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Synthesis and characterization of chiral stationary phases on hydride surface

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SYNTHESIS AND CHARACTERIZATION OF CHIRAL STATIONARY PHASES ON HYDRIDE SURFACE

A Thesis

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In partial Fulfillment

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by

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ABSTRACT

SYNTHESIS AND CHARACTERIZATION OF CHIRAL STATIONARY PHASE ON HYDRIDE SURFACE

by Shivanand Kamath

Chiral stationary phases (CSPs) have a wide application in pharmaceutical and biotech companies for separation of chiral compounds. In this research a new CSP was developed using 4-Allyoxybenzoic acid as the starting compound for bonding the chiral selector R(+)- (1)-Naphthylethylamine to the silica hydride surface. This is achieved by using dicyclohexyldiimide as a catalyst and N-hydroxysuccimide as an cleaving agent. The chiral stationary phase was characterized by DRIFT, NMR and HPLC. It was found that analytes such as derivatives of amino acids, amine and carboxylic acids are well separated on this CSP. For example 3,5 dinitrobenzoylalanine methyl ester has separation factor (α) value of 1.48 as compared to 1.09 obtained by other workers.

Acknowledgements

I would first like to acknowledge my thesis advisor Dr. J. Pesek and Dr. M. Matyska for rendering their valuable time in completing my research. They reviewed my manuscript and performed the ^{13}C and ^{29}Si nuclear magnetic resonance analyses for my silica samples. They even helped me out in interpretation of HPLC data and also explained troubleshooting problems. In addition I would also like to thank my committee members Dr. M. Scharberg and Dr. B. Stone for reviewing the manuscript for sitting in my seminar. I would to thank my collegeues who are still working under Dr.Pesek for sharing their ideas with me. Finally I would to thank Ray Berryesa, Richard Mercurio, Sam Monroe and Steve Capalloni for repairing or replacing the broken apparatus and giving chemicals required for my research, so that I could finish this project in a timely manner.

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CHAPTER I - INTRODUCTION

Molecules that are related to each other as identical in structure but non-superimposable are enantiomers or chiral compounds (from greek word *cheiro*, meaning hand); they are like a pair of hands. Stereoisomers are isomeric molecules with identical constitution but a different spatial arrangement of atoms. The symmetry factor classifies stereoisomers as either enantiomers or diastereoisomers. Diastereoisomers are stereoisomers that are not enantiomers. Structures (a) and (b) (Fig.1) are enantiomers because they are mirror images. Structures (a) and (c), or (b) and (d) are diastereoisomers because they do not have a mirror image relationship. Separation of chiral compounds are of utmost important in the pharmaceutical, agricultural and chemical industries[1]. Accurate assessment of the isomeric purity is important and critical, as an isomeric impurity may have unwanted toxicological, pharmacological, or other effects. Separation of enantiomeric impurities is difficult by conventional methods like thin layer chromatography or synthetic routes, as additional impurities are formed in these processes[2]. High performance liquid chromatography represents the best separation technique for stereoisomers.

A. High Performance Liquid Chromatography (HPLC)

In this technique analytes are separated by partitioning between a liquid mobile phase and solid or liquid stationary phase. Early column chromatographic separation took place in glass columns of 1 to 5 cm in diameter and 50 to 500 cm in length. The diameter

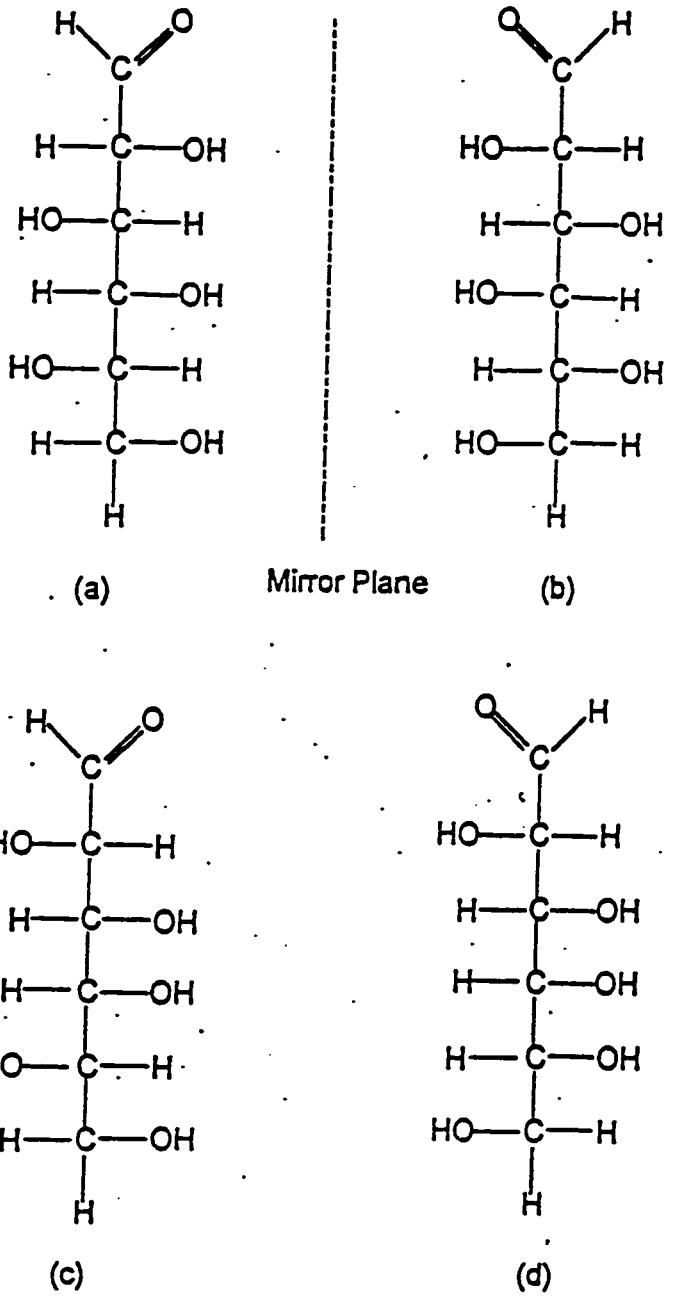


Figure 1. **Structures (a/b) are enantiomeres whereas structures (a/c; a/d; b/c;b/d) are diastereoisomeres.**

of the solid particles packed inside the column was between 50 to 200μm. Separation usually required several hours. Attempts to reduce the separation time were often accompanied by lower column efficiency. Later, by packing 3 to 10μm particles in smaller columns, the efficiency was improved.

Even though the reversed phase mode in which the stationary phase is non-polar (C18) and the mobile phase polar (water, acetonitrile, methanol, tetrahyrofuran....) is widely used in HPLC, the separation of chiral compounds usually involves the normal phase mode. In normal phase operations the mobile phase is a nonpolar solvent like hexane, heptane or dichloromethane and the stationary phase is polar. In this research the normal phase mode of separation is used in which the stationary phase is (R/S)-N-(1)-Napthylethylamine which is chemically bonded to the inert solid support (silica). To attain a reasonable flow rate, the mobile phase together with analytes are pushed through the column at high pressures (around 500 to 1000 psi). Physical adsorption of the chiral moiety on the support results in column bleeding. Consequently, chemical bonding is preferred. Solid supports commonly used are silica, zirconia, alumina and organic polymeric materials. Among these silica is the most popular support, since its chemistry is well studied. silica is spherical, mechanically sturdy, can withstand high pressure and is available in various sizes. The porous structure of silica allows greater analyte-stationary phase interaction[3]. Because of these advantages silica was chosen as the solid support in this research.

Since HPLC is now one of the most powerful separation techniques, resolution of enantiomers by HPLC is expected to develop rapidly. The major advantage of HPLC over conventional methods is that it can be applied for a wide range of compounds. Apart from this, the analysis time is reduced, a purified compound is obtained and preparative scale operation is possible.

Many factors contribute to the interaction of stereoisomeric molecules with the stationary phase They are electrostatic forces, inductive effects, dipole-dipole interactions, hydrogen bonding, hydrophobic interactions, structural rigidity/conformational flexibility, steric interaction, orientation and spacing of groups, ligand formation and temperature. Some of these factors can be used for the separation of stereoisomers.

A racemic mixture (equal amounts) of two enantiomers has always been a problem in separation science. Separation of chiral compounds basically can be performed by two different methods. The first technique, called the indirect method, basically involves derivatization of the components and separating them on achiral columns. The second, approach called the direct method involves separation of components by making the mobile phase chiral or the stationary phase chiral. The derivatization step can be the result of a chemical reaction between a pair of enantiomers with a chiral derivatizing agent (CDA) to afford chromatographic separation of diastereomers or it can be a short term interaction between the enantiomer and a CDA. The indirect approach is not the recommended method as it has many drawbacks such as, it often involves many steps for

the preparation of solutes, it is time consuming and lastly has a high cost/separation ratio. Thus analysts are now concentrating on direct methods for the separation of chiral compounds.

The first commercially available HPLC-CSP (high performance liquid chromatography chiral stationary phase) was introduced by Pirkle in 1981[4]. At present there are about 60 HPLC-CSPs on the market. The difficult question for the analyst now is which CSP to use. The classification of HPLC-CSP is based upon how the solute-CSP complexes are formed. The different types of stationary phases are

1. Pirkle-type and related phases,
2. Polysaccharide phases,
3. Cyclodextrin-bonded phases,
4. Chiral ligand-exchange phases,
5. Protein bonded stationary phases, and
6. Novel chiral phases (based on amide and cellulose).

Pirkle-type and related phases: Solute-CSP complexes are formed by attractive interactions such as hydrogen bonding, π-π interaction, dipole stacking. These are the types of CSPs developed by Pirkle[5] as well as those that operate in a similar fashion.

Polysaccharide phases: The primary mechanism for the formation of a solute-CSP complex is through attractive interaction but where an inclusion effect also plays an important role. These are cellulose-based CSPs described by Hesse et al.[6].

Cyclodextrin-bonded phases: The primary mechanism for the formation of the solute-CSP complex is the inclusion of the solute in the cavity of the CSP. These are the cyclodextrin-CSPs described by Armstrong and phenylmethacrylate polymers described by Okamoto[7].

Chiral ligand-exchange phases: The solute is the part of a diastereomeric metal complex. These are chiral ligand exchange CSPs.

Protein bonded stationary phases: The CSP is an immobilized protein and the solute-CSP complexes are based upon combinations of hydrophobic and hydrophilic attractions. These are derived mostly from bovine serum albumin and alpha-1-acid glycoprotein[8].

Novel chiral phases: The enantiomers are separated by three point interaction. These involve more than one chiral center on the stationary phase.

Looking at all the above techniques it is possible to reach to a conclusion that bonded HPLC materials should be capable of discriminating enantiomeric solutes by various strategies. The strategies employed are

(1) Charge transfer (π) interactions,

(2) Dipole-dipole interactions,

(3) Hydrogen-bonding interactions, and

(4) Steric interactions.

Given that a chiral packing may use more than one of these processes, the chromatographic mode employed may be varied between

(1) Ligand exchange LC,

(2) Normal phase (adsorption) LC,

(3) Reverse phase (partition) LC,

(4) Affinity or inclusion LC, and

(5) Ion-paring or ion-exchange LC.

It has been demonstrated that multiple competing chiral recognition mechanisms take place[9]. Taking into account the geometrical alignment of an enantiomer, it is seen that there should be a minimum of three points of simultaneous interaction between an enantiomer and the substrate. This chiral recognition is reciprocal, in that, if a molecule A(+) separates B(+) from B(-), then B(+) separates A(+) from A(-). This type of CSP has the ability to separate various amines, amino acids(as their ester and amide derivatives), amino alcohols, alcohols, carboxylic acids, diols and 3,5 dinitrobenzoyl derivatives of amino acids. All of these CSPs function somewhat through the same separation mechanism but may differ quantitatively in the level of performance for any given analyte. Some examples of novel chiral stationary phases based on the amide linkages are α– 6,7-dimethyl-1-napthyl 10-undecenyl amine, α-(6,7-dimethyl-1-napthyl)-5-hexenylamine and α-(6,7-dimethyl-1-napthyl)-11-dodecenylamine[10]. Such types of stationary phases interact with the analyte enantiomer by at least three simultaneous interactions, and all three are dependent on stereochemistry. It is also found that for the analyte to be resolved there should be a complementary functionality to that of the stationary phase. Hence, a successful CSP will have a certain class of resolvable analytes. It can be seen (Fig. 2) that analyte enantiomers 1A and 1B can be resolved by CSP 1 depending upon the interaction

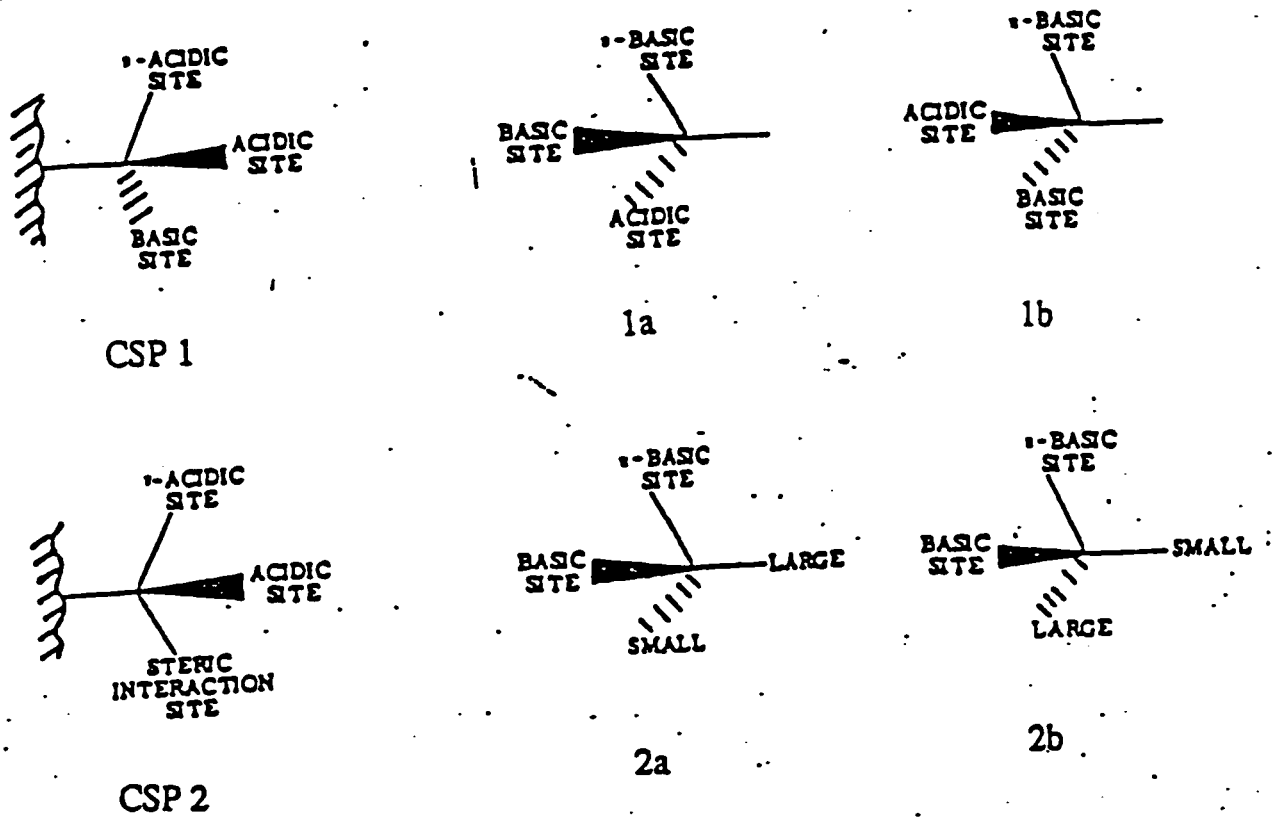


Figure 2. The diagram of the three point interaction represented by CSP 1 can separate the enantiomeres 1a from that of 1b, and CSP 2 can separate the enantiomeres of 2a from that of 2b.

that takes place. It can be deduced that 1A will have stronger interaction than 1B. If the CSP is of type 2 then enantiomers 2A and 2B can be resolved by the fact that 2A will have less steric interaction due to a smaller group pointing towards the chiral center than 2B which has a larger group. Thus depending upon the CSP different enantiomeric analytes can be resolved. Another important point to be noted is that each type of CSP is designed to interact in a 1:1 ratio.

The mechanism for separation can be further elaborated by taking into account that the mobile phase also plays an important role in resolving the solutes. Non-polar mobile phases like hexane-2-propanol mixtures make the major attractive interaction between the analyte and the CSP π-π bonding and antiparallel dipole 'stacking' of amide bonds. These interactions require a face to face approach of the analyte and the CSP. Since the bulky phenyl group is attached to the carbon atom it blocks the approach and therefore only one face is readily available.

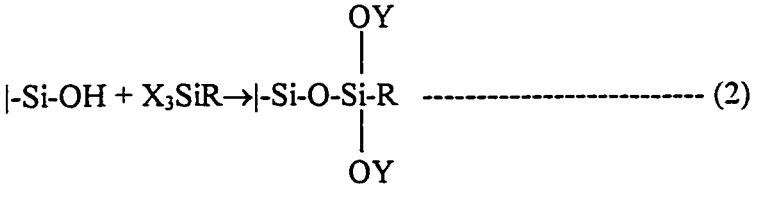
B. Synthetic Methods for Bonded Phases:

Advances in surface modification of sorbates have made HPLC a powerful technique of separation. HPLC has separated components ranging from low molecular weight species to biopolymers. Currently, the most common approach of preparing alkyl-bonded phases involves organosilanization, a reaction between fully hydroxylated silica and a dimethylchlorosilane as follows[11]:

$$\text{-Si-OH} + \text{ClSiMe}_2\text{R} \rightarrow \text{-Si-O-SiMe}_2\text{R} + \text{HCl} \quad \text{---------- (1)}$$

On reacting a chlorosilane with the hydroxyl group of the silica the hydrocarbon chain is attached by the stronger silicon-oxygen-silicon link. This is a fairly stable bonding sequence (more stable than the carbon-oxygen-silicon bond) but is easily broken at extremes of pH. This reaction results in a monomeric phase[12].

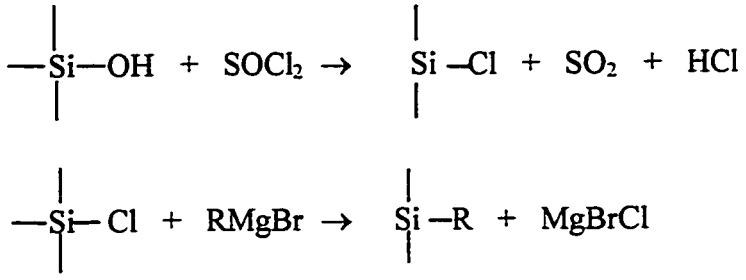
There are often a large number of unreacted silanol groups and they can interact with the solute that hinders the separation process. To overcome this difficulty a different approach which produces an Si-O-Si-C linkage on the silica support was developed (2) where silica was reacted with alkyoxyalkylsilanes in a similar manner as chlorosilanes but, as might be expected, required somewhat different reaction conditions. The ethoxy-and methoxysilanes are the most reactive of the alkyoxysilanes and, consequently, were the most commonly used reagent for the bonded phase synthesis. This reaction resulted in a polymeric phase[13] as shown below.



---------- (2)

X = halide, alkoxy or acyloxy groups and Y = H or Si

A entirely different method for bonding organic moieties to the surface of silica utilizes the Grignard reaction, a process pioneered by Halastz et al.[14]. The silica is first treated with thionyl chloride to replace the surface hydroxyl with a chlorine atom (chlorination). The chlorinated surface is treated with Grignard reagent and the chlorine atom is replaced with an alkyl group. The reaction is as follows:



R- C_nH_{2n+1}

The organic group is attached directly to the surface silicon atom with a Si-C bond which is very stable. It is less likely that the reagent is sterically hindered. The major drawback is that the reaction is very sensitive towards moisture.

A recent method for bonding an organic moiety like 1-octene and 1-octadecene involves catalytic olefin hydrosilylation using hexachloroplatinic acid as the catalyst[15], according to:

$$|\text{-Si-H} + CH_2 = CH\text{-R} \longrightarrow |\text{-Si-}CH_2\text{-}CH_2\text{-R} \quad (3)$$

Before this reaction the silanol groups are converted to silica hydride with the hydrolysis product of triethoxysilane which proceeds as follows:

$$|\text{-Si-OH} + (EtO)_3Si\text{-H} \longrightarrow |\text{-Si-O-Si(OY)(OY)-H} + 3EtOH \quad (4)$$

It is known that an Si-O-Si linkage is more hydrolytically unstable than an Si-C linkage. This instability leads to deleterious effects on the column and poor long term precision and potential contamination. It can be clearly seen that compared to conventional

organosilianization, olefin hydrosilinazation on an Si-H surface forms a stable bond. The superior stability of the new product is seen due to an Si-C linkage on the silica support rather than Si-O-Si-C linkage produced by the conventional method. Generally speaking, the selectivity of the new chromatographic phases resembles that of their conventional counter part, alkyldimethylsilyl-bonded phases(3). Compared to conventional phases under particularly aggressive eluent conditions, more specifically involving trifluroacetic acid containing aqueous-organic solvents, the new bonded phase has greater stability. The column longevity is improved along with long term precision in retention[16]. Therefore, this method was adopted for the preparation of the chiral stationary phase.

CHAPTER II - EXPERIMENTATION

A. Chemicals

(1) Chemicals with CAS Registry Numbers

Chemical Name	CAS Registry Number
Calcium chloride	[10043-52-4]
Chloroplatinic acid hexahydrate	[16941-12-1]
Dicyclohexylcarbondiimide	[538-75-0]
N,N- Dimethylformamide	[68-12-2]
Ethanol	[64-17-5]
Hydrochloric acid	[7647-01-0]
Diethyl ether	[60-29-7]
Isopropanol	[67-63-0]
Methanol	[67-56-1]
Tetrahydrofuran	[109-99-9]
Methylene chloride	[75-09-2]
Toluene	[108-88-3]
Thionyl chloride	[7719-09-7]
R(+)-(1)-(1-Naphthyl)ethylamine	[3886-70-2]
S(-)-(1)-(1-Naphthyl)ethylamine	[10420-89-0]

(2) Chemicals for Preparing 4-Allyloxybenzoic acid and R/S-Napthylethylamine Reaction Product

The chiral reaction product mentioned above was synthesized according to procedure described later in this thesis. 4-Allyoxybenzoic acid was prepared by other students who were working in the laboratory according to the procedure of (4). R/S-Naphthylethylamine (99%), Dicyclohexyldiimide (DCC), tetrahydrofuran, methanol, deionized water and N-Hydroxysuccimide (99%) were purchased from Aldrich Chemicals (Milwaukee, WI, USA). Concentrated hydrochloric acid was obtained in reagent grade. Triethylamine was purchased from Fisher Scientific (Fair lawn, NJ, USA).

(3) Chemicals for Bonding Chiral Compound to Silica Hydride

Methylene chloride, toluene and diethyl ether were obtained in reagent grade. Each solvent was distilled and dried over calcium chloride. Isopropanol was reagent grade and dried. Chloroplatinic acid hexahydrate (99.9%) was purchased from Strem Chemicals (Newburyport, MA, USA). The catalyst was prepared by quickly weighing the appropriate amount on an analytical balance (Mettler AE 200, Mettler Instrument Corp., Highstown, NJ, USA) and transferring the material to a 10 ml volumetric flask, dissolved and diluted to the mark with isopropanol. Vydac 101 HS (particle diameter - 6.5 μm, pore diameter 380 A°, specific surface area 280.4 m^2/g was donated by the Separations Group (Hesperia, CA, USA).

B. Instruments and Operating Procedures

(1) Diffuse Reflectance Infrared Fourier Transform Spectrometry (DRIFT) and Fourier Transform Infrared Spectroscopy (FTIR)

Liquid nitrogen was used to supply gaseous nitrogen (55-65 psi) to the sample compartment of the infrared spectrometer (Perkin-Elemer model 1800) to remove moisture and perform the experiment in an inert atmosphere. For the FTIR analyses, liquid samples were placed in-between potassium chloride pellets and were referenced against pure potassium chloride pellets. Double-beam FTIR spectra were scanned at a 2 cm^{-1} resolution and at 0.7 noise level. The infrared spectra of solid silica samples were obtained in the diffuse-reflectance mode. The diffuse reflectance accessory(Spectra Tech., Stanford, CT, USA) consisted of a 2 mm diameter and 2 mm depth sample cup. One part of silica sample was mixed with three parts of IR-grade potassium bromide (99.5%, Spectrum, Gardena, CA, USA). The mixture was ground into a fine powder and placed into the sample cup. The top was smoothed by a glass plate. The reflectance signal of the sample was compared against pure potassium bromide kept in a reference cell. Each spectrum was scanned 200 times at a resolution of 2 cm^{-1}. The output was connected to a computer system (Perkin-Elmer model 7500).

(2) Elemental Analyzer

The elemental analyzer measures the relative composition of carbon, nitrogen and hydrogen of a sample. First, a sample is combusted between 950 to 1000°C. Mainly

carbon dioxide and water are produced. The carbon and hydrogen contents are detected by comparing the difference in thermal conductivity before and after the removal of each product. Nitrogen is oxidized to various forms of oxides during combustion. To assure an accurate determination of the nitrogen content, nitrogen oxides are reduced to nitrogen between 650 to 700°C before the thermal conductivity measurements.

The elemental analyzer(Perkin-Elmer 240C) was connected to a chart recorder (Perkin-Elmer 56-3003) for recording the data obtained from the instrument. Highly pure compressed helium along with oxygen was supplied to the analyzer at 15-20 and 25-30 psi respectively. Cyclohexanone-2,4-dinitrophenylhydrazone from Perkin-Elmer was used as a calibration standard. 7-(2,3-dihydroxypropyl). Theophylline (99%) from Aldrich was used for instrument certification. Samples were weighed on a Cahn electro-microbalance. All determinations were performed in triplicate.

Surface Coverage, α_R, of the bonded silica was calculated as follows:

$$\alpha_R(\mu mol/m^2) = 10^6 P_c / (100 M_c n_c - P_c M_R) S$$

P_c - is the carbon-percentage difference in the modified silica and the silica hydride.

n_c - is the number of carbon atoms on the skeleton of the bonded material.

M_c & M_R - are the molecular weights of carbon and the bonded material.

$S(m^2/g)$ - is the specific surface area of the native silica as determined by the Brunauer, Emmet and Teller (BET) nitrogen adsorption method performed at the Chevron Research Center, Richmond, CA.

(3) Nuclear Magnetic Resonance Spectroscopy (NMR)

The ^{13}C and NMR spectra were obtained on a Bruker MSL 300 spectrometer. Cross Polarization magic angle spinning (CPMAS) techniques were employed to enhance the signal-to-noise ratio. Solid samples, spinning at 4700-5200 Hz, were placed in a ZrO_2 double bearing rotor. External glycine and polyhydridosiloxane samples were the reference standards for the ^{13}C and ^{29}Si chemical shifts respectively. Pulse widths of 6.5 and 5.0msec. were used for the ^{13}C and ^{29}Si spectra respectively. Both analyses were operated with a 5-msec contact time and a 5- μsec repetition rate; the probe temperature was 20±2°C.

(4) High Performance Liquid Chromatography (HPLC)

The Perkin Elmer HPLC consisted of a 200 IC series quaternary pump which was used to mix and pump the mobile phase through the column. A Perkin Elmer LC 295 was used as a multi-wavelength detector. Stainless steel tubes 250 mm in length and 4.6 mm i.d. were used for the columns. The sample was injected through a Rhenodyne 7125 six port injector valve with a 20 μL loop. The mobile phases used for analysis consisted of hexane, isooctane, 1,2 dichloroethane, ethanol and trifluroacetic acid. These solvents were filtered through a 4 mm millipore filter paper (Cole Palmer Instrument Co.) to remove any solid particles present. The solutes used for column testing were prepared in the laboratory. The dead time of the HPLC instruments was determined by the first peak

(solvent front). The flow rate for all the analyses was 1.0 ml/min, except in one case where 1.5 ml/min was used, in order to elute the compounds faster.

After packing the bonded silica into the stainless steel tubes (0.25" OD, 4.6 mm X 150 mm, Alltech), the column was equilibrated with pure methanol, ethyl acetate, dichloromethane and finally hexane. At the end of day the column was washed with a non-polar solvent, usually isooctane.

C. Synthetic Procedures

(1) Preparation of Silica Hydride

All the apparatus was dried in an oven at 110°C. Once the glassware was removed from the oven, the connections were greased and placed in a glove box. The preparation of a 1.0 M TES (Triethoxysilane) was performed in a nitrogen-filled glove box. The box was tightly closed form all the sides except from one end, in order to place the apparatus inside the box. After placing all the apparatus and the chemicals inside the box, the open end was sealed with a plastic. The box was purged alternatively by nitrogen and vacuum six times. TES (14.5 ml) was measured in a 25 ml volumetric cylinder and was then transferred to a 100 ml volumetric cylinder, in order to make up a final volume of 76 ml with 1,4-dioxane. The 1.0 M TES solution was then transferred to a pressure-equalizing-additional funnel. The pressure-equalizing-additional funnel was connected (Fig.3) to a side-neck of a 500 ml, three-necked round bottom flask. Then 7.0 g of Vydac101HS

silica was added along with 421±0.5 ml of distilled 1,4 dioxane and 17.0 ±0.5 ml of 2.3 M hydrochloric acid to the reaction flask containing a 0.5 inch magnetic stirring bar.

The mixture was stirred and heated to reflux, followed by dropwise addition of TES for 25 to 30 minutes. The solution was then heated for one hour. The supernatant solution containing dioxane was decanted into a waste beaker. The thick liquid remaining at the bottom was divided into four centrifuge tubes. Two-thirds of each tube was filled with a 1:1 mixture of teterahydrofuran (THF)/water. The tubes were stirred for 10 minutes with a stir bar inside the tube. All the tubes were centrifuged on a IEC HN-S centrifuge for 10 minutes at 1500 rpm. The supernatant solution was discarded. This procedure was repeated three times. The same procedure was carried out using pure THF and diethylether[17]. Finally, the silica hydride was transferred into an evaporating dish. The evaporating dish was covered with a speedy-vap and transferred into an oven maintained at a temperature of 110°C. The dish was left overnight to remove all the solvents. The silica hydride obtained was then characterized spectroscopically.

(2) Methods For Bonding Chiral Compounds to Silica Hydride

(a) Preparation of precursor (4-Allyoxybenzoylchloride)

The glassware was dried inside an oven since some compounds used in the synthesis are reactive with water. Fifty grams of 4-Allyoxybenzoic acid was placed into a round bottom flask with a condenser attached (Fig.3). Then 50 ml of thionyl chloride and 10 drops of dimethylformamide were added. The mixture was stirred at room temperature

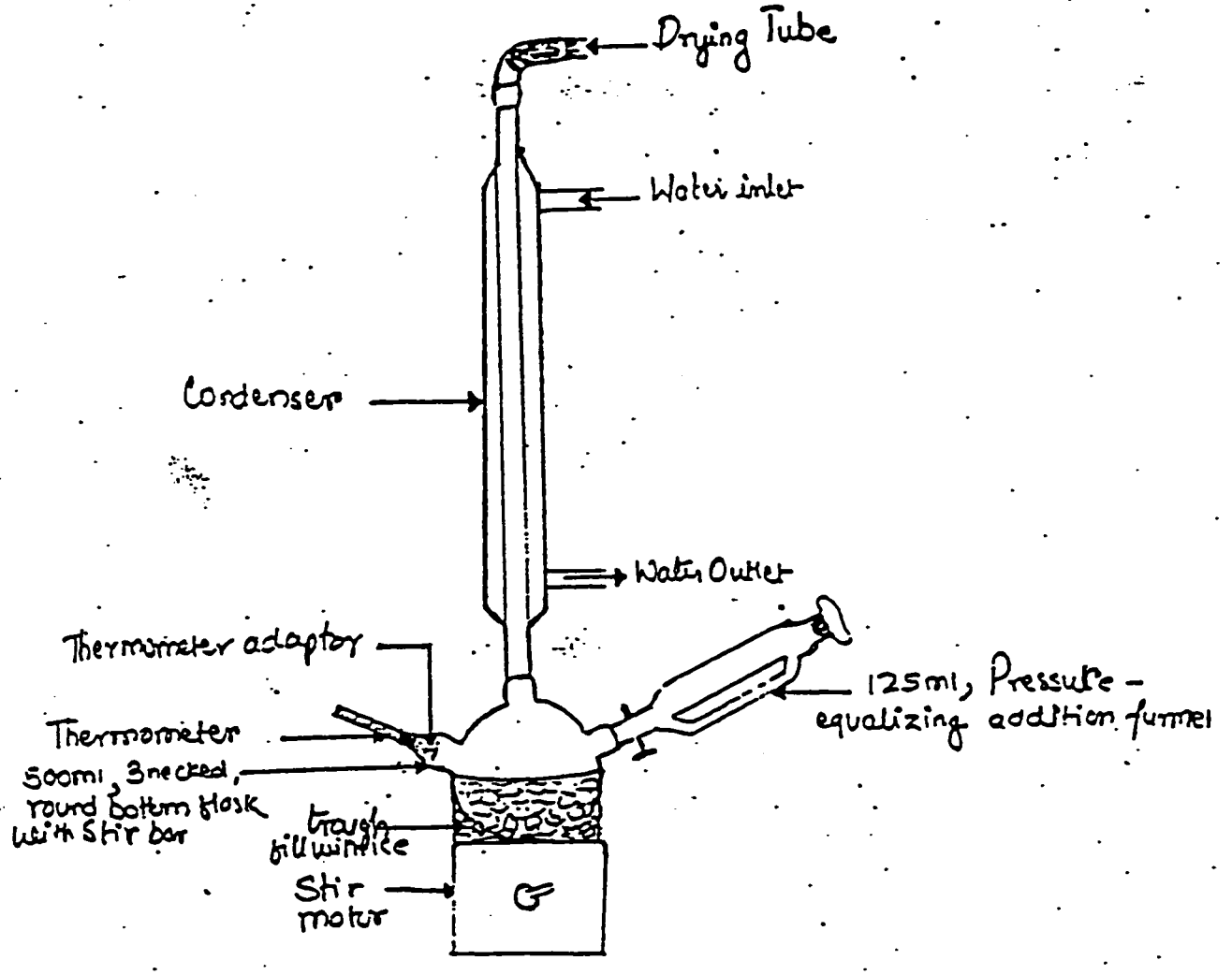


Figure 3. Assembly used for modification of silica.

overnight. The assembly was attached to a vacuum distillation apparatus to remove the excess thionyl chloride using a cold trap. The vapors were trapped inside a flask containing water. From time to time the pH of the water was tested. The complete removal of thionyl chloride was indicated by the pH of the water becoming neutral (~7). The product was a thick brown liquid. Since this liquid was light sensitive it was transferred into a amber colored bottle.

(b) Reaction I - Bonding of 4-Allyoxybenzoylchloride (4-ABC) to silica hydride followed by bonding of R/S - Naphthylethylamine (R/S-NEtA)

Step I: 1 g of 4-ABC was placed in a three-neck round bottom flask (Fig.3). 44 ml of dichloromethane was added to the flask. The reaction mixture was heated to 60°C for an hour. At this temperature dichloromethane started evaporating. In order to keep the volume constant, toluene was added. The reaction mixture was further heated for 4 hrs at the same temperature. After 5 hours, 2 g of silica hydride (Vydac 101 HS) was added to the reaction flask. The temperature was increased to 110°C and was maintained at these conditions for 5 days. After 5 days heating was stopped and the mixture was allowed to cool down for 30 minutes. The mixture was segregated into four centrifuge tubes and was centrifuged (IEC HN-S centrifuge) for 15 minutes at a rate of 1500 rpm. The supernatant solution was discarded and a fresh solution of toluene was added to each of the centrifuge tubes. The tubes containing a stirring bar were kept on a magnetic stirrer for 10 minutes to wash and remove impurities. The tubes were then placed in the centrifuge for 15 minutes to separate the suspended material from the solution. This

procedure of washing and centrifuging was done three times with each of the solvents toluene, dichloromethane and diethylether. At the end of the this process the compound was transferred to a evaporating dish. The dish was then covered with a speedy-vap and kept inside a oven at a temperature of 110°C overnight for drying.

Step II: This following procedure was used for bonding R/S- Naphthylethylamine to the compound obtained from Step I. The product obtained from Step I, 40 ml of dichloromethane and 1g of R(+)/S(-)-NEtA were placed in the previously described apparatus (Fig.3). 0.5 g of triethylamine was added to the mixture and stirred for 5 days under an inert atmosphere of N_2. The product obtained was extracted with acid and base, which was air dried overnight to obtain the required product. Characterization of the compound was done by FTIR and NMR. The complete reaction sequence is shown in Figure 4.

(c) Reaction II - Preparation of the compound (4-ABC and R/S - NEtA) and bonding the compound to silica hydride

Step I: A mixture of 1g of 4-ABC and 1g of R/S - NEtA in 1 ml of dichloromethane was added to the reaction flask. Then 0.5 g of trimethylamine (0.69 ml) was added to the mixture and the final volume was adjusted to 40 ml with dichloromethane. Nitrogen gas was passed into the flask to maintain an inert atmosphere. The reaction was carried out at room temperature for five days. The reaction mixture was extracted with 0.1N HCl and 0.1N NaOH.

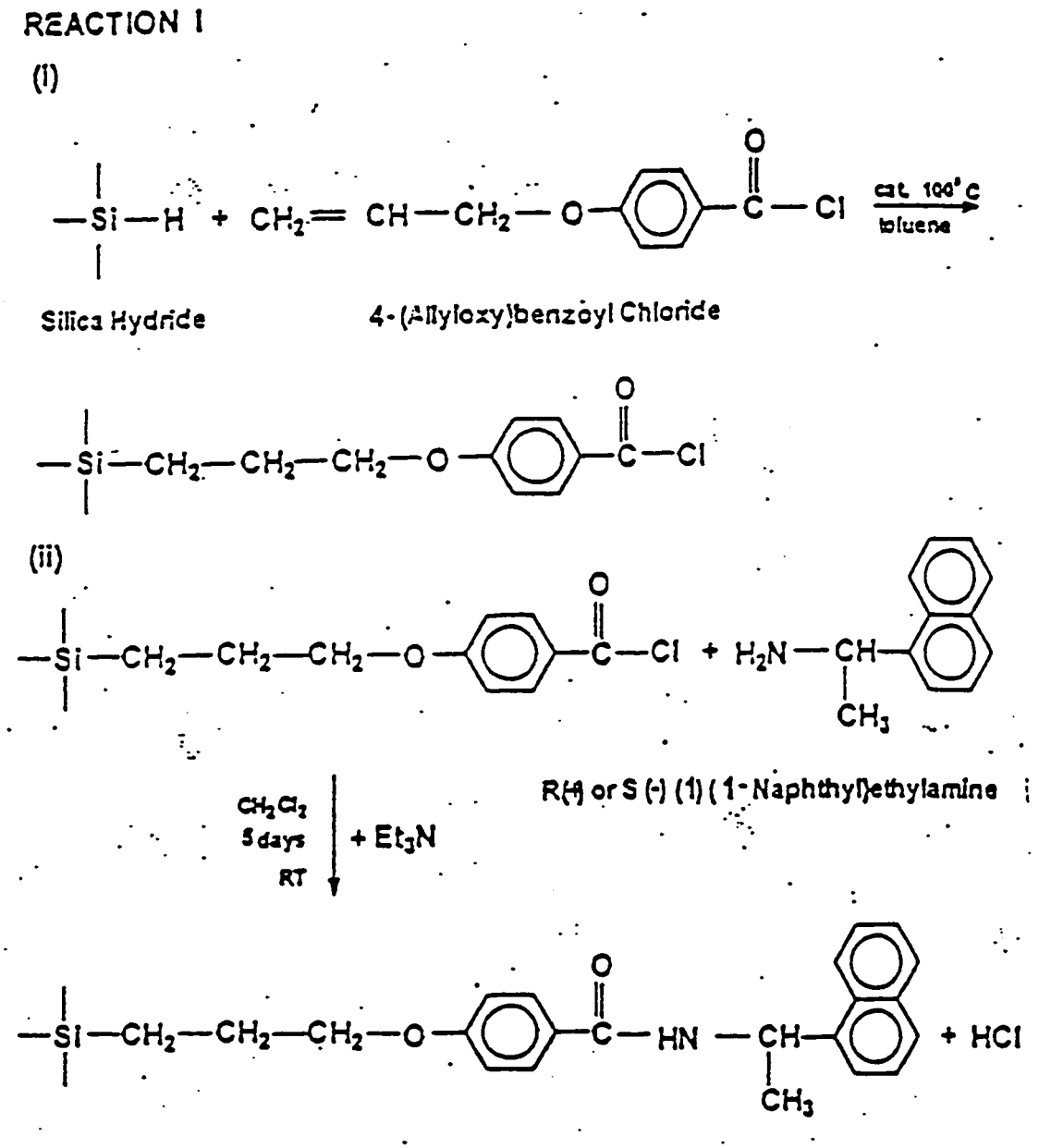


Figure 4. Schematic representation of silica modified using 4-Allyloxybenzoyl chloride as precursor and R(+)- Naphthylethylamine as chiral molecule (reaction I).

Step II: The product obtained from step I was placed in another flask. About 40 ml of toluene was added. The temperature was increased to 60°C in order to dissolve the compound. Then 1020 µl of 10 mM of hexachloroplatinic acid (catalyst) was added to the mixture and stirred for one hour. After one hour 2 g of silica hydride (Vydac HS) was added and the reaction temperature increased to 100°C for 5 days. The product obtained after 5 days was washed and centrifuged three times each with toluene, dichloromethane and diethylether and was transferred into a evaporating dish which was put in an oven for drying at 100°C overnight. The following day the product was removed from the oven and allowed to cool. The material was then characterized by FTIR and NMR. The complete synthesis scheme is shown in Figure 5.

(d) Reaction III - Reaction of 4-Allyoxybenzoicacid (4-ABA) and R/S - NEtA using Dicyclohexyldiimide(DCC) and bonding the compound to silica hydride

Step I: To a previously dried reaction flask 0.79 g of 4-ABA was added along with 0.83 g of dicyclohexyldiimide and 1.39 g of R/S NEtA. To the same flask 20 ml dichloromethane was added as a solvent. To carry out the reaction under an inert atmosphere nitrogen was passed for several minutes and then all the outlets were sealed to maintain the inert conditions. This reaction was continued overnight at room temperature. The product obtained was extracted first with 0.008 M HCl and then with 0.008 M $NaHCO_3$.

Step II: The compound obtained from step I was placed in a dried flask (Fig.3)

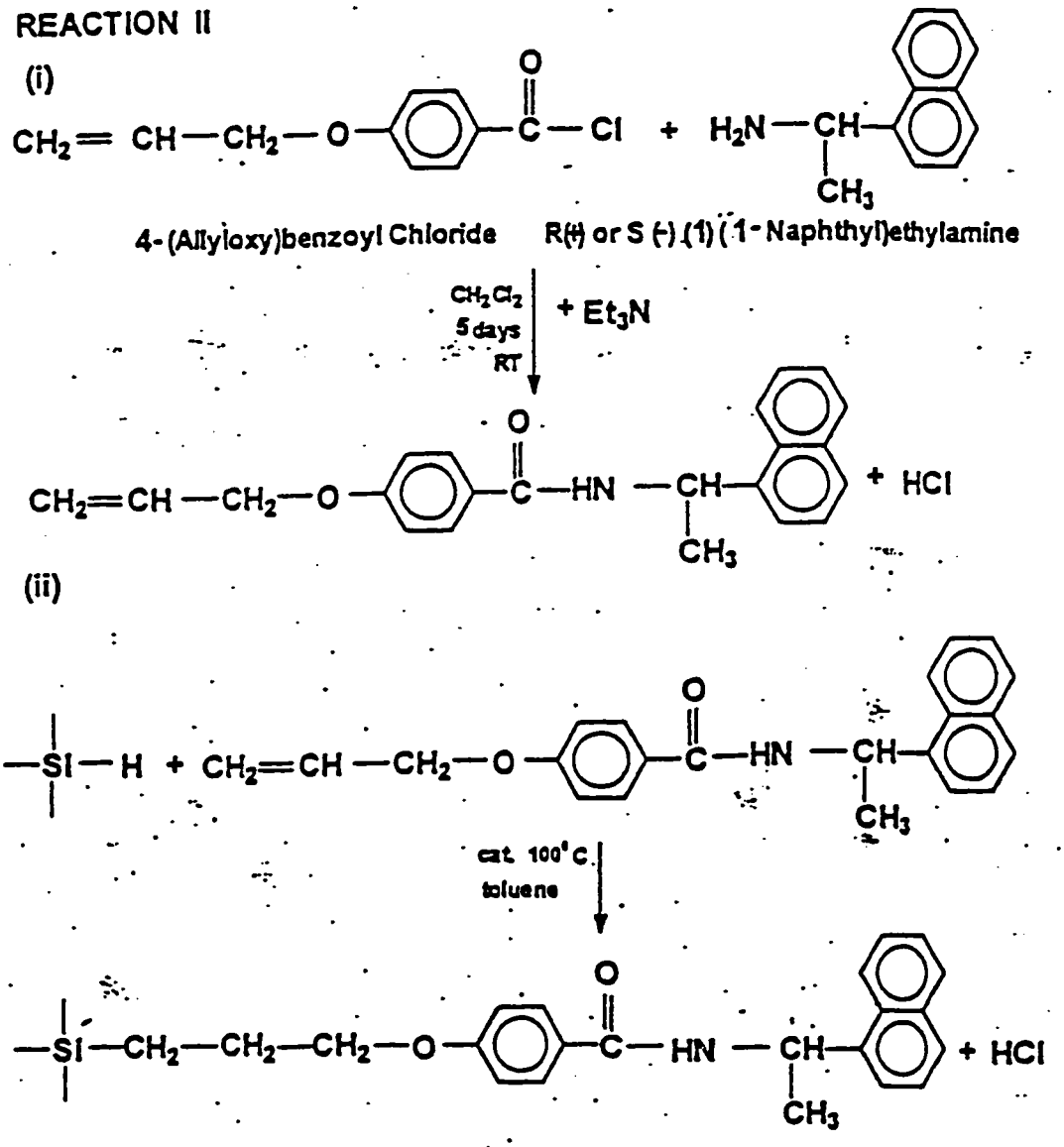


Figure 5. Schematic representation of silica modification by attaching 4-Allyloxybenzoyl chloride to R(+)- Naphthylethylamine and then attaching the chiral compound to the silica hydried (reaction II).

and 20 ml of toluene was added as a solvent. The reaction temperature was kept at 60°C and the mixture was stirred for one hour. Then 2 g of silica hydride was added and the reaction temperature was increased to 100°C. The reaction mixture was stirred for 5 days. The product obtained was washed and centrifuged with toluene, dichloromethane and diethylether and was transferred into an evaporating dish which was put in an oven for drying at 100°C overnight. The following day the product was removed from the oven and allowed to cool. It was then characterized by FTIR and NMR. The complete reaction scheme is shown in Figure 6.

(e) Reaction IV - Reaction of 4-Allyoxybenzoicacid (4-ABA) and R/S - NEtA using Dicyclohexyldiimide (DCC) and N-Hydroxysuccimide (N-OHSc) bonding the compound to silica hydride

Step I: To a previously dried reaction flask 0.79 g of 4-ABA was added along with 0.83 g of dicyclohexyldiimide, 1.39 g of R(+)/S(-) NEtA and 0.79 g N-hydroxysuccimide. To the same flask 20 ml dichloromethane was added as a solvent. The reaction temperature was maintained at 0°C. In order to sustain an inert atmosphere nitrogen was passed for several minutes through the system and then all the outlets were sealed. The reaction was allowed to proceed overnight. The mixture was allowed to settle for 30 minutes and the supernatant solution was discarded.

Step II: To the product obtained from step I, 40 ml of toluene was added in the assembly shown in Fig.3. The reaction temperature was increased to 60°C and the

REACTION III

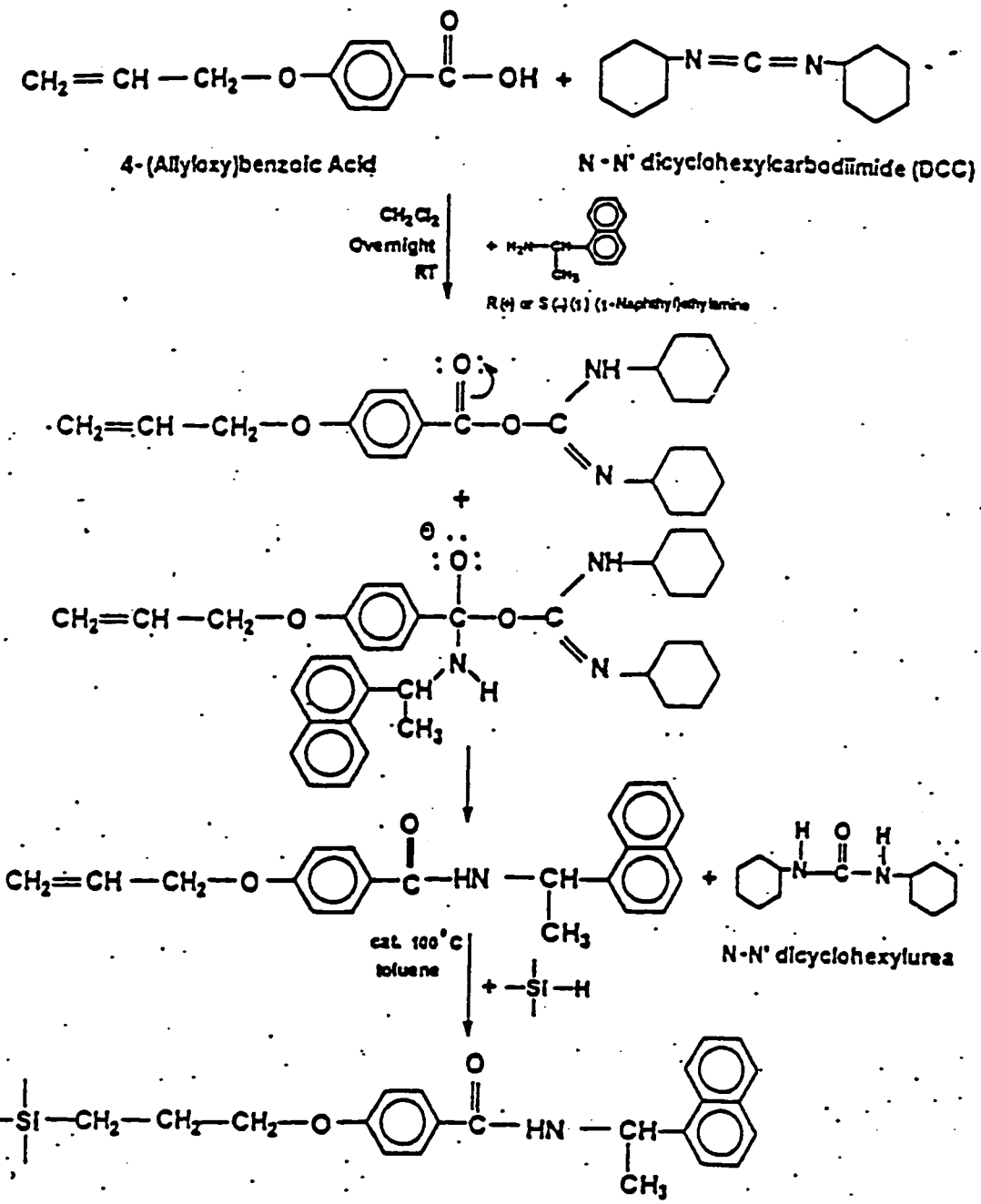


Figure 6.. Schematic representation of silica modified using 4-Allyloxybenzoic acid as precursor and R(+)- Naphthylethylamine as chiral molecule using dicyclohexyldiimide (DCC) (reaction III).

mixture was stirred for about an hour. Then 2 g of silica hydride (Vydac HS) was added and the reaction temperature was increased to 100°C. This mixture was kept stirring at the same temperature for five days. The product obtained was washed and centrifuged with toluene, dichloromethane and diethylether and was transferred into an evaporating dish which was put in the oven for drying at 100°C overnight. The following day the product was removed from the oven and allowed to cool. The sample was characterized by FTIR and NMR. The complete reaction sequence is shown in Figure 7.

(3) Column Packing Technique

The technique described is usually used for the preparation of analytical columns having particle diameters less than 20 μm and this procedure can be optimized to give an efficiency close to the maximum theoretically attainable for the material used. The scheme for slurry packing is as follows:

The column is cleaned with a series of solvents as recommended by Karger[18]:

(1) Chloroform, acetone, water, 10%v/v nitric acid, acetone and finally chloroform again. This treatment removes all greases, extrusions and lubricants that are present. (2) The packing material is made into a slurry mixture with a solvent which is sufficiently viscous so that no significant settling takes place. In this study a methanol/chloroform mixture in a 1:9 ratio was used. (3) The slurry was then ultrasonicated for 10 minutes in order to obtain a complete dispersion of the packing material in the slurry. During sonication vacuum was applied to the vessel to degas the slurry. (4) The column was filled with the

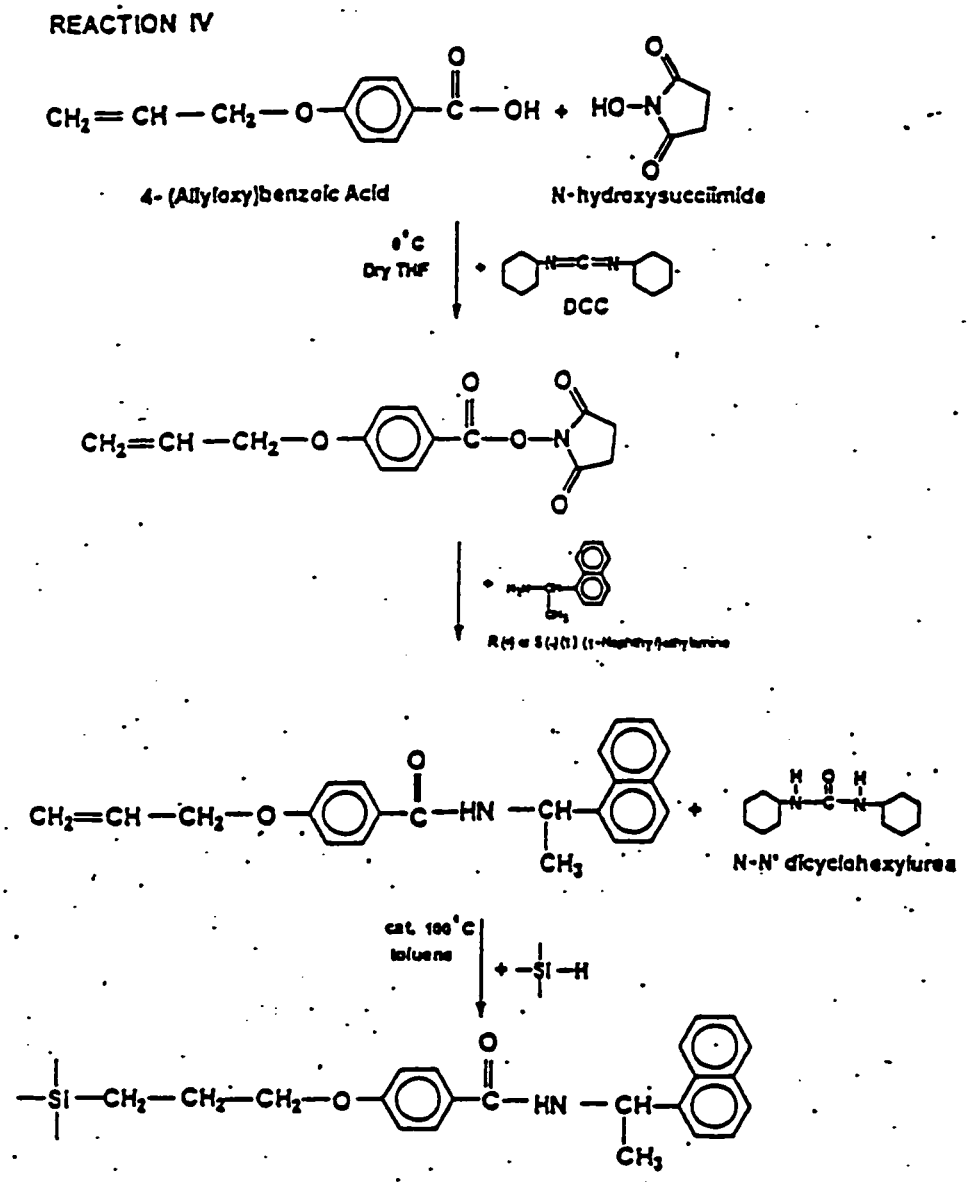


Figure 7. Schematic representation of silica modified using 4-Allyloxybenzoic acid as precursor and R(+)- Naphthylethylamine as chiral molecule using dicyclohexyldiimide (DCC) and N-hydroxysuccimide (reaction IV).

slurry solvent and the slurry was transferred to the packing reservoir. This step is carried out as rapidly as possible. (5) The tube from the valve to the reservoir was filled with solvent, the valve is closed and the tube is connected to the slurry reservoir. A shield is placed in front of the packing system to protect the operator from any accidents. (6) The pump was activated to force the liquid through the reservoir and the packed column until a constant flow rate is achieved. The valve was then turned off, the pump stopped and the excess pressure in the system is allowed to fall to zero. The packed column was ready for chromatographic analysis.

(4) Preparation of Solutes

A 0.1 mmol aliquot of amino acid was stirred with 1ml of 2.2 M hydrochloric acid in methanol at 100° C for 4 hrs. Subsequently, the solution was evaporated to dryness. The residue was suspended in 1 ml of ethyl acetate, and N-methylmorpholine (0.2 mmol) and 3,5-dinitrobenzoyl chloride (0.1 mmol) were added. After stirring at room temperature for 3-4 hrs. the mixture was washed with saturated sodium bicarbonate solution. The organic layer was dried over magnesium sulfate and evaporate to dryness[19].

CHAPTER III- RESULTS AND DISCUSSION

(A). Spectral Analysis for the Confirmation of Reactions

Reaction I: 4-Allyoxybenzoylchloride reacted with one mole of R/S-Naphthylethylamine. The byproduct of this reaction was hydrochloric acid. As the reaction proceeded, the HCl formed in the reaction being more electrophillic than the 4-Allyloxybenzoylchloride readily attacked the amine leading to an undesirable product. The compound which was assumed to be the chiral compound was not bonded to silica as shown by the FTIR spectrum. The peak at 2250 cm^{-1} is the Si-H stretch which is shown in the DRIFT spectrum (Fig.8) of silica hydride[15,20,21]. Both the small pore structure of silica and the steric hindrance of the new hydride bearing siloxane network prevented all hydroxides from reacting with TES. Therefore the hydroxide peak at 3440 cm^{-1} and surface-adsorbed water peaks are seen in the spectrum of silica hydride at 1620 cm^{-1}. The spectra of the modified silica (Fig.9) showed that there was no Si-H streching peak in the region of 2250 cm^{-1} and no peaks in the C-H stretching region (3200- 2800 cm^{-1}). This clearly shows that bonding had not taken place. To further confirm the result a ^{13}C CP-MAS spectrum of the bonded species was obtained (Fig.10). The spectrum shows a peak at 19 ppm which represents the methyl group (CH_3) attached to CH group of the amine. The peak at 45 ppm represents the CH_2-O group of the precursor (4-Allyloxybenzoic acid). The presence of the methine group (CH) can be determined from the peak at 70 ppm. The benzene ring is not clearly

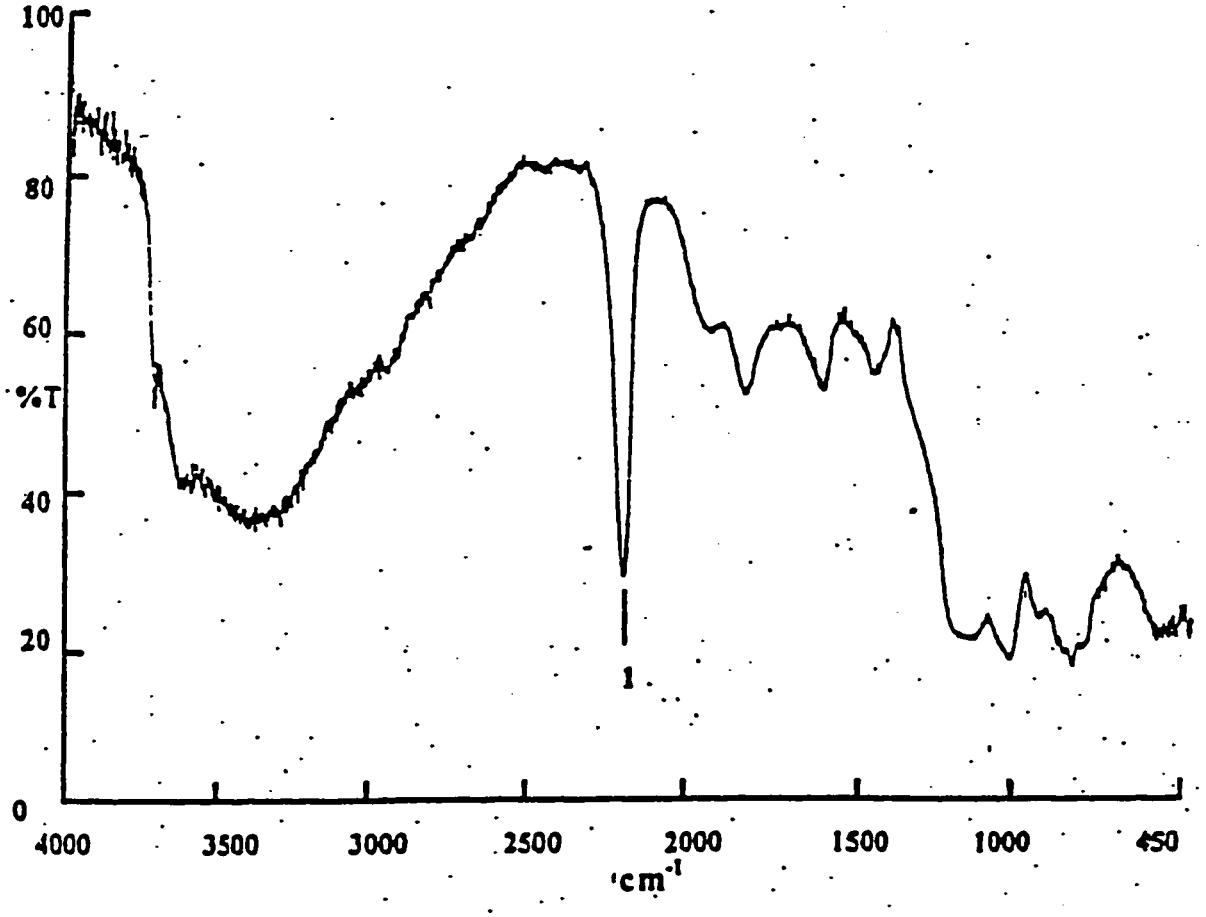


Figure 8. **The DRIFT spectrum of silica hydride (1) SiH - peak at 2250 cm^{-1}.**

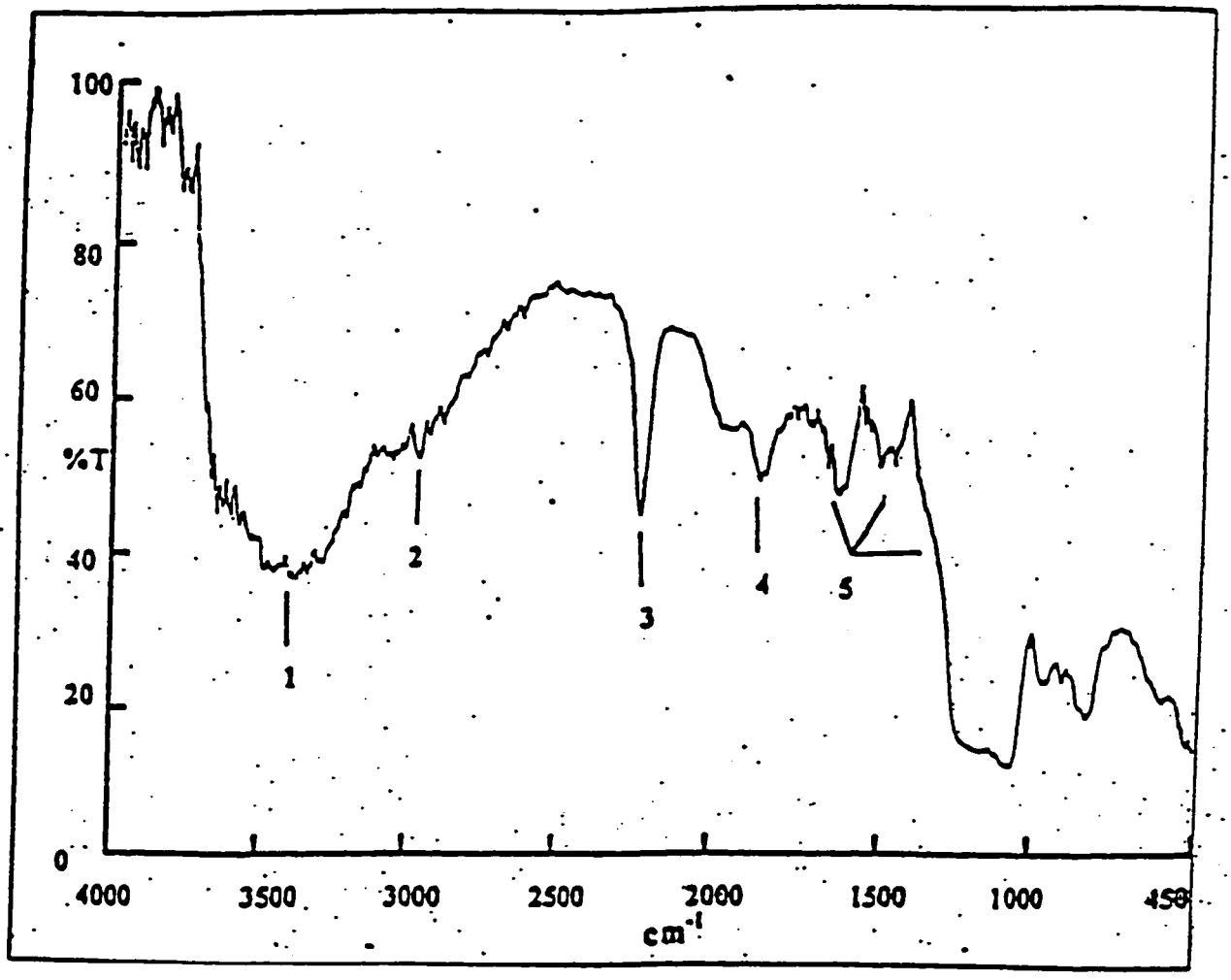


Figure 9. The DRIFT spectrum of bonded silica via reaction I (1) OH - Streching at 3440 cm^{-1} (2) CH - Streching due to aliphatic alkanes at 3000 and 2920 cm^{-1} (3) SiH - Streching at 2250 cm^{-1} (4) Ester group streches at 1720 cm^{-1} (5) Aromatic - Streching at 1610, 1500 and 1450 cm^{-1}.

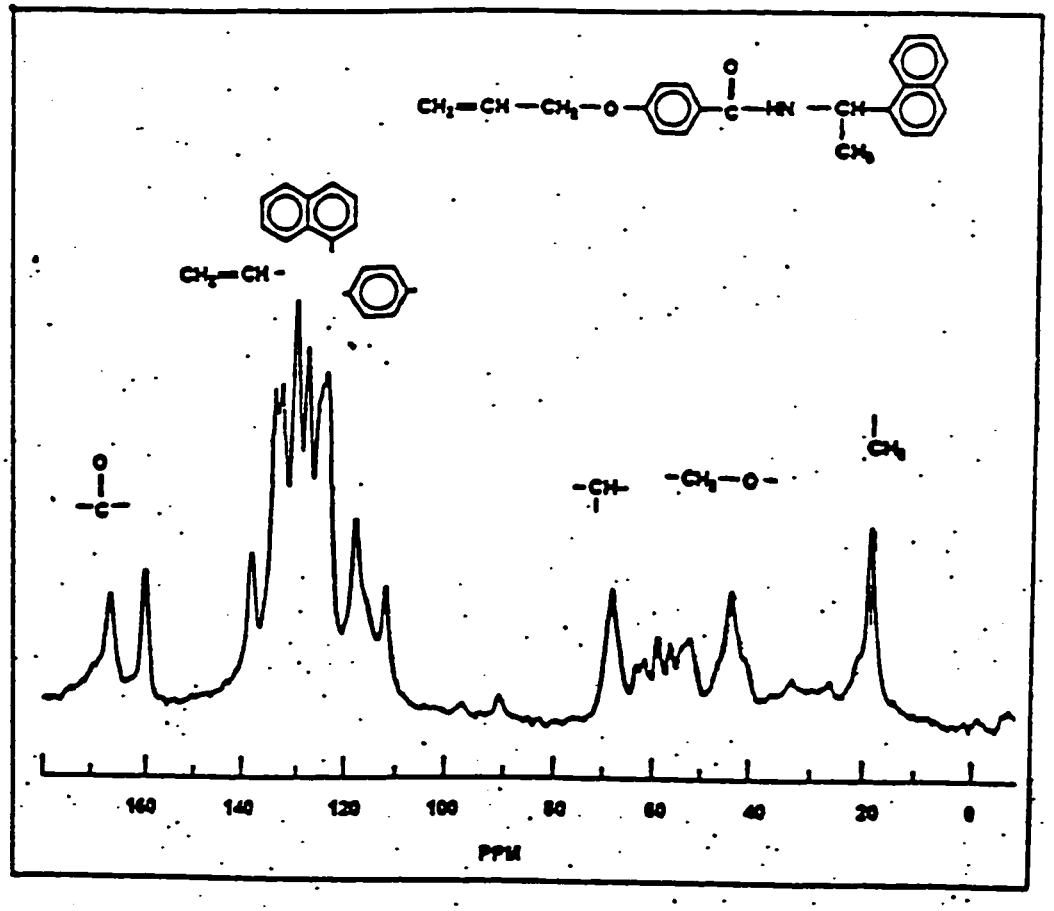


Figure 10. The ^{13}C NMR Spectrum of bonded silica via reaction I.

resolved which is represented by the peaks from 110 ppm to 120 ppm. The naphthyl group which is also not clearly resolved appears between 121 ppm to 139 ppm. Lastly the peak at 170 ppm represents the ketonic group. A peak near 10 ppm which would indicate an $Si-CH_2$ moiety [15,21] is not present in this spectrum therefore it can be concluded that this method is not suitable for bonding of a chiral compound to silica hydride.

Reaction II: This was proposed as an alternate method for silica modification. According to this scheme the chiral species was to be formed first and then the compound was to be bonded to silica hydride. Here again the same difficulty in obtaining the correct compound was encountered. The reason behind not obtaining the correct compound was the same as mentioned in reaction I. The DRIFT spectrum shown (Fig. 11) has no Si-H peak at 2250 cm^{-1} and also near 3000 cm^{-1} there were no significant peaks due to aliphatic C-H stretching. At 1720 cm^{-1} the peak was due to the ester group, peaks at 1610 cm^{-1}, 1500 cm^{-1} and 1450 cm^{-1} were due to aromatic CH- stretching. Overall it can be concluded that some how the chiral moiety has not been attached to silica hydride or it must attached through other functional groups which is not of importance in this research. The ^{13}C-NMR spectrum of the above reaction product shows (Fig.12) no peak at 10 ppm for the $Si-CH_2$ group. The $Si-CH_2$ peak confirms the bonding of reaction product to the silica hydride. The spectrum shows a peak at 19 ppm which represents the methyl group (CH_3) attached to CH group of the amine. The peak at 45 ppm represents the CH_2-O group of the precursor (4-allyloxybenzoic acid). The presence of the methine group (CH) can be determined from the peak at 70 ppm. The benzene ring is not clearly resolved

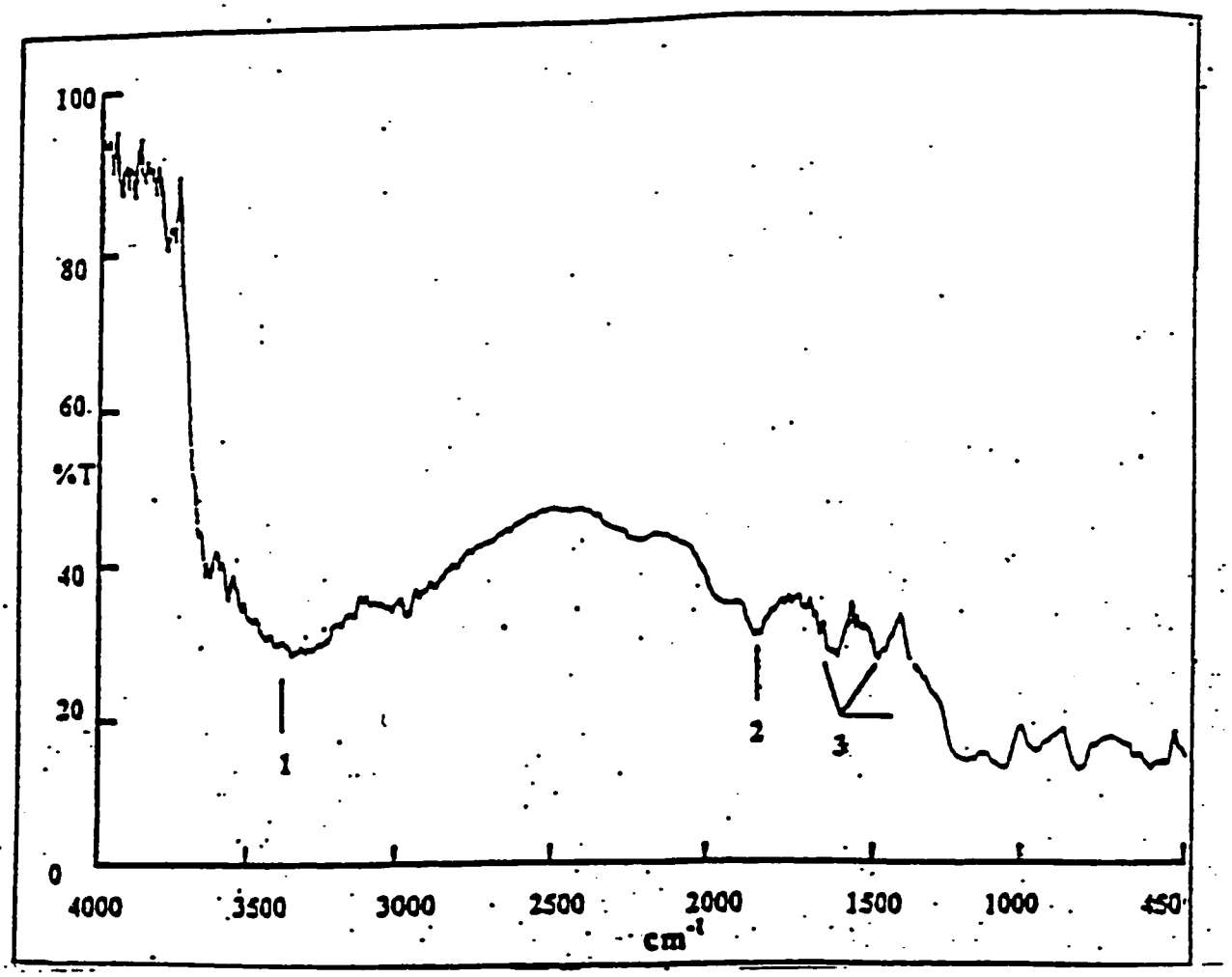


Figure 11. The DRIFT spectrum of bonded silica via reaction II (1) OH - Streching at 3440 cm^{-1} (2) Ester group streches at 1720 cm^{-1} (5) Aromatic - Streching at 1610, 1500 and 1450 cm^{-1}.

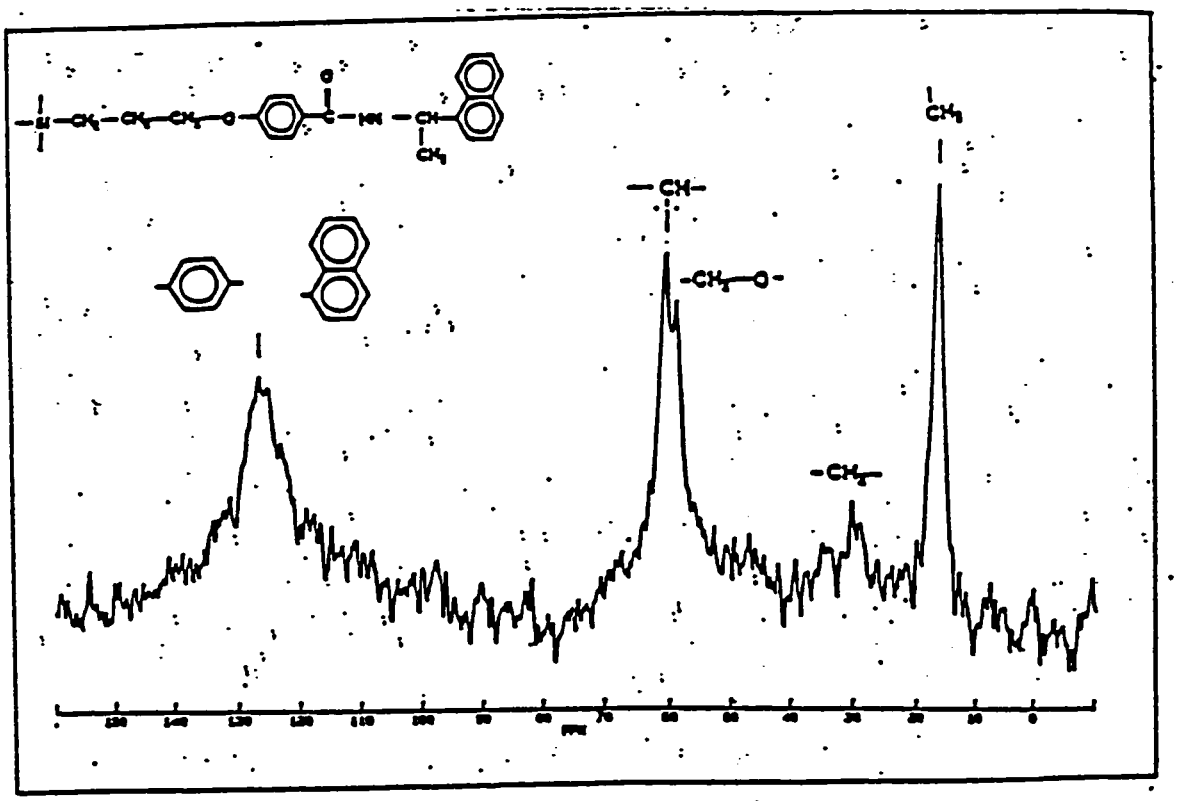


Figure 12. The ^{13}C NMR Spectrum of bonded silica via reaction II.

which is represented by the peaks from 110 ppm to 120 ppm. The naphthyl group which is not clearly resolved appears between 121 ppm to 139 ppm. Lastly the peak at 170 ppm represents the ketonic group. Absence of a peak at 10 ppm make this procedure of bonding inappropriate. In order to achieve a successful bonding of a chiral compound, a different approach was tested.

Reaction III (see Figure 6): The main problem in the previous reactions appears to be HCl formation. Therefore a reaction used for peptide formation which does not involve HCl formation was selected. This method for peptide bond formation involved the reaction of carboxylic acids with amines in the presence of DCC. An attempt was made to use this reaction for the bonding of silica with a chiral compound. After the completion of the reaction we did some spectral analysis of the final product. It was found that the desired product was not formed. There were two reasons that might have caused this problem. The first reason was that the expected cleavage of the intermediate compound was not taking place. The second possibility was that room temperature was too high for the formation of the desired product. From the FTIR spectrum (Fig.13) of the product obtained from this reaction it can be seen that the silica hydride peak at 2250 cm^{-1} had hardly changed its intensity and there were few aliphatic CH stretching peaks observed between 2800 cm^{-1} and 3000 cm^{-1}. The peak at 3440 cm^{-1} is due to OH stretching from water. The peak at 1720 cm^{-1} is due to the ester group and finally peaks at 1610 cm^{-1}, 1500 cm^{-1} and 1450 cm^{-1} are due to aromatic CH-stretching. This result indicates that

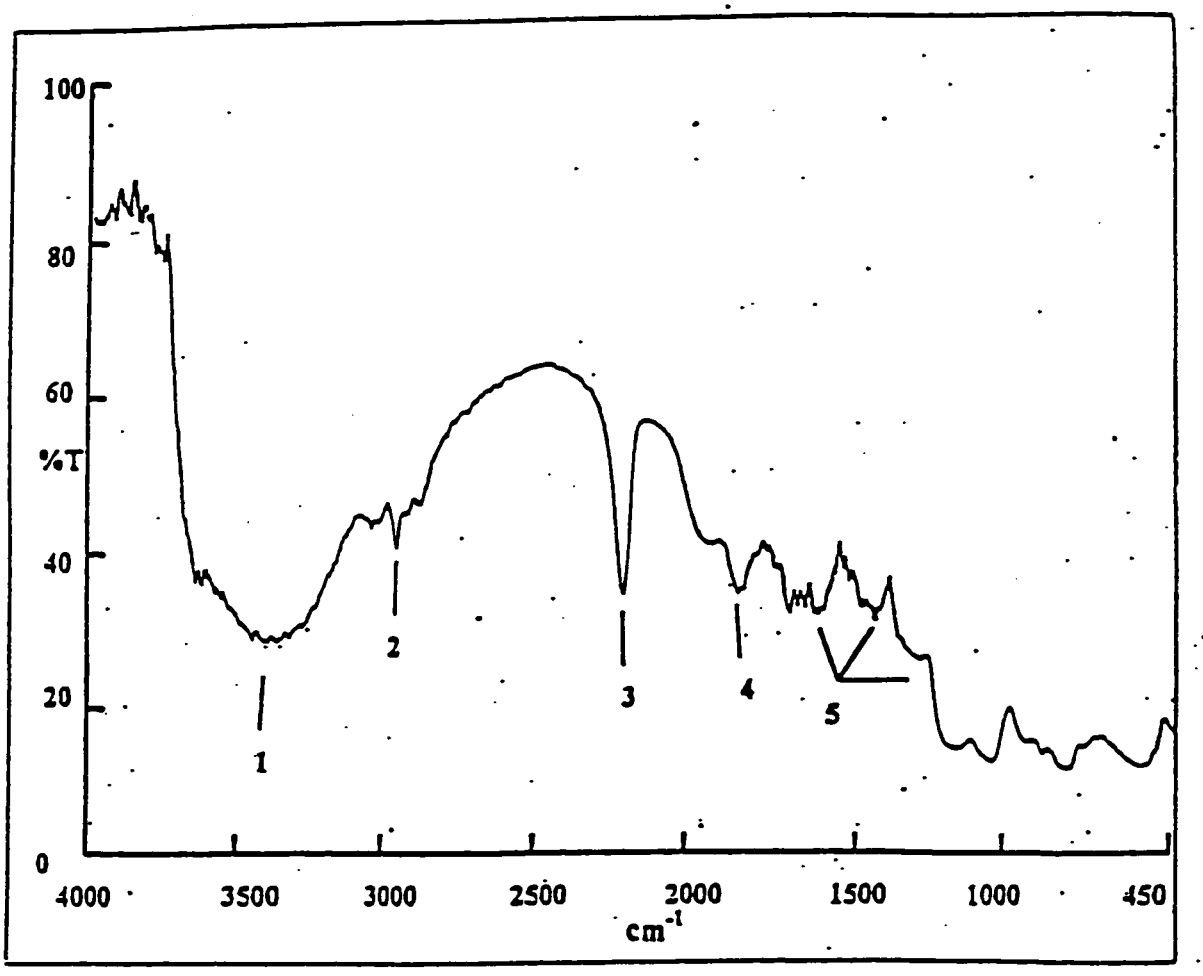


Figure 13. The DRIFT spectrum of bonded silica via reaction III (1) OH - Streching at 3440 cm^{-1} (2) CH - Streching due to aliphatic alkanes at 3000 and 2920 cm^{-1} (3) SiH - Streching at 2250 cm^{-1} (4) Ester group streches at 1720 cm^{-1} (5) Aromatic - Streching at 1610, 1500 and 1450 cm^{-1}.

occurred. The ^{13}C-NMR spectrum of the bonded silica was obtained to further (Fig.14) characterize the bonded material. The peak at 45 ppm represents the CH_2-O group of the bonded material. The peak at 70 ppm represents the CH methine group. From 110 ppm to 120 ppm, there are actually six peaks representing the benzene ring but are not clear as they have merged together. The peaks from 121 ppm to 139 ppm represent the twelve carbon peaks of the naphthyl group since the resolution was poor it was hard to identify those peaks as they have merged together. Absence of a peak at 10 ppm indicates that Si-CH_2 linkage has not taken place. A different approach was tried.

Reaction IV (see Figure 7): In this method a better cleaving agent like n-hydroxysuccimide was used. The reaction conditions were also altered with the temperature changed from 27°C to 0°C. By altering the conditions it was possible to bond the chiral product to the desired extent on silica hydride. As can be seen from the FTIR spectrum, (Fig.15) a considerable decrease in the intensity of the Si-H peak at 2250 cm^{-1} occurs and a corresponding appearance of aliphatic CH stretching peaks near 3000 cm^{-1} are observed. The ^{13}C CP-MAS NMR spectrum (Fig.16) of the bonded material confirmed the bonding of the chiral compound to the silica. The peak at 45 ppm represents the CH_2-O group of the bonded material. The peak at 70 ppm represents the CH methinine group. From 110 ppm to 120 ppm, there are actually six peaks representing the benzene ring but are not clear as they have merged together. The peaks from 121 ppm to 139 ppm represent the twelve carbon peaks of the naphthyl group since the resolution was poor it was hard to identify those peaks as they have merged together. In this

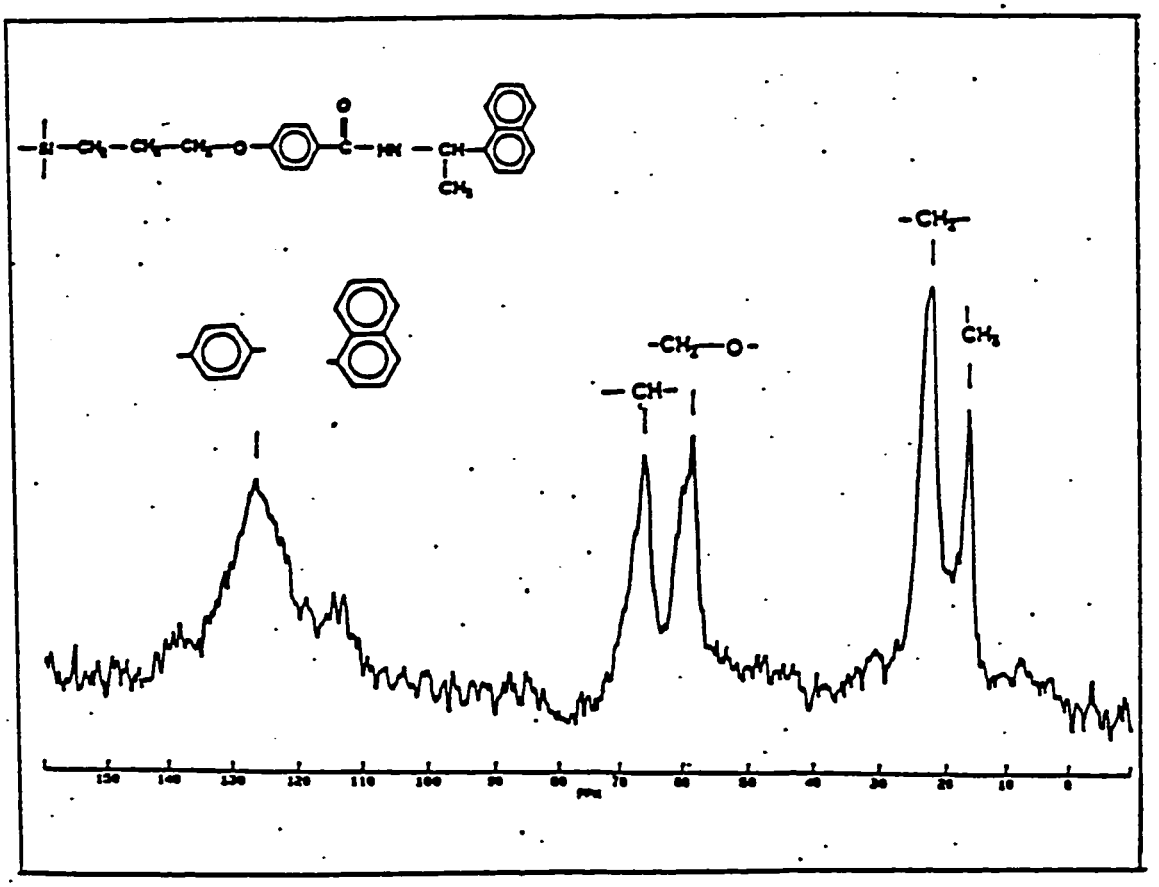


Figure 14 The ^{13}C NMR Spectrum of bonded silica via reaction III.

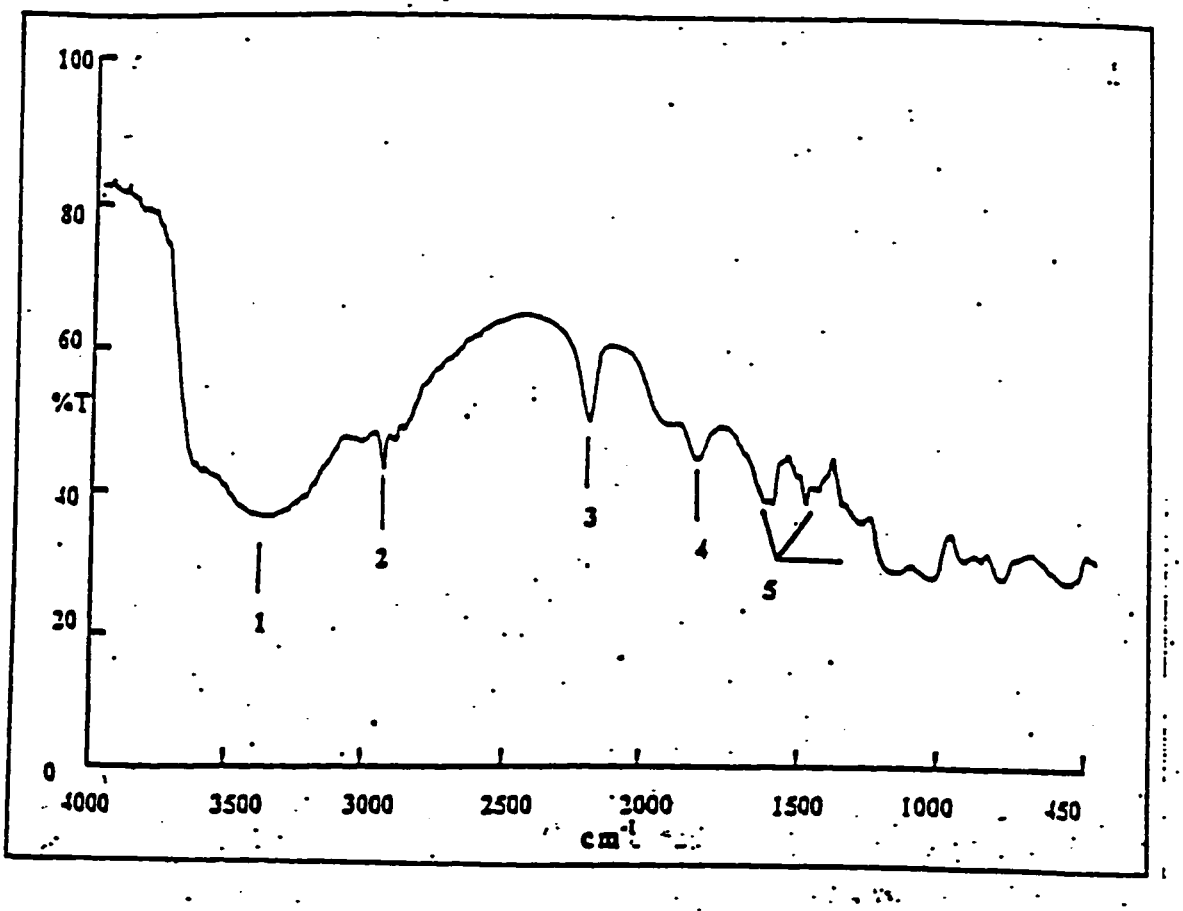


Figure 15. The DRIFT spectrum of bonded silica via reaction IV (1) OH - Streching at 3440 cm^{-1} (2) CH - Streching due to aliphatic alkanes at 3000 and 2920 cm^{-1} (3) SiH - Streching at 2250 cm^{-1} (4) Ester group streches at 1720 cm^{-1} (5) Aromatic - Streching at 1610, 1500 and 1450 cm^{-1}.

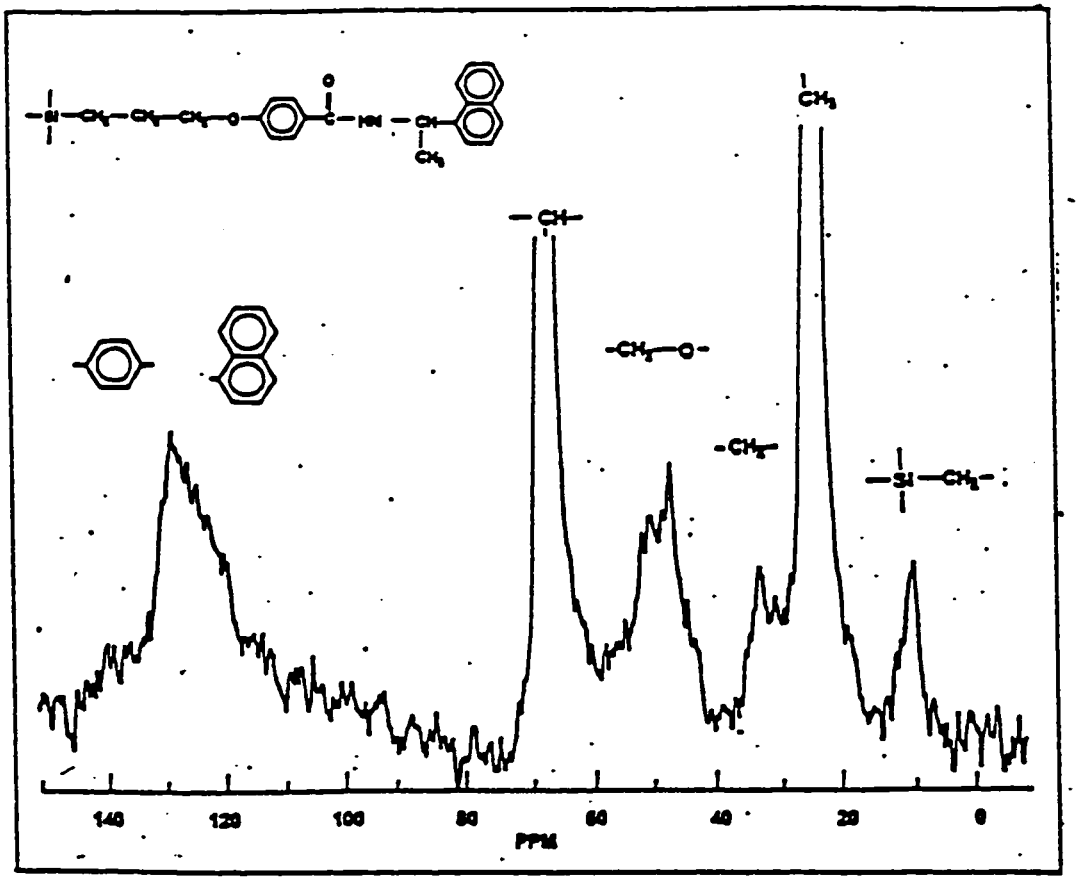


Figure 16. The ^{13}C NMR Spectrum of bonded silica via reaction IV.

spectrum a significant peak at 10 ppm has appeared which as mentioned before is due to Si-CH_2 peak. The appearance of the Si-CH_2 peak confirmed the bonding of the chiral compound to silica hydride.

To find out the surface coverage of the chiral compounds elemental analysis was performed on the modified silica. The carbon content on the surface was found to be 4.18% and the surface coverage was found to be 0.61 $\mu m/m^2$. The surface coverage obtained by other workers[24] was (1.2 $\mu m/m^2$) more than what was obtained in this research.

(B) HPLC Data Interpretation for Amino Acid Derivatives

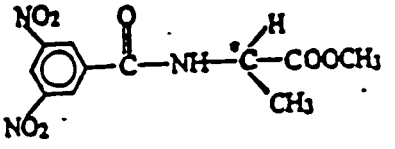
It was well known that both gas and liquid chromatography with optically active stationary phase are very useful for the separation of enantiomeres. Weistein et al.[22] reported that it was sufficient for a chiral stationary phase to contain an amide group with an asymmetric carbon attached to the nitrogen atom [e.g. $RCONHCH(CH_3)R'$] in order to show selectivity in its interaction with enantiomeric amides. The best efficiency was obtained when R' is aromatic. Oi et al.[23] have demonstrated that N-acetyl derivatives of R or S-1-(α–naphthylethylamine) have a high stereoselectivity for enantiomeric amides. Dappen et al.[24] have developed covalently bonded, π–donor stationary phases based on derivatives of optically active 1-(α–naphthylethylamine) and found that the best separation was obtained with the 3,5 dinitrophenyl group. Lloyd et al.[25] found that enatiomeric (α)-

1-naphtylethylamine has better resolution than a diastereomeric CSP. In this study a novel chiral stationary phase was prepared consisting of (R)-(1)-Naphthylethylamine chemically bonded to silica with 4-Allyloxybenzoic acid as the linking agent to the surface. The direct separation of the derivatives of some racemic amino acids was done. Further by the theory of reciprocality this CSP could also be applied to the separation of racemic amines and carboxylic acids.

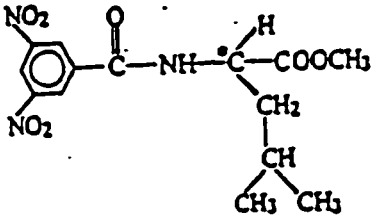
Some general statements on the retention and selectivity for all of the solutes can be made regardless of the CSPs and the mobile phases used. An increase in the size of the alkyl side chain (methyl, ethyl) or in branching (isopropyl) in the vicinity of the chiral center of the amino acid leads to a decrease in the capacity factor k' [26]. To confirm this statement we changed the R group of the solutes (3,5-dinitrobenzoyl derivatives of alanine, phenylglycine to phenylalanine) and the results (Fig.22) show that the k' value decreases as size of the alkyl chain increases. Results also show that isoleucine which has a branched side chain around the chiral center had higher k' value than leucine which has straight side chain around the chiral center. This contradicted the general statement. But it was found that if the α carbon atom next to the chiral center has bulkier group like ethyl and methyl groups(isoleucine) then greater is the steric interaction therefore larger k' value. In case of leucine the α carbon atom has two hydrogen atoms which are not bulky and so has less k' value which can be in Table 1. Probably the only inference that can be drawn is that the interaction in our CSP does depend upon the steric hindrance of R group. It was seen that solutes which have a different configuration from the CSPs are

retained more [R(+) stationary phase retains L(-) isomer of the solute] as they form a stable diastereomeric complex. To assign correctly absolute configuration from elution orders for a given CSPs of known absolute configuration, one must clearly understand the mechanism(s) by which the separation occurs. The three point interaction model has been widely used as a chiral recognition model (Fig.2). The predominant interactions seem to be dipole stacking, hydrogen bonding and π-π interaction around the chiral center. The rationalization used for separation of compounds can be put forward as exactly the same way as Oi et al.[23] have proposed. The CSP has 1-naphthyl group at the chiral center which is supposed to be a good π–donor (Fig. 23) group, along with it there is an amide group at the chiral center which interacts with the solute by hydrogen bonding process. Such types of CSPs can resolve analytes which have a π–acceptor group along with other suitably placed functionality [amide group]. Four different amino acid derivatives were used as analytes to demonstrate the efficiency of the separation. The solutes were derivatized with a dinitrobenzoylchloride(DNB). The dinitrobenzoyl group acts as a π–acceptor group in enantiomeric interactions with CSPs (Fig.17). Futhermore the dinitrobenzoyl group acts as a chromophore which can be detected in the UV at 254nm. The separation of various other derivatives of amino acids such as phenylthiohydantoin (PTH) and dansyl groups were tried without any success. It was found that these derivatives did not have π–acceptor groups so they do not have any π–π interaction for chiral recognition. This tells us that π–π interaction is one of the important interaction for chiral recognition. The k'

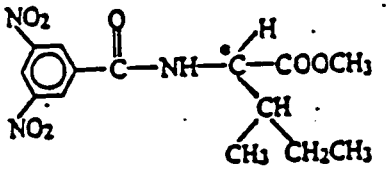
(PTH) and dansyl groups were tried without any success. It was found that these derivatives did not have π–acceptor groups so they do not have any π–π interaction for chiral recognition. This tells us that π–π interaction is one of the



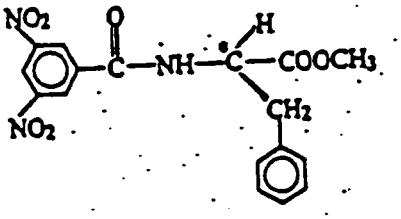
N-(3,5-Dinitrobenzoyl)-DL-Alanine Methyl Ester



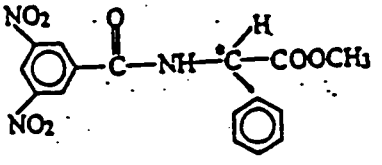
N-(3,5-Dinitrobenzoyl)-DL-Leucine Methyl Ester



N-(3,5-Dinitrobenzoyl)-DL-Isoleucine Methyl Ester



N-(3,5-Dinitrobenzoyl)-DL-Phenylalanine Methyl Ester



N-(3,5-Dinitrobenzoyl)-DL-Phenylglycine Methyl Ester

* - Chiral Center

Figure 17. Structures of derivatized amino acids used as solutes in chromatographic separations.

DNB derivatized amino acids. The values of the analytes summarized in Table.1 demonstrates that there is good retention of separation factor of these analytes are greater than those obtained by other workers[26]. For example Haurou et al. separate N-3,5 dinitrobenzoyl alanine methyl ester with a separation factor of 1.16, whereas the same analyte was separated with a separation factor of 1.48 on our CSP.

Better efficiency of separation was achieved by using this type of chiral stationary phase. The mobile phase used in this research was a ternary mixture of solvents (hexane:1,2 dichloroetane:ethanol) which had greater eluting strength. Previous workers had a binary solvent with less eluting strength. Another reason for efficient separation by this CSP to separate compounds effectively was due to the fact that the underlying silanol groups were modified to hydride which prevented the nitro group from reacting with the base silica. This reduced the secondary interaction and greater separation was achieved.

It was also seen that longer retention time leads to a better enantiomeric resolution if, and only if, the enantiomeric retention mechanisms are dominant. If, on the other hand, the dominant retention mechanism is not enantioselective, then an increase in the retention will not affect the enantiomeric resolution. The elution order and general chromatographic trends are consistent with an overall similarity in the fundamental mechanism of chiral recognition. It is also seen that amide-amide interaction can, a *priori*, take three forms: dipole-dipole interaction, hydrogen bonding of the CSP to the analyte and hydrogen bonding of the analyte to the CSP. Due to the acidity of the DNB amide hydrogen and the reduced basicity of its carbonyl oxygen, the second hydrogen bonding

process is expected to be more important than the first. The elution order of the solutes was determined by chromatography of samples whose configuration had been previously established and it can be seen that when the stationary phase was R(+) the first eluted enantiomer is D(+) (Fig 18,19,20,21). The converse was true too. That means if the CSP was the (S)-1-naphthylethyl amine moiety then the L(-) isomer would be eluted first.

(1) Influence of Mobile Phase

The mobile phase also plays an important role in separation. The optimization of the separation of racemates by manupilation of mobile phases rests on three principles: (1) solvent selectivity, (2) solvent strength, and (3) adsorption of solvent on sepicific sites of the CSP followed by displacment of solvent by solute.

Snyder's transformed Rohrschneider solubility data[28] into the more convenient and useful solvent selectivity triangle and it is recognized by all chromatographers today. The selectivity parameters were based upon the relative ability of the solvent to function as the proton acceptor, proton donor and strong dipole. Nonpolar hexane, an inert solvent can be modified by adding solvents form different parts of the triangle.

Once the mobile phase is fine-tuned by selecting a ternary mixture, the question of the polarity of the mixture arises and it is found that hexane, 1,2 dichloroethane along with ethanol gives the maximun selectivity. It seems possible to relate the dominant character of the polar modifier (1,2-dichloroethane) with its ability to favor hydrogen bonding or dipole-dipole interactions. Capacity factor are greater when ethylene dichloride is used

compared to 2-propanol although it has a larger polarity ($P'_{2,2,4\text{-TMP/2-PrOH}} = 17.6$, $P'_{2,2,4\text{-TMP/Ch}_2\text{Cl}_2} = 40$). This increase in retention generally leads to an increase in the separation factor. From the results obtained (Fig.22) it was seen that an increase in ethylene dichloride from 10% to 15% concentration in the mobile phase leads to an increase in capacity factor (k'). This leads to the conclusion that the high dipole character of 1,2-dichloroethane is the basis of interaction with the amide groups. However, solvation and/or the conformation of the solute and the CSPs are affected by a change in the polar modifier. The chiral recognition mechanism involves the establishment of dipole-diapole interactions and hydrogen bonding. These interactions do not seem to play an equivalent role in the chiral recognition mechanism. Ethanol being more polar is always used in a smaller percentage in the mobile phase. Because ethanol is a proton acceptor, it could interact preferentially with the NH moiety of the CSP (Fig.23) and the solute and apart from these interactions it may also have some charge transfer interaction with some groups of the stationary phase. This leads to the conclusion that the predominant interaction is hydrogen bonding in the chiral recognition mechanism along with $\pi-\pi$ interaction. Nevertheless, dipole-dipole interaction also plays a significant role.

The orientation of the analyte played an important role in the chiral mechanism. If the molecule orients itself in such a way that the DNB group is near the proximity of the naphthylgroup (Fig.23) and rest of the molecule is inside the mobile phase, then in such.

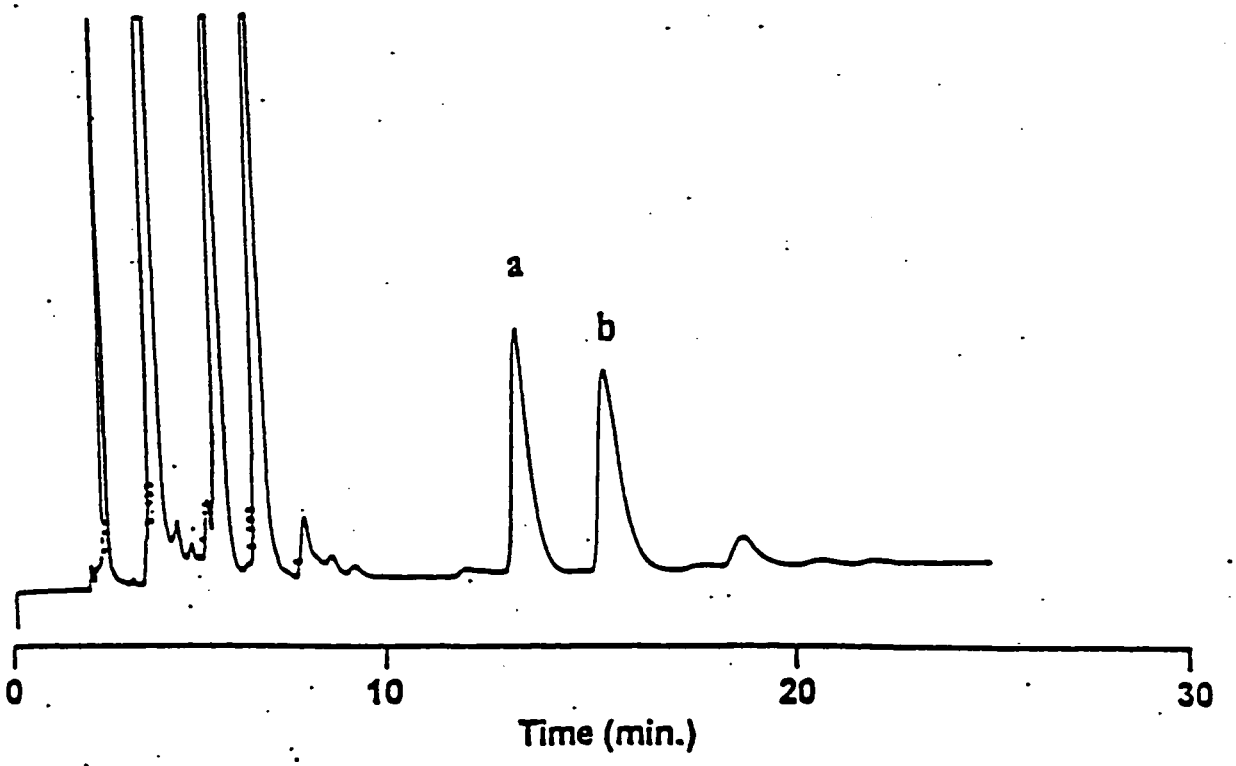


Figure 18. Isocratic separation of N-3,5DNB-(DL)-alaninemethylester on chiral column made from hydrosilation of 4-allyloxybezoic acid andR(+)-Naphthylethylamine on hydried intermediate. Mobile phase hexane/1,2dichloroethane/ethanol (87.5/12/0.5) (pH=6.5). Flow.rate of 1.0ml/min. Detected at 254nm.

a) N-3,5DNB-(D)-alaninemethylester; b) N-3,5DNB-(L)-alaninemethylester.

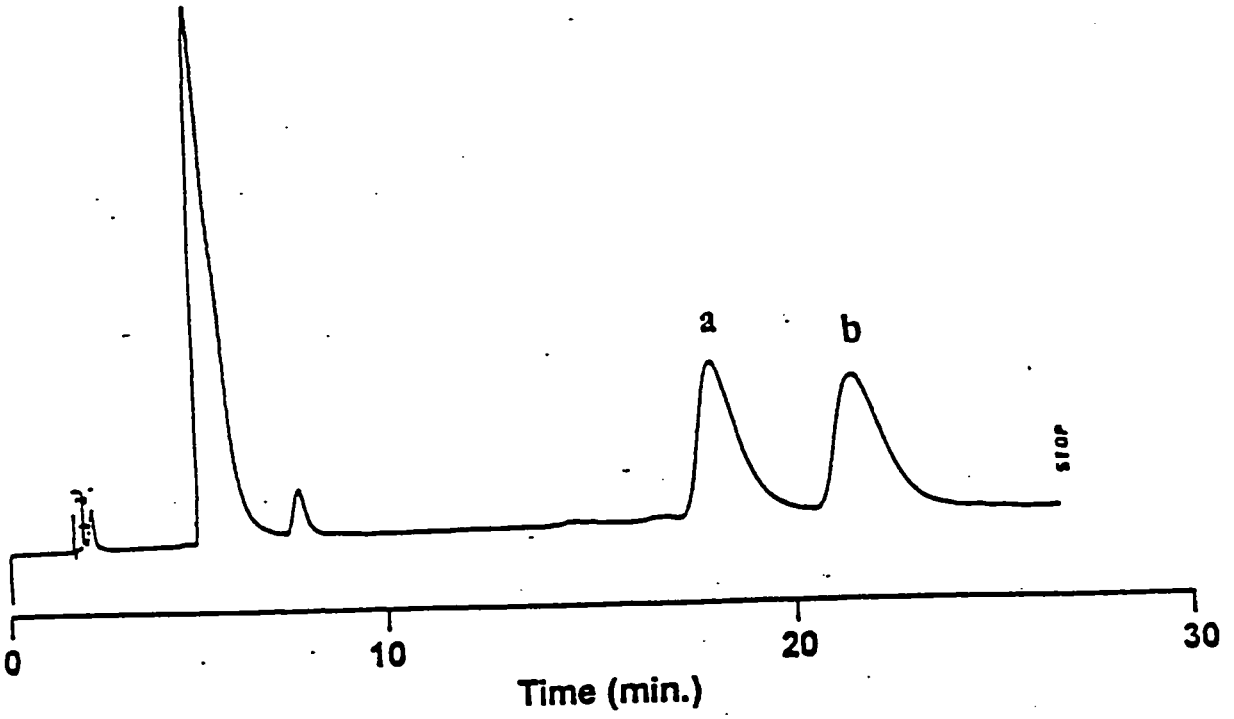


Figure 19. Isocratic separation of N-3,5DNB-(DL)-phenylalaninemethylester on chiral column made from hydrosilation of 4-allyloxybezoic acid andR(+)-Naphthylethylamine on hydried intermediate. Mobile phase hexane/1,2dichloroethane/ethanol (87.5/12/0.5) (pH=6.5). Flow rate of 1.0mL/min. Detected at 254nm.

a) N-3,5DNB-(D)-phenylalaninemethylester; b) N-3,5DNB-(L)-phenylalaninemethylester.

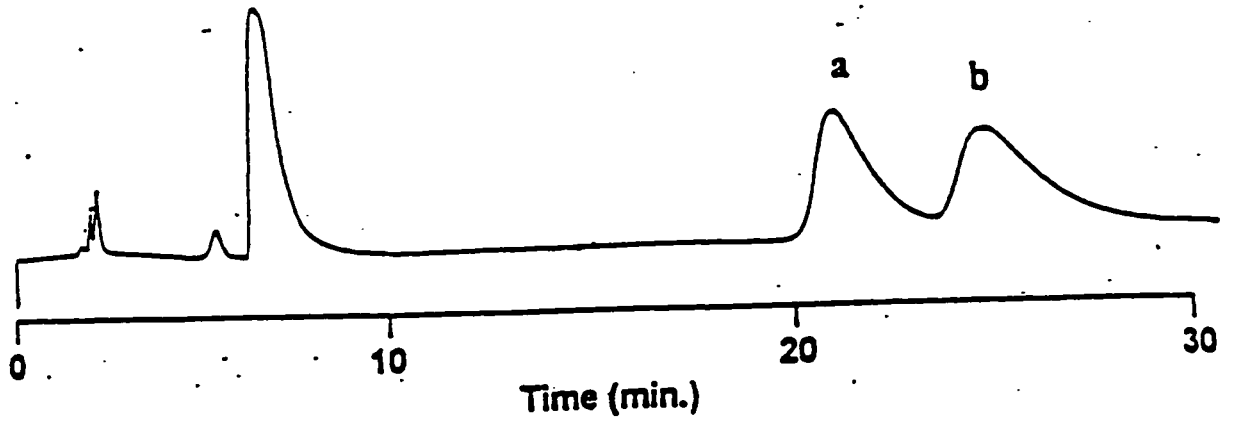


Figure 20. Isocratic separation of N-3,5DNB-(DL)-leucinemethylester on chiral column made from hydrosilation of 4-allyloxybezoic acid andR(+)-Naphthylethylamine on hydried intermediate. Mobile phase hexane/1,2dichloroethane/ethanol (87.5/12/0.5) (pH=6.5). Flow rate of 1.0ml/min. Detected at 254nm.

a) N-3,5DNB-(D)-leucinemethylester; b) N-3,5DNB-(L)-leucinemethylester.

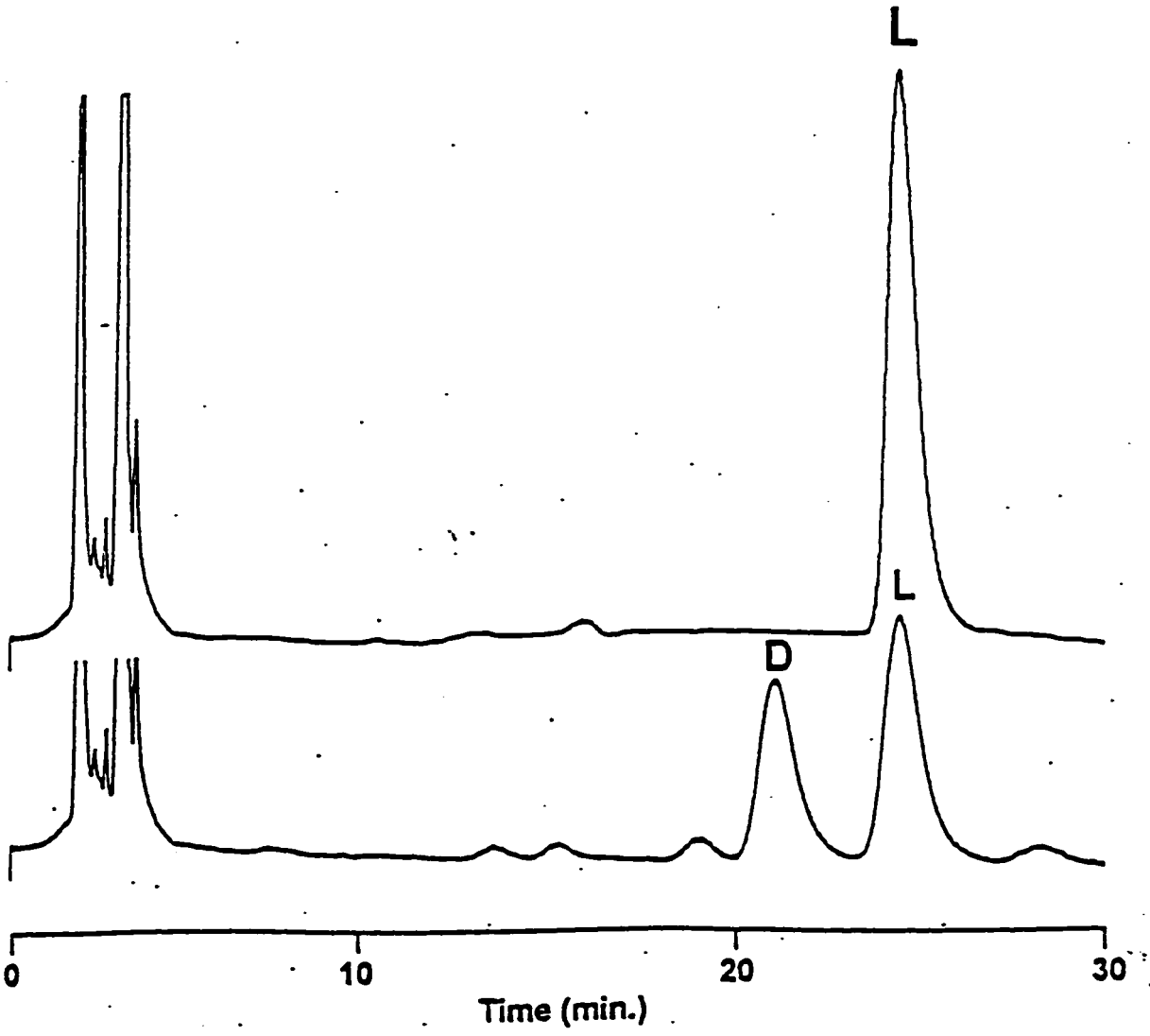


Figure 21.

Samples: N-3,5-DNB-isoleucine methyl ester (DL)
N-3,5-DNB-isoleucine methyl ester (L)
Column: R(+)- Naphthylethylamine
Mobile Phase: Isooctane:1,2-dichloroethane:ethanol (87.5:12:0.5)
Flow Rate: 1.0 mL/min.

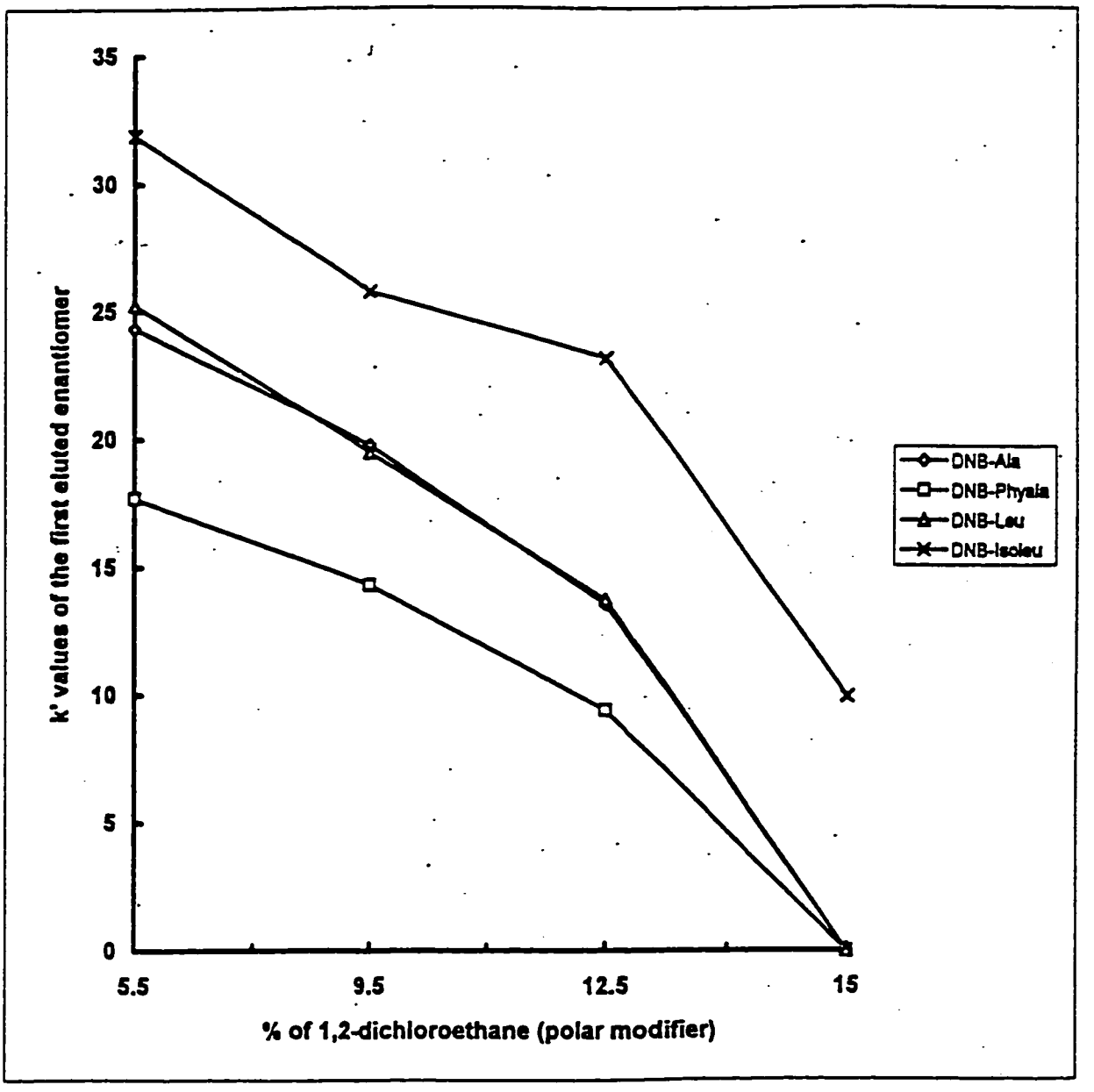


Figure 22 Plot of k' values of the first eluted enantiomer versus the % of 1,2-dichloroethane in the mobile phase.

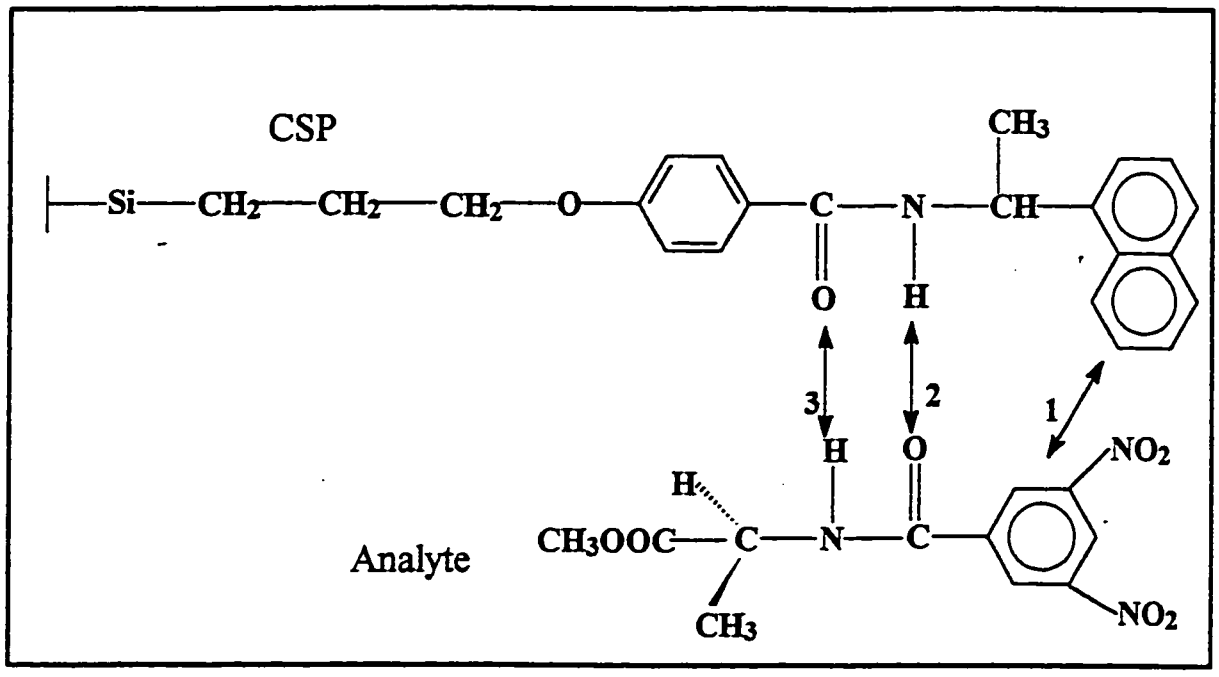


Figure 23. Three point interaction between the analyte (N-3,5 dinitrobenzoyl alanine methyl ester) and the chiral stationary phase (R(+)-(1)-naphthylethylamine) (1)π-π interaction between 3,5-dinitrobenzoyl group of analyte and naphthyl group of CSP; (2) hydrogen bonding interaction between aminyl hydrogen and the carboxyl oxygen; (3) hydrogen bonding interaction.between carboxyl oxygen and the aminyl hydrogen.

Table 1 The chromatographic separation data

Solutes	t_R1	t_R2	t_0	k'_1	k'_2	α	log k'_1	log k'_2	Mobile phase	α(Lit.)
N-3,5 DNB-DL- AME	19.75	26.46	1.89	9.45	14	1.48	0.97	1.14	1	1.16
N-3,5 DNB-DL- PAME	9.38	10.55	1.89	3.96	5.58	1.41	0.6	0.75	2	1.08
N-3,5 DNB-DL-IME	13.94	16.67	1.89	6.38	8.82	1.38	0.8	0.94	2	1.09
N-3,5 DNB-DL- ILME	23.16	26.56	1.89	11.25	14.06	1.25	1.05	1.15	2	1.07
N-3,5 DNB-DL- PGME	19.75	21.99	1.89	9.45	11.63	1.23	0.97	1.06	2	1.06

SOLUTES:
N-3,5 DNB-DL- AME; N- (3,5 - Dinitrobenzoyl)- DL-Alanine Methyl Ester
N-3,5 DNB-DL- PAME; N- (3,5 - Dinitrobenzoyl)- DL-Phenylalanine Methyl Ester
N-3,5 DNB-DL-IME: N- (3,5 - Dinitrobenzoyl)- DL-Leucine Methyl Ester
N-3,5 DNB-DL- ILME; N- (3,5 - Dinitrobenzoyl)- DL-Isoleucine Methyl Ester
N-3,5 DNB-DL- PGME; N- (3,5 - Dinitrobenzoyl)- DL-Phenylglycine Methyl Ester

Mobile Phases:
1. Isooctane: 1,2- dichloroethane: Ethanol (90:8:1)
2. Isooctane: 1,2- dichloroethane: Ethanol (87.5:12:0.5)

Stationary Phase:
R (+) - Naphthylethylamine bonded through reaction IV to Vydac Hydride
Flow Rate 1.0 ml/min

case the phenyl group has less interaction as compared to the methyl group and so the retention time of DNB-phenylalanine is less than DNB-alanine. Lastly it was found that decrease in retention time lead to a decrease in capacity factor.

CHAPTER IV - CONCLUSION

A new method was developed for synthesizing a chiral stationary phase from R/S-N-(1)-Naphthylethylamine with 4-Allyoxybenzoic acid as a precursor in the presence of DCC and N-Hydroxysuccimide. Even though the surface coverage was less when compared to other chiral stationary phases better capacity factors and separations were obtained. Solutes such as 3,5 dinitrobenzoyl derivatives of amino acids can be separated with better efficiency on this column. Separation factors (α) greater than 1 were achieved which again proves that this stationary phase has good resolving power for some racemic analytes. Reasonable retention of solutes was obtained (capacity factor k' more than 8.0) which shows a significant interaction between the stationary phase and the solutes even though the surface coverage is low. By optimizing reaction conditions higher surface coverage can be obtained which could enhance the isomeric separation. By a reciprocal approach it is easy to make many chiral stationary phases from amino acids which can further broaden the application.

CHAPTER V - REFERENCES

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