THE PREPARATION OF NUCLEOSIDE-FUNCTIONALIZED SILICONE AND OLIGONUCLEOTIDE-SILICONE COPOLYMERS

By

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ABSTRACT

Attempts to prepare silicone oligonucleotide copolymers are complicated by the large difference in hydrophobicity in the two materials. Two approaches were followed to overcome this challenge. Initially, highly sterically hindered tetraisopropyldisiloxanes were used to bind 5′-O-(4,4′-dimethoxytrityl)-thymidine at the 5′-OH. These compounds proved to be hydrolytically more stable than the analogous dimethylsiloxane compounds, which were also prepared. Alternatively, Si-C bonds, which are hydrolytically stable, can be used to bind the two species together. Introduction of allyl ether by traditional Williamson conditions was followed by hydrosilylation with hydride terminated (Si-H) silicone, catalyzed by using platinum complexes, to give the nucleoside-functionalized silicone. We also introduced an epoxy group to one end of a silicone chain and found it to be stable to hydrolysis. Once the epoxy group binds nucleoside-functionalized silicone to solid phase, it is expected that the nucleoside-functionalized silicone *via* a trimethylene spacer linkage might be a starter for preparation of oligonucleotide-functionalized silicones in future work.

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Chapter 1

INTRODUCTION

1.1 General Introduction

1.1.1 Silicone Fluids (Polysiloxane)

Silicones represent a class of materials with widespread use, ease of manufacturability and good biocompatibility. The name "silicone" denotes a polymer where n is between 0-3 and m is 2 or larger.

$$[R_nSiO_{4-n}/_2]_m$$

The term "silicone" arose from very old work in which the investigator erroneously assumed that the bonding between oxygen and silicon in (Ph_2SiO) was a double bond similar to that in ketones. Actually, "polysiloxane" is the proper name for silicones.

The simplest silicones are polydimethylsiloxanes. The repeating unit of the polymer, [Me_2Si-O], the dimethylsiloxane unit, is referred to as a D unit. The terminal unit [Me_3SiO], the trimethylsiloxy group, is referred to as an M unit. A very common silicone structure may be described as MD_nM.

$MD_nM = (CH_3)_3SiO[(CH_3)_2SiO]_nSi(CH_3)_3$

1.1.2 Physical properties and application of silicone materials

Silicone fluids consist of a broad range of different materials with the following characteristics:

- Wide service temperature range

- Low viscosity change vs. temperature
- Thermal stability
- Low flammability
- Shear stability
- Dielectric stability
- High compressibility
- Chemical inertness
- Low surface tension
- Low toxicity

The intermolecular forces in the methylsilicones are usually weak. This property is most important for understanding of the technological behaviors of these materials, which include: the relatively low surface tensions of liquid polydimethylsiloxanes, which in general are about 20 dynes cm^{-1}; the small variations of the physical constants with temperature, particularly the viscosity of the polydimethylsiloxanes; the fact that even the highest molecular weight, linear polydimethylsiloxanes are liquids (up to molecular weights on the order of 500,000 - this is their most striking property); their remarkably low freezing and pour points; their high compressibility, and their spreading ability. These features have facilitated the adoption of silicones as dielectric, hydraulic, heat transfer, power transmission and damping fluids. They have also found application as additives into plastics and rubbers as process and release aids, into coatings for flow and level control and into process streams as antifoams. Other unique properties have led to

their introduction in acoustical applications such as ultrasonic sensor and sonar buoys. Light refractive and index matching properties have allowed the use of silicones in fiber optics and optoelectronics.

Silicones make good elastomers because the bonds between a silicon atom and the two oxygen atoms attached to it in the backbone chain are very flexible. Dynamically, the angle formed by these bonds change easily (Figure 1 - 1) without much trouble. Polydimethylsiloxanes (PDMS) have virtually no energy barrier for torsional rotation. Nuclear magnetic resonance observations by Rochow and Le Clair[1] indicate that the hydrogen atoms of the methyl groups possess an unusually high mobility down to very low temperatures. At about 77 K this mobility arises mainly from rotation around the Si-C bond. It therefore appears that the methyl groups have considerable space requirements because of their mobility, thus keeping the mean distances between the molecules large. This is simply another way of saying that the intermolecular forces in silicones are small, which results in the observed low glass-transition temperatures. The polar siloxane backbone is well shielded by inert methyl groups, and thus it is generally thought that silicones are chemically unreactive except in the presence of strong acids or bases.

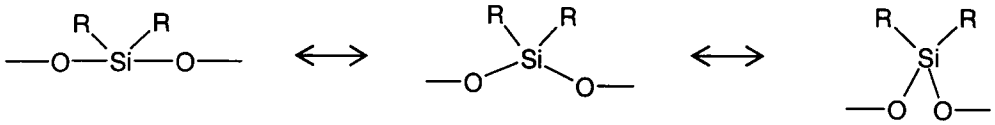


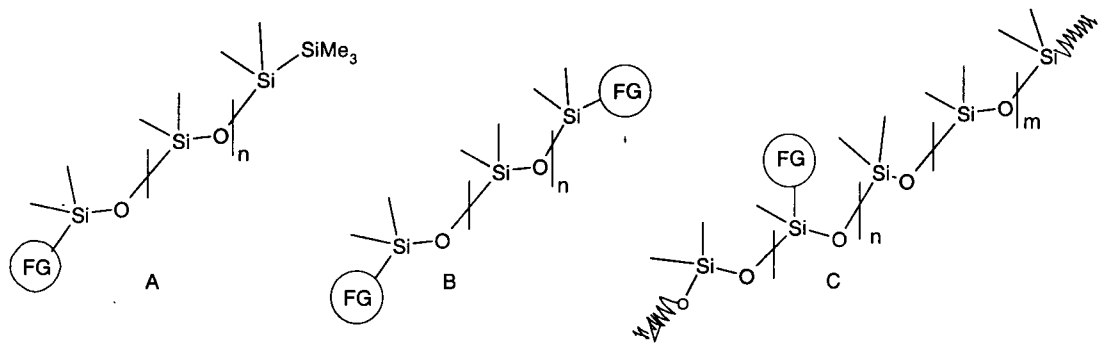
Figure 1 - 1: Dynamically, the angle formed by bonds between a silicon atom and the two oxygen atoms change easily.

The liquid surface tension of polydimethylsiloxane is lower than the critical surface tension of wetting (24 dynes/cm). This causes silicone polymers to spread over their own adsorbed films. Methylene chloride, chlorofluorocarbons, ethyl ether, xylene and methyl ethyl ketone are typical solvents for dimethylsiloxane.

1.1.3 Organofunctional siloxanes and their properties

Organofunctionalized siloxanes possess functional groups in lieu of a methyl group moiety. The feature common to all organofunctional systems is the presence of a functional group linked to the silicon through at least one carbon atom, although a 3 carbon spacer is more common. The groups normally used are classic organic functional groups exemplified by aromatic and non-aromatic unsaturated groups, halogens, hydroxyl, amino, nitro, and cyano groups.

Organosiloxanes were first prepared in commercial quantities in the early 1940s, after the discovery of the Mueller-Rochow-Process.[2] Approximately a decade later, with the advent of the transition metal-catalyzed hydrosilylation reaction,[3] organofunctional silanes became readily available. There are three general classes of functionalized silicones: those functionalized at one terminus or both termini, or as pendant groups along the chain (Figure 1 - 2).



FG = Functional Group

Figure 1 - 2: Silicone with functional termini M^X at one (A) or both (B) ends, or pendant along the chain D^X [X = H, – $CH{=}CH_2$, $-CH_2CH_2CH_2Cl$, $-CH_2CH_2CH_2OH$, – $CH_2CH_2CH_2CN$, – $CH_2CH_2CH_2NR_2$, etc.].

Although silicones containing simple functional groups are commercially available, there has been recent interest in incorporating more complex functional groups. For example, the introduction of a carbohydrate substituent into the nonpolar polysiloxane polymer matrix leads to a significant change in its solubility properties (Figure 1 - 3).[4,5] More complex, amines and imines of synthetic interest have been produced by hydrosilylation of C=N and C≡N bonds.[6] These polymers contain amine groups that more strongly adsorb onto a titania surface than the corresponding PDMS.[7]

Adding organic functional groups to siloxane polymers alters both the physical and chemical properties of the polymer. There is an arbitrary value assigned to organofunctional siloxanes to classify them into two groups; those containing less than

5mol% functional group incorporation, and those containing more than 5mol% functionality. The unique physical properties of these silicones have been described in detail.[8,9]

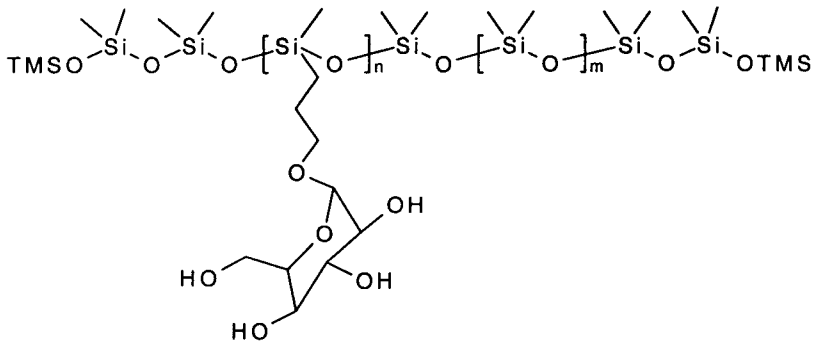


Figure 1 - 3: Carbohydrate modified silicones.

Interesting properties in silicone polymers arise when the intrinsic hydrophobic character of silicones is tempered by hydrophilic organic groups. The hydrophilicity of the polymers can be increased, depending on the degree of substitution, to a point where they can even become water soluble, in the extreme case.[6] The substitution can be through side chain functionalization or through the formation of siloxane copolymers[5] and networks.[10] The resulting properties of these polymers are mainly due to hydrogen bonding, both inter- and intramolecular.

1.1.4 Silicone rubbers and their properties

Silicone fluids are valuable materials. However, many applications call for materials that do not flow. Raw silicone rubber consists of non-crosslinked macromolecules in a

highly viscous liquid state; upon vulcanization it is converted into an elastic rubber. That is, to form coatings and elastomers, it is necessary to crosslink the silicones to give silicon resins and silicone rubber. A small amount of curing agent is required for the preparation of cross-linked or vulcanized silicones, which has the desired effect either during the polymerization or later, during the baking or vulcanization steps.

There are several possible routes to crosslink. The incorporation of tri- or tetrafunctional silanes that can react under ionic conditions, or the use of organic residues (methyl, vinyl, H groups) on the silicone polymers, can be used to form a network. *Hydrosilylation Cure* [11,12,13] is a very widely used process for the preparation of silicone elastomers. Two different, complementary polymers must be used: one containing Si-H groups, the other Si-CH=CH_2 groups. The resulting crosslinks (–Si–CH_2–CH_2–Si–) tie all the silicone chains together. Because they are tied covalently, essentially irreversibly, when the elastomers get hot, they can't flow past, nor around each other. So, these are thermoset materials, because all the polymer chains are covalently tied together. Crosslinking makes both elastomers and plastics stronger, but there is problem. Because crosslinked materials don't melt, it is very hard to recycle them. One answer to this problem is to create crosslinks that can be reversed or undone. We were interested in trying to exploit the twisted double strands of DNA as reversible crosslinking sites, via hydrogen bonds, in silicone polymers.

1.1.5 Nucleoside, Nucleotide and Oligonucleotide

A *nucleoside* consists of a nitrogenous base and a sugar (Figure 1 - 4). The sugar in a deoxyribonucleotide is deoxyribose. The nucleosides are *N*-glycosides in which the pentose C-1 atom is linked to the N-3 of purine or to the N-1 of pyrimidine. With reference to the pentose type involved, two different species of nucleosides are distinguished, *deoxyribonucleosides* containing 2-deoxyribose (DNA constituents), and *ribonucleosides* containing ribose (RNA constituents), with their respective nitrogenous bases:

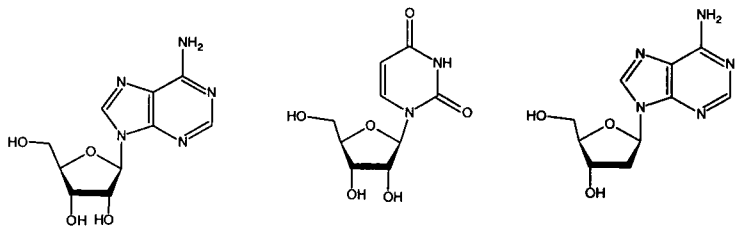
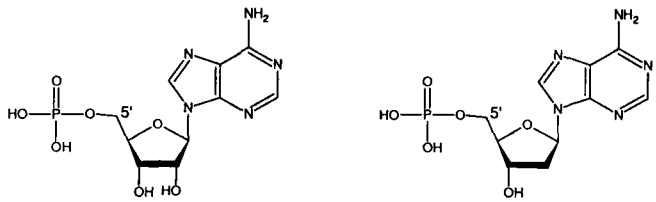


Figure 1 - 4: Nucleoside Structures.

Ribonucleosides	**Deoxyribonucleosides**
Adenine + ribose = adenosine	Adenine + deoxyribose = deoxyadenosine
Guanine + ribose = guanosine	Guanine + deoxyribose = deoxyguanosine
Cytosine + ribose = cytidine	Cytosine + deoxyribose = deoxycytidine
Uracil + ribose = uridine	Thymine + deoxyribose = deoxythymidine

Nucleotides are phosphoric acid esters of nucleosides (Figure 1 - 5). They are analogously divided into ribonucleotides, which contain ribose, and

deoxyribonucleotides, which contain 2′-deoxyribose. The phosphate group can add to the pentose ring at various positions: at positions 2′, 3′, and 5′ in ribonucleotides, and at positions 3′ and 5′ in deoxyribonucleotides.



Adenosine-5'-monophosphate

2'-deoxyadenosine-5'-mono-phosphate

Figure 1 - 5: Nucleotides are phosphoric acid ester of nucleosides.

DNA is a polymer of deoxyribonucleotide units. The primary structure of DNA and RNA is a linear polynucleotide chain made up of mononucleotides that are linked by 3′,5′-phosphodiester bonds. The assembly of both DNA and RNA primary structures follows the same basic principle (Figure 1 - 6): each pentose 3′-hydroxyl group of one mononucleotide is linked covalently to the pentose 5′-hydroxyl group of the neighbouring mononucleotide. Thus the name 3′, 5′-phosphodiester is derived: linear chains of DNA and RNA, whose lengths are determined by the number of constituent nucleotides, have two ends called the 3′ end and 5′ end. The chains of nucleic acids are polar and are directed either 5′ → 3′ or 3′ → 5′. The base sequence is written in the 5′→ 3′ direction.

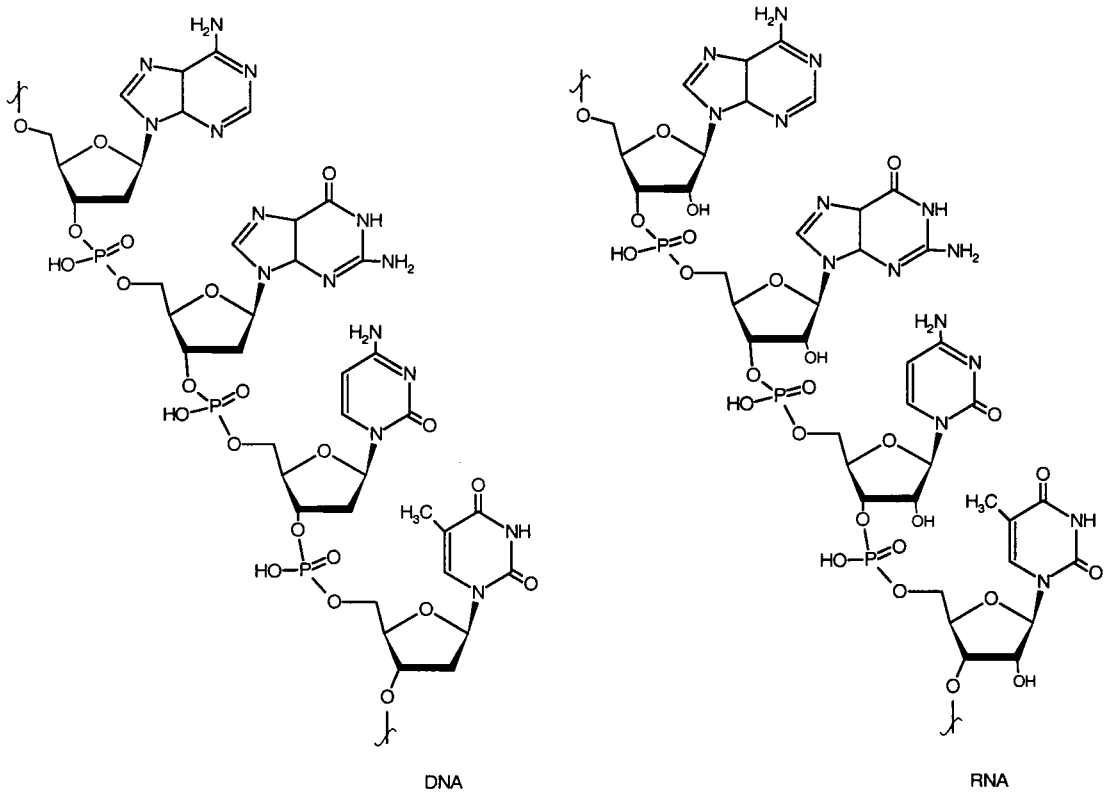


Figure 1 - 6: DNA and RNA primary structure.

1.1.6 The Watson-Crick DNA double helix

In 1953, James Watson and Francis Crick deduced the three-dimensional structure of DNA. Watson and Crick analyzed X-ray diffraction photographs of DNA fibers and derived a structural model. The important features of their model of DNA are:

a) Two helical polynucleotide chains coiled around a common axis, the chains run in opposite directions (Figure 1 - 7).

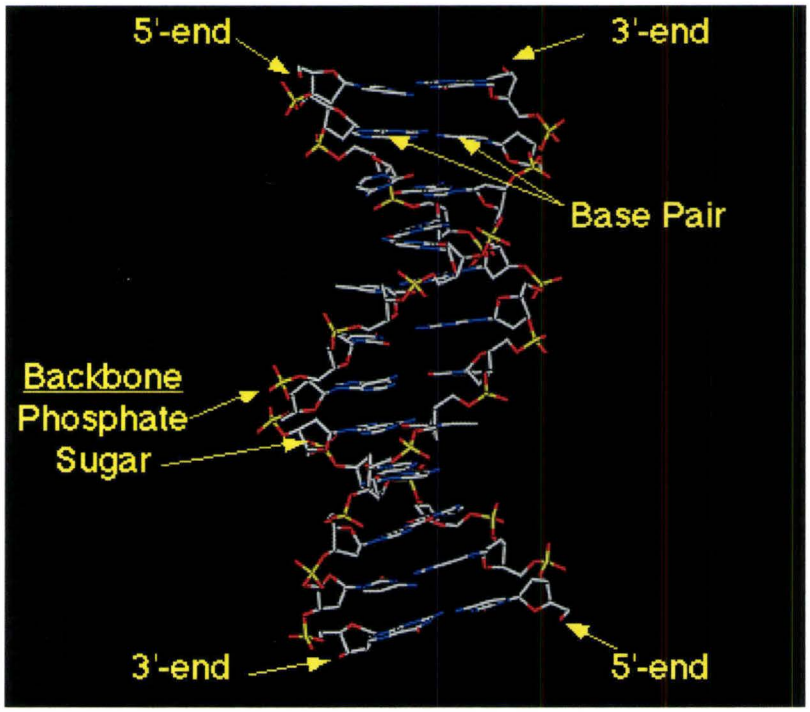


Figure 1 - 7: The Watson-Crick DNA double helix of DNA.

b) The purine and pyrimidine bases are on the inside of the helix, whereas the phosphate and deoxyribose units are on the outside. The planes of the bases are perpendicular to the helix axis.

c) The diameter of the helix is 20 Å. Adjacent bases are separated by 3.4 Å along the helix axis and related by a rotation of 36 degrees. Hence, the helical structure repeats after ten residues on each chain; that is, at intervals of 34 Å.

d) The two chains are held together by hydrogen bonds between pairs of bases. Adenine is always paired with thymine. Guanine is always paired with cytosine (Figure 1 - 8).

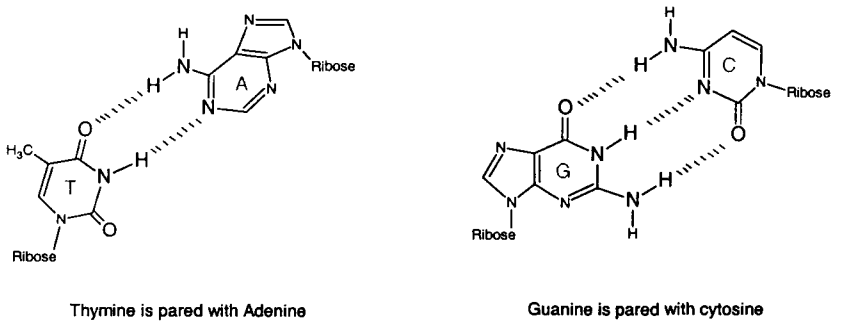


Figure 1 - 8: Watson and Crick base pairs.

The sequence of bases along a polynucleotide chain is not restricted in any way. The precise sequence of bases carries the genetic information. The most important aspect of the DNA double helix is the specificity of the pairing of bases. Watson and Crick deduced that adenine must pair with thymine, and guanine with cytosine, because of steric and hydrogen-bonding factors. The steric restriction is imposed by the regular helical nature of the sugar-phosphate backbone of each polynucleotide chain.

1.1.7 The reversibly melted double helix of DNA

The two strands of a DNA helix readily come apart when the hydrogen bonds between the paired bases are disrupted. This can be accomplished by heating a solution of DNA or by adding acid or alkali to ionize its bases. The unwinding of the double helix is called *melting* because it occurs abruptly at a certain temperature. The melting temperature (T_m) is defined as the temperature at which half of the helical structure is lost. The abruptness

of the transition indicates that the DNA double helix is a highly cooperative structure, held together by many reinforcing bonds; it is stabilized by the stacking of bases as well as by base pairing. The melting of DNA is readily monitored by measuring its absorbance of light at wavelength 260 nm. The unstacking of the base pairs results in increased absorbance, an effect called hyperchromism (Figure 1 - 9).

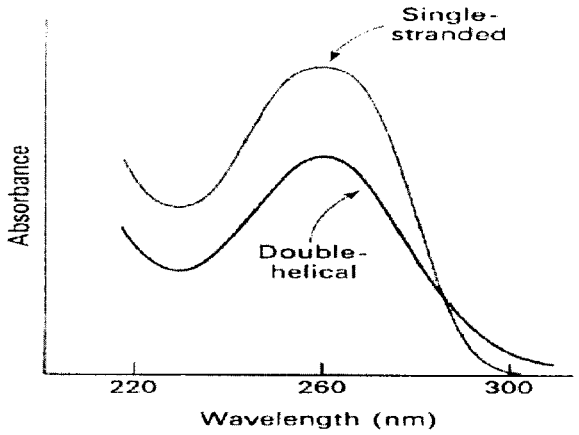


Figure 1 - 9: The absorbance of a DNA solution at wavelength 260 nm increases when the double helix is melted into single strands.

The melting temperature of a DNA molecule depends markedly on its base composition. DNA molecules rich in GC base pairs have higher T_m than those having an abundance of AT base pairs (Figure 1 - 10). GC base pairs are more stable than AT pairs because their bases are held together by three hydrogen bonds rather than by two. In addition, adjacent GC base pairs interact more strongly with one another than do adjacent AT base pairs. Hence, the AT-rich regions of DNA are the first to melt. The double helix

is melted *in vivo* by the action of specific proteins. Separated complementary strands of DNA spontaneously reassociate to form a double helix when the temperature is lowered below T_m. This renaturation process is sometimes called *annealing*. The facility with which double helices can be melted and then reassociated is crucial for the biological functions of DNA.

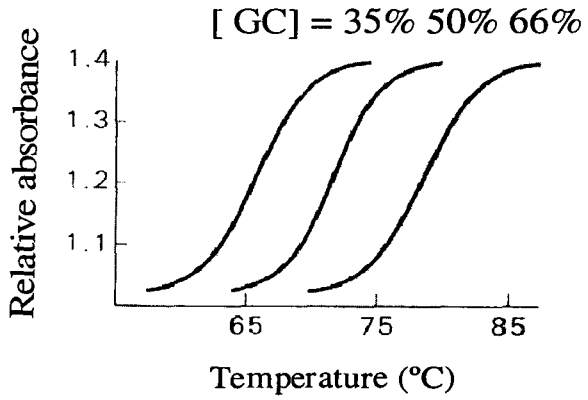


Figure 1 - 10: The absorbance is plotted against temperature (the wavelength of the incident light was 260 nm). The T_m is 69 °C for *E. coli* DNA (50% GC pairs) and 76 °C for *P. acruginosa* DNA (68% GC pairs).

1.1.8 Solid Phase DNA Synthesis

Natural DNA is an amphiphilic molecule, and is very soluble in water, mainly due to the presence of ionizable phosphate groups in the backbone and primary amino groups in cytosine, adenine and guanine bases. For chemical synthesis, however, it is generally necessary to use totally non-aqueous conditions, where natural DNA would be insoluble.

Furthermore, the presence of primary and secondary hydroxyl functions on the sugar moiety, the presence of primary amino groups on the base and the phosphodiester oxyanions, which are major targets for nucleophilic attack, would interfere with the process of forming an internucleotide linkage between a hydroxyl group of the deoxyribose of one nucleotide unit and the phosphate group in the other. In addition, the heterocyclic bases are prone to undergo a wide range of chemical reactions: the glycoside bond is readily hydrolyzed under acid conditions, and DNA is susceptible to attack by nucleases. All the nitrogen and oxygen atoms of the bases are potential sites for electrophilic attack, the N-7 position of purines being particularly vulnerable. Protonation occurs easily and leads to depurination, even more so when adenine and guanine are acyl protected.[14] It is clear, therefore, that successful chemical DNA synthesis can only be achieved if effective protection strategies are found for those functional groups that are not involved in condensations.

In 1965 Khorana introduced the phosphodiester approach for internucleotide bond formation, which was to dominate the field for twenty years. Oligonucleotide synthesis is a basic technique in molecular biology today. It finds application in almost all the biomedical sciences. The concept has four basic aspects.

The oligonucleotide is synthesized while attached covalently to a solid support. Excess soluble protected nucleotides and coupling reagent can drive a reaction near to completion.The reaction is carried out in a single reaction vessel to diminish mechanical losses due to solid support manipulation, allowing synthesis with minute quantities of

starting materials. The heterogeneous reactions are standardized, and these procedures are easily automated.

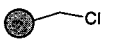
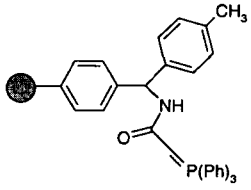
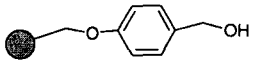
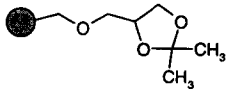
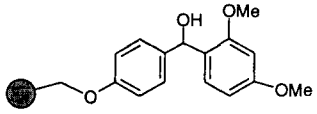
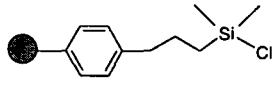
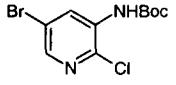
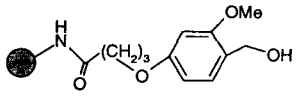
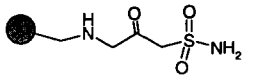
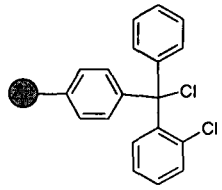
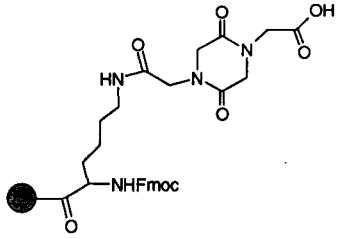
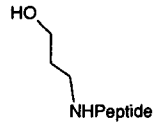
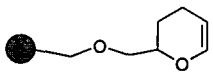
The most commonly used chemical route for solid-phase oligonucleotide synthesis is the phosphite triester method as modified by Beaucage and Caruthers.[15] The preparation begins with the linkage of the 3′-hydroxyl group of the first nucleotide to a solid support. Subsequently, the chain grows by nucleophilic attack of the 5′-hydroxyl of the immobilized oligonucleotide on the activated 3′- phosphate or phosphoramidite function of a soluble 5′-protected building block. The intermediate dinucleoside phosphite must be oxidized to the more stable phosphate before chain extension.

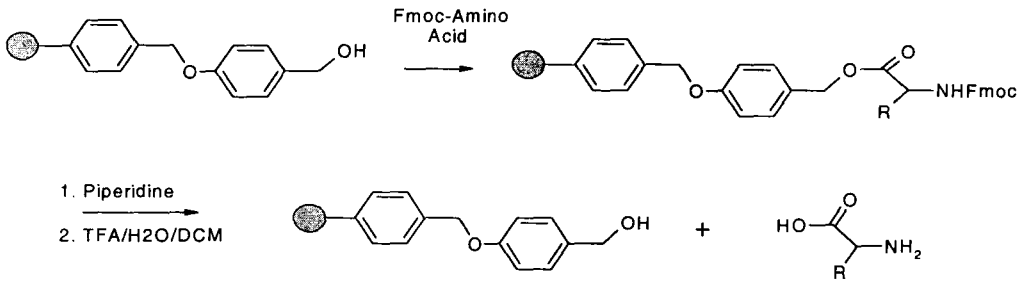
The nature of the solid support used is an important factor determining the choice of method. One type of support has been predominant over the last years -*Controlled Pore Glass (CPG)* - which consists of a glass matrix prepared uniformly with pores of defined size. New polystyrene-copolymer supports have been developed very recently and are available commercially.

One of the requirements of successful solid phase chemistry is a linker that attaches a substrate molecule to the solid support. There are many kinds of functionalized solid supports available (Table 1 - 1).[16,17] In the early days of solid phase synthesis, linkers provided nothing more than a means of immobilization and cleavage. In many ways, linkers bear many similarities to the protecting groups of solution phase chemistry and many of the early linkers were developed in analogy to these. For instance, one of the first linkers to emerge for the immobilization of carboxylic acids was based on the benzyl

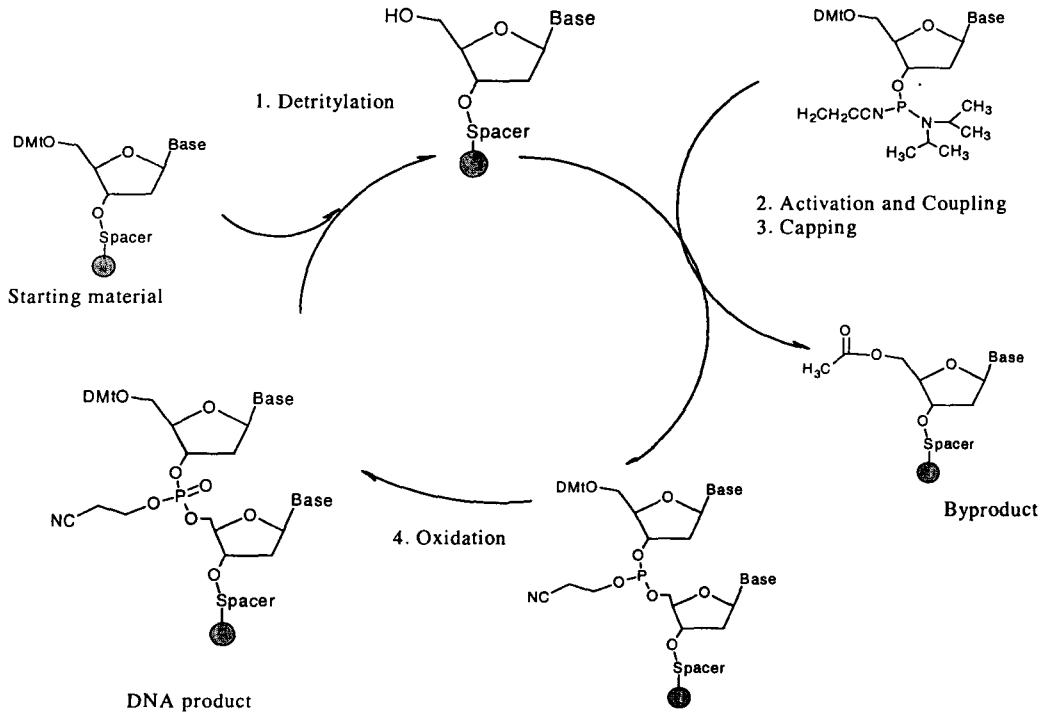
alcohol protecting group (Scheme 1 - 1).[17] Its ester linkage is typically cleaved by strong protic acid (e.g., trifluoroacetic acid).

Table 1 - 1: Functionalized Resins Showing Which Functional Groups are Conveniently Attached.

Functionalized/Linker-Derivatized Resins	Attached Group	Functionalized/Linker-Derivatized Resins	Attached Group
	RCOOH		RCHO
	RCOOH ROH		RCHO
	RCOOH		
	RCOOH		RCOOH
	ROH		
	ROH		



Scheme 1 - 1: Immobilization of carboxylic acids on solid phase.



Scheme 1 - 2: Solid-phase oligonucleotide synthesis according to the phosphite triester method.

The Phosphite-Triester Approach for Oligonucleotide Synthesis involves several steps (Scheme 1 - 2):

1) The Detritylation Step
2) The Coupling Step
3) The Capping Step
4) The Oxidation Step
5) The Final Deprotection and Cleavage Step

1.2 Reactions of the organic groups in silicone and polymer chemistry

1.2.1 Comparison of Si–X with C–X bond: Si-H vs C-H, and Si-C vs C-C

A comparison of Si–X and C–X bond strengths shows the two elements (Si and C) are quite different (Table 1 – 2).[18] Although bonds between silicon and the halogens or oxygen are exceptionally strong, the great reactivity of Si-halogen bonds is very noticeable, particularly in comparison with C-halogen bonds. This emerges from a comparison between the absence of reaction of water with CCl_4 and the strong and rapid reaction of water with $SiCl_4$.

To understand the unusual reactivity of Si-H bonds, one must compare the bond strength and polarity of the Si-H bond and the relative electronegativity of these elements with those of the carbon analogues. The bond energy of Si-H (Table 1 – 2, *ca.* 380 kJ/mol) is typically smaller than that of C-H (420 kJ/mol). The Si-H bond is much weaker and more reactive than would be expected, while the C-H bond is unusually strong. Since the electronegativity of hydrogen (2.20) is greater than that of silicon (1.74) and less than that of carbon (2.5), there is a reversal in the polarization of Si-H and C-H bonds.

$$Si^{\delta+} - H^{\delta-} \qquad C^{\delta-} - H^{\delta+}$$

In the Si-H bond, polarization makes the Si electron poor and the H electron rich. This results in the silicon atom being highly susceptible to nucleophilic attack. Another major difference between carbon and silicon, which allows for a very reactive Si-H bond, is the ability of Si to form hypervalent species. These compounds have a distinctive reactivity.[19,21]

Table 1 - 2: Approximate Bond Dissociation Energies and Bond Lengths for Si – X and C – X.

Bond	Compound	Bond energy (kJ mol^{-1})	Bond length (Å)	Bond	Bond energy (kJ mol^{-1})	Bond length (Å)
Si – C	$H_3Si–CH_3$	369	1.87	C – C	334	1.53
	$H_3Si–CMe_3$	376				
Si - H	$H_3Si–H$	378	1.48	C - H	420	1.09
	$Me_3Si–H$	378				
	$C_6H_5H_2Si–H$	369				
	$Cl_3Si–H$	382				
	$F_3Si–H$	419				
	$H_3SiH_2Si–H$	361	2.34	C – C	334	1.53
	$(Me_3Si)_3Si–H$	331				
Si – O	$Me_3Si–OH$	536	1.63	C – O	340	1.41
	$Me_3Si–OMe$	477				
	$Me_3Si–OEt$	484				
	$Me_3Si–OSiMe_3$	549				
Si – F	$Me_3Si–F$	665	1.6	C – F	452	1.39
	$H_3Si–F$	628				
	$F_3Si–F$	694		$F_3C – F$	544	
Si – Cl	$Me_3Si–Cl$	472	2.05	C – Cl	335	1.78
	$H_3Si–Cl$	451				
	$Cl_3Si–Cl$	449		$Cl_3C – Cl$	297	
Si – Br	$Me_3Si–Br$	402	2.21	C – Br	268	1.94
	$H_3Si–Br$	370				
	$Cl_3Si–Br$	368				
Si – I	$Me_3Si–I$	321	2.44	C – I	213	2.14
	$H_3Si–I$	321				
	$Cl_3Si–I$	276				

The Si-C bond is almost always polarized with the partial positive charge on the Si atom. Depending on the substituent groups on the silicon and carbon, the polarization

may be enhanced or weakened. Positively charged groups on the carbon (especially hydrogen) and negatively charged groups (especially oxygen) on the silicon decrease the bond polarization and stabilize the bond. Negatively polarized groups on the carbon substituent decrease the Si-C bond strength. For example, the F_3C and F_2HC groups can be removed from silicon even by cold water hydrolysis.[20] The silicon-carbon bond is, thermodynamically, nearly as strong as a single C-C bond. However, heterolytic fission of the Si-C bond usually occurs more readily than that of a C-C bond because of the greater ionic character of the former. Cleavage can be achieved by a nucleophilic attack on silicon, electrophilic attack on carbon, or a more or less concerted action of both types of attack. Radical reactions at silicon are much rarer than heterolytic cleavage reactions. We wish to exploit the reactivity of the Si-H bond where possible in our synthetic approach. We are also interested in the hydrolytic stability of Si-C bond in our study.

1.2.2 Reactions of silicon-halogen bond with compounds containing the hydroxyl group

The formation of Si-O bonds can be obtained by the reaction of chloro-terminated polysiloxanes with alcohols (eq. 1-1).

$$\equiv SiCl + ROH \rightleftharpoons \equiv SiOR + HCl \qquad (1\text{-}1)$$

The driving force behind these reactions is the energy gain that accompanies the change from Si-X to Si-O. Since the Si-O bond is much more stable than the Si-Cl bond, reactions often result in the formation of Si-O bonds. The equilibrium can be converted

into a unidirectional reaction by removal of the hydrogen halide formed.[21] This removal is more important in view of the fact that HX can react with the alcohol (eq. 1-2):

$$ROH + HCl \rightarrow RCl + H_2O \qquad (1\text{-}2)$$

The resulting water can hydrolyze the silicon halides and the silicon alkoxides to give silanols as by-products[22] (note that this process $MeOH + HCl \rightarrow MeCl + H_2O$ is used as a source of all methyl groups in silicones; MeCl is used in the Direct Process to convert Si metal into Me_2SiCl_2. Analogous reactions with phenols can be carried out without taking any special precautions, since hydrogen halides do not react with phenols. Pyridine accelerates the reaction.[23] The relative rates of the reactions between triisopropylchlorosilane and various alcohols are as follows:

isopropanol 1, ethanol 10^3, methanol 10^4

The influence of the structure of the silane on the reaction rate is shown by the fact that the rate of hydrolysis decreases along the series:[24]

$$SiCl_4 > RSiCl_3 >> R_2SiCl_2 > R_3SiCl$$

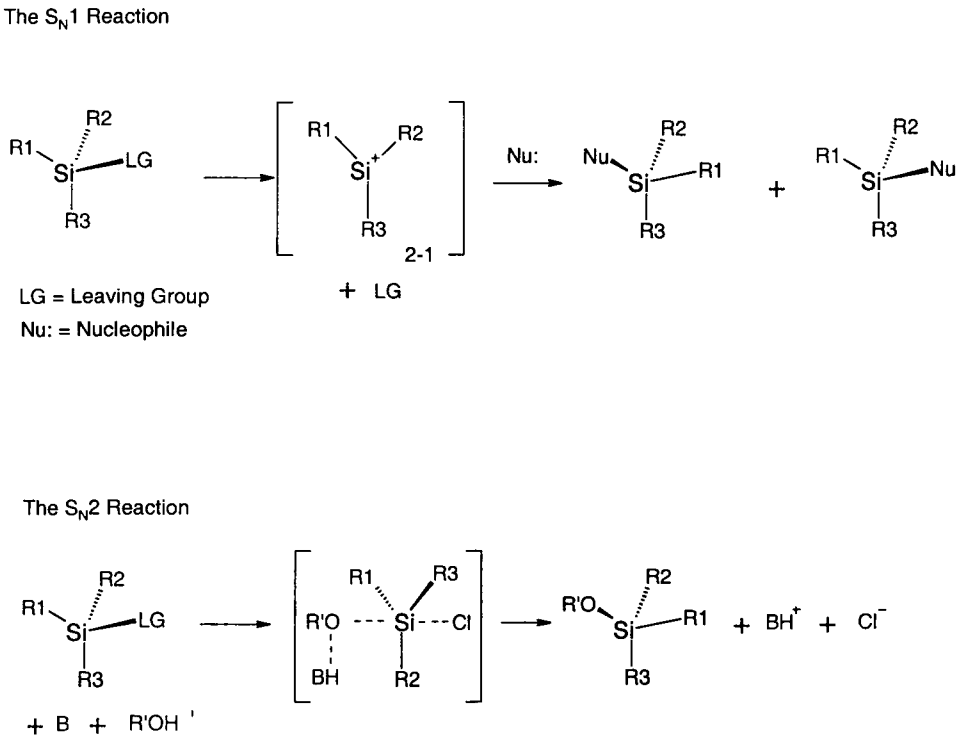
Electron-withdrawing substituents on the silicon atom increase the rate of substitution. Nucleophilic displacement of Si-Cl is one route by which silicone-nucleosides could be made.

1.2.3 Hypothesis for the nucleophilic substitution mechanism

The mechanism of nucleophilc substitution clearly involves a nucleophilic attack on the silicon (Scheme 1 - 3). The most common mechanism for such processes is S_N2; this

leads to inversion of configuration at silicon (although in this case of course the stereochemistry cannot be followed on this achiral silicon atom): where R´ is an organic group of hydrogen and B is a proton-acceptor such as the alcohol, water, or a base. The bonds to both the leaving group and incoming nucleophile are lengthened.[25,26,27]

Not all bimolecular reactions at silicon occur with inversion. Many retain the configuration at silicon, particularly if they involve a poor leaving group like hydride or alkoxide. The explanation proposed for the retention of configuration is that an intermediate is formed in which the leaving group is in an equatorial position. The transition state is set up by attack at the axial position of a trigonal bipyramid; Berri pseudorotation; and loss of the leaving group from the axial position to give net retention. Oxygen is powerful nucleophile for silicon, whereas nitrogen and organometallic carbon nucleophiles are less. So, S_N1 reactions at silicon are extremely rare, if they occur at all, largely because non-nucleophilic, non-basic polar solvents are used as the reaction medium and nucleophilic addition by solvent intervenes. Because the rate-determining step only involves bond cleavage, and thus has an intrinsically high barrier, the S_N1 reaction is generally observed only with carbon, not silicon atoms, and only when the intermediate (carbo) cation (2-1) is stabilized (tertiary, allylic, benzylic, etc).[28]



Scheme 1 - 3: The mechanism for nucleophilic substitution on silicon.

1.2.4 Reactivity of Si-H with compounds containing the hydroxyl group: formation of Si–O bonds

Nucleophilic attack by hydroxyl-containing compounds on the Si-H bond is favoured not only by the partial positive charge on the silicon, but also by the gain in energy (*ca.* 134 kJ/mol) that accompanies the change of Si-H to Si-O. The analogous attack on the C-H bond is not nearly as efficient for both steric and thermodynamic reasons.

The chemical reactivity of hydride-terminated silicones, or other compounds containing the Si-H functional group, makes it a useful entry point for organofunctional

silanes or silicones. A special feature of silicon hydrides is their ability to undergo alcoholysis leading to alkoxysiloxanes with the evolution of H_2 gas.

$$\equiv Si\text{-}H + HO\text{-}R \rightarrow \equiv Si\text{-}O\text{-}R + H_2 \qquad (1\text{-}3)$$

The rate of this reaction depends on the polarity of the Si-H bond, and the presence of either a nucleophilic or electrophilic catalyst.[29,30] Catalysts such as potassium and sodium hydroxides, organic bases, hydrogen chloride, metals, salts of metals and metal complexes have been used.[31,32] Only some hydrosilanes, such as monoorganosilanes[33,34,35] diorganosilanes[36,37] and arylfluorosilanes,[38] undergo dehydrocondensation with alcohols in the absence of catalysts. However, no reaction occurs when triethylsilane is boiled with anhydrous ethanol for 24 hours.[39] Reacting triethoxysilane and ethanol in a sealed tube at 100 °C during 125 hours brings about only a 50% conversion of triethoxysilane to tetraethoxysilane.[31]

The alcoholysis of hydrosilanes in the presence of alkaline catalysts has been studied in more detail than the reactions with other catalysts. However, it must be pointed out that KOH and NaOH-catalyzed solvolyses are frequently carried out in alcohol containing 5-7 vol % of water leading to the alcoholysis and hydrolysis of hydrosilanes simultaneously.[40,41,42,43,44] Numerous investigations have been dedicated to the kinetics of the alkaline solvolysis of hydrosilanes. As far back as 1947,[40] the effect of silane structure on the solvolysis of trialkylsilanes in 95% ethanol (catalyzed by KOH) was studied to give the following series of relative rates (Table 1 – 3).

Table 1 - 3: The relative rates for the reaction of trialkylsilane with ethyl alcohol.

	Et_3SiH	Et_2MeSiH	Me_2 *n*-PrSiH	Me *n*-Pr_2SiH	*n*-Pr_3SiH
$k\ (mol^{-1}S^{-1})$	100	169	391	115	44

A similar sequence was obtained for the reaction of trialkylsilanes with 94.5% ethyl alcohol in the presence of NaOH (Table 1 - 4).[42]

Table 1 - 4: The relative rates for the reaction of trialkylsilane with ethyl alcohol.

	Et_3SiH	*n*-Pr_3SiH	*n*-Bu_3SiH	*i*-Bu_3SiH	*i*-Pr_3SiH
$k\ (mol^{-1}S^{-1})$	100	40	27	4.1	1.6

This variation in the reaction rate constants is accounted for mainly by the steric effects of the substituents at the silicon atom.

A useful metal catalyst for the alcoholysis of hydrosilanes to alkoxysilanes should satisfy several criteria. The catalyst should be sufficiently active to dehydrogenatively couple even tertiary alcohols with the less reactive trialkylsilanes under mild, nonacidic and nonbasic conditions. This catalytic alcohol O-silylation activity should be selective in the presence of competing functional groups such as carbon-carbon multiple bonds and

carbonyl groups.[45,46] Further selectivity toward structurally different hydroxylic groups on the same molecule is also desirable. Finally, a useful hydrosilane alcoholysis catalyst should be easily accessible and moderate in cost.

Wilkinson's catalyst in combination with hydrosilane is a strong silylating agent for OH-groups,[47] hence it can be used for protection of OH-groups in terpenes, carbohydrates, etc. The reactivity of alcohols in the reaction with triethylsilane in the presence of catalyst, $Rh(PPh_3)_3Cl$, diminishes in the order:[46]

MeOH (100) > EtOH (64) > *i*-PrOH (11) > *t*-BuOH (0.8)

Dicobalt octacarbonyl $Co_2(CO)_8$, a new, more reactive catalyst, was proposed in 1970 for the silylation of alcohols with triethylsilane.[43] In its presence, the dehydrocondensation of triethylsilane with ethanol continues for several hours at 25 °C to yield 90% of triethylethoxysilane. $Co_2(CO)_8$ can be applied for selective silylation of alcohols. The reaction is described in eq. 1-4.

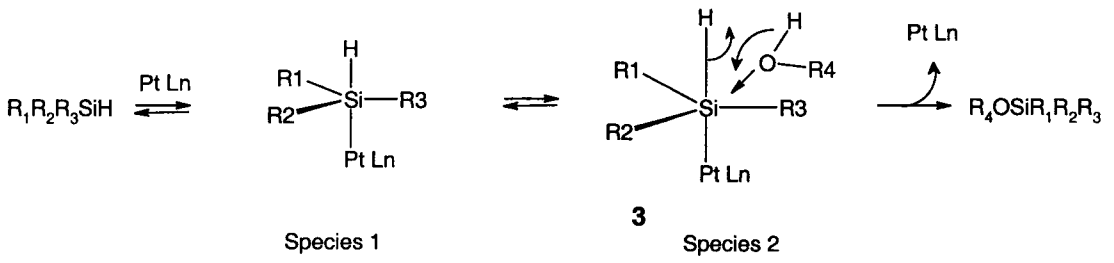
$$Co_2(CO)_8 + 2R_3SiH \rightarrow 2\,R_3SiCo(CO)_4 + H_2 \qquad (1\text{-}4)$$

The reaction of triethoxysilane with ethanol in the presence of $(Ph_3P)_3Co(N_2)H$ or $(Ph_3P)_3CoH_3$ proceeds during 2.5 hours with 80% yield of tetraethoxysilane.[44] With these two catalysts present, methanol reacts with triethoxysilane three times as fast as ethanol, whereas butanol fails to react.[48] Under the same conditions, triethylsilane does not react with alkanols, but the dehydrocondensation of polysiloxane $Me_3Si[OSi(H)Me]_n$ takes place easily. Many investigators[49,50,51,52,53] describe chloroplatinic acid ($H_2PtCl_6\ 6H_2O$) catalysis of dehydrocondensation. Despite the wide application of H_2PtCl_6 in synthetic

processes, the factors affecting these reactions are poorly understood. The kinetic study reported by Lukevics[53] is an exception, where the dehydrocondensation of triethylsilane with various alcohols was examined in the presence of $H_2PtCl_6 \cdot 6H_2O$ in different solvents. The iridium complex $IrCl(CO)L_2$ (L = $Ph_2P(CH_2)_2Si(OEt)_3$) has also been used as a heterogeneous catalyst in reactions of $HSiR_3$(R=Et, OEt) and $Me_3SiO[(Si(H)(Me)O]_nSiMe_3$ (PS 1, n = 1; PS 50, n = 50) with primary alcohols.[54]

1.2.5 Hypothesis for the Karstedt's-catalyzed Alcoholysis Mechanism

The alcoholysis reaction can be explained by a mechanism analogous to alkene hydrosilylation which involves, as the first step, activation of the Si-H bond.[55] In addition to this process, the reaction can be accelerated by direct coordination of the nucleophile to the silicon atom to give a more reactive pentacoordinate species 1 (Scheme 1 - 4).[56,57,58] This mechanism is consistent with the observations that a pentacoordinate silicon species is more reactive than a tetracoordinate silicon species and also that a pentacoordinate silicon species may undergo nucleophilic attack.[59]



Scheme 1 - 4: Proposed alcoholysis mechanism.

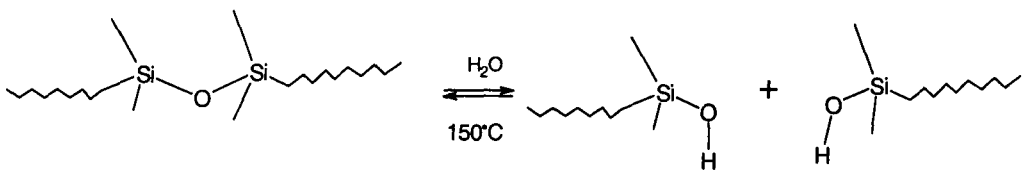
The alcoholysis of potassium hydridosilicates in the presence of 18-crown-6, giving another pentacoordinate species[60] also supports this mechanism very well since this reaction, a nucleophilic displacement at a pentacoordinate silicon species, would also reasonably be expected to take place through a hexacoordinate intermediate.

$$[HSi(OR)_4]^-K^+ + ROH + 18\text{-crown-6} \longrightarrow [Si(OR)_5]^-[K, 18\text{-crown-6}]^+ + H_2 \quad (1\text{-}5)$$

1.2.6 Stability of silicone backbone and the relative rate of Si-O cleavage

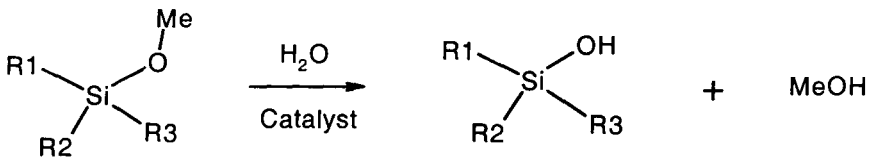
The existing literature data on the hydrolytic stability of the siloxanes shows that the cleavage of the siloxane bond by water is accelerated in the presence of alkali metal hydroxides[61] or acids[62] with a reaction rate constant $\geq 10^{-2}$ (mol^{-1} S^{-1}). Under the influence of acids or bases the hydrolysis of siloxane bonds proceeds easily.

Siloxanes are neither impervious to water vapor, nor completely stable to water. At higher temperatures (>150 °C) in the absence of catalysts, or more rapidly in the presence of acids or bases, silicone chains undergo hydrolytic scission, producing lower-molecular-weight, silanol-terminated polymers (Scheme 1 - 5).



Scheme 1 - 5: Under certain conditions silicone chains undergo hydrolytic scission.

Literature shows that the rate of nucleophilic attack (Scheme 1 - 6) at silicon decreases for steric reasons as the size of spectator groups increases. The rates of acid and base hydrolysis have been measured (Table 1 - 5).[63,64,65,66] The rate of cleavage of a TMS ether ($SiMe_3$) by solvolysis is about 10^2 faster than a TES ($SiEt_3$) and 10^4 faster than the analogous TBS ether ($SiMe_2t$-Bu). Apparently, steric factors suppress the cleavage process by increasing the difficulty with which chains approach each other. The larger the organic groups on the silicon, the more stable the silicone backbone is. The steric factor plays such an important role on the hydrolytic stability of Si-O bond that we consider it as a clue to make a more stable Si-O bond linkage between nucleosides and silicone portion.



Scheme 1 - 6: Nucleophilic attack at silicon atom.

Table 1 - 5: Relative Stabilities ($1/k_{rel}$) towards hydrolysis/relative rate of Si-O cleavage.

$R_1R_2R_3Si$	Acidic hydrolysis	Basic hydrolysis
Me_3Si	1	1
Et_3Si	64	1.3×10^3
t-$BuMe_2Si$	2×10^4	2×10^4
i-Pr_3Si	7×10^5	10^5
t-$BuPh_2Si$	5×10^6	10^3-10^4

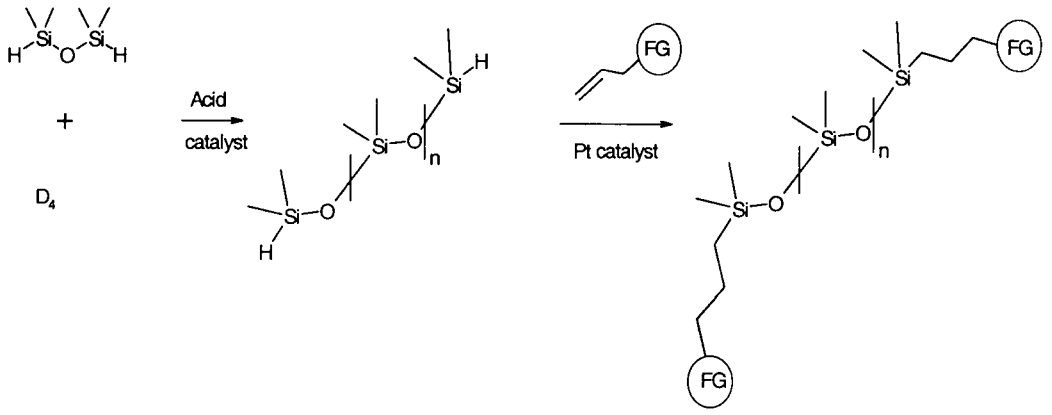
1.2.7 Reactivity of Si-H with unsaturated carbon-carbon bond: formation of Si–C bonds through hydrosilylation process

Hydrosilylation (eq. 1-5), by definition, is the addition of a Si-H bond to unsaturated bonds.[67] Normally, hydrosilylation generally refers to the facile addition of a hydrosilane to C=C or C≡C bonds. Hydrosilylation[3,68,69,70,71,72] takes advantage of the relatively weak Si-H bond.

$$R_3Si\text{-}H + H_2C = CHR' \rightarrow R_3Si - CH_2 - CH_2R' \qquad (1\text{-}6)$$

The process may be initiated by radical initiators, or, more frequently, transition metal catalysts. The soluble platinum complex catalysts are exceptionally efficient in the hydrosilylation reaction. One of the most commonly used catalysts for this purpose is Karstedt's catalyst $[Pt_2(H_2C{=}CHSiOSiCH{=}CH_2)_3]$.[73] Under certain conditions, some functional groups (OH, NH, etc.) are not compatible with hydrosilylation reactions. Plueddeman' s work showed that epoxide are compatible with hydrosilylation. If the platinum catalyst is carefully used during hydrosilylation, the epoxide group will not conduct the ring-opening polymerization.[74]

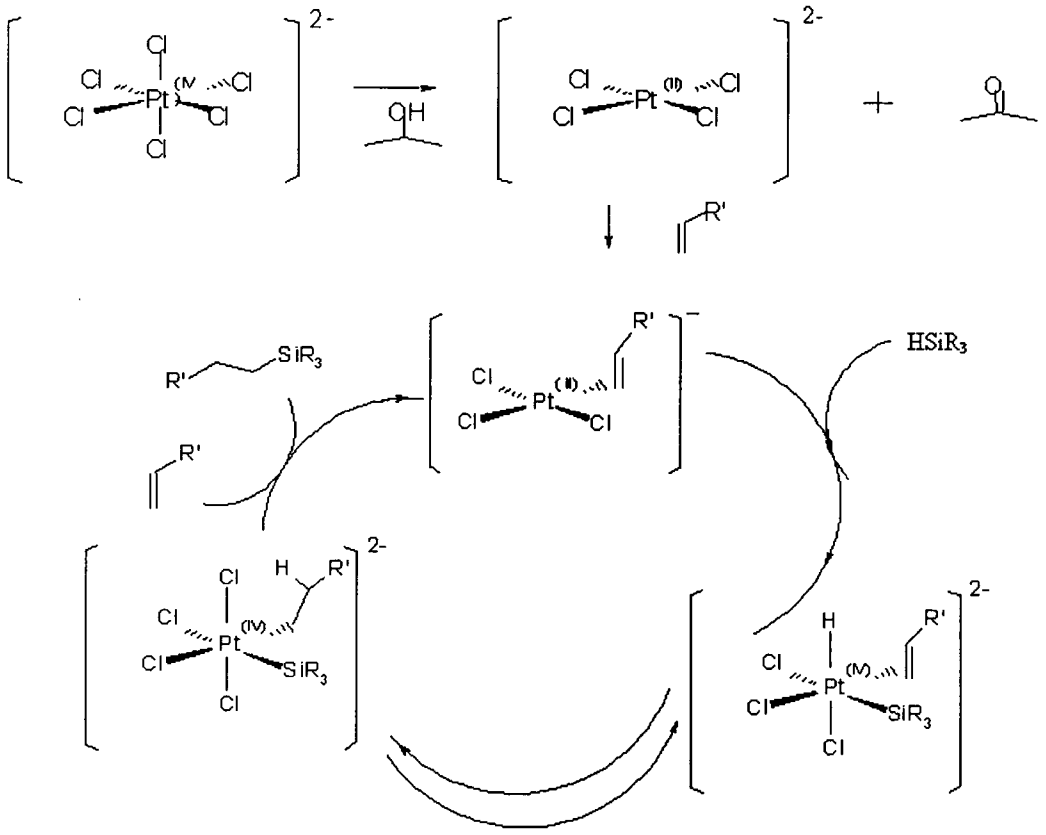
One important application of hydrosilylation of the Si-H group with allyl derivatives is to make a wide variety of functional silicones with special organic reactivity (Scheme 1 - 7).[75]



Scheme 1 - 7: The equilibration of cyclics (D_4 or D_3) with silicone and the addition of Si-H across a site of an unsaturation.

1.2.8 Mechanism of Hydrosilylation: Chalk-Harrod Mechanism

For many years the accepted mechanism has been the Chalk-Harrod mechanism (Scheme 1 - 8).[76] Starting from a Pt(II) species, it involves oxidative addition of Si-H; olefin insertion in the Pt-H bond; reductive elimination of the product; and regeneration of the catalyst.



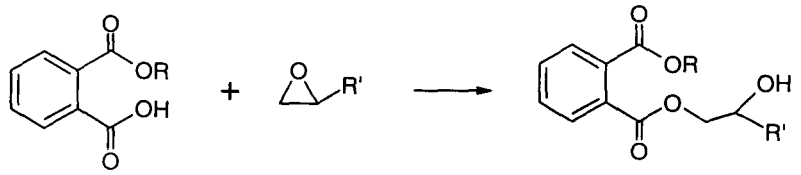
Scheme 1 - 8: Chalk-Harrod Hydrosilylation Mechanism.

1.2.9 Properties of the Epoxy group

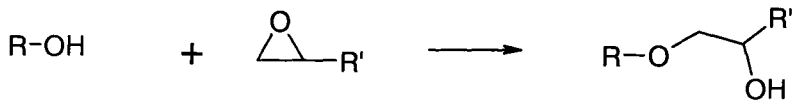
Epoxy groups can perform a variety of reactions in polymer chemistry. We are interested in those properties of the epoxy group as we may apply them to our project.

Epoxides are an unusual sub-class of ethers. While they contain the C–O–C unit, the 3-membered heterocyclic system is distinguished from other ethers by their special reactivity. Both C–O bonds are polar due to the high electronegativity of the O atom. The three membered ring is highly strained and thus the ring system is reactive to attack

that will relieve ring strain by opening the ring. Usually, weaker nucleophiles attack the C of the C–O bond causing the C–O bond to break (Scheme 1 - 9, and Scheme 1 - 10).[77,78]

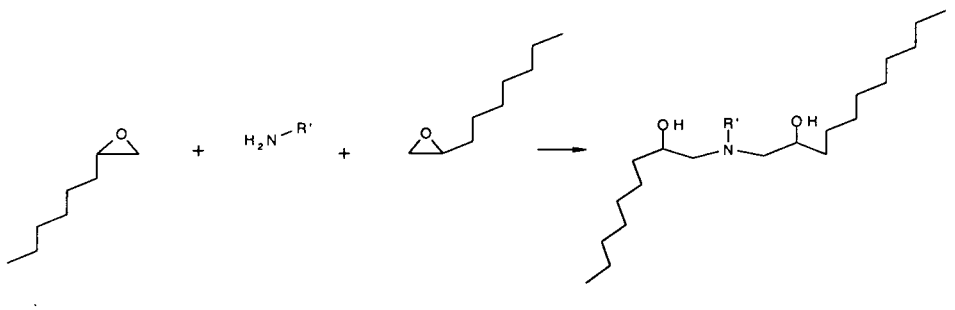


Scheme 1 - 9: Esterification of the epoxy group.

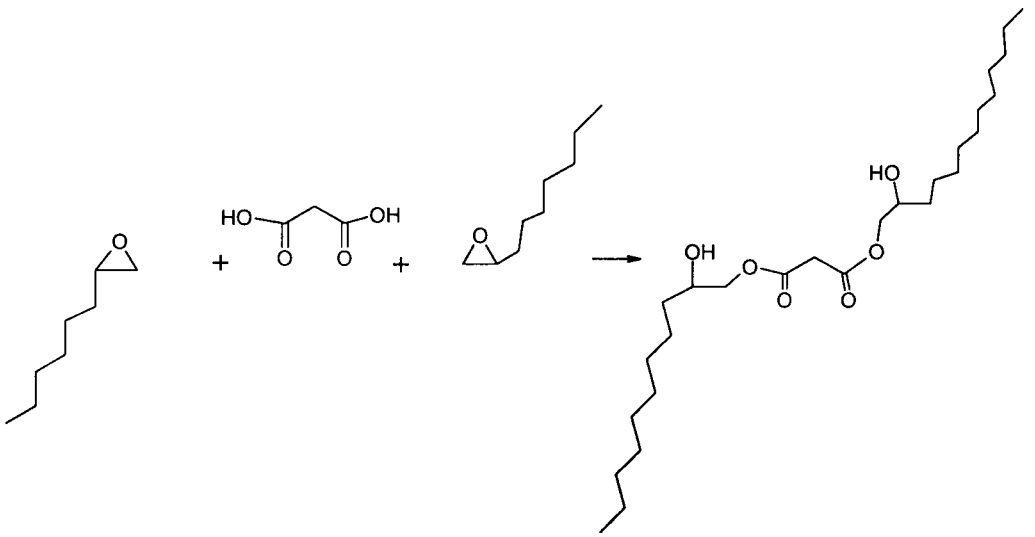


Scheme 1 - 10: Etherification of the epoxy group.

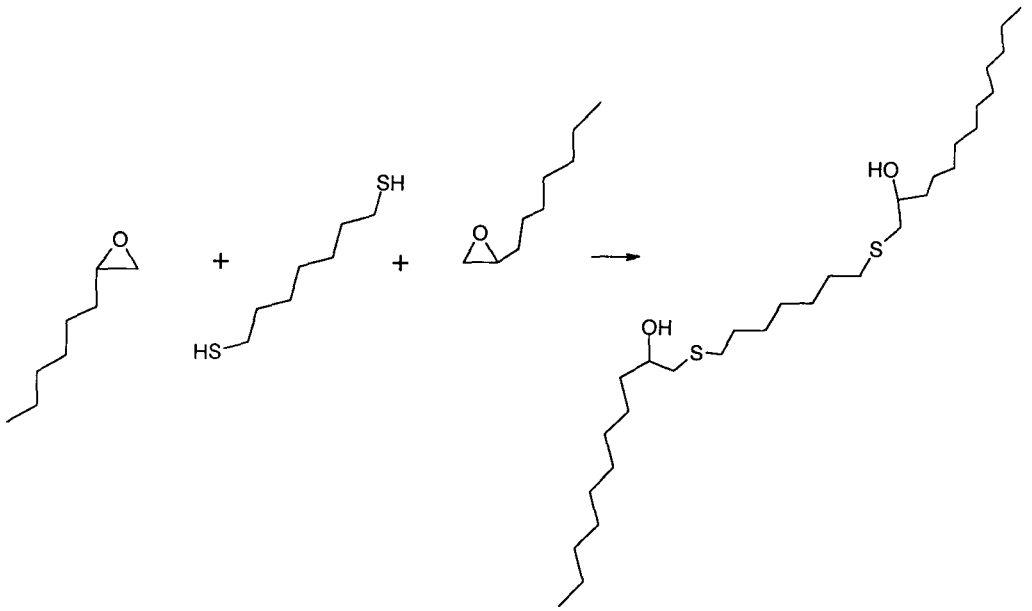
The epoxy functionality provides excellent synthetic flexibility and thus conveys significant freedom in polymer design. Cross-linking can be carried out by making use of the epoxy groups and nucleophiles including amines (Scheme 1 -11),[79] with acids (Scheme 1 - 12)[80] which link across the epoxy units and become part of the structure, or other bifunctional nucleophiles. In another polymer design, polysulfides (HS-R-SH with MW ~1000) can react with the epoxy group and act as a chain extender (Scheme 1 - 13).[81]



Scheme 1 -11: Crosslinking of epoxy functionalized polymer chains by amine.

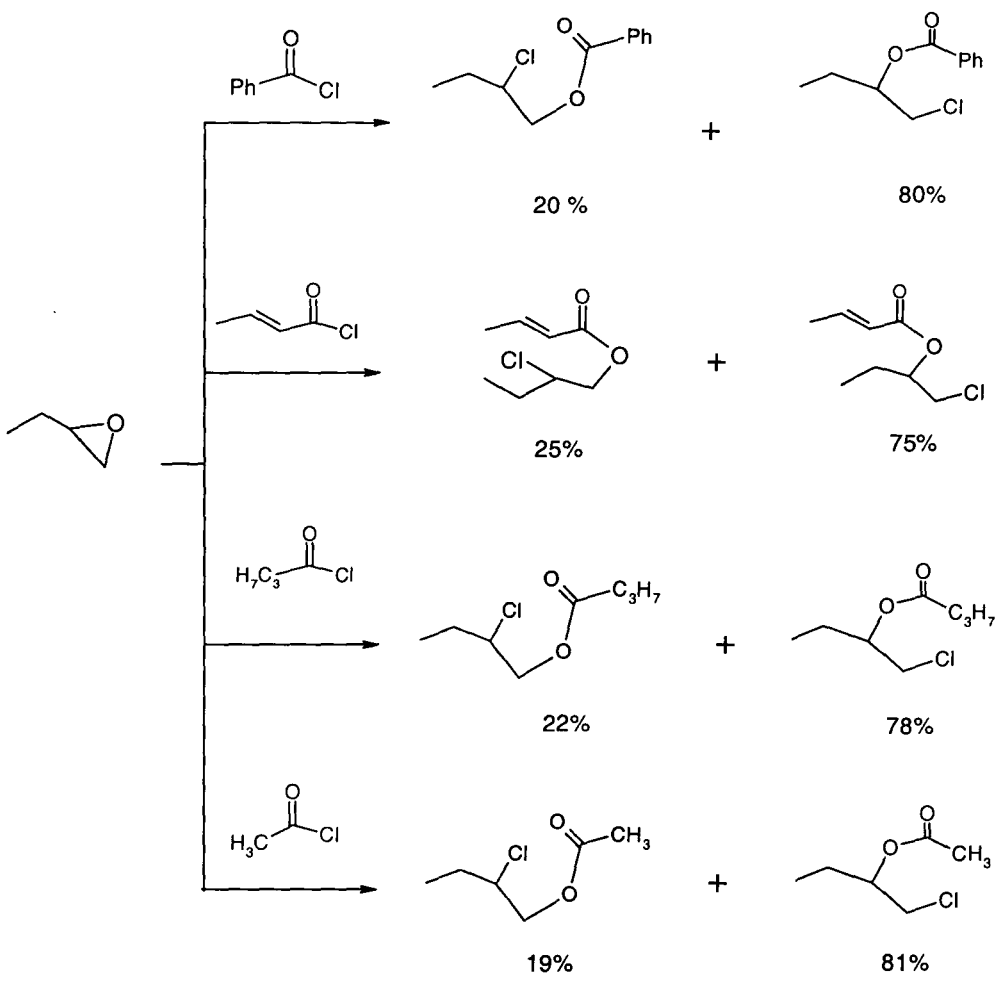


Scheme 1 - 12: Crosslinking of epoxy functionalized polymer chains by acid.

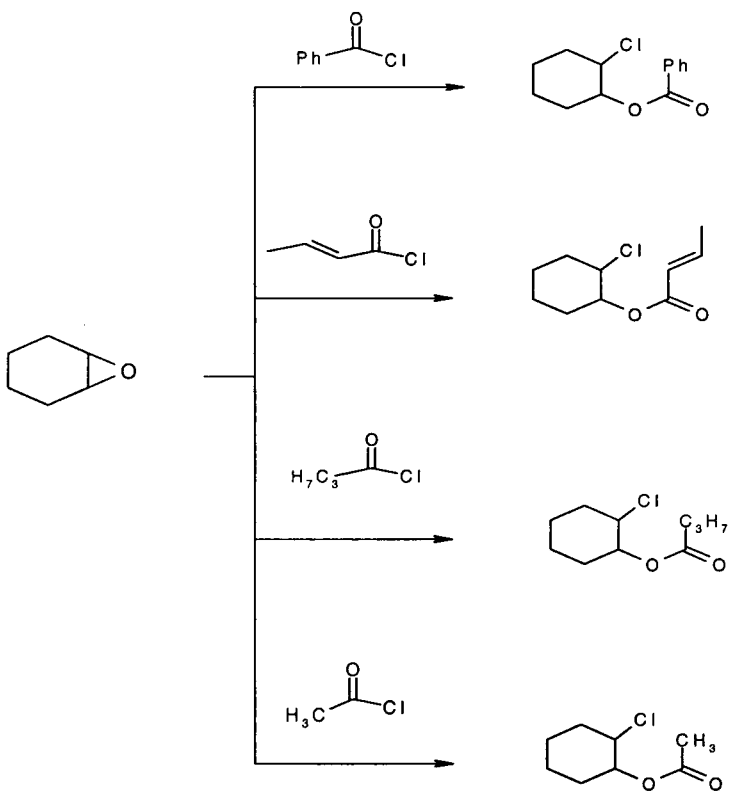


Scheme 1 - 13: Polysulphides react with epoxy functionalized polymer chairs.

The three membered epoxide rings can be cleaved with an acid chloride in the presence of catalytic cobalt (II) in acetonitrile at 30 °C (Scheme 1 - 14 and Scheme 1 -15).[82,83] These reactions are quite efficient, as a variety of oxiranes can be converted to the corresponding esters and chlorides in high yields under very mild conditions. Oxiranes can be efficiently cleaved in a highly regioselective manner to give the corresponding β-chloroesters in excellent yields. One regioisomer is obtained preferentially due to attack of chloride ion on the less substituted carbon atom, although 1,2-epoxybutane yields a mixture of both regioisomers (Scheme 1 - 14).[84] However, cyclohexene oxide gave only the trans-β-chloroester (Scheme 1 -15).[85]



Scheme 1 - 14: 1,2-epoxybutane yields a mixture of both regioisomers.



Scheme 1 -15: Cyclohexene oxide gave only the trans-β-chloroester.

1.3 Objectives of this study

In the area of medical products, the design of biocompatible polymers represents a major area of interest due to the potential broad application in both acute and chronic medical applications. In this study, we are interested in reactions of a range of nucleosides with polysiloxanes. The aim is to develop a kind of thermoplastic elastomer that contains a hydrophobic (siloxane) and a hydrophilic (oligonucleotide) portion as described in Figure 1 - 11. The oligonucleotide can provide reversible crosslinking to polysiloxane chains through hydrogen bonding.

The significance of this study is that the hydrophobicity of the silicone can be changed by the introduction of nucleosides. And, depending on the ratio of the siloxane and the oligonucleotide portions, the solubility in water or more weakly polar solvents, such as alcohols, can vary within wide limits. Finally, it is anticipated that the nucleoside functionalized silicones could be promising biomaterials because the biocompatibility of the nucleoside and oligonucleotide constituents has been proven.

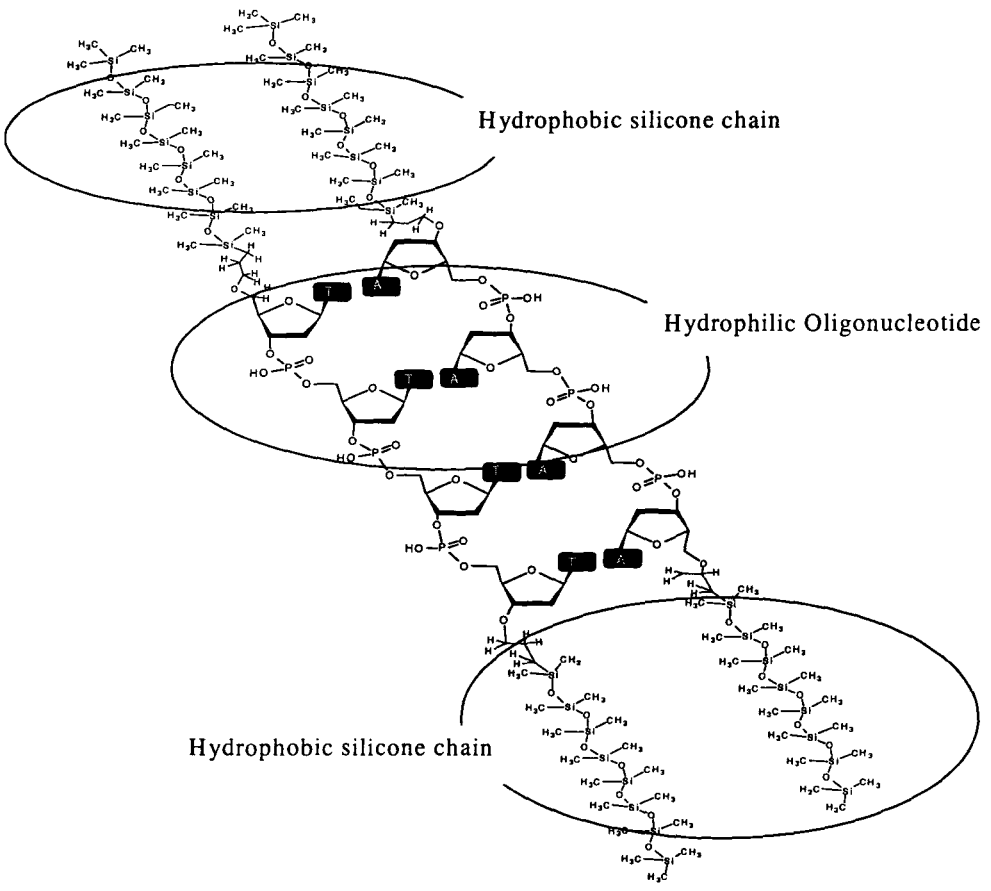
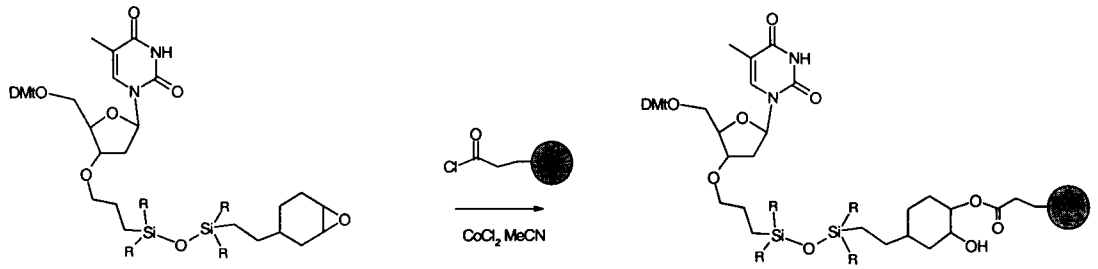


Figure 1 - 11: Design of *thermo-reversible* binding behaviour between oligonucleotide-Silicone Copolymer chains.

In this study, we explored several reactions that could be applied to the preparation of the designed nucleoside functionalized silicone and oligonucleotide silicone copolymers. The processes required to prepare this kind of copolymer are:

1) Bind the nucleoside to silicone directly, or through a three-carbon spacer. The linkage of the silicone and nucleoside will be achieved through a Si-O-C or a Si-C bridge. Si-O-C linkage could be formed, for example, through reactions of either chloride-terminated silicone or a hydride terminated silicon with 5′-O-(4,4′-dimethoxytrityl) thymidine. The Si-C linkage between the siloxane and the oligonucleotide could be formed through the hydrosilylation of Si-H bond with an allyl group that was previously bound to the nucleoside.
2) Introduce an epoxy group to one terminus of silicone. We expect the epoxy group can be used to attach the nucleoside-functionalized silicone to a solid support (Scheme 1 - 16). This would make it possible to perform Solid Phase Synthesis of an oligonucleotide using the nucleoside on the silicone as a starter group.



Scheme 1 - 16: Solid phase bound silicone through reaction of epoxy with acid chloride.

Chapter 2

RESULTS AND DISCUSSION

The objective of the research, as noted in Chapter 1, is to assemble silicone-nucleoside copolymers under conditions that could ultimately be exploited using standard solid phase synthesis. The experimental protocols that were developed thus first assessed the reactivity of candidate silicones under the nucleotide synthesis conditions, and then utilized the most promising candidates in the assembly of siloxane nucleosides.

Our initial experiments focused on the simplest synthetic steps using commercially available materials. As will be seen, the products did not have sufficient hydrolytic stability. Therefore, more bulky silanes were developed using Si-O linkages to graft the silicone to the nucleoside. Finally, we discuss the use of more stable Si-C linkers.

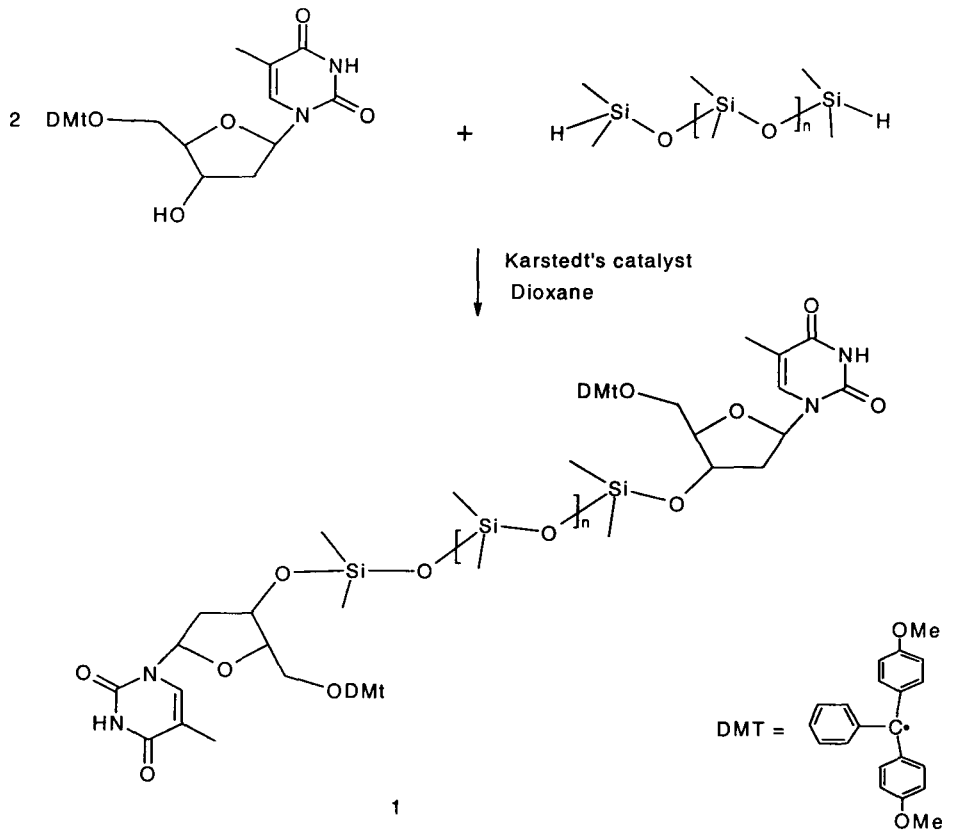
2.1 Formation of Si-O bonds between thymidine and silicone and the hydrolytic stability of Si-O bonds

2.1.1 Synthesis of 5′-O-(4,4′-dimethoxytrityl) thymidine-terminated silicone from hydride-terminated polydimethylsiloxane starting materials

Long chain polydimethylsiloxanes terminated with hydrogen are commercially available. Therefore, we decided to use the alcoholysis of the Si-H bond of silicones as a simple synthetic approach to nucleoside-modified silicones. The synthesis of 5′-O-(4,4′-dimethoxytrityl) thymidine-terminated polydimethylsiloxane was performed by

employing the reaction of hydride-terminated polydimethylsiloxane with 5′-O-(4,4′-dimethoxytrityl) thymidine. The reaction occurred exclusively at the 3′-OH group of thymidine, because the 5′- OH of thymidine was blocked with the 4,4′-dimethoxytrityl group (Scheme 2 - 1). The data on the solvents used in the alcoholysis, reaction times, reaction temperatures and yields are shown in Table 2 - 1. The efficiency of solvents in promoting the reaction was found to be the following:

18-crown-6 ether/dioxane > dioxane > ethylene glycol dimethyl ether > THF >> CH_2Cl_2



Scheme 2 - 1: Synthesis of 5′-O-(4,4′-Dimethoxytrityl) thymidine-terminated polydimethylsiloxane.

Table 2 - 1: Solvent effect of Karstedt's catalyzed alcoholysis of hydride-terminated polydimethylsiloxane with dimethoxytrityl thymidine.

Solvent	Time (min)[a]	Temperature (°C)[b]	yield (%)[c]
18-Crown-6 Ether/Dioxane (1:5, w/w)	245	110	95
Dioxane	305	100	90
Ethylene glycol dimethyl ether	435	80	86
THF	540	65	42
CH_2Cl_2	540	40	45

a. Reaction time (min).

b. Reaction temperature.

c. Only thymidine functionalized silicone was detected by ^1H NMR spectroscopy. The chemical yield was calculated by NMR spectroscopy.

We found that the bond between dimethoxytrityl (DMt-) and the 5′-oxygen atom of thymidine was stable to the alcoholysis conditions. And, because the 5′-hydroxyl group of thymidine was blocked by dimethoxytrityl group, only the 3′-hydroxyl group of thymidine reacted with the H-Si group of the hydride-terminated silicone: no trace of any free dimethoxytrityl group was detected while monitoring the reaction by UV during and after the reaction.

The process of the Karstedt-catalyzed alcoholysis was followed by monitoring the disappearance of the Si-H bond stretching vibration band in the FTIR spectrum (Figure 2 - 1). The incorporation of the polysiloxane to thymidine was indicated by the Si-O-Si absorption at 1100 ~ 1060 cm^{-1}. Additional proof was provided by the presence of the shoulders at 770 cm^{-1} and 757 cm^{-1}, which are attributed to the Si-Me bonds. The Si-H absorption at *ca.* 2200 cm^{-1} is diagnostic for Si-H groups and in a region where few other peaks are found. The most obvious proof of reaction was the disappearance of the strong band at 2127 cm^{-1}. After 5 h, approximately 95% of the Si-H had reacted.

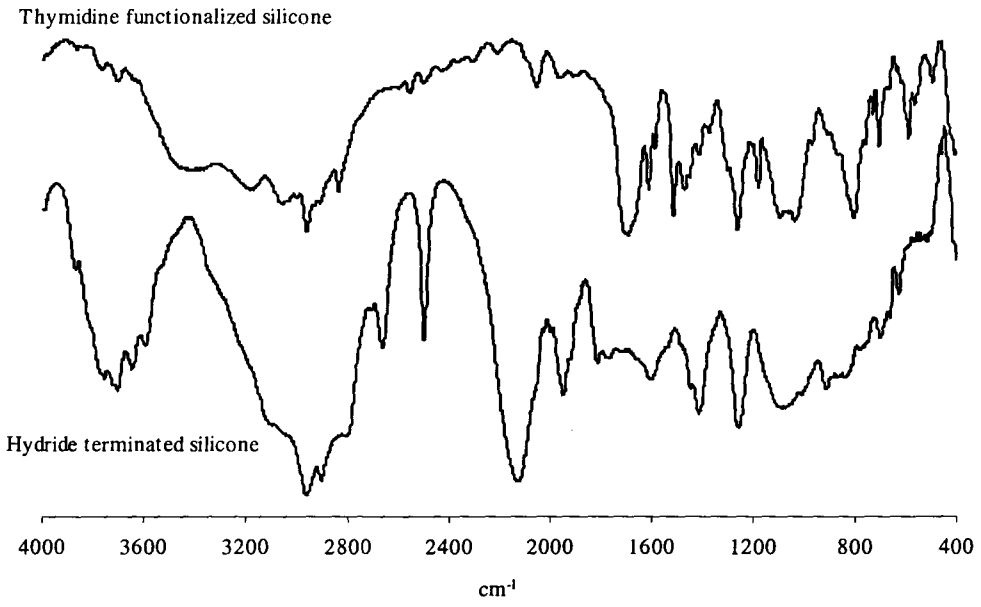


Figure 2 - 1: FTIR spectra of 5´-O-(4,4´-Dimethoxytrityl)-thymidine terminated silicone and hydride-terminated silicone.

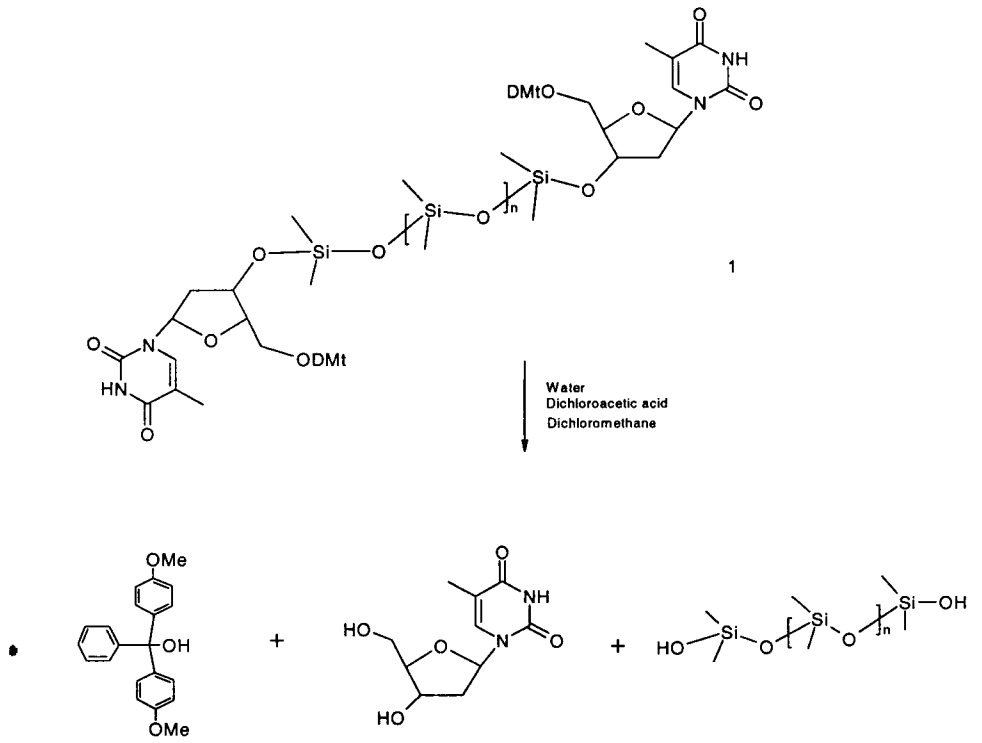
The molecular ion peak in the mass spectrum indicated the presence of the product. Additional evidence for the attachment of the siloxane onto the nucleoside was obtained from ^{1}H NMR and ^{13}C NMR spectral analysis, including the disappearance of the hydrogen of the Si-H group in the ^{1}H NMR.

In these experiments, the usual catalyst concentration was 2.64×10^{-4} g platinum per 1 gram of silicone. One advantage of this synthetic approach is that the silicone can be functionalized with thymidine, and processed in one step. The approach made it possible for us to form the Si-O bond linkage easily between nucleoside and silicone.

2.1.2 Tests on the hydrolytic stability of the 5′-O-(4,4′-dimethoxytrityl)thymidine-terminated polydimethylsiloxane

The product 1, 5′-O-(4,4′-dimethoxytrityl) thymidine- terminated polydimethylsiloxane, was treated with water in the presence of dichloroacetic acid (DCA). We performed the hydrolysis (Scheme 2 - 2) using similar conditions to that used for the detritylation of oligonucleotides in *Solid Phase Synthesis*, in order to test if our product 1 could survive the harsh acid conditions. After reaction, all compounds were separated from each other. All compounds including the 4,4′-timethoxytrityl alcohol were identified by ^{1}H NMR. The 4,4′-trimethoxytrityl alcohol was identified by UV and isolated by separation on a silica column. Unfortunately, accompanying detritylation was the cleavage of the silicone from the compound. It is important to note that cleavage

occurred at the C-O-Si linkage. This is not surprising, given the relatively small steric barrier provided by the dimethylsilyl groups.[86] In order to suppress this hydrolysis reaction, we chose sterically bulky silanes.



Scheme 2 - 2: Hydrolysis of the 5′-O-(4,4′-Dimethoxytrityl) thymidine-terminated polydimethylsiloxane.

2.1.3 Stability test of the tetraisopropyldisiloxane

Because the bulky organic groups (isopropyl) on the silicon atoms are expected to contribute stability, the commercially available tetraisopropyldisiloxane was employed as

a starting material for our project. An initial assessment of the stability of tetraisopropyldisiloxane was made under the conditions typically used in solid phase synthesis. Thus, the tetraisopropyldisiloxane was exposed to various concentrations of dichloroacetic acid (DCA). Results (Table 2 - 2) showed no new peaks in the GC chromatogram of ultrasonicated samples.

Table 2 - 2: Si-O-Si backbone is stable to oligonucleotide synthesis conditions.

Sample	Mixed and Ultrasonicated		Sonicated 1 hr	Sonicated 2 hr
	DCA/DCM v/v%	TIPDS μl		
1	1	100	stable	stable
2	2	100	stable	stable
3	2.4	100	stable	stable
4	3	100	stable	stable
5	5	100	stable	stable

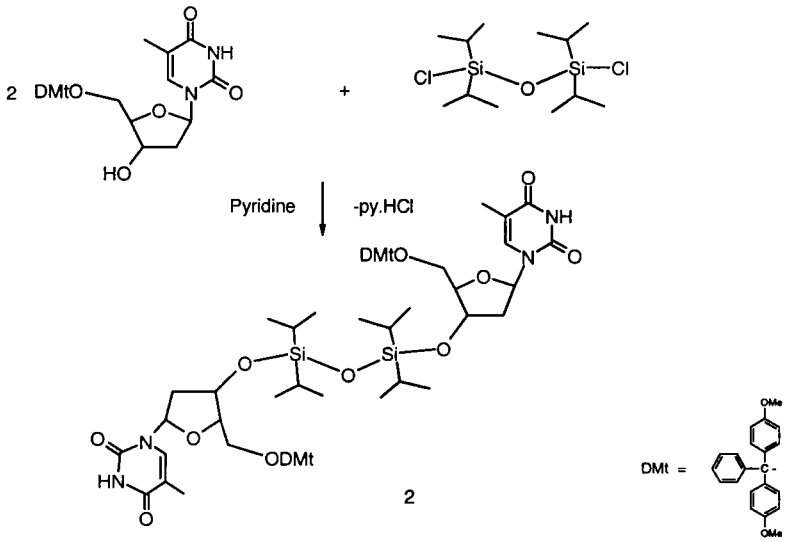
So, from the results of the above experiments, we expected that this Si-O-Si backbone of silicone portion with bulky isopropyl substituent on silicon atom would survive the oligonucleotide synthesis conditions, particularly the acid-catalyzed cleavage of dimethoxytrityl-protecting groups.

The bulky isopropyl substituent on silicone atoms contributes to the stability of Si-O-Si, because the isopropyl group sterically hinders the hydrolysis. Due to the same steric hindrance effect, the isopropyl group will suppress the alcoholysis. As was discussed in

section 1.2.3(a) (page 23), the silane substituent in the alcoholysis exerted such a great effect on the rate of alcoholysis that the rate of the reaction of *i*-Pr_3SiH with ethanol is much lower to that of Et_3SiH (the relative rate is 1.6 to 100).[40,42] So, we didn't think the reaction of hydride terminated tetraisopropyldisiloxane to 3′- hydroxyl group of 5′-O-(4,4′-dimethoxytrityl) thymidine would proceed as fast as hydride-terminated polydimethylsiloxane did (see 2.1.1). To make the reaction of tetraisopropyldisiloxane with the 3′- hydroxyl group of 5′-O-(4,4′-dimethoxytrityl) thymidine occur, we used more active chloride-terminated tetraisopropyldisiloxane as reagent, instead of hydride-terminated tetraisopropyldisiloxane. Because the reactivity of the Si-Cl bond is higher than that of Si-H, and -Cl is a better leaving group, we expected the substitution reaction on the silicon atom would occur easily. And, in this substitution reaction, pyridine was used. When chloride was substituted by the 3′-oxygen of 5′-O-(4,4′-dimethoxytrityl) thymidine, the resulting HCl formed a stable salt with pyridine. Because the chloride was consumed, the equilibrium of the reaction was shifted far away to the direction of product making (Scheme 2 - 3). The process worked very well, as we described below in the synthesis of 5′-O-(4,4′-dimethoxytrityl) thymidine-terminated tetraisopropyldisiloxane (see 2.1.4).

2.1.4 Synthesis of 5′-O-(4,4′-dimethoxytrityl) thymidine-terminated tetraisopropyldisiloxane from chloro-terminated tetraisopropyldisiloxane starting materials

A nucleophilic displacement reaction was performed to bind nucleoside to silicone. As expected, the secondary 3′-OH group of the 5′-O-(4,4′-dimethoxytrityl) thymidine reacted efficiently with chloro-terminated tetraisopropyldisiloxane (Scheme 2 - 3) to give product 2. To drive the reaction to completion, pyridine was employed to form a salt with the released HCl.



Scheme 2 - 3: Synthesis of 5′-O-(4,4′-dimethoxytrityl) thymidine-terminated tetraisopropyldisiloxane.

Usually, polysiloxanes have a very strong infrared band in the 1130 to 1000 cm^{-1} region due to the asymmetric Si-O-Si stretching vibration. As the siloxane chain becomes

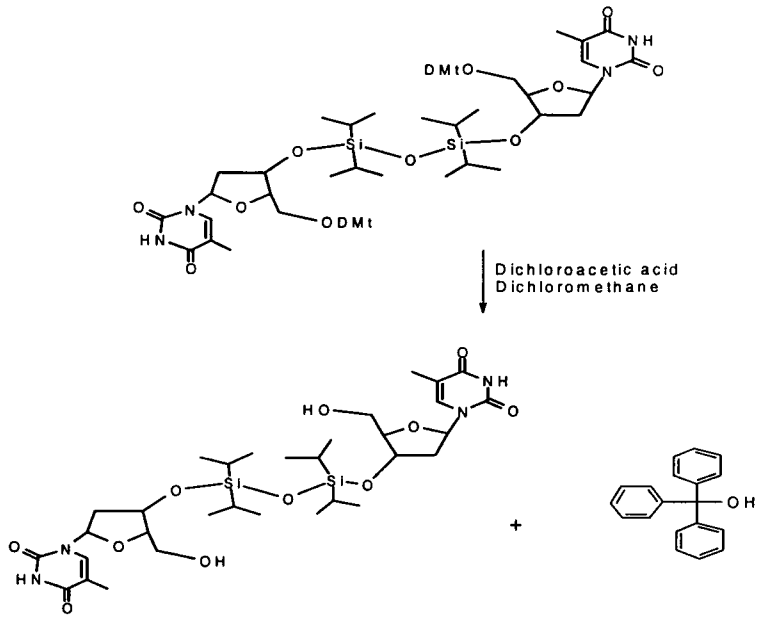
longer, this band splits into two or more overlapping absorptions. In the case of disiloxanes, the position of this band can vary over the entire region (1130-1000 cm^{-1}) depending on the mass and the inductive effects of the other groups on the silicon. For our isopropyl substituted disiloxanes, the band fell at 1070 to 1050 cm^{-1}, which was different from the starting material that exhibited this band at 1113 cm^{-1}.

The ^1H NMR spectrum of the isopropyl group, $SiCH(CH_3)_2$, in either product structure or starting material, showed a complex AB_6 pattern in which there was no clear separation between the CH and CH_3 protons.

The mass spectrum of the product exhibited no molecular ion, which is a common situation for siloxanes: silicon compounds usually show a peak resulting from cleavage of the smallest group attached to silicon leaving a silicon cation. Mass spectra showed that two isopropyl groups were cleaved from the product molecule, 5′-O-(4,4′-dimethoxytrityl) thymidine-terminated tetraisopropyldisiloxane, giving a signal at mass 1110, as the highest molecular weight fragment in the spectrum. In this experiment, the disiloxane was used as a model substance for long chain polysiloxanes, because it is much easier to isolate and characterize these species by GC and NMR, respectively. We demonstrated that Si-Cl is reactive to the 3′-hydroxyl group of nucleosides leading to the formation of Si-O bonds between silicone and the nucleoside.

2.1.5 Tests on the hydrolytic stability of the 5′-O-(4,4′-Dimethoxytrityl) thymidine-terminated tetraisopropyldisiloxane

The product, 5′-O-(4,4′-Dimethoxytrityl) thymidine-terminated tetraisopropyldisiloxane, was treated with water in the presence of dilute dichloroacetic acid (DCA) dichloromethane solution. We performed the hydrolysis under the conditions much similar to those used in the *Solid Phase Synthesis* of oligonucleotide (Scheme 2 - 4). After reaction, 4,4′-dimethoxytrityl alcohol was identified by UV and isolated by passing the mixture through a silica column. The isolated 4,4′-dimethoxytrityl alcohol was further identified by performing ^{1}H NMR. We found the DMt- removal was achieved using this detritylation process.



Scheme 2 - 4: Detritylation and hydrolytic stability of the 5′-O-(4,4′-dimethoxytrityl) thymidine-terminated tetraisopropyldisiloxane.

As expected, the bulky isopropyl substituents on silicon depressed hydrolysis processes effectively such that the silicone backbone remained intact: Si-O-Si and Si-O-C linkages were found in the product. However, the yield of the detritylation was not as high as we expected. In addition, it became clear through other work in the laboratory that increasing the molecular weight of the silicone chain, without affecting the C-O-Si group, was going to be problematic.

2.1.6 Discussion on the Si-O bond linkage between thymidine and silicone

It was found that the Si-O bond linkage with a small methyl substituent on the silicon atom was easily achieved through the alcoholysis process. However, we didn't think the alcoholysis process was applicable to the case with a bulky isopropyl substituent. The bulky isopropyl group can steric hinder the alcoholysis as we demonstrated in the hydrolysis process. To effect the Si-O bond linkage with a bulky isopropyl substituent on the silicon atom, we took the advantage of the good leaving group -Cl of Si-Cl which made the nucleophilic substitution happen. The Si-O bond linkage between thymidine and tetraisopropyldisiloxane could be easily achieved through the displacement of -Cl with the 3′- oxygen of the nucleoside.

We demonstrated the reaction of alcoholysis or nucleophilic substitution could be employed to prepare nucleoside-functionalized silicone. Thus, we have achieved one of our primary goals.

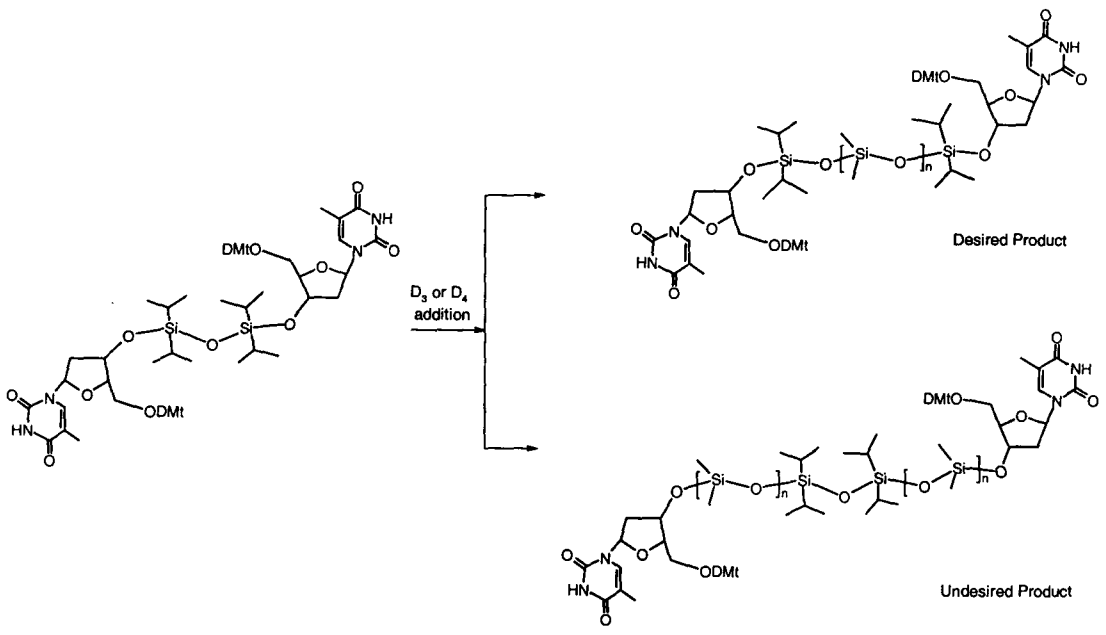
We found in Scheme 2-2 that the C-O-$SiMe_2R$ groups are not stable to hydrolysis. As we expected in Chapter 1 that the product 1 (5′-O-(4,4′-dimethoxytrityl) thymidine-terminated polydimethylsiloxane) was not hydrolytically stable, mainly because the small methyl groups on silicon atom are not sterically hindered enough to depress water nucleophilic attack at silicon.[66] So, we decided not to prepare oligonucleoside functionalized silicone from the first nucleoside which was directly bound to polydimethylsiloxane *via* Si-O linkage, because we worried about the hydrolytic stability of the Si-O bond linkage.

To increase the hydrolytic stability of the silicone backbone, we designed our silicone portion to have bulky isopropyl groups on silicon atoms. Indeed, the second product (see Scheme 2 - 3) derived from chloride-terminated tetraisopropyldisiloxane and 5′-O-(4,4′-dimethoxytrityl)-thymidine was stable at Si-O-C to the conditions necessary for removal of the dimethoxyltrityl group. It is obvious that the bulky isopropyl on the silicon atom accounted for the stability of Si-O-C linkage between silicone and nucleoside. So far, we developed a more stable Si-O-C linkage between silicone and nucleoside. This kind of Si-O-C linkage was different to that we previously made. In this Si-O-C linkage the alkyl isopropyl groups, which provide steric hindrance, were on silicon atom, instead of the small methyl groups.

In order to achieve the final oligonucleotide functionalized silicone, one issue must be considered. That is the stability of Si-O-C linkage to silicone chain redistribution. We realized we could not leave the Si-O-C linkage with bulky isopropyl substituent on silicon

atom untouched, if we wanted to perform the subsequent silicone chain-enlarging process (Scheme 2 - 5). In preparing high-molecular-weight silicones,[87,88,89,90,91,92] which involve a series of nucleophilic substitutions at the disiloxane and D_4, the conditions are sufficiently vigorous that it would not be possible to prepare the desired compounds in which the bulky silicon resides near the ring 2 of thymidine (see the Undesired Product in Scheme 2 - 5). In fact, the hydrolytically unstable compound (see the Undesired Product in Scheme 2 - 5) would form. In other word, it is very hard to control the insertion of cyclics (D_3 or D_4) into linear silicone at the desired position (the middle of silicone backbone).[93] Silicone redistribution may occur in an uncontrolled way. That means we may get the unstable Si-O-C linkage with small methyl groups on the silicon atom. This kind of linkage might be cleaved, when oligonucleotide is grown from the first nucleoside of the nucleoside-functionalized silicone.

Thus, although we partially achieved our objectives, we were left with compounds that were susceptible to damaging side reactions under acidic and basic conditions. The classic solution to this dilemma is to remove Si-O-C linkages from the system, and replace them with linkers, in which much stronger bonds are utilized: that is Si-O-C linkages are replaced with Si-O-Si and C-O-C linkages. The utilization of this approach is discussed below.

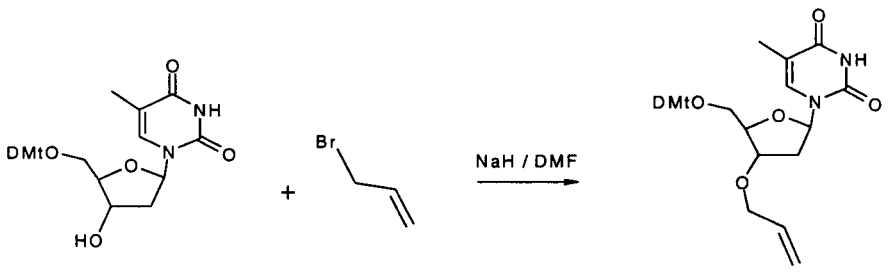


Scheme 2 - 5: D_3 or D_4 addition to thymidine-functionalized tetraisopropyldisiloxane.

2.2 Formation of Si-C bond linkages between nucleosides and silicones through C=C hydrosilylation

2.2.1 Synthesis of 3′-O-allyl - 5′-O-(4,4′-dimethoxytrityl) thymidine

The allyl group was introduced to 5′-O-(4,4′-dimethoxytrityl)-thymidine at the 3′-O position (Scheme 2 - 6) using traditional Williamson conditions. Only the 3′-hydroxyl group of thymidine reacted with allyl bromide because the 5′- hydroxyl group of thymidine had been blocked by the dimethoxytrityl group before. We found that the bond between dimethoxytrityl (DMt-) and 5′-oxygen atom of thymidine was stable to the Williamson conditions. No trace of free dimethoxytrityl alcohol could be detected by UV during or after the reaction.



Scheme 2 - 6: Allyl group bound at the 3′-O position of thymidine.

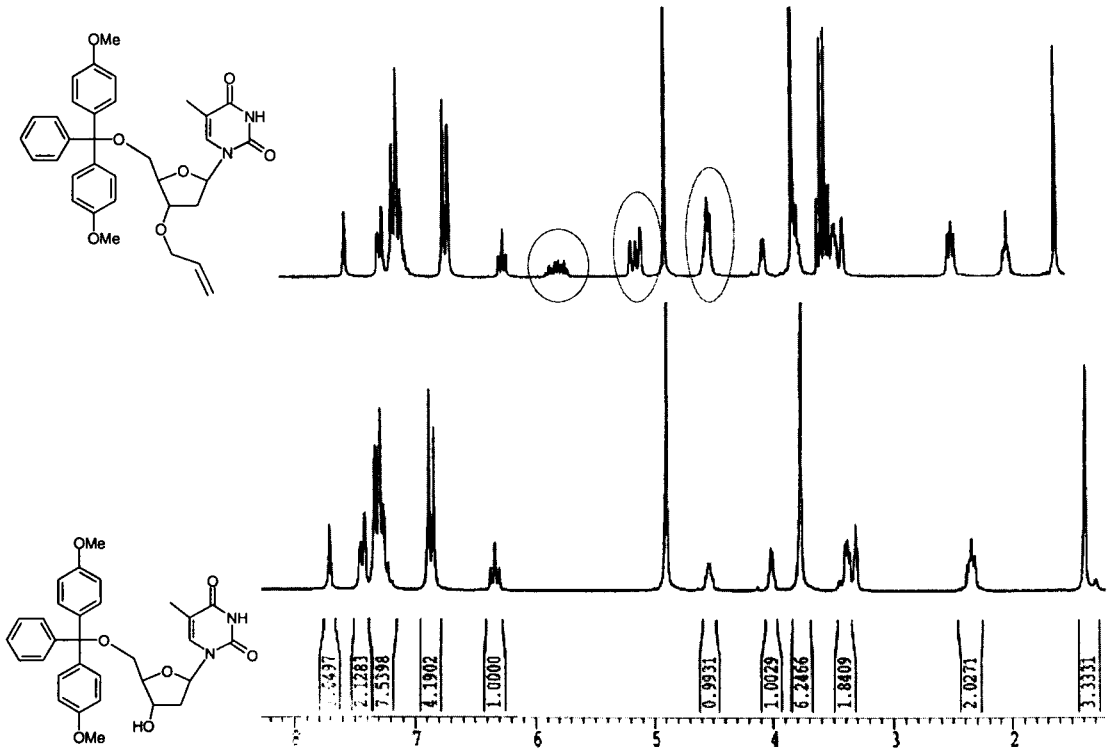


Figure 2 - 2: ^{1}HNMR spectra of 3′-O-allyl - 5′-O-(4,4′-dimethoxytrityl)-thymidine and 5′-O-(4,4′-dimethoxytrityl)-thymidine.

The completion of the reaction attaching the allyl group onto the thymidine could be determined by performing ^{1}H NMR measurements. For example, the new peaks (Figure 2 - 2) in the upper ^{1}H NMR assigned to the protons of allyl group shows the reaction occurred. In the work of Montembault and Lebreton,[94] the 5′- hydroxyl group of thymidine was protected first with *tert*-butyldimethylsilyl (TBDMS) group, allowing then the selective introduction of an allyl group in the 3′- oxygen of thymidine.

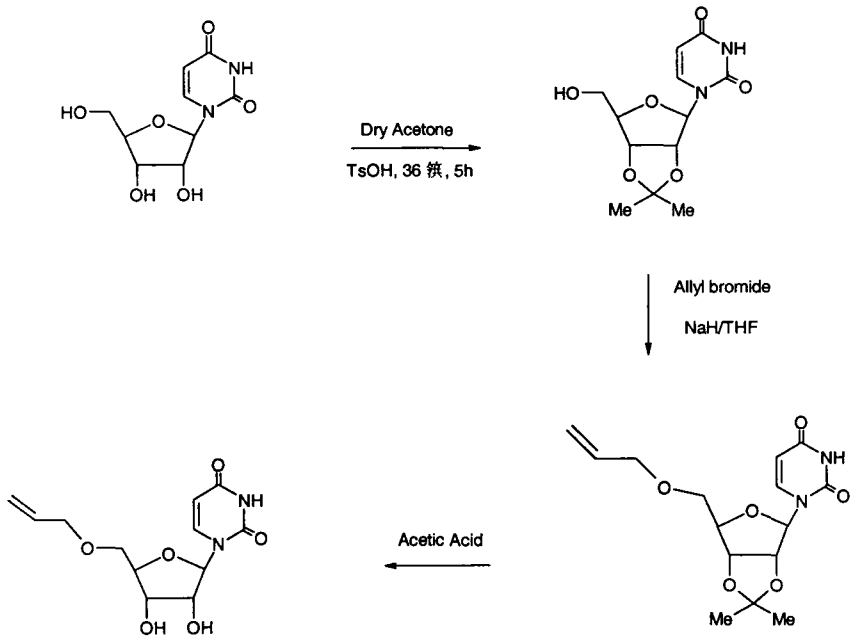
Signals (chemical shift = 164.4 and 151.9) in ^{13}C NMR which can be assigned to the two carbons of C=O of thymine proved that the ally group was linked to the 3′- oxygen of ribose, not to the nitrogen atom of thymine. Under this condition, no evidence has been found on the possible formation of the N-alkylated derivative. From our spectrum data and the previous work described, we believed we have successfully bound allyl group to 3′-oxygen.

The existence of the molecular ion peak, along with other molecular fragment peaks, in the mass spectrum also confirmed the molecular structure.

2.2.2 Synthesis of 5′-O-allyl uridine

Prior to the preparation of 5′-O-allyl uridine, the 2′- and 3′- hydroxyl groups were first protected as an acetonide, following which the allyl group was introduced to the 5′-O position of uridine. As is normally the case, preparation of the 5-membered ring acetonide from the *cis*-hydroxyl groups is favored over the analogous 6-membered ring that would

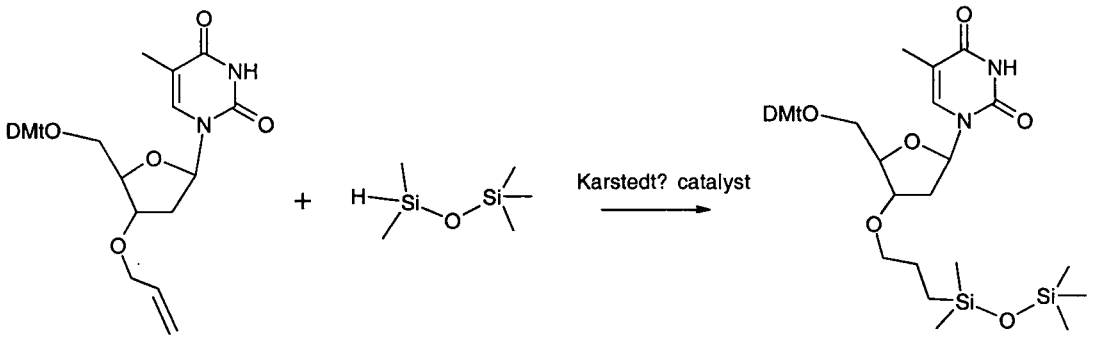
result from 3'-5' protection. After protection of the 2′- and 3′-hydroxyl groups, it was possible to graft the allyl group selectively to the 5′-oxygen (Scheme 2 - 7).



Scheme 2 - 7: Allyl group was bound to uridine at the 5′-O position after 2', 3' protection.

2.2.3 Synthesis of 3′-O-(propyl)- 5′-O-(4,4′-dimethoxytrityl) thymidine-terminated pentamethyldisiloxane

Once the allyl group was bound to the nucleoside, the hydrosilylation reaction was optimized using the monofunctional compound pentamethyldisiloxane, an analogue of our desired silicone polymer (Schem 2 - 8). The Si-H bond effectively added to the alkene, linking the nucleoside through a three-carbon spacer to the siloxane.



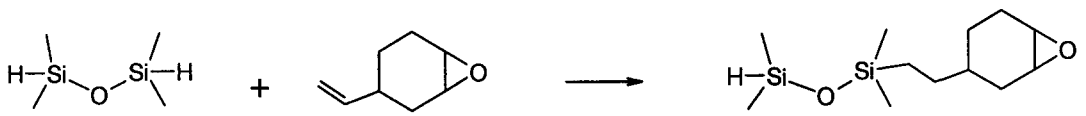
Scheme 2 - 8: Hydrosilylation reaction of pentamethyldisiloxane with allyl-grafted thymidine.

Evidence for the attachment of the disiloxane onto the nucleoside was directly obtained from the ^{1}H NMR. The peak at 0.4 ppm may be assigned to the protons of the methylene carbon adjacent to silicon. The methylene carbon serves as one part of a three-carbon spacer connecting the disiloxane to the thymidine. The peak between 0.10 ppm and 0.05 ppm is due to the protons of the disiloxane methyl groups.

From the FTIR spectrum, the characteristic double bond absorption at 888 cm^{-1} is lost from the IR spectrum, as is the characteristic Si-H band absorption at 2160 cm^{-1}, and the Si-O-Si absorption at 1090 cm^{-1} indicated the incorporation of tetramethyldisiloxane. The occurrence of a peak at 699 cm^{-1} is also consistent with the presence of the nitrogenous base ring in the silicone phase. Mass spectral results also confirm the formation of the product.

2.2.4 Synthesis of cyclohexene 1,2-epoxide terminated tetramethyldisiloxane

Simple hydrosilylation of vinylcyclohexene epoxide led to the formation of an asymmetric disiloxane 3 (Scheme 2 - 9). The efficiency of product formation depended on the ratio of disiloxane to vinylcyclohexene epoxide. The higher the amount of hydride-terminated silicone, the more the monofunctional disiloxane was produced. We found the use of a five fold excess of tetramethyldisiloxane over the 4-vinyl-1-cyclohexene-1,2-epoxide ensured the formation of the mono-epoxy terminated silicone.



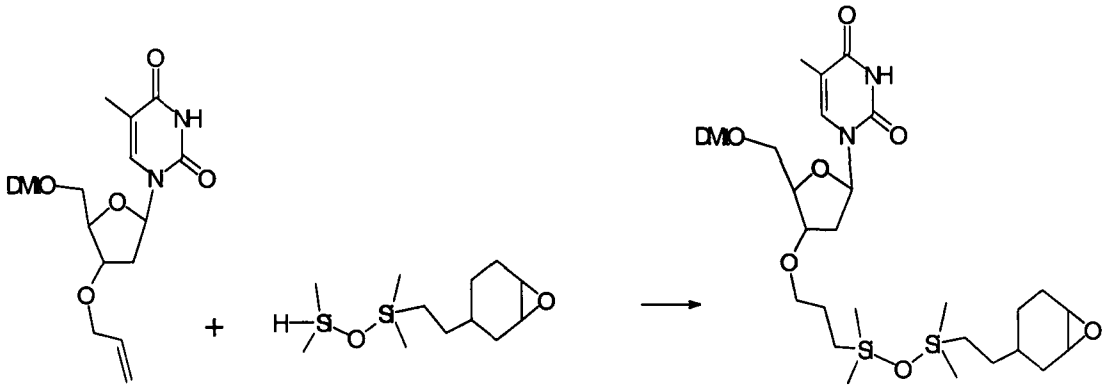
Scheme 2 - 9: Reaction of 4-vinyl-1-cyclohexene 1,2-epoxide with 1,1,3,3-tetramethyldisiloxane.

The structure of this compound could be shown by spectroscopic techniques. The characteristic peak at 0.4 ppm1H in the NMR spectrum is assigned to the two protons of the $-CH_2-Si \equiv$. The protons of cyclohexene-1,2-epoxide appear at essentially the same chemical shift in the product as in the starting material. The molecular structure determination is also supported by the existence of the molecular ion peak in the mass spectrum.

2.2.5 Attempted synthesis of nucleoside and epoxy bifunctionalized silicone

We carried out hydrosilylation to bind allyl-thymidine to the cyclohexene 1,2-epoxide (Scheme 2 - 10). Through ^{1}H NMR observation, it was found that the epoxide was stable to the hydrosilylation process. The ^{1}H NMR of the crude product showed the expected set of signals.

Unfortunately, the crude ^{1}H NMR exhibited silicon methyl peaks of approximately twice than that expected. This could be the result of hydrosilylation of the NH or C=O groups of the base. This reaction caused some silicone to link to the base of nucleoside (Figure 2 - 3).



Scheme 2 - 10: Attempted hydrosilylation of allyl-bound thymidine with mono-cyclohexene 1,2-terminated tetramethyldisiloxane.

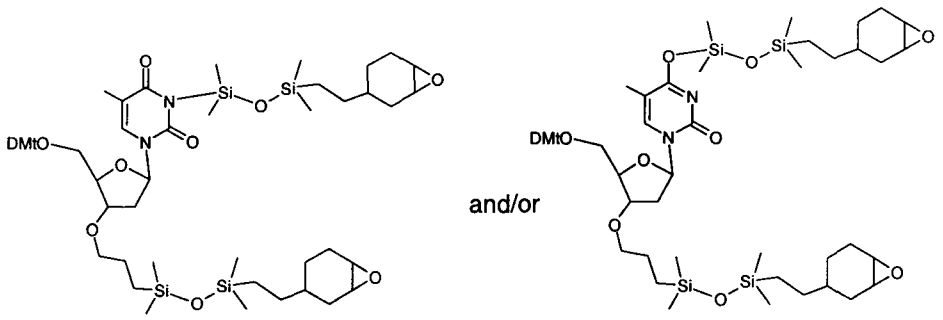


Figure 2 - 3: Possible structures of the unexpected products.

We found it was very hard to purify the crude product, because of its higher boiling point. Attempts to purify the material by classical column chromatography have so far been unsuccessful. We expected the procedure could be modified to hydrosilylate only C=C bond (instead of C=O, or NH) in future work.

2.2.6 Discussion on Si-C bond between nucleosides and silicones, and the significance of introduction of epoxide group to silicone in our project

We demonstrated that hydrosilylation offered a method to attach the nucleoside to silicone via a trimethylene spacer. One advantage of taking the trimethylene spacer is that the three-carbon spacer[95]will allow the nucleoside to retain functionality much similar to the independent precursor. The obvious benefit of the Si-C bond linkage is its relative stability.

The reactivity of Si-C bears a strong resemblance to C-H,[66] although, silyl group are more hydrophobic, more electrophilic, and bulkier than protons. The Si-C bonds in

organosilanes usually remain dormant in the presence of nucleophiles, with the exception of fluoride. Generally, Si-C bonds can withstand a wide variety of reaction conditions and reagents. Oxidations frequently occur at organic functional groups without affecting the C-Si bond.

In the synthesis of nucleoside and epoxy bifunctionalized silicone, we demonstrated the epoxide group was introduced to silicone by hydrosilylation process. As described in Chapter 1, the three member ring of epoxide can be cleaved with an acid chloride to form ester bond under very mild conditions. And, the requirement of a successful solid phase synthesis of oligonucleotide is to bound first nucleoside covalently to the solid support. After oligonucleotide is synthesized on the solid support, it will be cleaved from the solid support. The most easily handled cleavage in solid phase synthesis is the readily cleavable ester bond linkage. Considering the future work on the solid phase synthesis of oligonucleotide, we synthesized cyclohexene 1,2-epoxide terminated tetramethyldisiloxane first, and then tried to prepare nucleoside and epoxy bifunctionalized silicone whose epoxy functional group has the potential to bound nucleoside terminated silicone to solid support *via* a cleavable ester bond.

We predicted other functional groups (–COOH, –OH, $–NH_2$) are not as compatible with hydrosilylation as epoxide. This was another reason why we chose the epoxy group as the functional group to react with the commercially available propionyl chloride-functionalized resin (solid support).

2.3 Status of the Project

We had explored several reactions, such as nucleophic substitution on silicon atom, Pt-catalyzed alcoholysis and hydrosilylation. We found those reactions can be applied to our preparation of nucleoside-functionalized silicone. However, the practical solid phase bound procedures have not been set up. Once a practical solid phase bound procedure is established, the oligonucleotide-functionalized silicone will be synthesized with an automatic synthesizer.

2.3.1 Finished step on preparation of nucleoside-functionalized silicone

Applying Pt-catalyzed alcoholysis process, we prepared nucleoside-functionalized polydimethylsiloxane. The advantage of the alcoholysis process is the one-step procedure. However, we found the product is not stable to the hydrolysis process. To increase the hydrolytic stability of the nucleoside functionalized silicone, we took advantage of the bulky isopropyl group which depressed hydrolysis effectively and succeeded to make more stable Si-O linkage that had isopropyl substituents on silicon atoms. With this Si-O linkage, the nucleoside and silicone was combined together.

However, the Si-O linkage with isopropyl substituent cannot survive the rigorous condition of silicone chain enlarging process. There is a problem of silicone chain redistribution. Our solution to the problem is to set up Si-C linkage between siloxane and nucleoside, instead of Si-O linkage.

Employing hydrosilylation process, the Si-C linkage between the siloxane and the nucleoside was formed. Through the hydrosilylation of Si-H bond with an allyl group that was previously bound to the nucleoside, we prepared another kind of nucleoside-functionalized silicone. With one nucleoside bound upon the end of silicone *via* a three-carbon spacer, it was ready to grow oligonucleotide chain from the first nucleoside.

2.3.2 Unfinished step on preparation of oligonucleotide-functionalized silicone

Usually, *Solid Phase Synthesis* process is a standard method to synthesize oligonucleotide. One requirement for the *Solid Phase Synthesis* procedure is to bind the target nucleoside to a solid phase support. After solid phase binding, oligonucleotide can be prepared from the first nucleoside (in our case, from the nucleoside-functionalized silicone) automatically with a synthesizer. The most common linkage to solid phase is the easily handled ester bond, because it is ready to be cleaved from solid support after growth of oligonucleotide.

To meet the requirement, we succeeded to introduce epoxide group to one terminus of silicone. However, we need to do some work on the purification of the crude product, and to determine the exact molecular structure of epoxy and thymidine bifunctionalized silicone. We have not developed a protocol in which the epoxy group, as an anchor point, attaches the nucleoside-functionalized silicone to a solid support (see Scheme 1 - 16). Due to the complex heterogeneous reaction phase and uneasiness to determine molecular

structure on solid phase surface, right now, we are not sure the nucleoside-functionalized silicone was bound to solid phase.

Once the nucleoside functionalized silicones were bound to solid phase, it should be easy to perform *Solid Phase Oligonucleotide Synthesis* from the first nucleoside on the silicone.

Chapter 3

EXPERIMENTAL

3.1 Reagents and Physical Methods

The following materials were obtained from Aldrich: Karstedt's catalyst PC072 $Pt_2[(H_2C{=}CHMe_2Si)_2O]_4$, 3.5 % platinum concentration in xylene, neutral, 5′-O-(4,4′-dimethoxytrityl) thymidine, 4-vinyl-1-cyclohexene-1,2-epoxide 89%, uridine, allyl bromide, dichloroacetic acid, tetrahydrofuran (THF, 99.5% BDH), hexanes, DMF (anhydrous), pyridine (anhydrous), 1,4-dioxane (anhydrous, 99.8%), CH_2Cl_2, $NaHCO_3$, $MgSO_4$, methanol, dichloromethane and chloroform. Dichlorotetraisopropyldisiloxane (TiPDSCl), pentamethyldisiloxane and GC-detectable low molecular weight silicone standards were purchased from Gelest. These included hydrogen functional materials including PDMS-H (2-3cs, 500 cs and 10,000 cs) and 1,1,3,3-tetramethyldisiloxane 97%. Silicones are sold by viscosity in centistokes: the approximate molecular weights of these samples are *ca.* 400-500, 17,200 and 62,700, for PDMS-H 2-3cs, 500 cs and 10,000 cs, respectively. The purity of these materials was checked with ^{1}H NMR immediately before use. Chloroform-*d*, acetone-d_6 and methanol-d_4 were bought from Cambridge Isotope Laboratories, Inc.

All solvents were thoroughly dried before use: pentane and acetonitrile were dried over P_2O_5; THF was dried from K/benzophenone. All reactions were carried out in dry

apparatus under a nitrogen atmosphere with the use of septa and syringes for the transfer of reagents.

^{1}H NMR spectra were recorded on a Bruker AC-200 (at 200 MHz for protons) Fourier transform spectrometer. ^{13}C NMR was performed on a Bruker AC-200 (50 MHz). Chemical shifts are reported with respect to $CDCl_3$ as an internal standard for protons, set at 7.24 ppm. Coupling constants (*J*) are recorded in Hertz (Hz). The abbreviations s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, m = multiplet, are used to report spectra.

Electron impact (EI) and chemical ionization (Cl, NH_3) mass spectra and GC/MS analyses were recorded on a Hewlett-Packard 5890II gas chromatograph equipped with a HP-5971A mass selective detector and a DB-5 fused silica capillary column (30m x 0.25 mm; Chromatographic Specialities, Inc.). Infrared spectra were run as KBr pellets or as liquid films on KBr discs (as indicated) on a Perkin-Elmer 283 spectrometer or on a BIORAD FTS-40 spectrometer as a neat film. Gas chromatographic (GC) analyses were carried out using a Hewlett-Packard 5890A gas chromatograph equipped with a conventional heated detector, a flame ionization detector, a Hewlett-Packard 3396A integrator, and a DB-1 megabore capillary column (30m x 0.54mm Chromatographic Specialties, Inc.).

3.2 Synthesis of 5′-O-(4,4′-dimethoxytrityl) thymidine-terminated silicone from hydride-terminated polydimethylsiloxane starting materials

All alcoholysis reactions were carried out under a dry nitrogen atmosphere. Glassware was dried either under a stream of nitrogen just prior to use or dried in a 120 °C oven overnight. Solvents and reagents were purified and dried by standard methods prior to use. The alcoholyses were conducted with several kinds of solvent (Table 2 - 1) at a variety of temperatures. In the case of dioxane, Karstedt's catalyst (two drops) was added to H-Si(Me_2)-[O-(Me_2)Si]$_n$-O-(Me_2)Si-H (n ~ 7, 129.6 mg, 0.199 mmol) in anhydrous 1,4-dioxane solution (15 mL), which was then added to 5'-O-(4,4'-dimethoxytrityl)-thymidine (216 mg, 0.397 mmol) in dioxane (5 ml). After a 20-30 second induction period, pronounced gas evolution from the pale yellow solutions was observed. The mixture was refluxed for 4 h at 100 °C in a 50 mL reaction flask. Once the reactions ceased evolving gas, the solution was kept at reflux for another hour. The solvent was removed under reduced pressure and gave the product. Yield: 310 mg, (90%).

^{1}H NMR ($CDCl_3$, 200 MHz): δ = 7.65 (m, 1H, 6-H), 7.20-7.40 (m, 9H, protons in phenyl ring), 6.80 (2 x d, 4H, J = 9.0 Hz, protons in phenyl ring), 6.43 (dd, 1H, 1′-H, $J_{1,2a'}$ = 7.2 Hz, $J_{1,2b'}$ = 2.6 Hz), 4.57 (m, 1H, 3′-H), 4.07 (m, 1H, 4′-H), 3.76 (s, 6H, OCH_3), 3.42 (m, 2H, 5′-H), 2.34 (m, 2H, 2′-H), 1.43 (s, 3H, protons of methyl in the ring 1), 0.075 (m, 27H, Si-Me).

^{13}C NMR ($CDCl_3$): δ = 162.7 (C-4), 158.5 (Cq DMt), 149.9 (C-2), 140 (Cq DMt), 139.9 (C-6), 135.4, 135.1 (Cq DMt), 112.9 (CH DMt), 108.7 (C-5), 86.0 (Cq DMt), 84. 9

(C-4′), 84.2 (C-1′), 68.1 (C-3′), 60.5 (-OCH_3), 58.9 (C-5′), 39.7 (C-2′), 15.6-16.2 (CH_3 PDMS).

FTIR (neat, KBr disc) ν (cm^{-1}): 2970, 1710, 1510, 1390, 1260, 1180, 1100, 1060, 808, 770, 757.

MS (ES): *m/z*, 1737 (M^+, 1), 1111 (5), 567 (20), 338 (70), 303 (100).

3.3 Tests of the hydrolytic stability of the 5′-O-(4,4′-dimethoxytrityl) thymidine-terminated polydimethylsiloxane

The 5′-O-(4,4′-dimethoxytrityl) thymidine-terminated polydimethylsiloxane (300 mg, 0.17 mmol) was dissolved into dichloromethane (8 ml). Water (0.5 ml) and dichloroacetic acid water solution (0.05 M, 200 μl) was added into the above solution. The mixture above was ultrasonicated for 20 min. After hydrolysis, each component was separated by passing through a flash silica column (CH_2Cl_2/CH_3OH, 98/2, v/v). The first fraction with its characteristic color (orange) was identified as 4,4′-dimethoxytrityl alcohol from its ^1H NMR observation. The second and the final fraction were identified as polydimethylsiloxane and thymidine, respectively.

3.3.1 Isolated hydrolysis product

3.3.1a 4,4′-Dimethoxytrityl alcohol

^1H NMR ($CDCl_3$, 200MHz): 7.20-7.40 (m, 4H, protons in *m*-position to methoxyl group and 5H protons in the third aryl ring), 6.83 (d, 4H, J = 9.0 Hz, protons in *o*-position to methoxyl group), 3.76 (s, 6H, OCH_3).

^{13}C NMR ($CDCl_3$): δ = 145.2 129.6, 127.8, 127.6, 81.3.

FTIR (neat, KBr disc) ν (cm^{-1}): 3474, 3080, 1491, 1401, 1331,1182, 1001, 938, 891, 684.

MS: (ES) *m/z* 303 $(M-17)^+$,(85), 123 (15), 81(25), 80 (100).

3.3.1b Thymidine

^1H NMR ($CDCl_3$, 200MHz): δ = 7.71 (m, 1H, 6-H), 6.18 (dd, 1H, 1′-H, $J_{1,2a'}$ = 7.2 Hz, $J_{1,2b'}$ = 2.6 Hz), 4.25 (m, 1H, 3′-H), 3.77 (m, 1H, 4′-H), 3.76 (s, 6H, OCH_3), 3.55 (m, 2H, 5′-H), 2.08 (m, 2H, 2′-H), 1.78 (s, 3H, protons of methyl in the base ring).

^{13}C NMR ($CDCl_3$): δ = 167.2 (C-4), 152.4 (C-2), 138.3 (C-6), 112.1 (C-5), 87. 3 (C-4′), 85.88 (C-1′), 71.2 (C-3′), 62.0 (C-5′), 39.4 (C-2′), 12.34 (C-methyl group).

FTIR (neat, KBr disc) ν (cm^{-1}): 3158, 3020, 2996, 1660, 1466, 1401, 1391, 1026, 1001, 1710, 968, 871.

MS: (ES) *m/z* 544 (M^+, 25), 506 (40), 438 (13), 303 (90), 123 (20), 80 (100).

3.4 Stability test of the tetraisopropyldisiloxane

Dichloroacetic acid (DCA) was dissolved into dichloromethane (DCM) to prepare a solutions (DCA/DCM) at concentrations of 1%, 2%, 2.4%, 3%, or 5% (v/v). To the DCA/DCM mixture (1000 μl), tetraisopropyldisiloxane (100 μl, 00.57 μmol) was added. The mixtures of DCA/DCM solution with tetraisopropyldisiloxane were ultrasonicated for 1 hour or 2 hours and analyzed by gas chromatography (GC, Table 2-2).

3.5 Synthesis of 5′-O-(4,4′-dimethoxytrityl) thymidine-terminated tetraisopropyldisiloxane

To a solution of 5′-O-(4,4′-dimethoxytrityl)thymidine (181.5 mg, 0.33 mmol) in dry pyridine (15 ml) was added 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (52.6 mg, 0.17 mmol). After the mixture was stirred for 2h, it was extracted with CH_2Cl_2 and washed with sat. aqueous $NaHCO_3$ and brine. The organic layer was dried ($MgSO_4$) overnight and filtered. The solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel with MeOH/$CHCl_3$ (1:100, v/v) to give compound (white powder). Yield 165 mg (0.15 mmol, 86%).

^{1}H NMR ($CDCl_3$) δ = 7.65 (m, 1H, 6-H), 7.20-7.40 (m, 9H, protons in aryl ring), 6.82 (d, 4H, J = 9.0 Hz, protons in *o*-position to methoxyl group), 6.43 (dd, 1H, 1′-H, $J_{1',2a'}$ = 7.2 Hz, $J_{1',2b'}$ = 2.6 Hz), 4.57 (m, 1H, 3′-H), 4.07 (m, 1H, 4′-H), 3.76 (s, 6H, protons of –OCH_3), 3.42 (m, 2H, 5′-H), 2.34 (m, 2H, 2′-H), 1.43 (s, 3H, protons of methyl in the ring 1), 1.00 (m, 14 H, protons of isopropyl connected to silicon).

^{13}C NMR ($CDCl_3$): δ = 162.7 (C-4), 158.5 (Cq DMt), 149.9 (C-2), 144.5 (Cq DMt), 139.9 (C-6), 135.4, 135.1 (Cq DMt), 113.1 (CH DMt), 108.7 (C-5), 86.7 (Cq, DMt), 84. 9 (C-4′), 84.2 (C-1′), 68.1 (C-3′), 60.5 (-OCH_3), 58.9 (C-5′), 39.7 (C-2′), 16.6-17.1 (CH_3 TiPDS), 12.2-13.1 (CH TiPDS).

FTIR (neat, KBr disc) ν (cm^{-1}): 3500, 2970, 1700, 1558, 1250, 1180, 1070, 1050, 911, 885, 828, 802, 759, 705, 625, 480.

MS: (ES) *m/z* 1289 ($M-43^+$, 15), 1110 (78), 1026 (15), 971 (12), 927 (100), 861(25) and 845(22).

3.6 Test of the hydrolytic stability of the 5′-O-(4,4′-dimethoxytrityl) thymidine-terminated tetraisopropyldisiloxane

The 5′-O-(4,4′-dimethoxytrityl)thymidine-terminated tetraisopropyldisiloxane (150 mg, 0.11 mmol) was dissolved into dichloromethane (5 ml) and cooled to 0 °C. Water (200 μl) and dichloroacetic acid water solution (0.01M, 100μl) were added to the above solution. The mixture was ultrasonicated for 3 min. Each component was separated by passing through a flash silica column (CH_2Cl_2/CH_3OH, 95:5, v/v). One fraction with its characteristic color (orange) was identified as 4,4′-dimethoxytrityl alcohol by its ^1H NMR. Another was identified as thymidine-terminated tetraisopropyldisiloxane.

3.6.1 Isolated hydrolysis product

3.6.1a 4,4′-Dimethoxytrityl alcohol

^1H NMR ($CDCl_3$, 200MHz): 7.20-7.40 (m, 4H, protons in *m*- position to methoxyl group and 5H protons in the third aryl ring), 6.80 (d, 4H, J = 9.1 Hz, protons in *o*-position to methoxyl group), 3.76 (s, 6H, OCH_3).

^{13}C NMR ($CDCl_3$): δ = 145.2 129.6, 127.8, 127.6, 81.3.

FTIR (neat, KBr disc) ν (cm^{-1}): 3474, 3080, 1491, 1401, 1331,1182, 1001, 938, 891, 684.

MS: (ES) *m/z* 303 $(M-17)^+$ (85), 123 (15), 81(25), 80 (100).

3.6.1b Thymidine-terminated tetraisopropyldisiloxane

^{1}H NMR ($CDCl_3$) δ = 7.65 (m, 1H, 6-H), 6.43 (dd, 1H, 1′-H, $J_{1',2a'}$ = 7.2 Hz, $J_{1',2b'}$ = 2.6 Hz), 4.57 (m, 1H, 3′-H), 4.07 (m, 1H, 4′-H), 3.42 (m, 2H, 5′-H), 2.34 (m, 2H, 2′-H), 1.43 (s, 3H, protons of methyl in the ring 1), 1.00 (m, 14 H, protons of isopropyl connected to silicon).

^{13}C NMR ($CDCl_3$): δ = 162.7 (C-4), 149.9 (C-2), 139.9 (C-6), 108.7 (C-5), 84. 9 (C-4′), 84.2 (C-1′), 68.1 (C-3′), 58.9 (C-5′), 39.7 (C-2′), 16.6-17.1 (CH_3 TiPDS), 12.2-13.1 (CH TiPDS).

MS: (ES) *m/z* 683 (M-43$^+$, 10), 543 (40), 418 (54), 371 (68), 126 (15), 81 (12), 79 (100).

3.7 Synthesis of 3′-O-allyl, 5′-O-(4,4′-dimethoxytrityl) thymidine

In a flask, sodium hydride (60% suspension in mineral oil, 80.0 mg, 2.0 mmol) was washed three times with DMF (3×10 ml, anhydrous). 5′-O-(4,4′-Dimethoxytrityl)-thymidine (816.9 mg, 1.5 mmol), which was previously dissolved in DMF (15 ml, anhydrous), was added to the flask. The mixture was kept stirring for 5 h at 0 °C. Allyl bromide (217.7 mg, 1.8 mmol), which was previously dissolved in DMF (5 ml), was added into the flask. After stirring for 20 h, the reaction was quenched. The excess sodium hydride was consumed by adding aqueous $(NH_4)_2SO_4$ (5 ml). The aqueous layer was extracted with EtOAc (3×8 ml). The combined organic layers were washed with brine (3×5 ml) and dried on $MgSO_4$ (anhydrous). Finally, the solvent was removed under

reduced pressure. The residue was purified by flash chromatography column to give the product (light yellow powder). Yield: 0.76 g (86%).

^{1}H NMR ($CDCl_3$): δ = 7.65 (m, 1H, 6-H), 7.20-7.40 (m, 9H, protons on aromatic ring), 6.80 (d, 4H, J = 9.0 Hz, protons in aromatic ring), 6.43 (dd, 1H, 1′-H, $J_{1,2a'}$ = 7.2 Hz, $J_{1',2b'}$ = 2.6 Hz), 5.85 (m, 1H, proton of allyl group), 5.15 (m, 2H, protons of allyl group), 4.50 (d, 2H, J = 4.3 Hz, protons of allyl group), 4.47 (m, 1H, 3′-H), 4.01 (m, 1H, 4′-H), 3.74 (s, 6H, protons of $-OCH_3$), 3.42 (m, 2H, 5′-H), 2.34 (m, 2H, 2′-H), 1.41 (s, 3H, protons of methyl in the ring 1).

^{13}C NMR ($CDCl_3$): δ = 164.4 (C-4), 159.0 (Cq, DMTr), 151.9 (C-2), 144.5 (Cp, DMTr), 135.6 (C-2′′, propene carbon), 135.4, 135.1 (Cq, DMTr), 134.9 (C-6), 132. 9 ~ 127.8 (CH arom), 114.5 (C-1′′, propene carbon), 113.1 (CH DMTr), 108.7 (C-5), 77. 9 (C-4′), 77.2 (C-1′), 71.5 (C-3′′, propene carbon), 69.1 (C-3′), 60.5 ($-OCH_3$), 58.8 (C-5′), 39.7 (C-2′).

FTIR (neat, KBr disc) ν (cm^{-1}): 2934, 2860, 1672, 1515, 1448, 1392, 1261, 1097, 870, 660.

MS: (ES) *m/z* 584 (M^+, 25), 271 (38), 259 (13), 244 (18), 228 (20), 215 (65), 180 (50), 165 (65) 152 (100), 105 (65), 77 (68).

3.8 Synthesis of 5′-O-allyl uridine

Step 1: Uridine (300 mg, 0.81 mmol) was mixed with dry acetone (58 mg, 1.0 mmol) and ethylene glycol dimethyl ether (10 ml). This mixture was cooled to 0 °C and TsOH (3

mg, 0.02 mmol) was added as an acid catalyst. The stirred reaction mixture was allowed to warm to room temperature and kept at this temperature for 2h. The reaction was then cooled (ice-bath) and a 10% sodium carbonate solution (7 ml) was slowly added. The resulting suspension was filtered, and the filtrate evaporated under reduced pressure.

Step 2: In a flask, sodium hydride (60% dispersion in mineral oil, 38.4 mg, 0.96 mmol) was washed three times with DMF (3×10 ml, anhydrous). 2′,3′-Acetonide protected uridine (180.0 mg, 0.64 mmol) prepared in Step 1 was added to the flask. The mixture was allowed to stir for 5 hours at 0 °C. Allyl bromide (92.0 mg, 0.76 mmol), which was previously dissolved in DMF (5 ml), was added to the flask. After stirring for 20 h, the reaction was complete.

Step 3: Acetic acid (80%, 90 mg, 1.5 mmol) was added dropwise into the flask and the mixture was heated to reflux for 30 min. The reaction was quenched by the slow addition of aqueous NH_sH_2O (5 ml). The aqueous layer was extracted with EtOAc (3×5 ml) and the combined organic layers were washed with brine (3×5 ml) and dried on $MgSO_4$ (anhydrous). Finally, the solvent was removed under reduced pressure. The residue was purified by flash chromatography. Yield: 92.8 mg (54%).

^{1}H NMR (Methanol-d_4): δ = 8.0 (m, 1H, 5-H), 5.92~5.90 (m, 2H, 1′-H and one proton of the allyl group), 5.70 (m, 1H, 6-H), 5.11 (m, 2H, two protons of allyl group), 4.50 (d, 2H, J = 1.5 Hz, H_2C=CHCH_2-), 4.16 (dd, 1H, 2′-H), 4.13 (dd, 1H, 3′-H), 4.00 (m, 1H, 4′-H), 3.82 (m, 2H, 5′-H).

^{13}C NMR (D_2O): δ = 166.7 (C-4), 150.9 (C-2), 139.9 (C-6), 134.4 (C-2′′, propene carbon), 119.0 (C-1′′, propene carbon), 108.7 (C-5), 84. 9 (C-4′), 84.2 (C-1′), 71.0 (C-3′′, propene carbon), 70.1 (C-3′), 61.9 (C-5′), 39.7 (C-2′).

FTIR (neat, KBr disc) ν (cm^{-1}): 3341, 2927, 2863, 1665, 1508, 1389, 1254, 1098, 869, 761, 660.

MS: (ES+) *m/z* 285 (M^+, 78), 223(42) 151 (5), 105 (4) 90 (5), 73.7 (100).

3.9 Synthesis of 3′-O-(propyl), 5′-O-(4,4′-dimethoxytrityl) thymidine-terminated pentamethyldisiloxane

Karstedt's catalyst, platinum-divinyltetramethyldisiloxane complex in xylene, (5 drops) was added to the pentamethyldisiloxane (81.5 mg, 0.57 mmol) and mixed vigorously. After the colloid was formed, as indicated by the characteristic yellow color of the solution, the pentamethyldisiloxane solution was added to 3′-O-(allyl)-5′-O-(4,4′-dimethoxytrityl)thymidine (260.0 mg, 0.48 mmol) which was previously dissolved in THF(8 ml). Subsequently, the mixture was refluxed at 67 °C. During the entire process, a moisture-protecting atmosphere (to keep water, but not oxygen, from the system) was applied in order to avoid hydrolysis. Samples were collected for analysis at different time intervals. A maximum reaction time of 12 h was utilized. The catalyst was removed via filtration through activated carbon. The solvents and excess pentamethyldisiloxane were removed under vacuum. Yield: 187g, 45%.

^{1}H NMR ($CDCl_3$): δ = 7.73 (m, 1H, 6-H), 7.21-7.43 (m, 9H, protons in phenyl ring), 6.84 (d, 4H, J = 9.2 Hz, protons in *o*-position to methoxyl group), 6.35 (dd, 1H, 1′-H, $J_{1',2a'}$ = 7.2 Hz, $J_{1',2b'}$ = 2.6 Hz), 4.47 (m, 1H, 3′-H), 4.07 (m, 1H, 4′-H), 3.76 (s, 6H, –OCH_3), 3.42 (m, 2H, 5′-H), 3.37 (t, 2H, 3′′-H, J = 2.5 Hz, protons of methylene connected to 3′-O), 1.5 (m, 2H, 2′′-H, protons of methylene), 2.34 (m, 2H, 2′-H), 1.45 (s, 3H, protons of methyl in the ring 1), 0.4 (m, 2H, 1′′-H, Si-CH_2-CH_2CH_2), 0.10 ~ 0.05 (15 H, Si-Me).

^{13}C NMR ($CDCl_3$): δ = 164.4 (C-4), 159.0 (Cq, DMTr), 151.9 (C-2), 144.5 (Cp, DMTr), 135.4, 135.1 (Cq, DMTr), 134.9 (C-6), 132. 9 ~ 127.8 (CH arom), 113.1 (CH DMTr), 108.7 (C-5), 77. 9 (C-4′), 77.2 (C-1′), 71.5 (C-3′′, methylene carbon connected to 3′-O), 69.1 (C-3′), 60.5 (-OCH_3), 58.8 (C-5′), 39.7 (C-2′), 24.3 (C-2′′, methylene carbon), 15.4 (C-1′′, Si-CH_2-CH_2CH_2).

FTIR (neat, KBr disc) ν (cm^{-1}): 3450, 2970, 2920, 1710, 1510, 1410, 1260, 1090, 833, 708, 699.

MS: (ES+) *m/z* 732 (M^+, 8), 630 (10), 554 (21), 478 (5), 447 (20), 404 (20), 377 (30), 358 (5), 303 (100), 132 (15), 117 (30).

3.10 Synthesis of cyclohexane-1,2-epoxide-terminated tetramethyldisiloxane

1,1,3,3–Tetramethyldisiloxane (7.6 g, 56.6 mmol) was dissolved in dry pentane (20 ml) in a round-bottomed flask and Karstedt's platinum-divinyltetramethyldisiloxane complex in xylene, (3 drops) was added dropwise to the flask, and then the solution was heated to

50 °C. Finally, 4-vinyl-1-cyclohexane-1,2-epoxide (1.4 g, 11.3 mmol), which was previously dissolved in dry pentane (10 ml), was added dropwise to the flask. The mixture was heated to reflux during which time a bright yellow solution resulted, which slowly turned green. Aliquots (0.25-0.5 ml) were removed for NMR analysis at convenient intervals to monitor the reaction. Each aliquot was distilled under reduced pressure before conducting NMR analysis. The reaction was manipulated so that hydrosilylation would only occur at one terminus of tetramethyldisiloxane by using a large excess of the siloxane. The catalyst was removed by filtering through activated carbon. Finally, the product was obtained through distillation under reduced pressure. Yield: 2.6 g (85%).

^{1}H NMR ($CDCl_3$): δ = 4.64 (s, 1H, Si-H), 3.08 (m, 2H, 5-H and 6-H, epoxide protons), 2.12 ~ 1.10 (m, 7H, cyclohexene protons), 0.85 (m, 2H, 2-H, HC=CH), 0.40 (m, 2H, 1-H, Si-CH_2), 0.12 (m, 12H, Si-Me).

^{13}C NMR ($CDCl_3$): δ = 59.8, (C-6), 58.5 (C-5), 32.7 (C-4), 30.4 (C-3), 28.7 (C-8), 25.9 (C-2), 25.0 (C-7), 18.6(C-1), 5.3 (Si-CH_3).

FTIR (neat, KBr disc) ν (cm^{-1}): 2362, 2100, 1390, 1260, 1240, 1130, 1070, 910, 870, 805, 760, 750.

MS: (ES^+) *m/z* 258 (M^+, 5), 244 (100), 228 (10), 207 (62), 195 (32) 181 (95).

3.11 Synthesis of thymidine and epoxy bifunctionalized silicone

Karstedt's catalyst, platinum-divinyltetramethyldisiloxane complex in xylene (4 drops) was added to the cyclohexane-1,2-epoxide-terminated tetramethyldisiloxane (387.8 mg,1.50 mmol) and then mixed vigorously. After the colloid was formed, as indicated by the characteristic yellow color of the solution, the cyclohexene-1,2-epoxide-terminated tetramethyldisiloxane was added to the 3′-O-(allyl)- 5′-O-(4,4′-dimethoxytrityl)-thymidine (1.17 g, 2.0 mmol) which was previously dissolved in THF (25 ml). Subsequently, the mixture was refluxed at 67 °C under a CaH_2 protected atmosphere (to keep water, but not oxygen, from the system) for a maximum reaction time of 12 h: samples were collected for analysis at different time intervals. The catalyst was removed by addition of activated carbon to the reaction mixture and then filtration. The solvents were removed under reduced pressure. The excess of reactant, 3′-O-(allyl)- 5′-O-(4,4′-dimethoxytrityl)-thymidine, was washed out using methanol. Yield: 0.65g, 45 %.

^{1}H NMR ($CDCl_3$): δ = 7.73 (m, 1H, 6-H), 7.21-7.43 (m, 9H, protons in phenyl ring), 6.84 (d, 4H, J = 9.1 Hz, in *o*-position to methoxyl group), 6.35 (dd, 1H, 1′-H, $J_{1',2a'}$ = 7.2 Hz, $J_{1',2b'}$ = 2.6 Hz), 4.47 (m, 1H, 3′-H), 4.07 (m, 1H, 4′-H), 3.76 (s, 6H, –OCH_3), 3.42 (m, 2H, 5′-H), 3.37 (tri, 2H, 3′′-H, CH_2-3′-O), 3.08 (m, 2H, 5′′′-H and 6′′′-H), 2.34 (m, 2H, 2′-H), 2.12 ~ 1.10 (m, 7H, cyclohexene protons), 1.5 (m, 2H, 2′′-H), 1.45 (s, 3H, CH_3), 0.85 (m, 4H, 2′′-H and 2′′′-H, CH_2), 0.40 (m, 4H, 1′′-H and 1′′′-H), 0.10 (m, 31H, Si-CH_3).

^{13}C NMR ($CDCl_3$): δ = 164.4 (C-4), 159.0 (Cq, DMTr), 151.9 (C-2), 144.5 (Cp, DMTr), 135.4, 135.1 (Cq, DMTr), 134.9 (C-6), 132. 9 ~ 127.8 (CH arom), 113.1 (CH DMTr), 108.7 (C-5), 77. 9 (C-4′), 77.2 (C-1′), 71.5 (C-3′′), 69.1 (C-3′), 60.5 (-OCH_3), 59.8, (C-6′′′), 58.8 (C-5′and C-5′′′), 39.7 (C-2′), 32.7 (C-4′′′), 30.4 (C-3′′′), 28.7 (C-8′′′), 25.9 (C-2′′′), 25.0 (C-7′′′), 24.3 (C-2′′), 15.4 (C-1′′), 18.6 (C-1′′′), 5.2 (Si-C).

FTIR (neat, KBr disc) ν (cm^{-1}): 2920, 1700, 1610, 1510, 1390, 1260, 1110, 1050, 777.

MS: (ES) *m/z* 1180 (60), 1106 (100), 1056 (30), 979 (30), and 920 (52).

Chapter 4

CONCLUSION

4.1 Summary of the Study

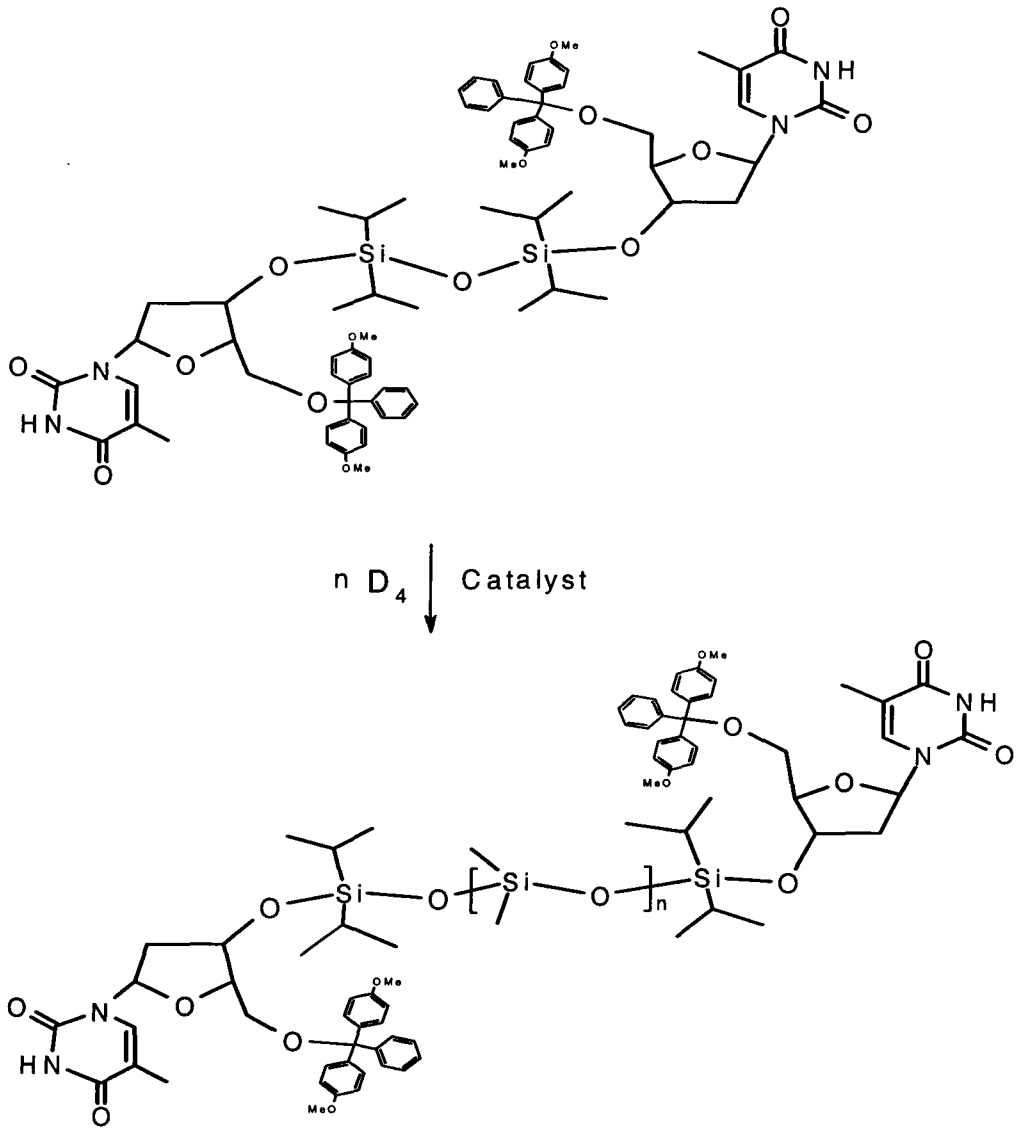
We have demonstrated a strategy to prepare nucleoside-functionalized silicones. In order to take advantage of *Solid Phase Synthesis* processes, we introduced an epoxy group to one end of a silicone chain using hydrosilylation process. Through our study, we found the epoxy group was stable to hydrolysis. In addition, the literature[85~88] shows that epoxy groups can react with acyl halides group to form ester bonds in a homogeneous phase. It was our plan, using heterogeneous conditions that mirror traditional solid phase protocols, to bind the nucleoside functionalized silicone to a support *via* reaction of the epoxide with an acyl halide functionalized resin. However, we have not yet demonstrated effective surface grafting and to submit the resin to standard conditions that will permit the preparation of an oligonucleotide-functionalized silicone. Performing this synthesis constitutes future work.

4.2 Future Work

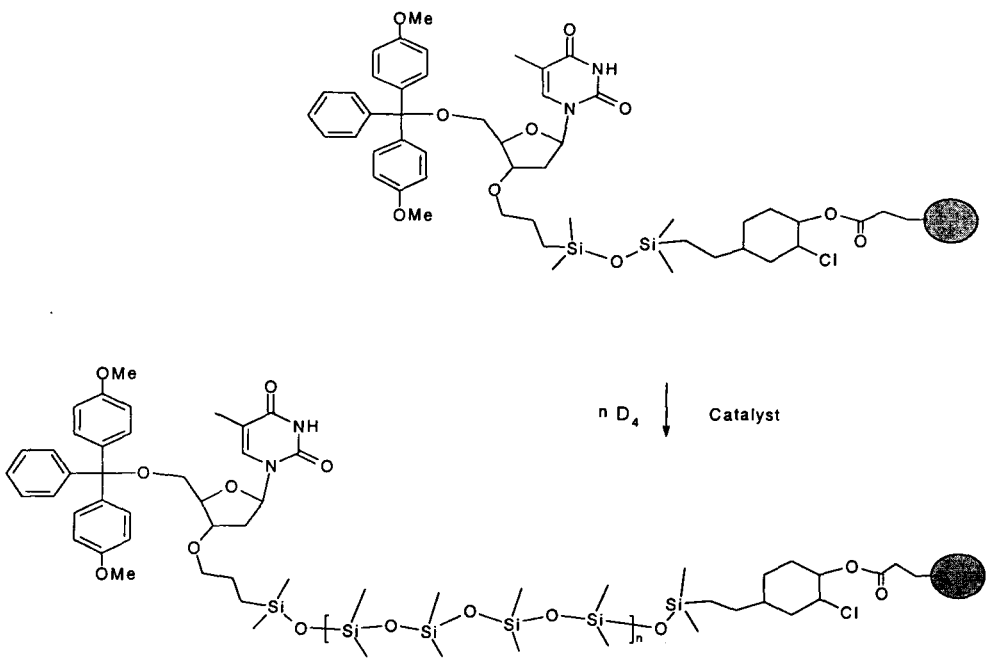
4.2.1 Orthogonal Chemistries

The hydrophobic part of the oligonucleotide-silicone copolymer will be increased by taking advantage of the redistribution of cyclic and chain silicone (Scheme 4 - 1, Scheme 4 - 2). The process involves initiating polymerization of octamethylcyclotetrasiloxane

(D_4) with a base or acid catalyst. So, both acids and bases will be examined as catalysts for the silicone chain growing process.



Scheme 4 - 1: The redistribution of cyclic and chain silicones.



Scheme 4 - 2: The redistribution of cyclic and chain silicones on a solid phase support (● = functional resin).

4.2.2 Characterization of the complementary binding between oligonucleotide-silicone copolymer chains

We shall compare the properties (melting point, surface tension, viscosity) of single stranded silicone-oligothymidine polymers with that of double strands prepared from silicone-oligothymidine-silicone and silicone-oligoadenine-silicone polymers (Figure 4 - 1). It is anticipated that the non-selective hydrogen bonded affinity of polymers, containing only one type of nucleoside, will be significantly lower than the complementary hydrogen bonding between the two different polymers. The affinity of the two strands, compared to the homopolymers, can be established using calorimetry: the

temperature of dehybridization is a good guideline for the strength of the interaction. The melting temperature (T_m) will hopefully indicate that the copolymer double chains are a highly cooperative structure.

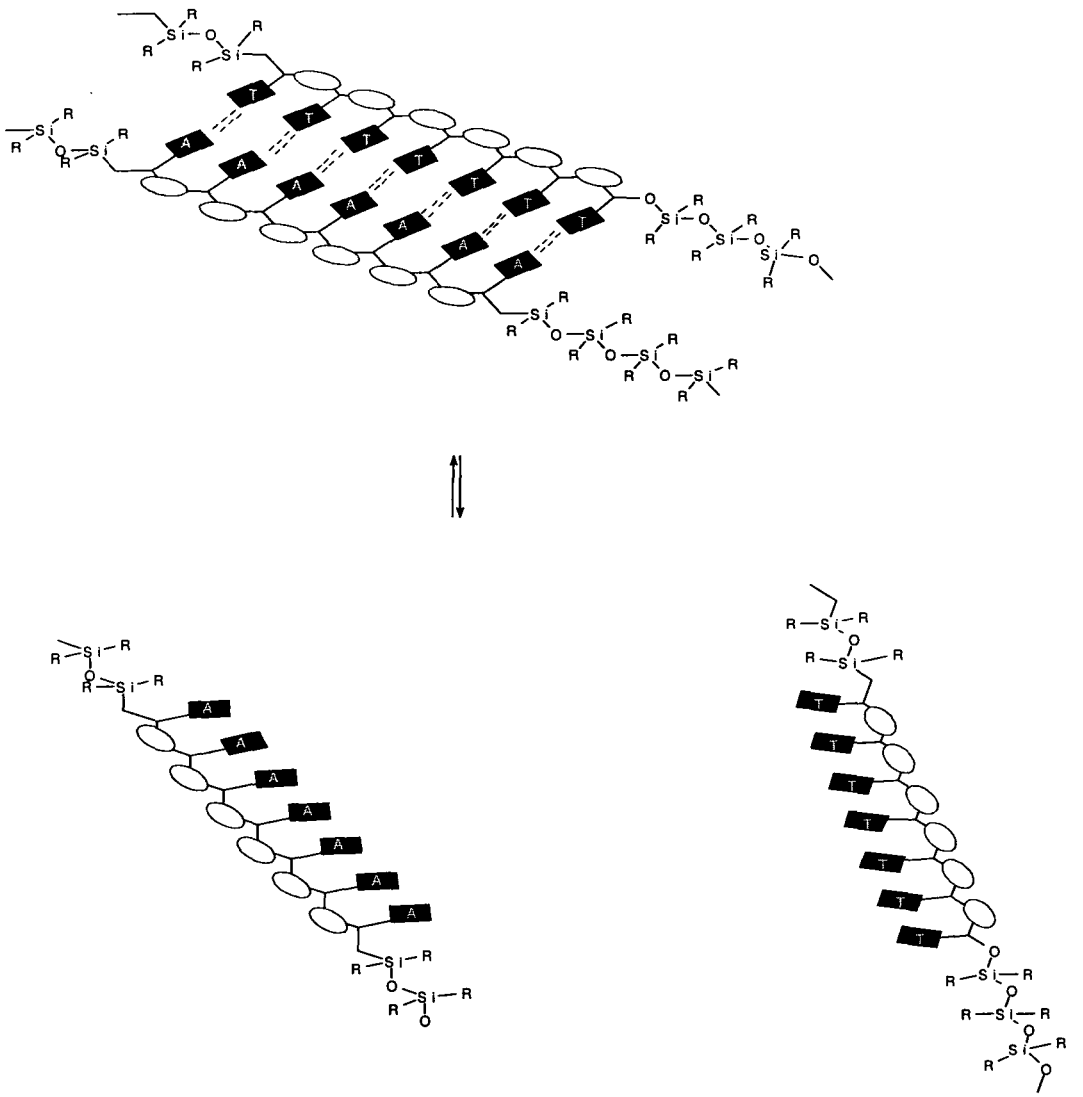


Figure 4 - 1: Complementary binding between oligonucleotide-silicone copolymer chairs.

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