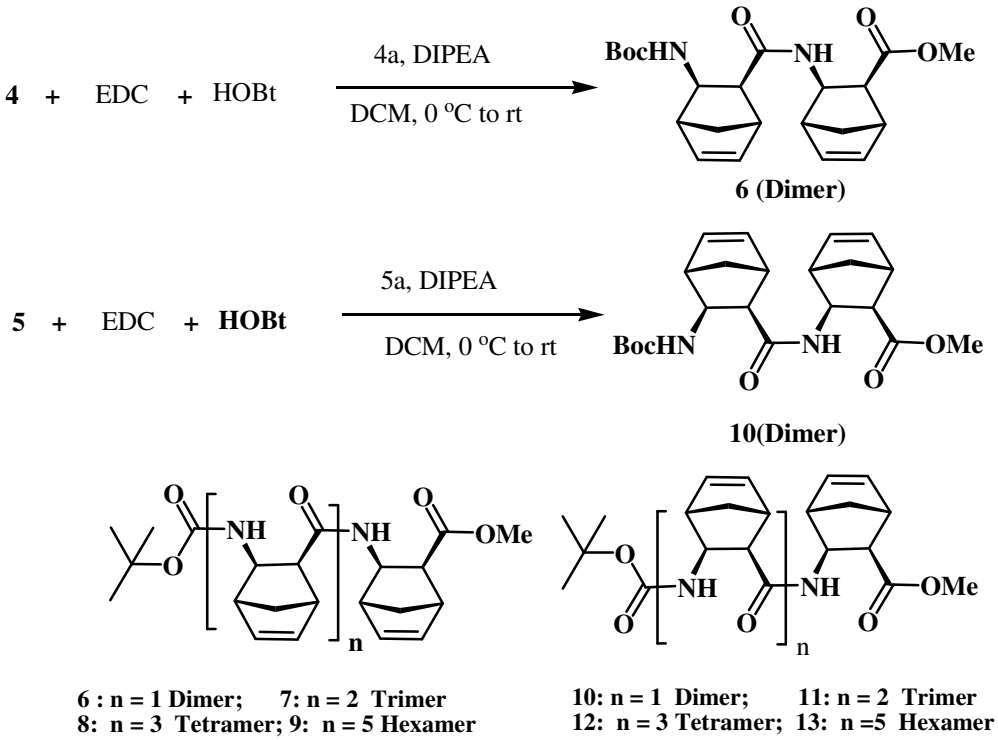
exo-amino carboxylate **2**. This was further converted into *N*-Boc protected acid **3**. Separation of enantiomers of *N*-Boc protected acid by fractional crystallization of diastereomeric salts that they form with enantiomerically pure phenylethylamine. Fractional crystallization of the amino acid derivative **3** and *S*(-) phenylethylamine was dissolved in ethylacetate and the solution was kept overnight at room temp. The resulting salt was filtered off and recrystalized from ethyl acetate. The fractional recrystalization procedure has been repeated until the value of optical rotation become constant. Treatment of finally obtained resolved salt with 2N HCl afforded *exo*-(1*S*,2*R*,3*S*,4*R*)-**4**. Similar method was used to obtain another isomer *exo*-(1*R*,2*S*,3*R*,4*S*)-**5**. Acids **4** and **5** were separately converted into methyl amino carboxylate hydrochloride salt **4a, 5a** respectively by treatment of HCl (gas) in MeOH (Scheme 1).



Scheme 5

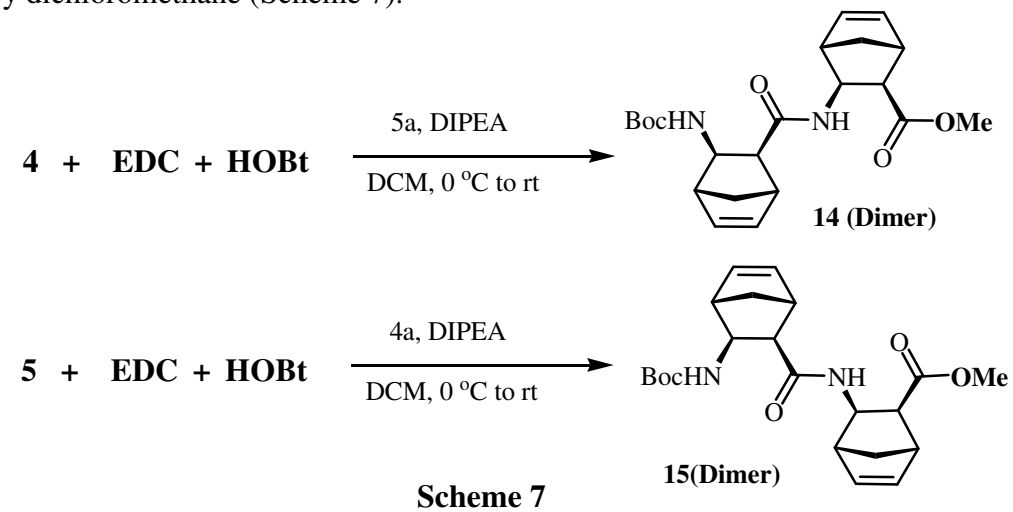
Having successfully synthesized new class of *β*-amino acid monomers, Norbornene amino acid residue, the attention was then focused to synthesize the new class of β-peptides. Hence, a new design to provide conformational freedom and steric strain, was envisaged based on the use of *cis,exo*-(2*S,*3*R*)-norborna-5-ene-amino acid and *cis exo*-(2*R*,3*S*)-amino acids with self and mixed, and studied the secondary structure pattern. In the synthesis of rigid bicyclo-β-peptides di-, tri-, tetra-, and hexa peptides were prepared with β-amino acid **4 / 4a** with **5 / 5a** using requisite sequence by adopting segment condensation method. Dipeptides were prepared by condensation of two

monomers. Tripeptides were prepared by coupling of dipeptide acid and monomer amine. Tetrapeptides were prepared by coupling of dipeptide acids and amines. Hexapeptides were synthesized by condensing tripeptide acid with tripeptide amine (Scheme 6).



Scheme 6

The synthesis of oligomers **14-20** involved peptidation of monomers **4/5a** and **5/4a** in the requisite sequence using standard coupling reagents EDCI, HOBt and DIPEA in dry dichloromethane (Scheme 7).



Scheme 7

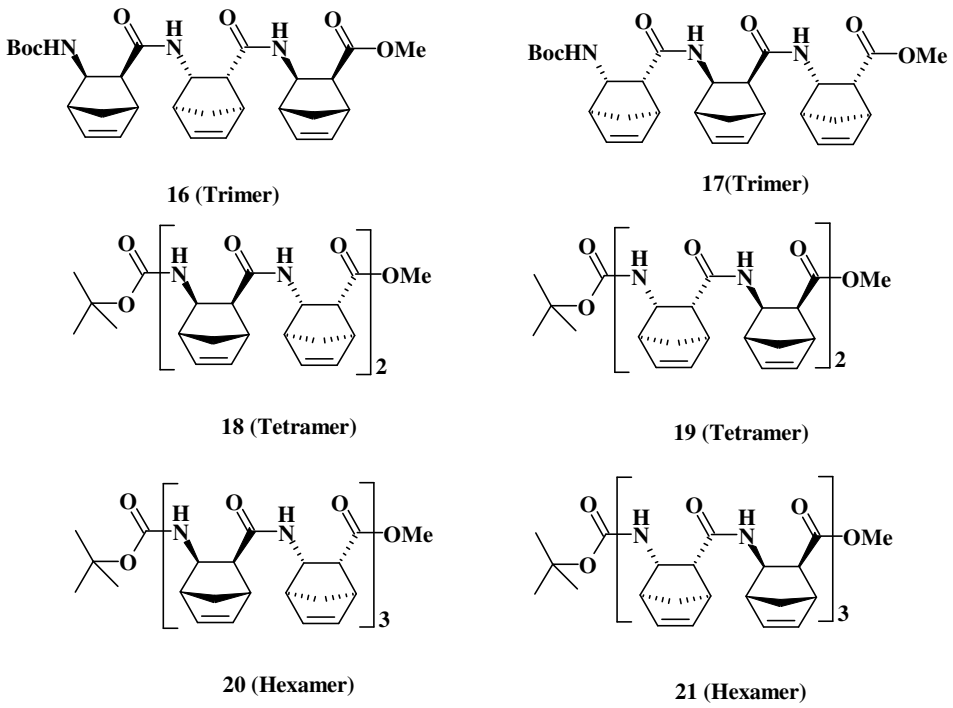


Fig 2

In summary, we have been synthesized novel β-peptides and carried out detailed characterization of β-strand mimetics derived from the oligomerization of *exo*-norborn-5-ene amino acid residues. In this series of peptides, β-strand structure was observed in the dipeptides **6** and **10**, tetrapeptides **8** and **12** and hexa peptides **9** and **13**. From the ^{1}H NMR, CD, molecular dynamics studies of peptides **6**, **8**, and **9** shows right-handed strand and left-handed strand formation in the oligomers of **7**, **12**, and **13**. Furthermore, we synthesized another series of mixed β-peptides using both the enantiomers of *exo*-norborna-5-ene residue by usual coupling reaction. Mixed peptides of **14**, **16**, **18**, and **20** were synthesized by using mononer acid **4**, amine **5a** and another series of mixed β-peptides **15**, **17**, **19** and **21** synthesized by using mononer acid **5**, amine **4a** (Fig 2)**.** Preliminary structural studies showed extended β-strand conformations and work in this direction is under progress. Applications of these peptides in chemical biology programme are currently underway.

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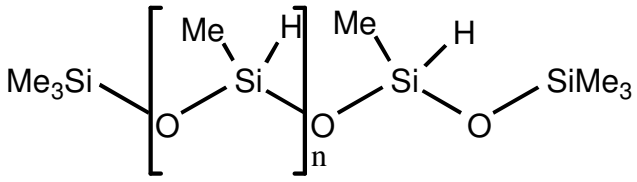
CHAPTER III: This chapter deals with development of new reduction procedures using polymethylhydrosiloxane (PMHS).



1

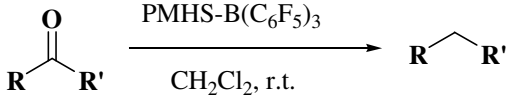
Reduction provides an important method for functional group inter conversion in organic synthesis. The development of cleaner and safe synthetic methods and technologies for reduction to meet ever-stricter environmental regulations remains an active area of organic chemical research. Silicon hydrides are an important class of reducing agents, reasonably stable under normal conditions, requiring activation with transition metal complex, fluoride ion or Lewis acid. Polymethylhydrosiloxane (PMHS) **1** is such an attractive reducing reagent for environmentally benign reductive processes because it is inexpensive, non-toxic, and stable to air and moisture. This is in marked contrast to commonly used reducing agents such as lithium aluminium hydride, borane and hydrogen, which are all clearly hazardous. This chapter is further divided into two sections.

Section I: Tris(pentafluorophenyl) borane [$B(C_6F_5)_3$] as a catalytic activator for polymethylhydrosiloxane (PMHS).

$B(C_6F_5)_3$ has been used as a non-conventional Lewis acid catalyst to activate PMHS in wide variety of functional group transformations such as carbonyl to methylene and reductive etherification of carbonyl compounds with alkoxysilanes.

Defunctionalization of carbonyl group to methylene with polymethylhydrosiloxane-$B(C_6F_5)_3$: Defunctionalization of organic functional groups is an equally desirable achievement as compared to fictionalization. There is a great need to discover new methodologies for Defunctionalization especially for conversion of polyfunctional natural products to useful building blocks and bioactive molecules. A careful literature survey reveals that, there are only a very limited protocols available for removal of a certain functional group *viz.*, the carbonyl group can be defunctionalized to methylene group by Clemensen or Wolff-Kishner reduction both of which require very

drastic reaction conditions. All these while offering some advantages also suffer from disadvantages. Most of these methods are generally restricted to aromatic systems, are some times harsh and need pyrophoric hydride source for reduction, low yielding and require longer reaction hours with careful workup procedures for quenching the excess reagent. Others and we have been exploiting the usefulness of very safe and polymeric hydride source polymethylhydrosiloxane (PMHS), a co-product of the silicone industry as an excellent source of reduction of various organic functional groups. The quest to find newer activators for this rather inert polymer resulted in identifying tris (pentafluoro phenyl) borane as an excellent catalyst for vigorous activation of PMHS. $B(C_6F_5)_3$ is a non conventional and relatively unexplored Lewis acid. This combination of PMHS-$B(C_6F_5)_3$ is found to be a versatile carbonyl defunctionalization system with very short reaction times (Scheme 8).



R= alkyl, aryl; R'= H, alkyl, aryl

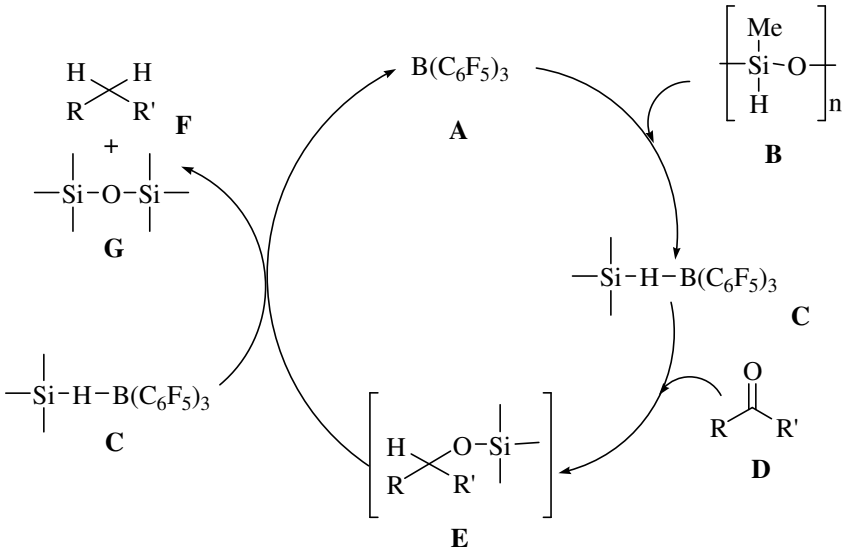
Scheme 8

Interestingly, this combination establishes a powerful "catalytic switch", *viz.*, our initial studies using $ZnCl_2$ as an activator resulted in reduction of ketone to alcohol whereas this new catalyst reduced the same substrate to methylene. The procedure is so simple that reaction monitoring is done only by observing the effervescence. Once the effervescence is seized indicates reaction completion (5 to 20 minutes).

In order to establish the optimum reaction conditions, the reaction was first studied on readily available benzophenone **2a** reduction to diphenyl methane **2b** in 88% isolated yield (entry 2) in 10 minutes of commencement of the reaction. Another substrate phenyl propanaldehyde **1a** was reduced to *n*-propyl benzene **2b** in 90% yield (entry 1) in almost 8 minutes. These two examples demonstrate that not only benzylic ketone (a very easily reducible carbonyl), but also the other extreme example aliphatic aldehyde succumbs to present protocol (Table 1).

We propose that the complex **C**, which is formed from $B(C_6F_5)_3$ **A** and PMHS **B** is responsible for the reduction of carbonyl functionality. The complex **C** would react

with carbonyl group **D** to form **E** (not isolated), which would produce the reaction product, hydrocarbon **F**, silyl ether **G** and would regenerate **A** (Scheme 9).

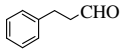
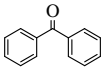
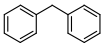
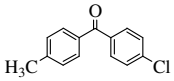
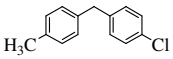
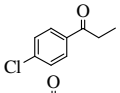
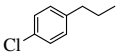
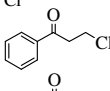
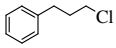
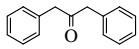
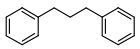
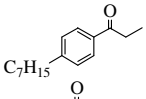
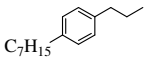
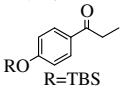
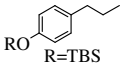
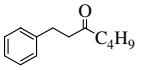
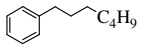
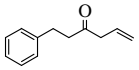
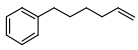
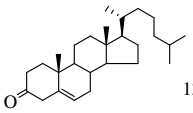
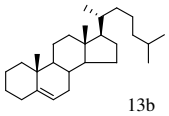
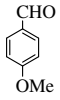
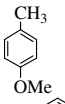
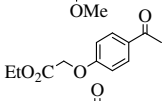
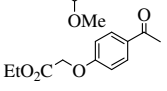
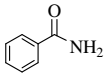


Scheme 9

There is literature precedence, that Ph_3SiH and Et_3SiH also operates in a more or less similar pathway, and the intermediate **E** is isolable when one equivalent of Ph_3SiH is used and longer reaction times (20 hours) where Et_3SiH is used. However in the case of PMHS, even though one equivalent of reagent is used, the intermediate **E** could not be isolated and instead 45-50% hydrocarbon conversion was observed within 5 to 20 minutes and remaining starting carbonyl was isolated. This clearly indicates that PMHS is a more powerful reducing agent than Ph_3SiH and Et_3SiH.

In conclusion, we have demonstrated for the first time, a direct rapid conversion of carbonyl to methylene under very mild conditions with high yields. The procedure is so simple that reaction can be monitored by visualization without any analytical support. The shorter reaction times in all the cases studied are an added advantage. It is a first report wherein non-conventional Lewis acid $(C_6F_5)_3B$ is utilized for activation of inert PMHS in reduction.

Table 1: Defunctionalization of carbonyl compounds

entry	substrate		product[a]		yield (%)[b]
1		1a		1b	90
2		2a		2b	88
3		3a		3b	85
4		4a		4b	83
5		5a		5b	84
6		6a		1b	86
7		7a		1b	89
8		8a		8b	88
9		9a		9b	82
10	 R=TBS R= THP	10a 10a'	 R=TBS R= THP	10b 10b'	90 84
11		11a		11b	90
12		12a		12b	88
13		13a		13b	87
14		14a		14b	65
15		15a		15b	82
16		16a	No Reaction		

[a]All the products were characterized by ^{1}H NMR and mass spectroscopy

[b]Isolated yields

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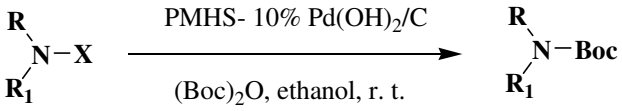
Section II: Paladium hydroxide as the catalytic activator for polymethylhydrosiloxane (PMHS).

PMHS and Paladium hydroxide combination has been used in widely for the safe and convenient reduction, deprotections and hydrogenation reactions. PMHS is a best hydrogen and hydride source in presence of Pd/C and $Pd(OH)_2$.

Single step conversion of N-benzyl, N-trityl and N-diphenylmethyl amines to t-butyl carbamates using polymethylhydrosiloxane

The protection, deprotection and interconversion of protecting groups forms an integral part of a multistep organic synthesis. A direct method for interconversion of one protecting group to other is often a much desired transformation as it is increases synthetic efficiency by avoiding the intermediate steps. Amines are found to be an important part of many bio-active natural products and they are useful intermediates both in protected as well as unprotected forms in basic as well as applied research. Among various N-protecting groups, tert-butyloxy carbonyl is one of the most commonly and frequently used protecting group due to its reasonable stability and ease of cleavage under mild conditions. Besides this, Boc protection in Taxol side chain (Taxotere ®) made it a block buster in cancer chemotherapy.

A literature search shows that, although, there are few examples of conversion of *N*-benzyl and *N*-trityl amines to tert-butyl carbamates. There are, to our knowledge, no report presenting the direct conversion of N-diphenylmethyl amines to t-butyl carbamates. Consequently, the development of new and general methods for these purposes being pursued. In our development of new reduction procedures for the synthesis of t-butyl carbamates, we are especially interested in exploring the potential use of polymethylhydrosiloxane (PMHS) as a versatile reductant in organic synthesis, which is gaining prominence as a safe and economic reagent in the recent times. In continuation of these studies, herein, we wish to report a new and efficient protocol for the one-pot reductive transformation of *N*-benzyl, *N*-trityl and *N*-diphenylmethyl amines to the corresponding t-butyl carbamates using polymethylhydrosiloxane and di tert-butyl dicarbonate in the presence of $Pd(OH)_2$/C catalysis (Scheme 10).



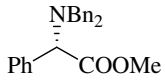
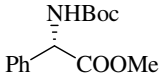
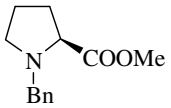
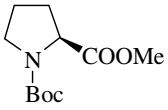
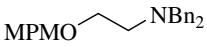
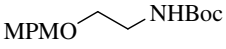
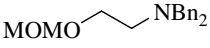
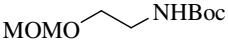
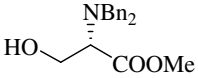
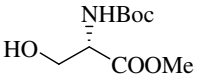
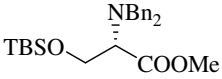
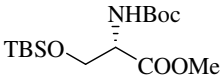
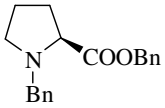
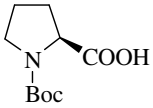
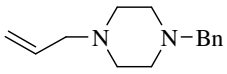
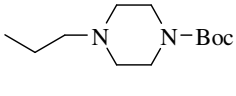
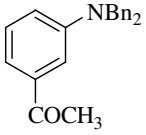
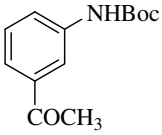
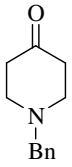
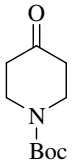
X= Bn or Tr or DPM **R, R_1= Aryl,alkyl,H**

Scheme 10

The scope and generality of this reagent system is illustrated with several examples and the results are summarized in Table 2 and Table 3. Initially, N, N-dibenzyl phenyl glycine methyl ester was treated with PMHS and $(Boc)_2O$ in the presence of $Pd(OH)_2/C$ in absolute ethanol at room temperature to afford the corresponding t-butyl carbamate in 90% isolated yield, with ester functionality being unaffected (entry 1, Table 2). Interestingly it was found that, the transformation proceeded smoothly without affecting much used hydroxyl protecting groups MPM, MOM and TBS Encouraged with these findings we have extended the reaction conditions to *N*-trityl and *N*-diphenylmethyl amine substrates and it was found that this protocol equally holds well with respect to these aliphatic as well as aromatic substrates(Table 3).

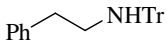
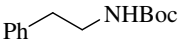
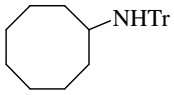
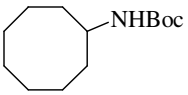
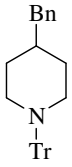
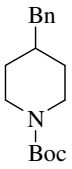
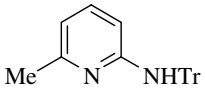
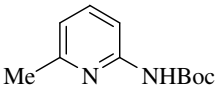
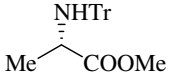
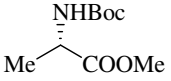
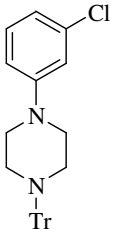
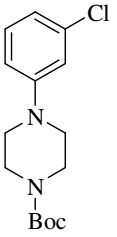
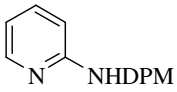
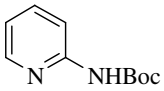
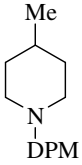
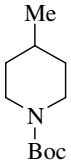
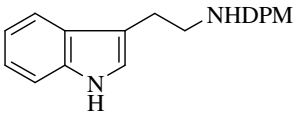
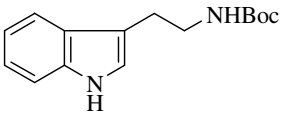
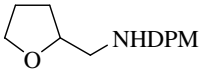
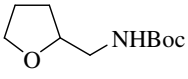
For the present work, it may be pertinent to state that, a new and convenient method for the direct conversion of *N*-benzyl, *N*-trityl and *N*-diphenylmethyl amines to more useful *t*-butyl carbamates. We firmly believe that the present protocol has potential utility in organic synthesis due to its chemoselectivity, efficiency, economy, simplicity and safety.

Table 2: Conversion of -NBn_2 to -NHBoc

entry	substrate	time (h)	product	yield (%)a
1		3		90
2		4		88
3		3		84
4		3		82
5		4		88
6		4		92
7		5		86
8		5		90
9		3		85
10		5		86

aAll the yields refer to pure isolated products, characterized by 1H NMR and mass spectroscopy.

Table 3: Conversion of -NHTr and -NHDPM to -NHBoc

entry	substrate	time (h)	product	yield (%)[a]
1		2		92
2		5		88
3		8		90
4		6		86
5		5		86
6		8		87
7		5		88
8		5		89
9		4		88
10		3		87

[a]All the yields refer to pure isolated products, characterized by ^{1}H NMR and mass spectroscopy.

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