

University of Warwick institutional repository: <http://go.warwick.ac.uk/wrap>

A Thesis Submitted for the Degree of PhD at the University of Warwick

<http://go.warwick.ac.uk/wrap/3994>

This thesis is made available online and is protected by original copyright.

Please scroll down to view the document itself.

Please refer to the repository record for this item for information to help you to cite it. Our policy information is available from the repository home page.

STUDIES OF HEXAHELICENE
BONDED PHASES
FOR THE HPLC RESOLUTION
OF ENANTIOMERS

by

AURORA DEL ALAMO

A thesis submitted in partial fulfilment of the
requirements for the degree of Doctor of Philosophy
at the University of Warwick

Department of Chemistry
University of Warwick

June, 1995

CONTENTS

<u>Chapter One</u>	Pg
LITERATURE REVIEW: CHIRAL LIQUID CHROMATOGRAPHY	
1.1 Introduction	1
1.2 Resolution of Enantiomers	3
1.3 Preparation of HPLC Chiral Stationary Phases	4
1.4 Development of Commercially Available Direct Chiral Resolution Phases	5
1.4.1 Chiral Ligand Exchange Chromatography (LEC)	6
1.4.2 Synthetic Multiple Interaction CSPs	8
1.4.3 Protein Chiral Stationary Phases	13
1.4.4 Cyclodextrin CSPs	18
1.4.5 Polysaccharide Phases	31
1.4.6 Synthetic Polymer CSPs	36
1.4.7 Chiral Crown Ethers	40
<u>Chapter Two</u>	
LITERATURE REVIEW: SOME ASPECTS OF THE CHEMISTRY AND PROPERTIES OF HELICENES	
2.1 Helicenes	45
2.2 Syntheses of Hexahelicenes: Methods and their Problems	46
2.2.1 Non-Photochemical Syntheses	46
2.2.2 Photochemical Syntheses	48
2.2.3 Asymmetric Syntheses of Helicenes:	56
2.2.3.1 Asymmetric Synthesis with Circularly Polarised Light	56
2.2.3.2 Asymmetric Synthesis in Chiral Solvents	56
2.2.3.3 Asymmetric Synthesis in Cholesteric Liquid Crystals	58
2.2.3.4 Chemically Induced Asymmetric Synthesis	58
2.2.4 Problems in the Syntheses of Helicenes	59

2.3	Structural Determinations	59
2.4	Chiroptical Properties	61
	2.4.1 Optical Resolution	61
	2.4.2 Thermal Racemisation	72
2.5	Spectral properties	74
	2.5.1 ¹ H NMR Spectroscopy	74
	2.5.2 ¹³ C NMR Spectroscopy	75
	2.5.3 Photoelectron and Ultraviolet Spectra	76
	2.5.4 Charge-transfer Complexation	77
	2.5.5 Photophysical Properties	78
	2.5.6 Mass Spectrometry	78
2.6	Chemical Reactions	79
	2.6.1 Intramolecular Reactions	79
	2.6.2 Intermolecular Reactions:	81
	2.6.2.1 Electrophilic Reactions	81
	2.6.2.2 Oxidation	81
2.7	Photoreactions	82
2.8	Conclusions	82

Chapter Three

LITERATURE REVIEW: CHROMATOGRAPHIC SEPARATION OF ENANTIOMERS ON HEXAHELICENE BONDED PHASE.

3.1	Resolution of Enantiomers of Electron-deficient Compounds.	83
-----	--	----

Chapter Four

OBJECTIVES AND STRATEGY

4.1	Introduction	91
4.2	Objectives	92
4.3	Strategy	93

Chapter Five

RESULTS AND DISCUSSION

5.1	Synthesis of Hexahelicen-7-ylacetic acid methyl ester.	95
5.2	Resolution of Hexahelicen-7-ylacetic acid methyl ester Derivatives.	128
5.3	Preparation of (+/-)-Hexahelicen-7-yl 1'-methyl-1'-phenylmethanamide.	129
5.4	Chromatographic Resolution of the Diastereoisomers of (+/-)-Hexahelicen-7-yl 1'-methyl-1'-phenylmethanamide on a 3,5- Dinitrobenzoyl phenylglycine APS Semipreparative Column	130
5.5	Chromatographic Resolution of the Enantiomers of (+/-)-Hexahelicen-7-ylacetic acid methyl ester on a Polysaccharide Column.	132
5.6	Hydrolysis of the Hexahelicen-7-ylacetic acid methyl ester Enantiomers.	136
5.7	Bonding the Hexahelicen-7-ylacetic acid to Hypersil APS	139
5.7.1	Calculation of the Surface Coverage	140
5.8	Separations on the (+)- and (-)-Hexahelicene Bonded Phases	142
5.8.1	Design of the Chiral Test Analytes	142
5.8.2	Development of the Chromatographic Conditions for the Resolution of the Test Analytes	143
5.8.3	Discussion of Results	144
5.8.4	Retention Mechanism of the Hexahelicene Chiral Stationary Phases	148
5.8.5	Comparison of the Resolution of the Chiral Test Solutes on (+)- and (-)-Hexahelicene Phases	149
5.8.6	Order of Elution	150
5.8.7	Discussion	150

Chapter Six

EXPERIMENTAL

6.1	Synthesis of (+/-)-Hexahelicen-7-ylacetic acid methyl ester	154
6.1.1	Diethyl 2-(1'-naphthylmethylene)malonate	154
6.1.2	1-Naphthylmagnesium bromide	155
6.1.3	Diethyl 2-(1,1' -dinaphthylmethyl)malonate	155
6.1.4	2-(1,1' -Dinaphthylmethyl)-1,3-propanediol	156
6.1.5	2-(1,1' -Dinaphthylmethyl)-1,3-propanediol di-mesyl ester	157
6.1.6	3-(1,1' -Dinaphthylmethyl)glutaronitrile	159
6.1.7	3-(1,1' -Dinaphthylmethyl) glutaric acid	159
6.1.8	[1,2,3,4-Tetrahydro-4-(1' -naphthyl)-1-oxo-3-phenanthrenyl]- -acetic acid	160
6.1.9	[1,2,3,4-Tetrahydro-4-(1' -naphthyl)-3-phenanthrenyl]- -acetic acid	162
6.1.10	7,8,8a,9,10,16c-Hexahydro-7-oxohexahelicene	163
6.1.11	3,4,4a,5,6,12c-Hexahydro-3-hydroxy-phenanthro[3,4-c] phenanthren-3-ylacetic acid methyl ester	164
6.1.12	4a,5,6,12c-Tetrahydrophenanthro[3,4-c]phenanthren- -3-ylacetic acid methyl ester	166
6.1.13	Hexahelicen-7-ylacetic acid methyl ester	166
6.2	Synthesis and Resolution of (+/-)-Hexahelicen-7-yl 1'-methyl-1'phenylmethanamide.	167
6.2.1	Hydrolysis of the Racemic Hexahelicen-7-ylacetic acid methyl ester	167
6.2.2	Synthesis of (+/-)-Hexahelicen-7-yl 1'-methyl-1'-phenyl methanamide	168
6.2.3	Resolution of (+/-)-Hexahelicen-7-yl 1'-methyl-1'-phenyl methanamide on 3,5-Dinitrobenzoylphenylglycine	168

6.2.4	Hydrolysis of (+)- and (-)-Hexahelicen-7-yl 1'-methyl- -1'-phenylmethanamide.	169
6.3	Direct Resolution of (+/-)-Hexahelicen-7-ylacetic acid methyl ester	169
6.3.1	Analytical Resolution of (+/-)-Hexahelicen-7-ylacetic acid methyl ester on Carbohydrate Carbamate Columns	169
6.3.2	Preparative Resolution of (+/-)-Hexahelicen-7-ylacetic acid methyl ester	170
6.3.3	Hydrolysis of Hexahelicen-7-ylacetic acid methyl ester Enantiomers	170
6.4	Bonding of (+)-Hexahelicen-7-ylacetic acid and (-)-Hexahelicen -7-ylacetic acid to APS	171
6.5	Packing of (+)- and (-)-Hexahelicen-7-ylacetic acid Bonded Stationary Phases	172
6.6	Synthesis of the Chiral Analytes for Testing	172

Chapter Seven

REFERENCES

ABBREVIATIONS

HPLC:	High Performance Liquid Chromatography
CSP:	Chiral Stationary Phase
LEC:	Ligand Exchange Chromatography
CMP:	Chiral Mobile Phase
TFAE:	2,2,2-Trifluoro 1-(9-anthryl)ethanol
BSA:	Bovine Serum Albumin
HSA:	Human Serum Albumin
AGP:	α -Glycoprotein
OV:	Ovomucoid protein
AUFS:	Absorbance Units Full Scale
LC:	Liquid Chromatography
CD:	Cyclodextrins
IPA:	Isopropanol alcohol
THF:	Tetrahydrofuran
TEAA:	Triethyl ammonium acetate
MCT:	Microcrystalline Triacetate
Co(acac) ₃ :	Cobalt (III) tris (acetylacetonate)
PTrMA:	Poly (triphenylmethyl methacrylate)
TrMA:	Triphenylmethyl methacrylate
(+)-DDB:	Dimethoxy-1,4-bis (dimethylamino) butane
μm :	micron
Å:	Angstrom
CE:	Crown Ethers
IR:	Infrared Spectroscopy
¹ H NMR:	Proton Nuclear Magnetic Resonance
¹³ H NMR:	Carbon Nuclear Magnetic Resonance
UV:	Ultraviolet

EI-MS:	Electron Impact Mass Spectrometry
FAB-MS:	Fast Atom Bombardment Mass Spectrometry
COSY:	Correlated Spectroscopy
NOE:	Nuclear Overhauser Effect
NORD:	Noise Off-Resonance Decoupling Technique
st:	Stretch
bd:	Broad doublet
d:	Doublet
dd	Doublet of doublets
DHP:	Dyhydrophenanthrene
CPL:	Circularly Polarised Light
GLC:	Gas Liquid Chromatography
TLC:	Thin Layer Chromatography
TAPA:	2-(2,4,5,7-tetranitro-9-fluorenylideneaminoxy) propionic acid
BPA:	Binaphthyl-2,2'-diyl Hydrogen Phosphate
APS:	Aminopropyl Silica
APC:	Amylose tris (phenylcarbamate)
CPC:	Cellulose tris (phenylcarbamate)
ADMPC:	Amylose tris (3,5-dimethylphenylcarbamate)
CDMPC:	Cellulose tris (3,5-dimethylphenylcarbamate)
DCC:	Dicyclohexyl carbodiimide
CDI:	1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-4-toluenesulphonate
DNB:	Dinitrobenzoyl
EEDQ:	N-ethoxy carbonyl-2-ethoxy-1,2-dihydroquinoline

ACKNOWLEDGEMENTS

I would like to express my profound gratitude to my supervisor, Professor S. A. Matlin, for his advice and encouragement throughout this work.

I would also like to express my great appreciation to Dr Ana Belenguer and Sally Grieb for their invaluable discussion and their assistance in the preparation of this thesis. I am also indebted to Elizabeth Tiritan for lending me the preparative column used in the separation of the enantiomers of hexahelicen-7-ylacetic acid methyl ester.

Grateful thanks are due to the students and members of staff at City University and at Warwick University, in particular Paul Crick, Dr Charles Thomas and Vivian Stacey who have contributed and assisted during this work.

**PAGE
NUMBERING
AS ORIGINAL**

ABSTRACT

The thesis presents a review of the literature on the HPLC resolution of enantiomers using chiral stationary phases and discusses the mechanisms of separation and the utility of these phases. The synthesis and chemical, chiroptical and spectroscopic properties of helicenes are also reviewed and the potential utility of helicene-based for the HPLC resolution of enantiomers is discussed.

The work carried out involved the preparation of a hexahelicene-based chiral stationary phase and demonstrations of its utility for the resolution of chiral analytes. This phase was chemically bonded rather than physically coated, in order to make it stable to hydrolysis and solvent stripping, and therefore to permit its employment with a wide range of mobile phases.

This thesis describes the synthesis of hexahelicen-7-ylacetic acid methyl ester, including confirmation of the structure of the key synthetic intermediates by spectroscopic analysis, and the investigation of several procedures for the resolution of hexahelicen-7-ylacetic acid methyl ester into its enantiomers, one of which enabled around 100 mg amounts of each of (+) and (-) enantiomers of hexahelicen-7-ylacetic acid to be obtained in highly chemically and optically pure form. The thesis also gives an account of several synthetic approaches to covalently bonding of the chiral selector (hexahelicen-7-ylacetic acid) to a modified silica stationary phase.

Chiral stationary phases were prepared from each of the enantiomers in sufficient amount to permit the packing of analytical columns of these phases. Chiral solutes containing nitroaryl functionalities were synthesised to investigate the chiral resolving power of these novel phases. Adequate separations were obtained with both chiral stationary phases and, as anticipated, the eluting order of chiral analytes was reversed between the (+) and (-) stationary phases. The work demonstrated the utility of these columns for the resolution of nitroaryl-containing chiral analytes.

Chapter One

LITERATURE REVIEW: CHIRAL LIQUID CHROMATOGRAPHY

1.1- Introduction.

Many natural and synthetic molecules are chiral, i.e. they can exist in two non-superimposable mirror image forms, called enantiomers. In the case of chiral drugs the interaction of each enantiomer with its target site (biological structures such as enzymes, DNA or receptors which are themselves large, chiral structures) are generally different. Usually, only one enantiomer will "fit" the target receptor and produce the required response, whilst its mirror image may largely fail to interact or give an effect at this receptor and is often assumed to be "inert".

At the present time, more than half the pharmaceuticals in use are chiral, but only about a quarter of these are sold as a single enantiomer, mainly because of the inconvenience and expense of manufacturing a pure enantiomer. The individual isomers of racemic mixtures frequently differ in pharmacological or metabolic activities, and often only one isomer is therapeutically active^[1].

During the last three decades there has been growing evidence that the assumption of biological inertness of the "wrong" drug enantiomer is often incorrect. The most dramatic example is that of the sedative drug thalidomide (Fig. 1), which about thirty years ago caused severe physical deformities in the offspring of pregnant women who were prescribed the drug. It is now clear that most, if not all, of the teratogenic effects reside in the opposite enantiomer to that having the sedative action [2].

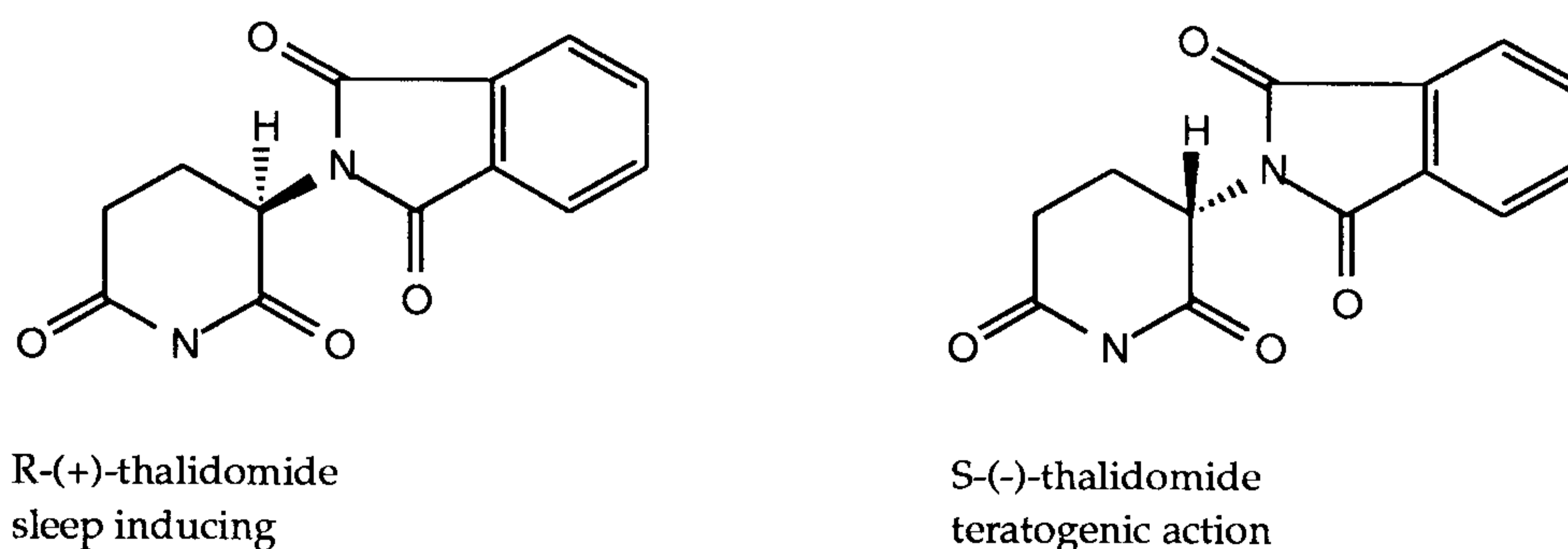


Fig. 1 The enantiomers of thalidomide

In most cases, the effects of the wrong enantiomer may be much less significant, but there is a growing recognition by drug regulatory agencies of a need for clear evidence of this and a sharp decline in the use of racemic drugs is now expected by the pharmaceutical industry. Consequently, the ability to separate enantiomers for analytical and preparative purposes has become critically important. Major advances in technology for doing this by high performance liquid chromatography (HPLC) have taken place in the last decade [3].

The classical approach to resolution has been to prepare derivatives of racemates using a resolved chiral reagent, which generates diastereoisomers that differ in physical properties. Although such diastereoisomers can be resolved by HPLC on conventional (normal or reverse phase) columns, they are tedious to prepare and represent an indirect approach to the problem.

A direct approach involves the synthesis of a chiral stationary phase (CSP), where a chiral selector is immobilised onto the surface of the support. The CSP interacts diastereoselectively with the enantiomers in the racemate so that their elution times are different.

Another direct approach involves adding a chiral selector to the mobile phase and using an achiral stationary phase. Chiral resolution occurs when the chiral mobile phase additive forms *in situ* diastereomeric complexes with the solute enantiomers and these interact differently with the achiral stationary phase. This method will not be treated in any detail in this thesis, which will concentrate on the preparation of chiral stationary phases.

There has been substantial success in achieving direct enantiomer resolutions in the last ten years and some commercial columns are now available for chiral HPLC separations. However, none of the chiral phases is able to resolve all classes of compounds and there is an urgent need for the development of additional phases.

1.2- Resolution of Enantiomers.

Enantiomeric chemical substances, although consisting of non-superimposable mirror images, display identical physical and chemical properties except when interacting with another chiral entity.

The difference in the interaction of a chiral selector with the two enantiomers is called enantioselectivity [4]. Dalgleish proposed a model to explain the concept of enantioselectivity between chiral drugs and receptors. This model is called the "three point interaction model". The model states that two enantiomeric structures can only be recognised by the chiral selector if at least three active positions of that agent simultaneously interact with the appropriate active positions of one enantiomer and that there must be an enantioselective element in one of these interactions. These interactions can be repulsive as well as attractive. The chiral discrimination of the chiral selector results from the preferential enantioselective interaction with one enantiomer and not with the other.

Pirkle used this model to explain the enantioselective retention mechanism of chiral stationary phases and his group later used this model to design chiral selectors for chromatography. Pirkle stated [5] that for chiral resolution to take place, there must be at least three simultaneous interactions between a chiral stationary phase (CSP) and the most retained solute enantiomer, assuming the enantioselective interaction is attractive. The solute enantiomer forming the stronger diastereomeric complex with the CSP would be retained longest by the column (Fig. 2). The separation or discrimination power of the chiral selector towards the chiral solutes is therefore determined by the difference in the strength of the diastereomeric complexes formed with each of the solutes enantiomers.

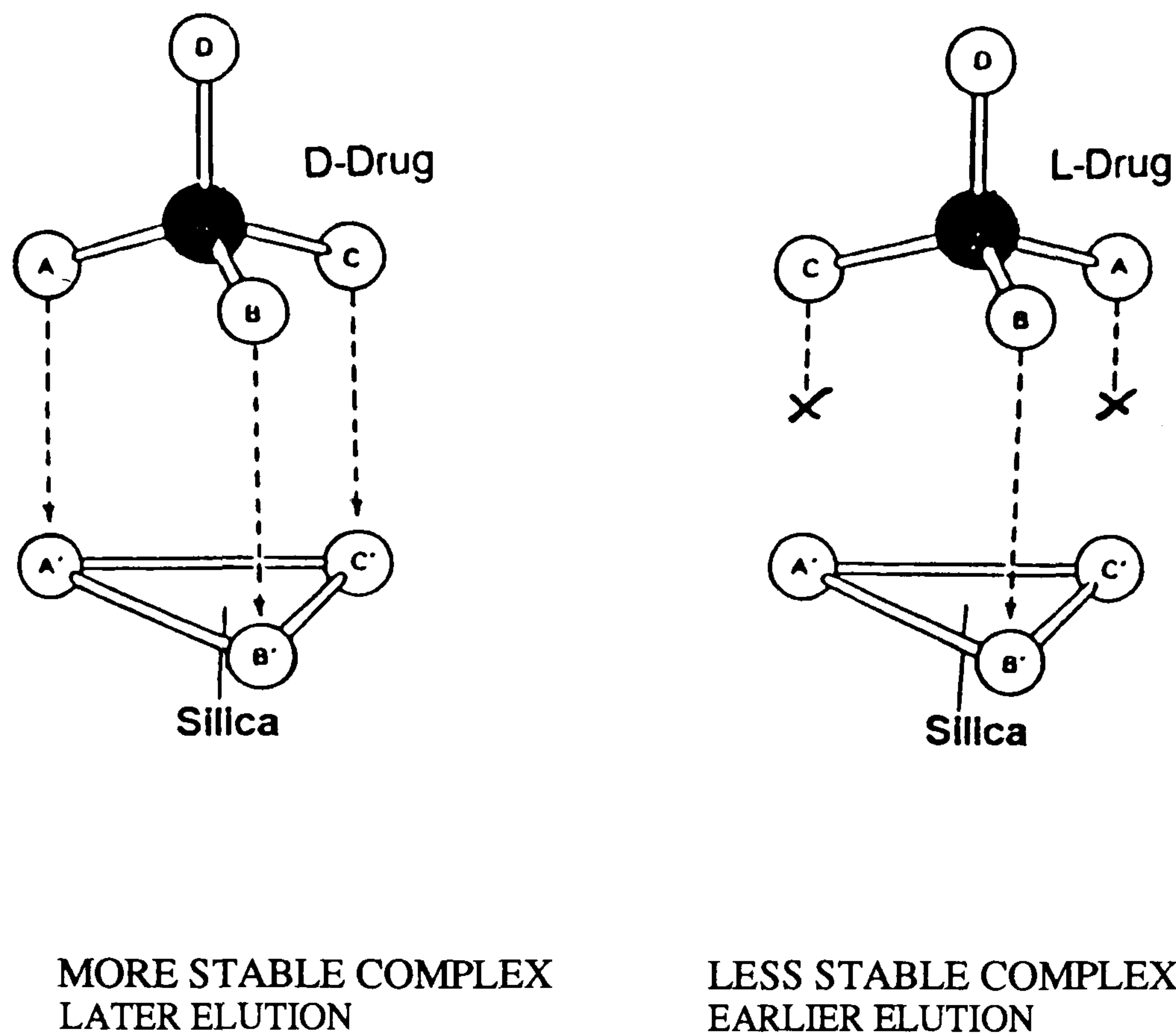


Fig. 2 Three point interaction model

Typical intermolecular interactions involved in the chiral recognition process are π - π interactions, hydrogen bonds and dipole-dipole, hydrophobic and steric interactions. Other interactions involved in chiral discrimination are metal coordination complex formation, inclusion complexing and ionic interactions.

1.3- Preparation of HPLC Chiral Stationary Phases.

The chiral stationary phases commercially available have been prepared by three methods (Fig. 3).

- Covalently bonding the chiral selector to modified silica, e.g. via amide, ether or sulphide linkages.
- Ionically bonding the chiral selector to charged modified silica, as e.g. an ammonium carboxylate salt.
- Coating the chiral selector onto modified silica: A solution of the chiral selector is evaporated in the presence of the modified silica.

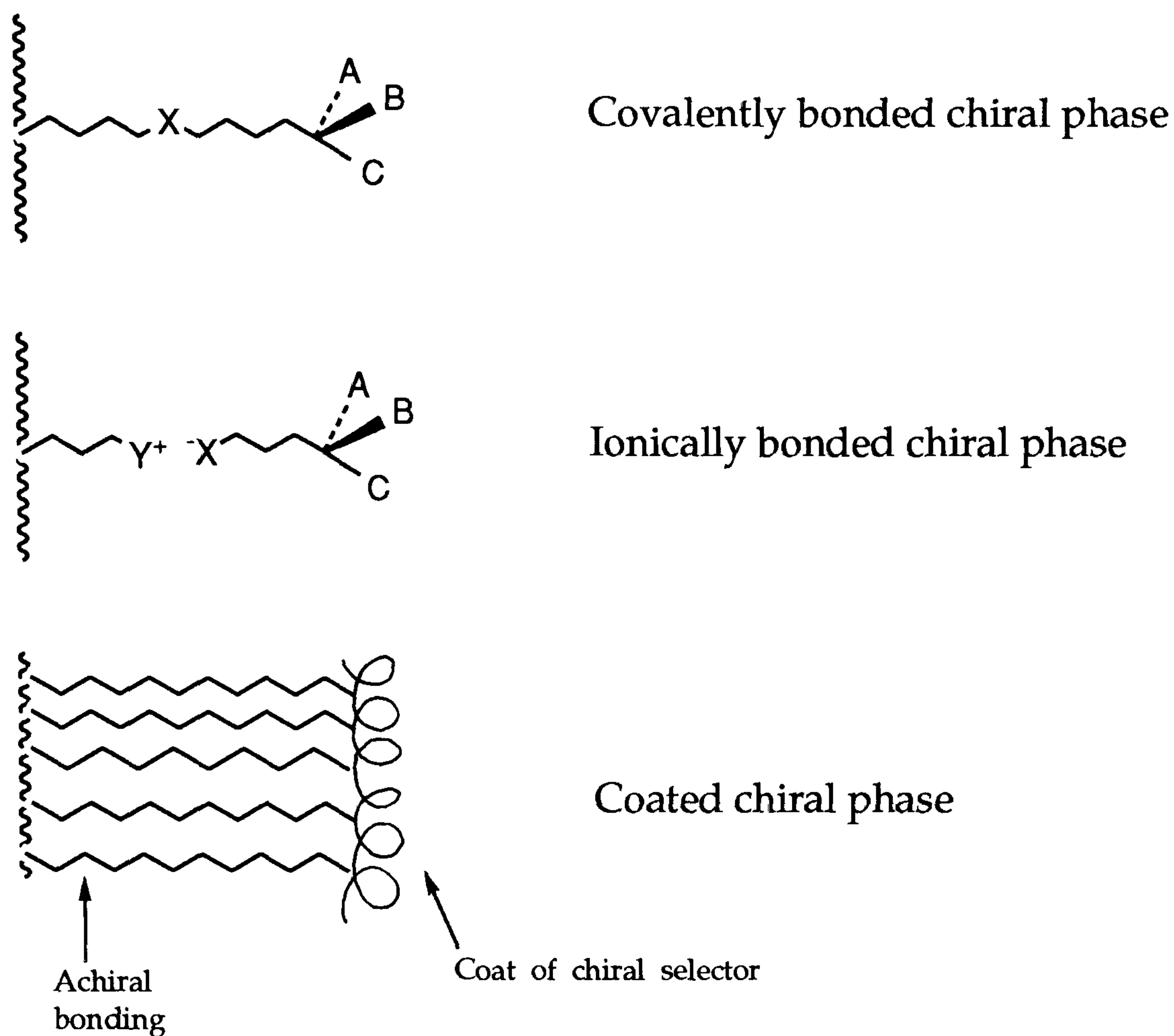


Fig. 3

1.4- Development of Commercially Available Direct Chiral Resolution

Methods.

The methods of resolution of enantiomers in chiral liquid chromatography, in the approximate order in which they became available, are as follows:

- 1.4.1- Chiral Ligand Exchange Chromatography (LEC).
- 1.4.2- Synthetic Multiple Interaction CSPs.
- 1.4.3- Protein Chiral Stationary Phases.
- 1.4.4- Cyclodextrin CSPs.
- 1.4.5- Polysaccharide Phases.
- 1.4.6- Synthetic Polymer CSPs.
- 1.4.7- Chiral Crown Ethers.

1.4.1- Chiral Ligand Exchange Chromatography (LEC).

LEC was the first direct chiral resolution method to become easily accessible^[6], and subsequently proved a powerful technique for the chiral resolution of underivatized amino acids, whose electron-donating primary amino and carboxylate groups can act as bidentate ligands towards a transition metal ion. If an additional functionality in the amino acid can coordinate with the metal ion, this compound can then act as a tridentate ligand.

In ligand exchange chromatography, the chiral stationary phase is formed by covalently bonding an α -amino acid, acting as a ligand, to a resin or to a silica phase. In the presence of transition metal ions, preferably copper (II), and a chiral solute, acting as a bidentate ligand, the stationary phase forms a mixed labile coordination complex. Chiral resolution occurs by fast exchange of solute ligands, the enantiomer forming the most stable diastereomeric complex with copper (II) and the chiral stationary phase being retained longest.

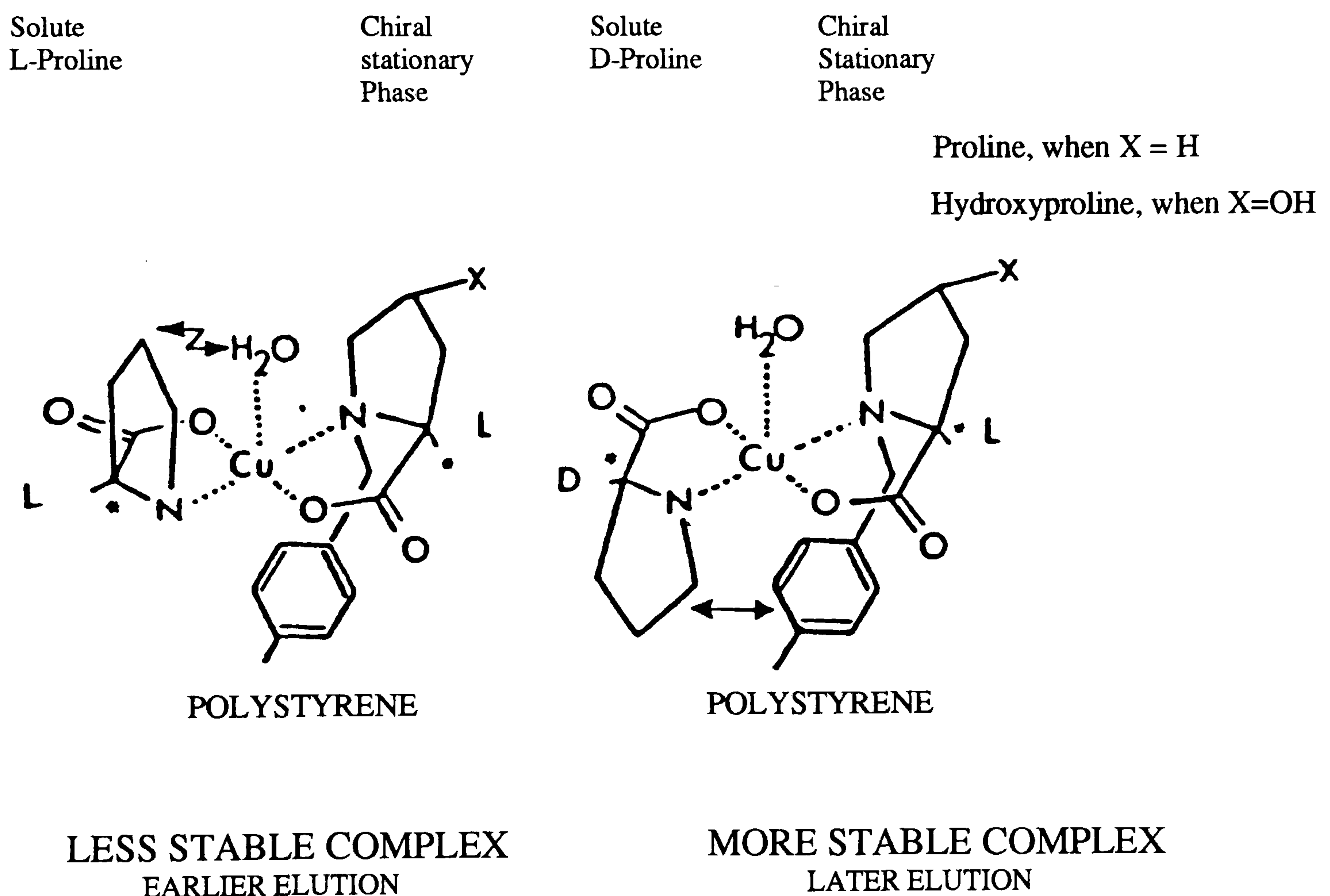


Fig. 4 Stereoselective interaction between the chiral phase and the enantiomers of proline by ligand exchange.

In the example of Fig. 4, the complex of the D-enantiomer of proline shows increased stability over that of the L-enantiomer, by allowing the copper (II) to coordinate at the unhindered axial position to a molecule of water. This stability is further enhanced by the hydrophobic attraction between the proline residue of the solute and the phenyl group of the stationary phase in the aqueous environment. In this case the L-enantiomer will elute before the D-enantiomer.

A large number of chiral ligand-exchange resins have been described, often containing chiral amino acids as the ligands, coupled with transition metal ions such as Cu(II) or Ni(II) [7]. In some cases, ammonia, pyridine, or an amine solution have been added to the eluting solvent [7]. As these chiral phases have been found to be quite inefficient, overall resolution can be increased with column temperature. These phases are often used between 40 and 60°C.

The process of ligand exchange can be applied to any compound that contains electron-donating heteroatoms (e.g. nitrogen, oxygen, sulphur) which are adequately situated in a spacial arrangement to allow them to be incorporated into the metal ion coordination sphere giving shell structures characteristic of noble gases. The complex generated must be kinetically labile so that it is dissociated and re-formed many times during the chromatographic process.

Three major variants of LEC of optical isomers have been developed so far [8]. The first uses chiral stationary phases (CSP) in which the chiral ligand is covalently bonded to a polymeric sorbent or silica; the second employs a conventional, achiral column packing containing a chiral metal chelate strongly adsorbed on its surface; and the third variant employs the chiral mobile phase additive technique (CMP), in which an achiral packing material is combined with an eluent containing a chiral metal chelate additive. The latter method [9] involves the addition of a chiral bidentate ligand (usually L-proline or L-hydroxyproline) to a polar mobile phase along with a metal ion, usually Cu^{2+} or Zn^{2+} .

In the first two cases, the diastereomeric ternary complexes comprising metal ion, chiral selector and each of the two enantiomers being resolved are formed in the stationary phase alone, while in the case of the CMP additive technique, complex formation and ligand-exchange reactions proceed both in the mobile and stationary phases.

Ligand-exchange is especially reliable for the resolution of potentially bidentate solute ligands, such as amino acids, amino alcohols, hydroxy acids and diamines, as well as some types of their derivatives. However, it shows little applicability to other classes of chiral compounds.

Commercially available stationary phases are:

Chiral phase	Description	Manufacturer
Chiral ProCu	L-proline	Serva
Chiral ValCu	L-valine	Serva
Nucleosil Chiral-1	L-hydroxyproline	Macherey Nagel
ChiralPak WM	Unspecified ligand	Daicel
ChiralPak WH	L-proline	Daicel
ChiralPak WE(-)	Unspecified ligand	Daicel
MicroPak Optimer L1	Unspecified ligand	Varian

All these chiral ligands are covalently bonded to silica via an alkyl/aromatic spacer.

1.4.2- Synthetic Multiple Interaction CSPs.

The first synthetic multiple interaction CSPs to become commercially successful were those which were initially synthesised by Pirkle. They were developed as practical applications of the "three point interaction model".

These phases are synthesised on a rational design where desired functional groups are incorporated into the stationary phase in order to interact simultaneously through different mechanisms with complementary functionalities contained in a

target group of chiral solute molecules (Fig. 5). Chiral resolution is achieved because, for stereochemical reasons, some of the interactions can only take place with one enantiomer and not with the other.

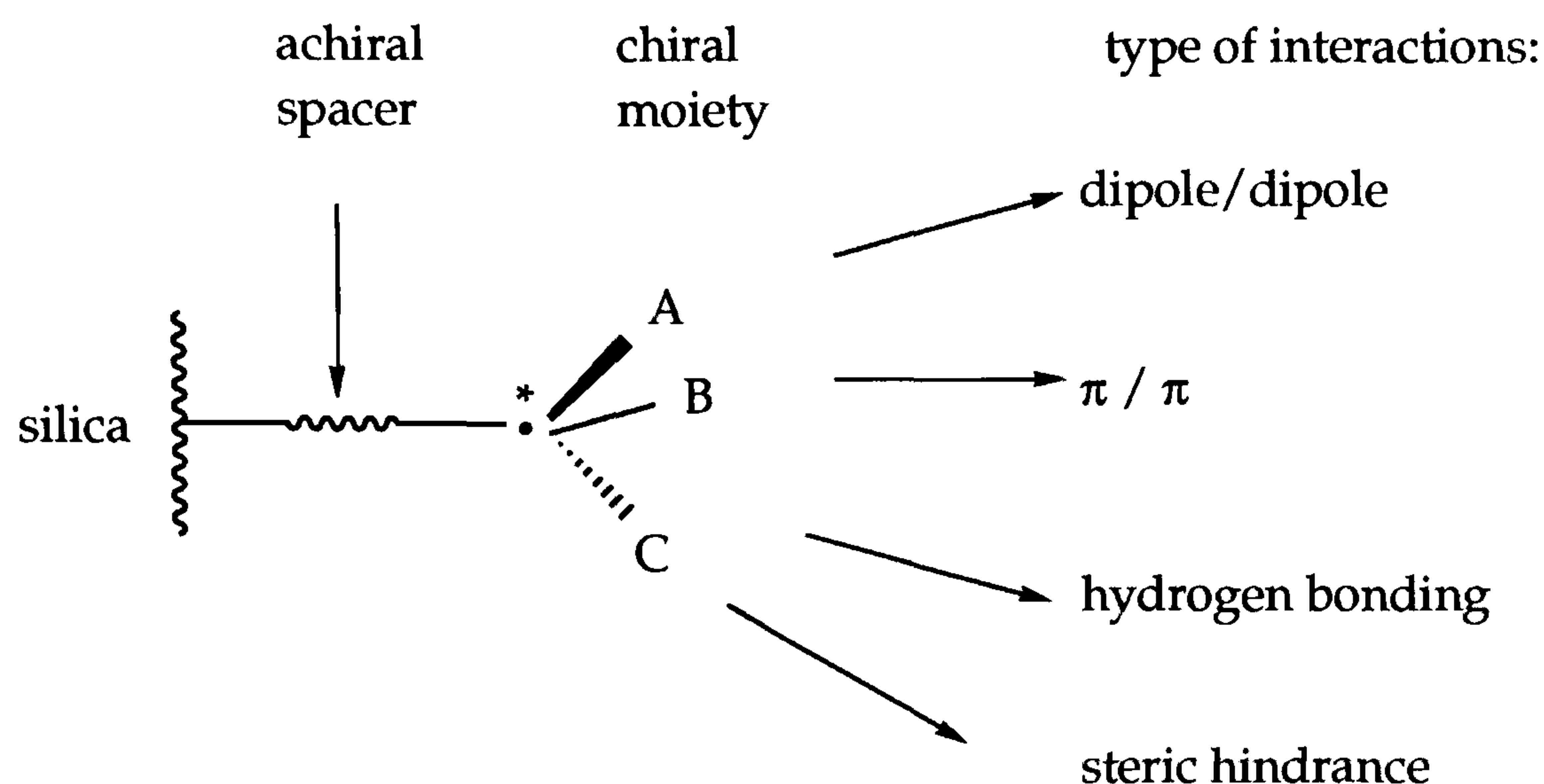


Fig. 5 Types of interaction that can be involved in chiral recognition

An example of the kind of functionality that can be designed into the Pirkle phase is illustrated in Fig. 6

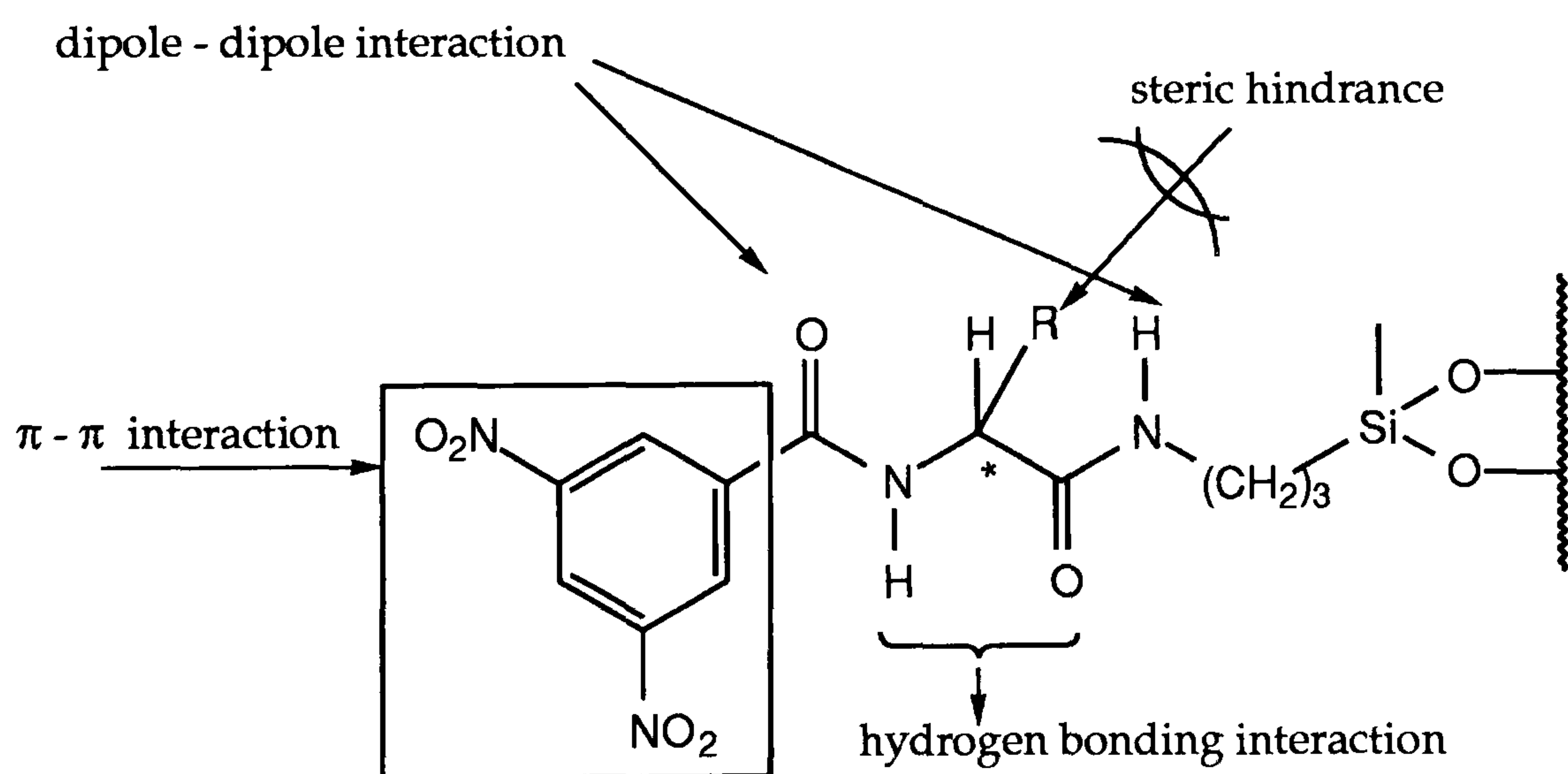


Fig. 6 Possible stereoselective sites of interaction on a Pirkle-type chiral stationary phase

The first phase that Pirkle designed was 2,2,2-trifluoro 1-(9-anthryl) ethanol (TFAE) covalently bonded to silica.

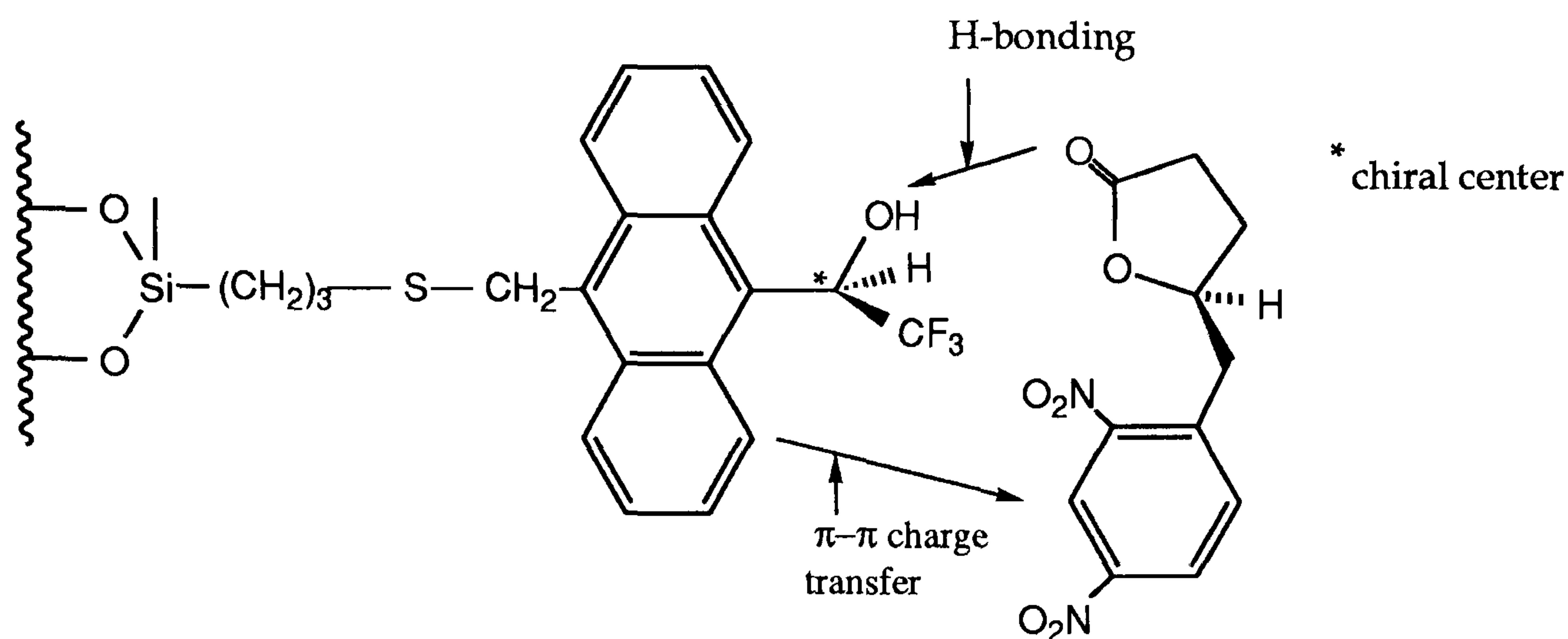


Fig.7 Interaction taking place on a π -base Pirkle phase.

This phase, which contains a π -base moiety, was designed to resolve solutes containing a π -acid functionality by a combination of π - π charge transfer and other interactions. Many chiral compounds containing nitro-substituted aromatic groups were successfully resolved.

A π -base is an electron donor such as a polynuclear aromatic group (e.g. naphthyl, anthryl) or an aromatic group with electron donating substituents (e.g. amino-, hydroxyl-, alkoxy-, alkyl- and phenyl groups). A π -acid is an electron acceptor, such as an aromatic molecule with electron-withdrawing substituents such as nitro or halogen groups.

The anthryl alcohol CSP (Fig. 7) gave good resolution of dinitrobenzoyl phenylglycine enantiomers. At this stage, Pirkle introduced the "reciprocity concept" [10] as follows: "The diastereomeric interactions that allow a column containing, as a chiral selector, an enantiomer of A to resolve a racemic compound B should allow a column containing, as a chiral selector, an enantiomer of B to resolve a racemic compound A" i.e. if the best resolved π -acid chiral solutes are bonded to silica to form π -acid phases, these phases should resolve solutes containing π -base functionalities. This evolutionary phase selection can be carried on, and each generation of chiral phase will have improved resolution with respect to an earlier generation.

The second generation of designed multiple interaction phases were π -acids such as various nitroaromatic chiral compounds, bonded to aminopropyl silica. A multitude of chiral solutes containing a π -base functionality were successfully separated. Fig. 8 shows the 3 point interactions between a π -acid chiral phase, dinitrobenzoyl phenylglycine, and two different solutes containing a π -base functionality.

(a) 2-carboxyindoline.

(b) N- (2-naphthyl) phenylglycine methyl ester.

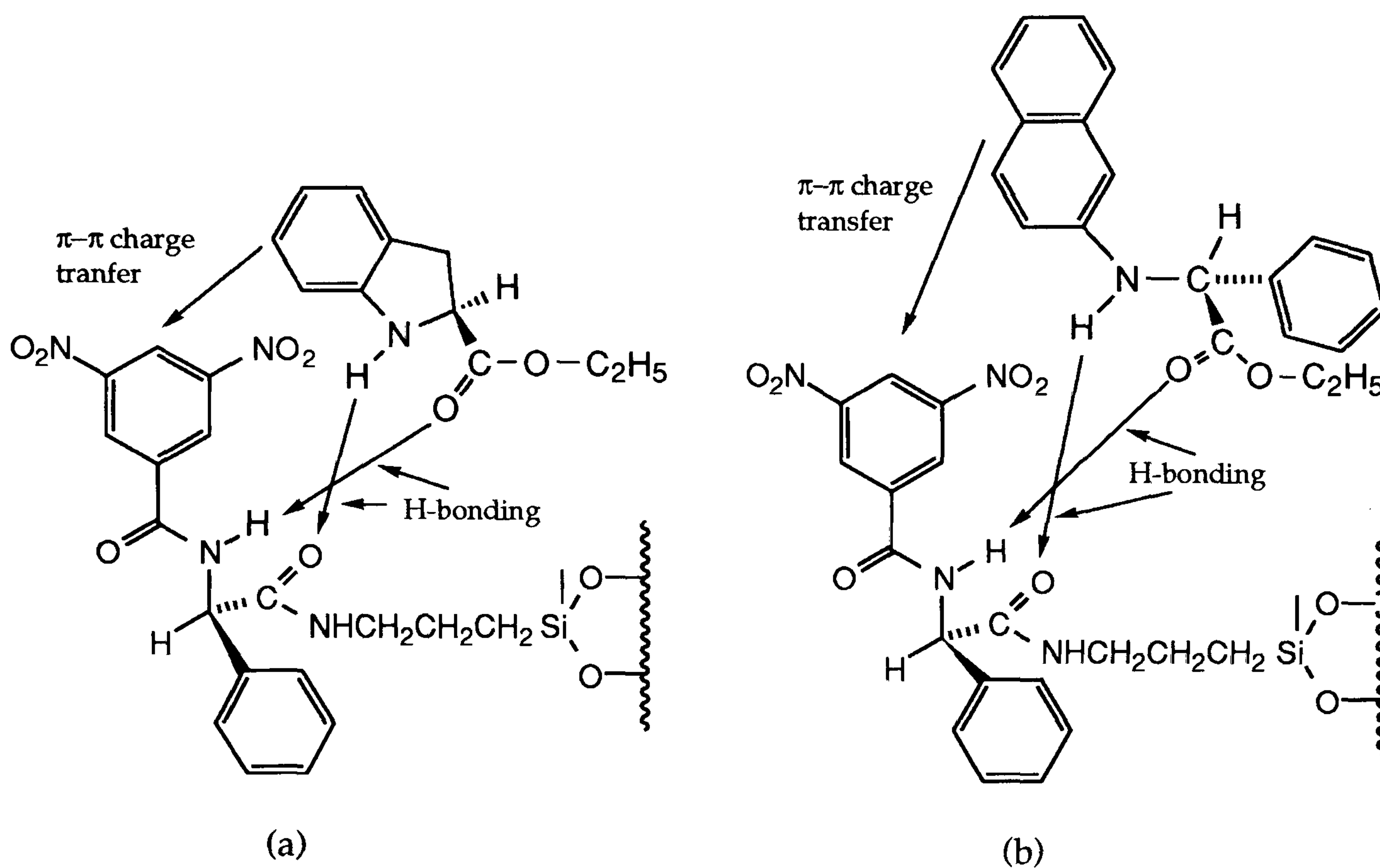


Fig. 8 Three point interaction in π -acid Pirkle phases

The third generation of Pirkle type phases incorporated a series of π -base functionalities. They were prepared by bonding polynuclear aromatic compounds to silica. Many chiral molecules containing a π -acid functionality such as aromatic nitro compounds, were successfully resolved.

Designed multiple interaction chiral phases have been traditionally used in normal phase mode. They are suitable for preparative work due to the good loading capacity and the high efficiency displayed by the stationary phases. Although achieving a poorer enantioseparation, many chiral separations can also be performed

under reverse phase conditions. A typical mobile phase used is methanol containing small amounts of water [11].

At present, the literature describes about a hundred structurally and configurationally distinct multiple-interaction CSPs, many of which are commercially available. Some CSPs involve difficult synthesis, but there are a number which can be prepared with no chemical manipulation other than bonding to silica, e.g. naphthylethylurea CSP [12] Fig. 9 (a), or with a very easy synthesis, e.g. N-naphthylalanine and valine CSPs [13] Fig. 9 (b).

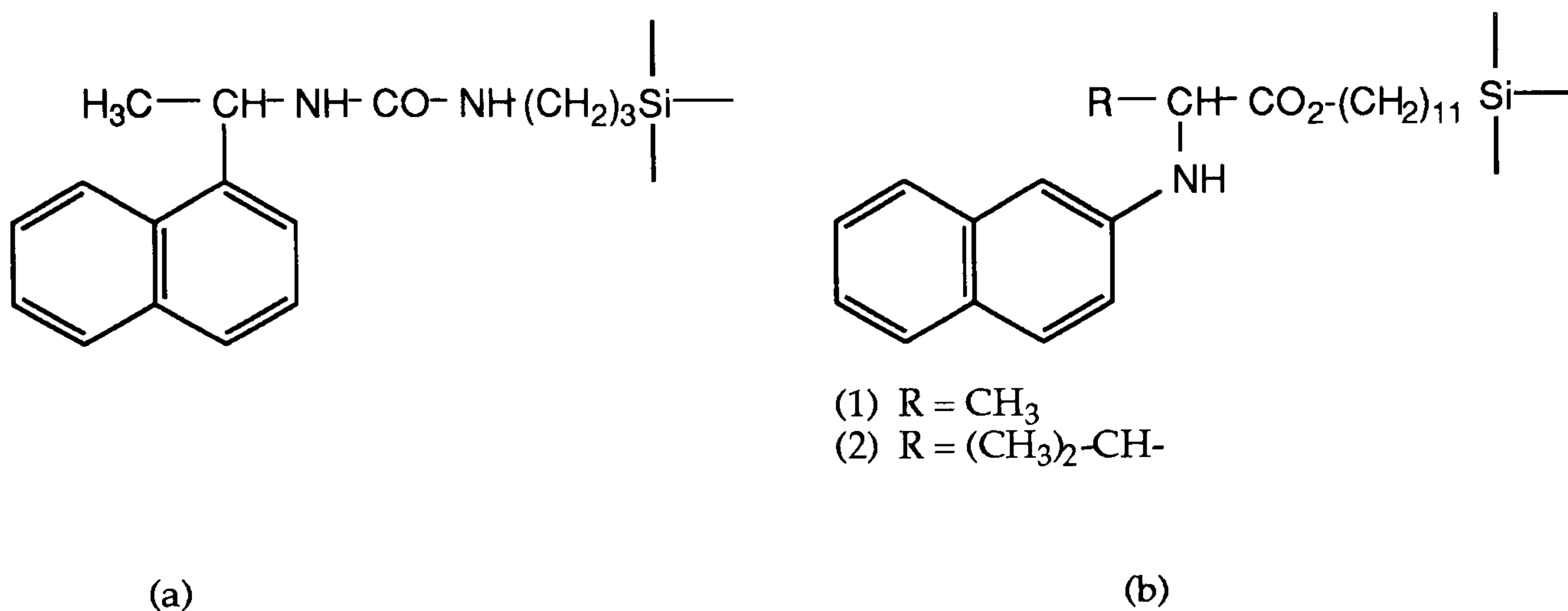


Fig. 9 (a) naphthylethyl urea CSP
(b) N-naphthyl alanine (1) and valine (2)

The most popular CSPs are N-3,5-dinitrobenzoyl phenylglycine or leucine bonded to aminopropyl silica, either covalently through an amide bond or ionically as an ammonium salt (Fig. 10).

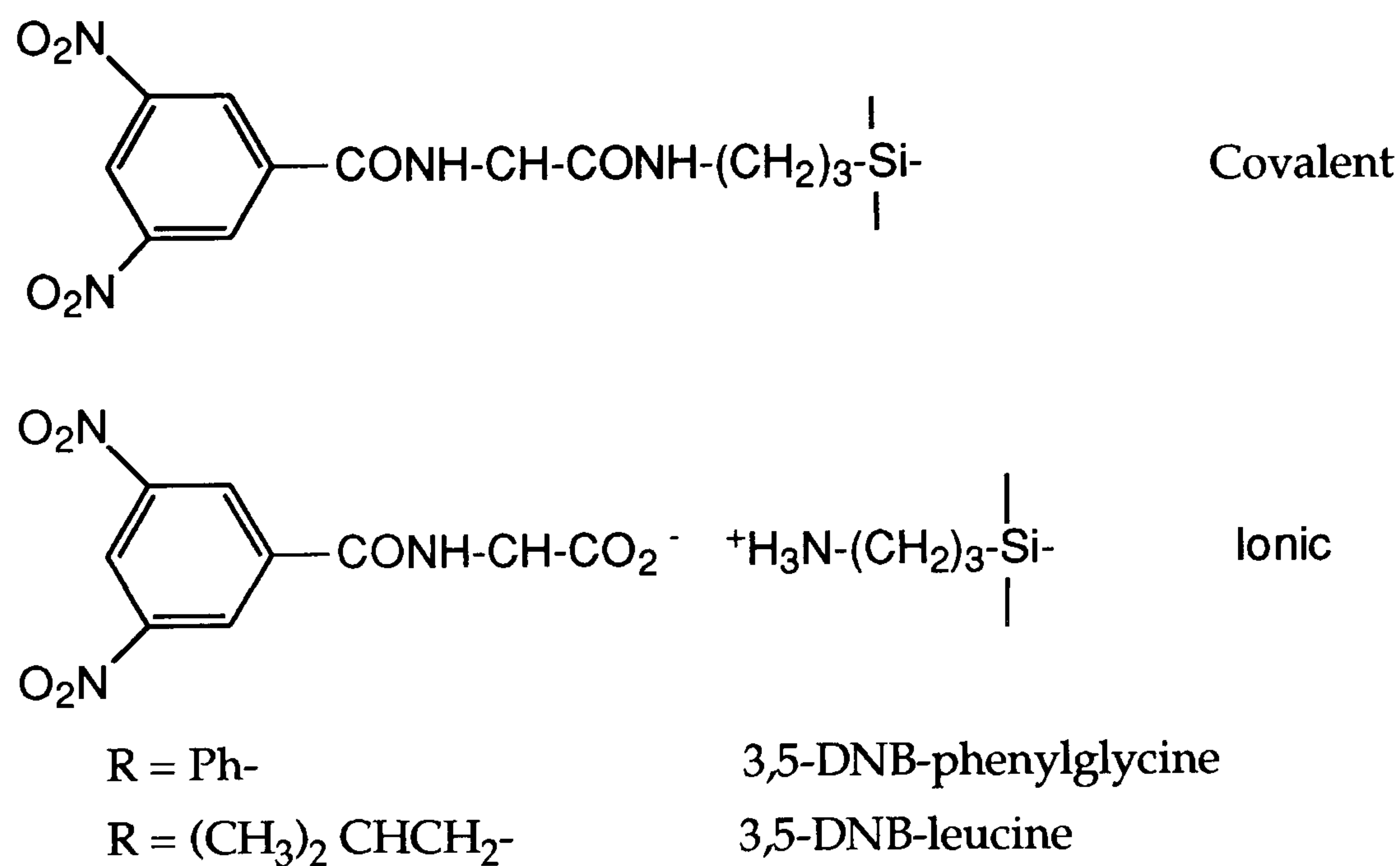


Fig. 10 Dinitrobenzoyl phenylglycine or leucine ionically and covalently bonded to aminopropyl silica

Despite their wide popularity, multiple interaction CSPs are not necessarily the most effective CSPs currently available. They have some limitations:

- a). There is frequently a need for derivatisation of the solutes to be resolved, because they do not have the required π -base or a π -acid functionality.
- b) There is usually a requirement for relatively lipophilic solutes and for normal-phase elution (hexane-isopropanol as mobile phase). This requirement is not absolute, as multiple-interaction CSPs have occasionally been utilised in reverse-phase mode [11] for the resolution of chiral solutes of high polarity.
- c). At least one of the interacting groups should be proximate (usually, adjacent) to the chiral centre.

1.4.3- Protein Chiral Stationary Phases.

Proteins are naturally occurring chiral polymers with a high molecular weight, composed of chiral subunits of L-amino acids linked through -CONH-bonds. The complexity of the molecular structure of proteins makes them very interesting from a chromatographic point of view with regard to molecular recognition. Originally, this technique was used for protein isolation via selective interaction with an immobilised ligand (i.e. affinity chromatography)[14]. However, the principle of reciprocity suggests that a reversal of the selector and solute (ligand and protein) should produce a method to retain a particular low molecular weight compound on a column containing the complementary protein as the stationary phase.

These phases consist of proteins immobilised on silica, in most cases by covalent bonding. Due to the complex nature of proteins, they have numerous recognition sites, making them applicable to a wide range of enantiomeric compounds. Although the chiral recognition mechanism is largely unknown, protein chiral phases recognise hydrogen bonding, polar, ionic and hydrophobic groups as well as the three dimensional structure of the sample molecule. It is difficult to

predict the resolution of a racemic mixture because the nature of the bonding sites responsible for the stereoselective interactions is generally unknown.

Several proteins have proved useful for the separation of enantiomers, including bovine serum albumin (BSA) and human serum albumin (HSA) and some glycoproteins, (α -glycoprotein AGP and ovomucoid, OV). Some of them have given rise to commercially available packing materials [15] and they can be classified into two groups:

a)- Albumin derivatives: BSA and HSA.

b)- Glycoproteins: AGP and OV.

a)- **Albumin derivatives:** Bovine serum albumin (BSA) is a globular, hydrophobic protein with a molecular weight of about 66,000 Daltons. The protein contains a single 581 amino acid chain with 17 intrachain disulphide bridges forming nine double loops. It is the principal plasma binding protein for weakly acidic drugs. Stewart and Doherty [16] reported the chromatographic resolution of D,L-tryptophan using BSA bonded to agarose, confirming that L-tryptophan had an affinity for albumin 100 times greater than that of D-tryptophan [17].

Several versions of BSA columns have been produced, ranging from the original work of Stewart in 1973 [16] using Sepharose support with a succinoylaminoethyl linkage to Allemark's immobilisation work on 10 μm silica [18]. BSA-CSP's can resolve a wide variety of anionic and neutral enantiomeric molecules.

A BSA column commercialised by Macherey Nagel is known as Resolvosil. The BSA material can be used with aqueous buffer mobile phases for a wide range of analytical-scale chiral separations [19] including sulphoxides, e.g. 2-methylsulphonyl benzoic acid, benzodiazepines and coumarins such as warfarin and N-benzoylamino acids, e.g. N-benzoyl-D,L-alanine [20]. The α value obtained for the sulphoxide resolution increased quite significantly with decreasing pH and concentration of the phosphate buffer used as the mobile phase. Addition of large

amounts of methanol and acetonitrile denatures albumin and has to be avoided with the BSA-phase. The use of small, rigid silica particles increases column efficiency and permits the use of higher column pressures. The effect of changes in pH are complicated because they may also affect the binding properties of albumin [21] and thus the stereoselectivity. The main interactions between BSA and the solute enantiomers are hydrophobic and electrostatic interactions, although hydrogen bonding or charge transfer interaction can also contribute to chiral selection. The solute must contain aromatic and polar moieties [22]. Therefore, nonaromatic amino acids must be derivatised, generally through the amine function.

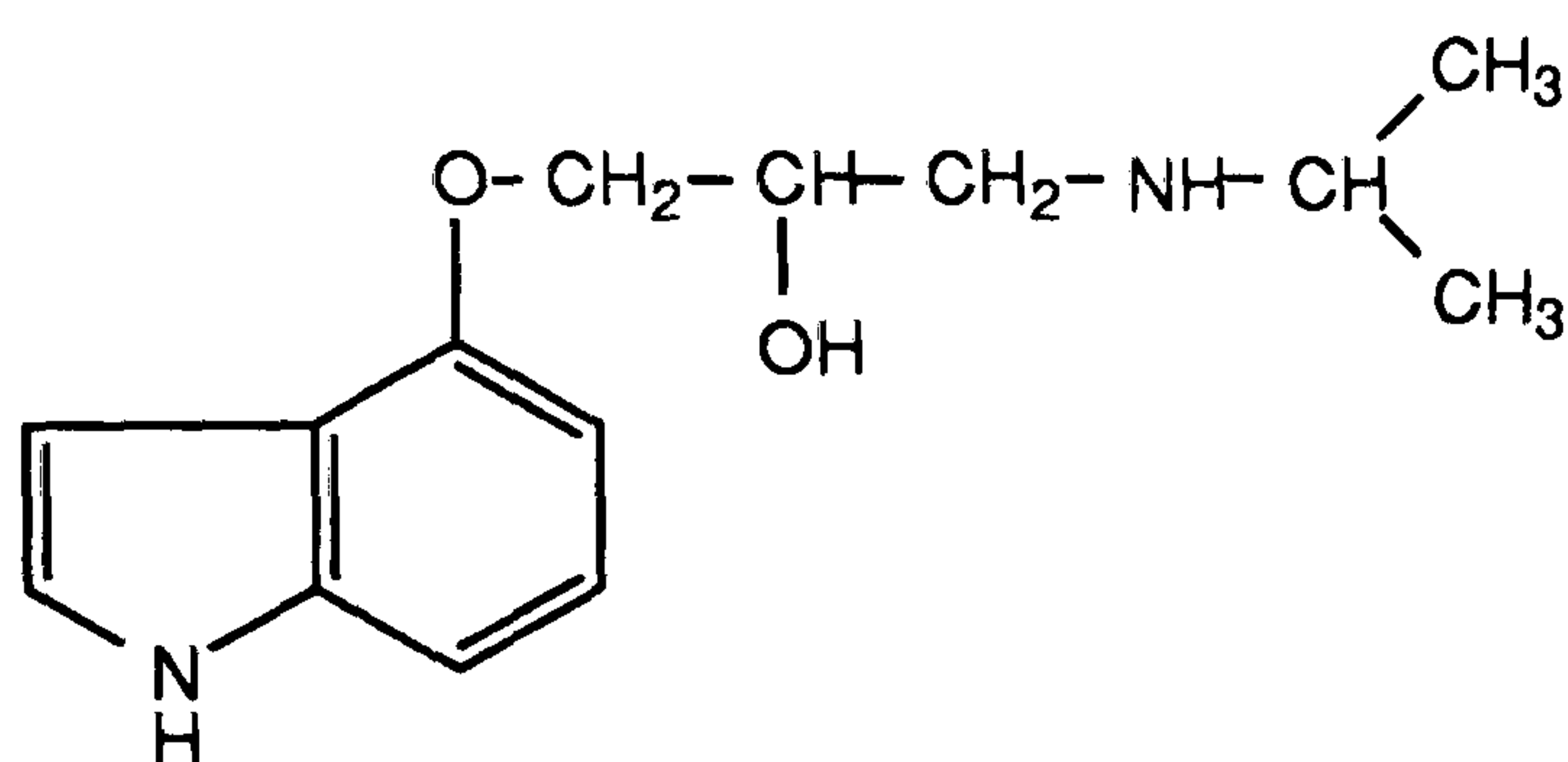
Human serum albumin (HSA) is a carbohydrate-free polypeptide with a molecular weight of 69,000 Daltons. The protein was immobilised on a commercially available diol column which had been activated with 1,1-carbonyldiimidazole [23].

Whilst BSA and HSA both resolve the same chiral compounds, they often display different affinities and stereoselectivities [24]. For example, the enantiomeric elution orders on HSA-CSP for leucovorin [25] and N-benzoyl-phenylalanine [15] are the same as on BSA-CSP, whereas the elution order for the N-benzoyl-alanine enantiomers is reversed. For warfarin, the R enantiomer is the first eluted on HSA-CSP and the second eluted on BSA-CSP [26].

b)- **Glycoproteins:** The major plasma binding protein for basic drugs is α -acid glycoprotein (α -AGP) [27]. It is a very acidic glycoprotein which is composed of many proteolytic groups both in the peptide chain and in the carbohydrate moiety. Anionic drugs resolved include naproxen [28] and 2-phenylbutyric acid [29]. The AGP chiral stationary phase is prepared by first ionically binding the glycoprotein to diethylaminoethyl silica and then crosslinking the proteins using a process which involves the oxidation of the alcohol groups of the sugar residues to aldehydes, the formation of Schiff bases and reduction of the resulting enamines to secondary amines [30]. This forms a chiral stationary phase which is said to be stable for

months [31]. Addition to the mobile phase of an amine that can interact with the glycoprotein will decrease the retention of the enantiomers [31].

Another type of protein phase is based on ovomucoid [32] (OV), which is a relatively abundant glycoprotein which can easily be purified from chicken egg whites. It is an acid glycoprotein with a molecular weight of 55,000 Daltons and a stereoselectivity for resolving a number of different cationic compounds (amines) such as chlorpheniramine and pindolol (Fig. 11) and anionic compounds (carboxylic acids) such as flurbiprofen and ketoprofen.



Column:	ULTRON ES-OVM
Column Size:	150 x 4.6mm
Mobile Phase:	20mM KH ₂ PO ₄ (pH=5.5):C ₂ H ₅ OH = 100/3
Flow Rate:	1.0 ml/min
Detection:	UV-220nm (0.16AUFS)

Fig. 11 Resolution of the β -blocker pindolol on an ovomucoid CSP.

Optimisation of chiral separations on protein CSPs (Fig. 12) can be achieved by varying the buffer pH or ionic strength and shorter retention times are obtained by adding an organic modifier such as propanol to the mobile phase^[33].

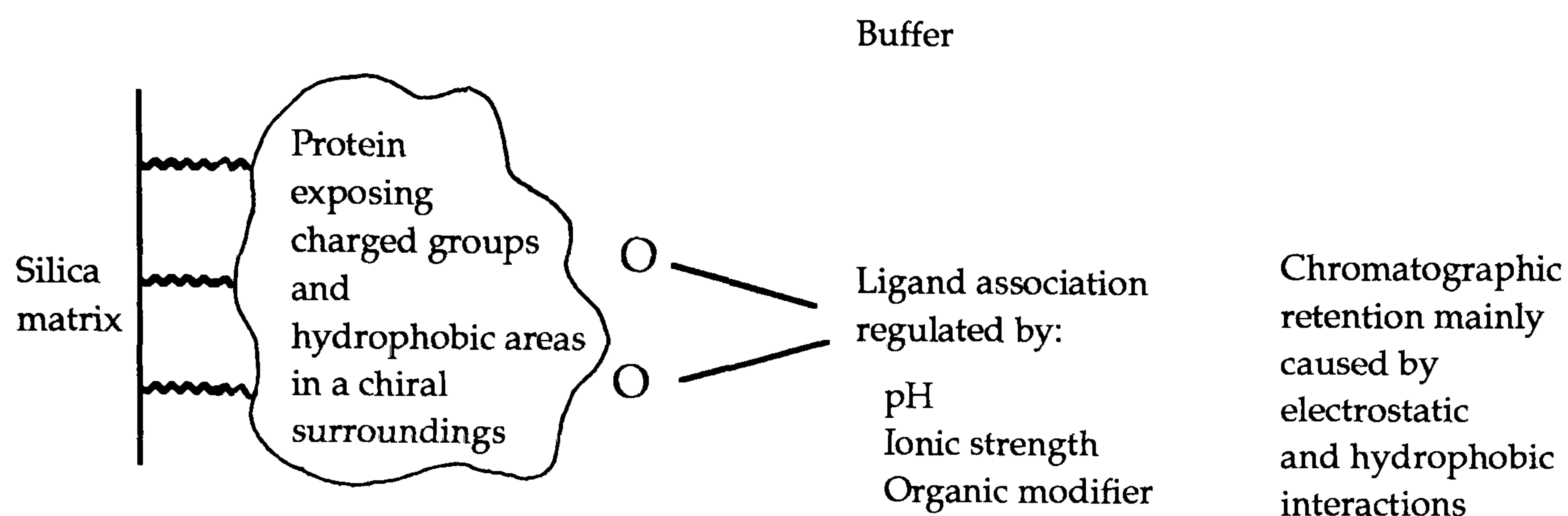


Fig. 12 Basic types of interactions between a ligand analyte and an immobilised protein in aqueous media

The protein itself is not particularly durable, being subject to degradation over time and under extremes of temperature and pH ^[15]. As the protein CSPs have low sample capacity (less than 2ng per injection), they have not been used for preparative-scale separations.

A further way to use the stereoselective discrimination of macromolecules such as proteins is to add them to the mobile phase as complexing agents and use an achiral stationary phase. The enantiomer with the highest affinity to the complexing agent will elute first from the column. This principle has been used with albumin as the complexing agent in the mobile phase for separation of enantiomers of tryptophan ^[34] as well as for the resolution of aromatic mono- and dicarboxylic acids^[35] and warfarin. The same principle was also used for separation of enantiomeric amines with α -glycoprotein as the complexing agent ^[36].

1.4.4- Cyclodextrin CSPs.

The next significant advance in chiral LC was the commercial introduction of cyclodextrin CSPs, which employ inclusion complexing to achieve chiral selectivity: the cyclodextrin molecule acts as the host and the chiral solute enters as the guest.

The cyclodextrins (CD) are a group of cyclic oligosaccharides, produced by enzymatic degradation of starch, containing from six to twelve D-(+)-glucopyranose units, which are bonded through α -(1,4) linkages [37]. Three CDs, α -cyclodextrin (cyclohexamylose), β -cyclodextrin (cycloheptamylose) and γ -cyclodextrin (cyclooctamylose) (Fig. 13), are available commercially. They contain 6, 7 or 8 glucose units respectively. They are all available in the native and in the acetylated form. β -cyclodextrins are available in many derivatised forms.

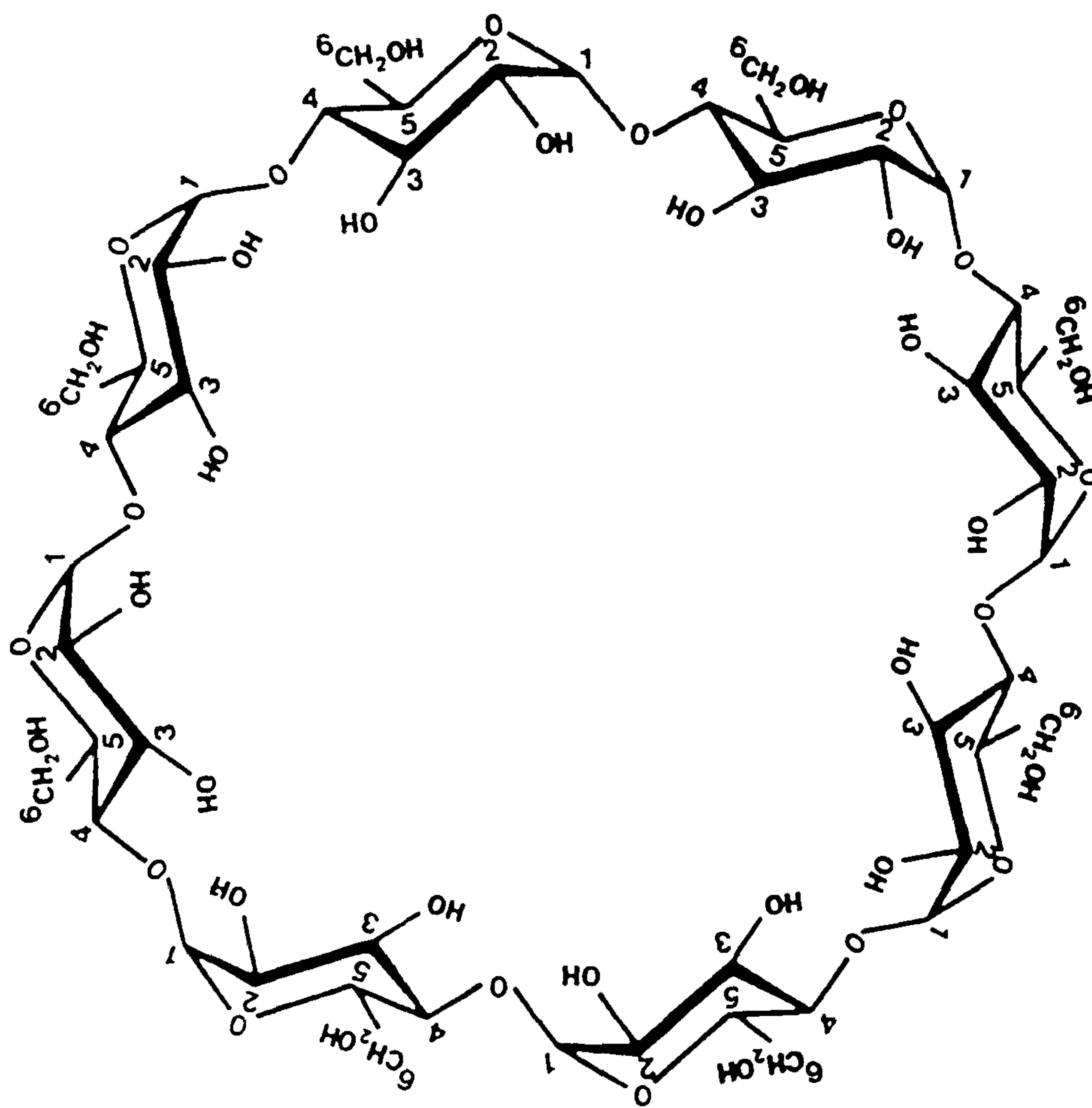
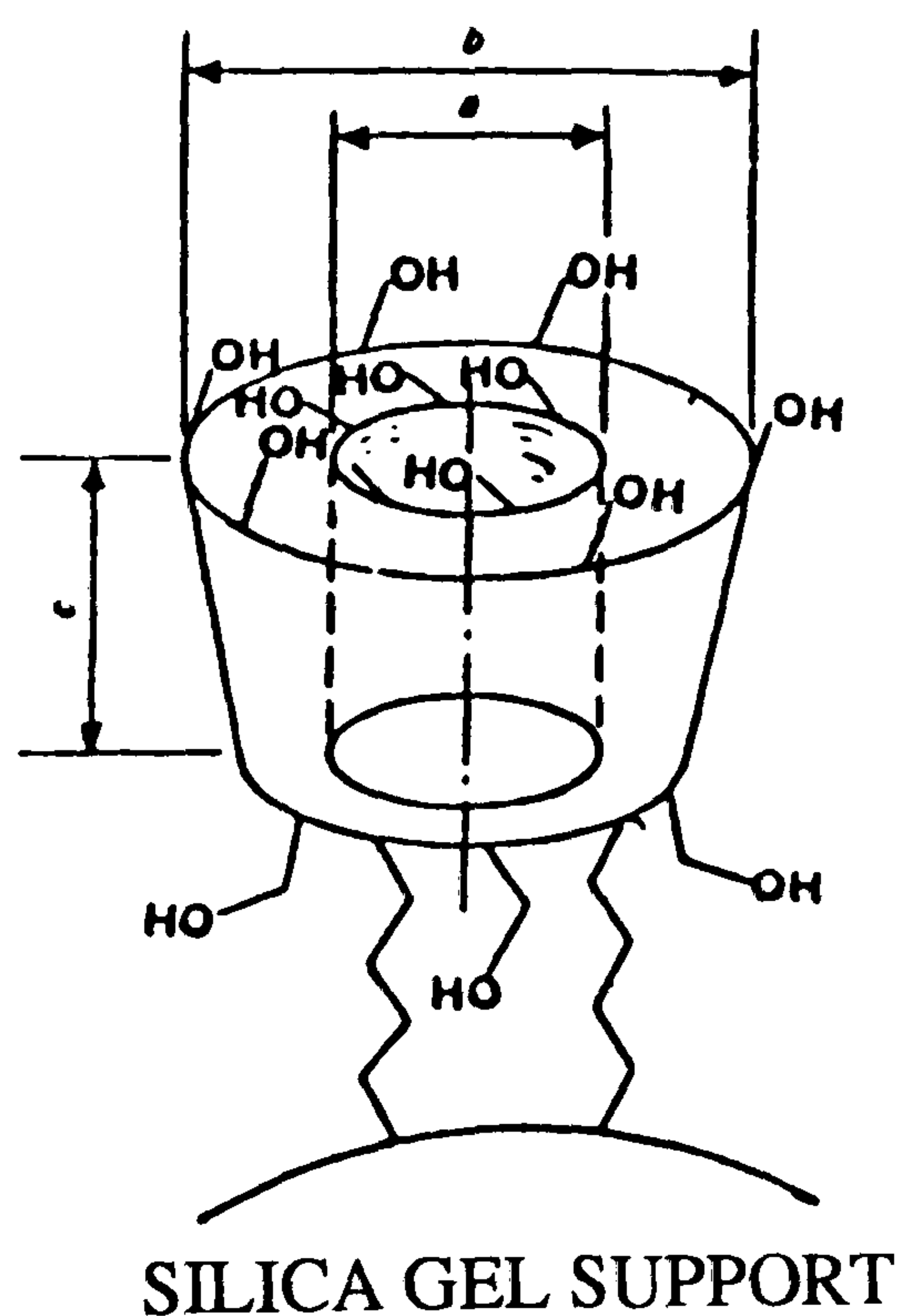


Fig. 13 Cyclodextrin molecule

They are not regular cylindrical molecules but have the shape of a hollow truncated cone. Each cyclodextrin possesses a chiral cavity whose diameter at the wide end is proportional to the number of glucose units present e.g. 8Å for β -cyclodextrin. From crystal data, their structures appear to be quite rigid and inflexible and stable to a wide variety of conditions. The materials are capable of forming inclusion complexes with a variety of racemates. All the hydroxyl groups of the glucose units are situated on the outside of the molecule so the inside of the cavity is relatively hydrophobic as it is composed of the glucoside oxygens and methylene hydrogens. The 1,4-glucoside linkage bonds of α -cyclodextrin appear weaker than those of β and γ CD and are capable of stretching. The cavity acts as a host to aromatic and alkyl groups, which enter and exit by diffusion. The secondary 2- and 3-hydroxyl groups are situated on the large opening of the truncated cone. They are orientated in a fixed direction. The 2-hydroxy groups are orientated clockwise while the 3-hydroxyl are orientated anticlockwise. The primary hydroxyl groups, which rotate freely, are situated around the small opening. Some of these hydroxyl groups are used to attach this molecule to the silica gel. The inclusion complex is formed within the hydrophobic chiral cavity of the cyclodextrin and involves various hydrophobic and hydrogen bonding forces [38].



CD Dimensions			
Å			
	a	b	c
α	5.7	13.7	7.8
β	7.8	15.3	7.8
γ	9.5	16.9	7.8

Fig. 14 Cyclodextrins stationary phases: physical shape and dimensions

The size and geometry of the guest molecule in relation to that of the cyclodextrin cavity is an important factor in inclusion complex formation. If the smallest dimension of a molecule is larger than the mouth of the CD cavity, no inclusion complex can be formed. Size does not preclude a molecule from forming hydrogen bonds to the CD's external hydroxyl groups, or other types of association. Molecules that have a much smaller volume than the CD cavity can still form inclusion complexes, but stereoselectivity is usually then lost [38].

Chiral separations require the solute molecule to enter the hydrophobic cavity in such a way as to place the centre of asymmetry in association with the polar hydroxyl groups at the edge of the cavity. Where there is no association between these polar groups and the groups attached to or near the solute's chiral centre, separation is minimal or nil. Generally, it is not the degree or nature of the penetration into the cyclodextrin cavity that is the main criterion for resolution to occur, but the existence of interaction between the secondary hydroxyls and the guest molecule.

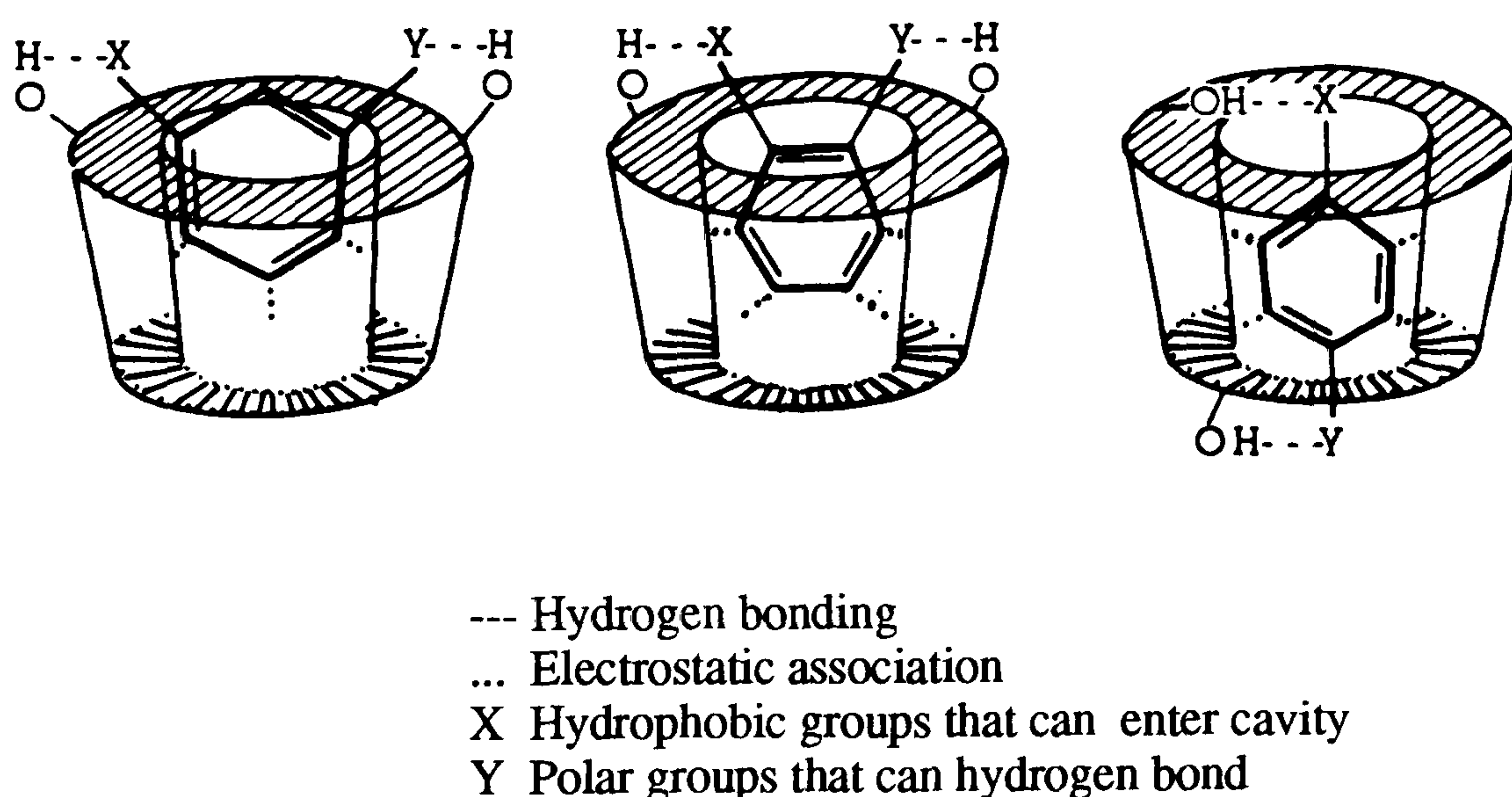


Fig. 15 Inclusion complexes

For a racemic molecule to be resolved into its enantiomers there must be a difference in stability of the inclusion complex formed for each isomer.

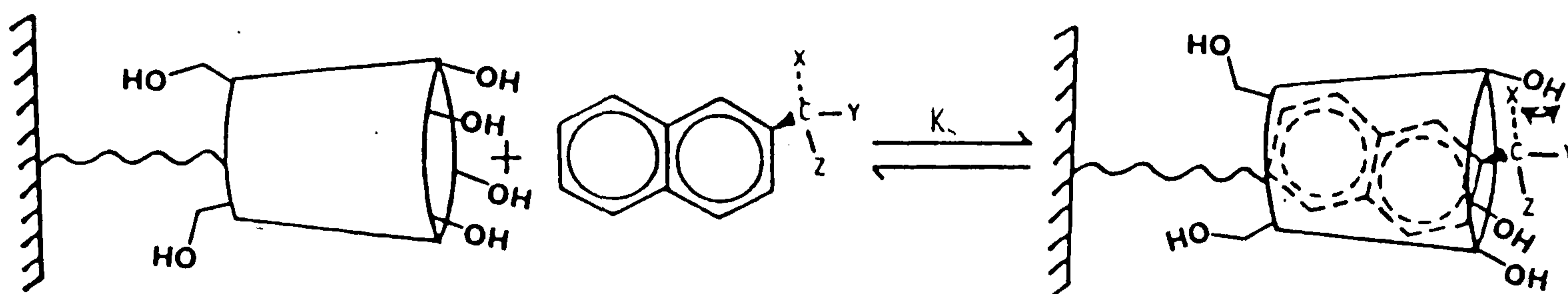


Fig. 16 Reversible formation of an inclusion compound

Since inclusion complexes are formed easily in aqueous organic solvents, CD bonded phases columns have been used predominantly in the reversed-phase mode. Complex stability constants usually have greater values in water and tend to decrease upon addition of sufficient quantities of organic modifier [39]. In spite of this, some enantiomer resolutions have been reported using a high concentration of organic modifier (up to 100%) and long retention times have been observed. This is an indication that CD-bonded phases might be useful in normal phase and in polar organic modes. In the reverse phase mode, chiral recognition is independent of solvent strength as the latter affects only the displacement of the analyte from the cavity.

The enantioselective mechanism of cyclodextrins stationary phases is dependent on the mobile phase used [40]. Three different modes can be used with some of cyclodextrin chiral phases:

- . Reverse phase mode (host-guest inclusion mechanism).
- . Normal phase mode (resembles Pirkle type mechanism).
- . Polar organic phase mode (stereoselective H-bonding with secondary OH groups).

Reverse phase mode:

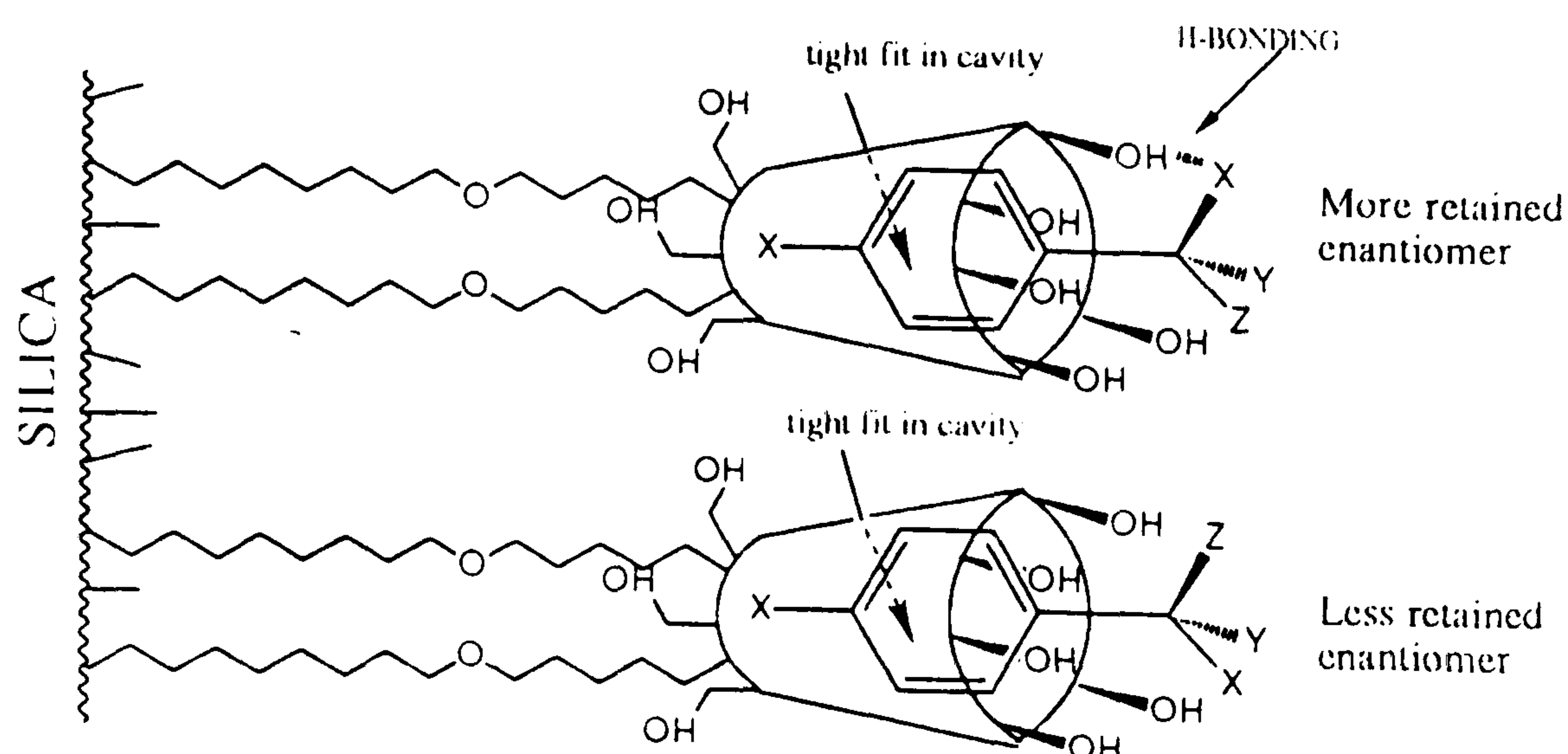


Fig. 17 Resolution of chiral analyte in cyclodextrin column under reverse phase conditions

In this aqueous environment, the lipophilic part of chiral solutes enters the hydrophobic cavity of the cyclodextrins. As the interior of the cavity is lined with ether glycosidic oxygen atoms this tends to orientate any aromatic moiety. For chiral discrimination to take place, the lipophilic part of the solute needs to have a tight fit in the cyclodextrin cavity and hydrogen donor or hydrogen acceptor functionalities of the chiral solutes need to just protrude out of the mouth of the cavity enough to form hydrogen bonds with the secondary hydroxyl groups of cyclodextrin [41].

Ethanol, propanol, dioxane, dimethylformamide, methanol and acetonitrile have been used as organic modifiers for reverse phase elution with cyclodextrins, the last two being the most common.

In reverse phase, in many cases, pH and ionic strength of the aqueous portion of the mobile phase are as important as the choice of modifier. It is often advantageous to change the pH or add salt mixtures to effect or enhance a separation. By increasing the hydrophobic character of the solute, the retention times increases. Adding a small amount of salt (e.g. triethylammonium acetate) retention and resolution can be improved. In some cases, resolution of enantiomers can be obtained at one pH but not another. For example, racemic N'-benzylornicotine[42]

resolves only at $\text{pH} \geq 6$, while 4-benzyl-2-oxazolidone resolves better at a pH of 4.1^[43]. It appears that the stronger the inclusion, the more effective are higher concentrations of buffer. Buffer is known to include into the CD cavity and as the buffer concentration increases, peaks become sharper and retention decreases.

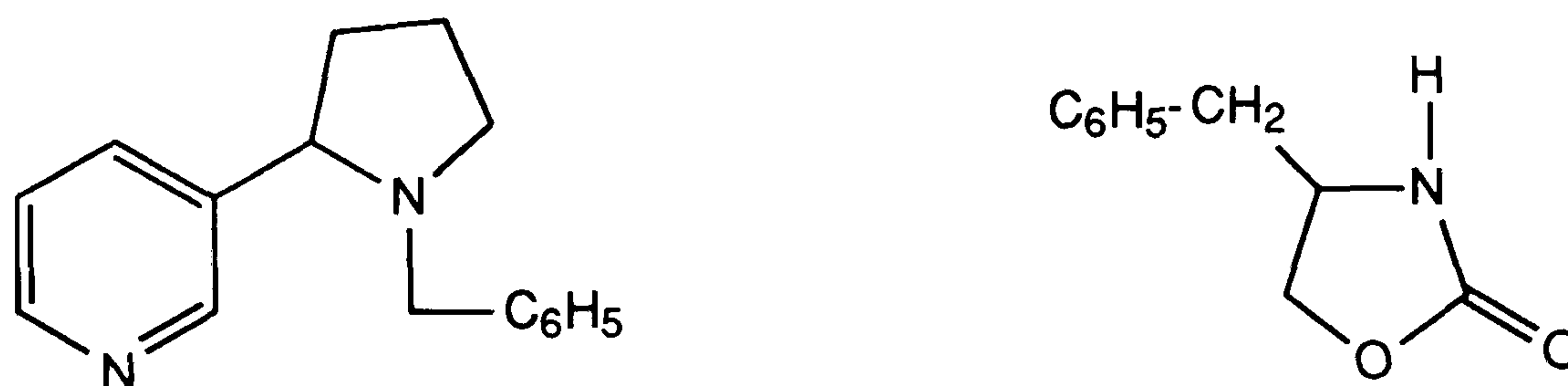


Fig. 18 N'-benzylnormicotine and 4-benzyl-2-oxazolidinone.

Normal phase mode

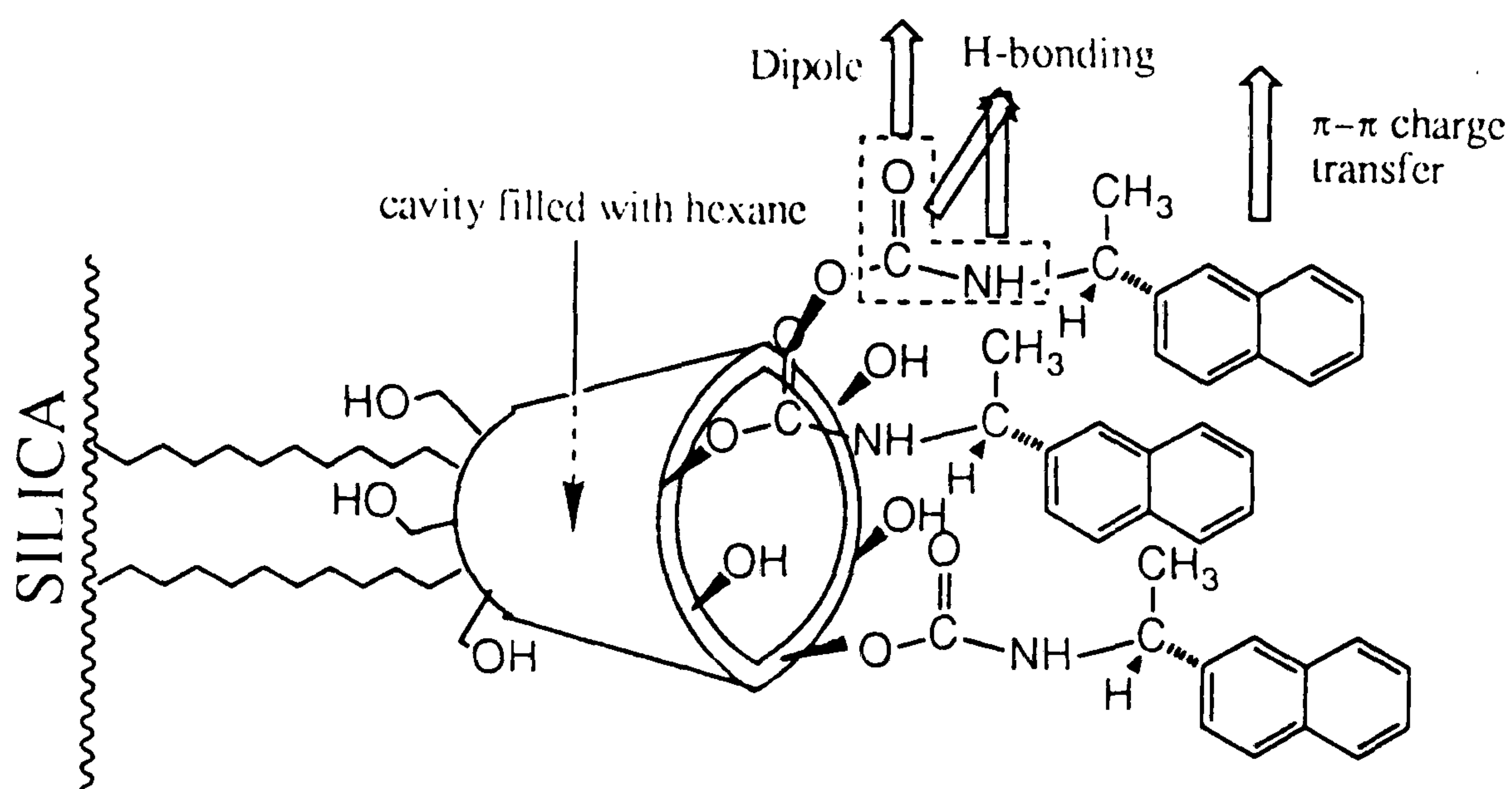


Fig. 19 (Naphthylethyl)carbamoylated β -cyclodextrin.

In the normal phase mode, the solvents used are hexane typically modified with IPA^[44], acetonitrile, THF or methanol. The apolar character of hexane encourages this solvent to occupy the cavity of the cyclodextrine. Therefore, in normal phase mode enantioseparation does not involve inclusion complexing. Chiral resolution of analytes occurs through multiple interactions resembling those of the Pirkle type columns. Chiral analytes that can be separated on this mode are those

containing π -base or π -acid functionalities additional to other functionalities able to form hydrogen and dipole interactions.

In general, within a class of compounds, retention tends to decrease with increasing chain length. This also seems to be the case for branched compounds. The reduction in capacity factor with increasing chain length may be due to the increased hydrophobic character of the solute with increasing chain length leading to increased partitioning into the non-polar mobile phase. Aromaticity also contributes to increased retention.

Organic polar mode

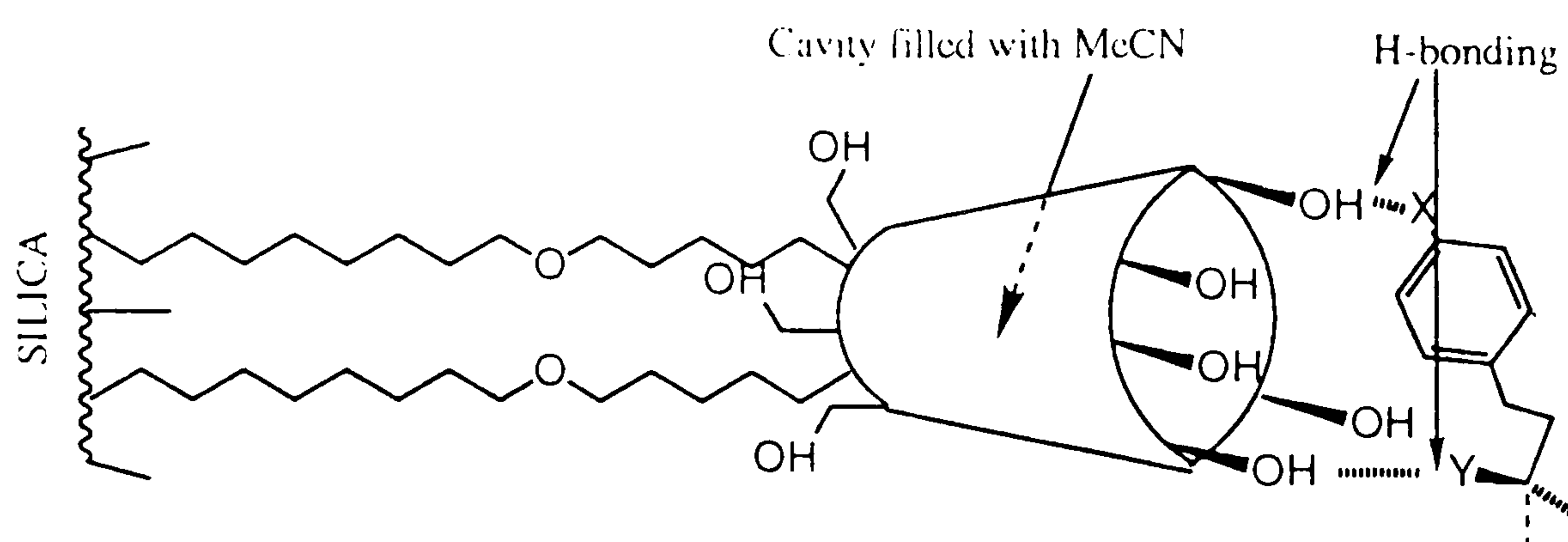


Fig. 20 Chiral separation with cyclodextrin using polar organic mode.

Solvents used in this mode are anhydrous organic solvents to which a small amount of base or acid have been added. Typical organic solvents are acetonitrile to which less than 5% methanol has been added to adjust retention. A typical acid used is trifluoroacetic acid and typical bases used are triethylamine or diethylamine. The acids and bases [45] are used to control hydrogen bonding. The cyclodextrin cavity is filled with acetonitrile in this mode. Chiral recognition comes from stereoselective hydrogen bonding across the mouth of the cyclodextrin cavity (Fig. 20).

Some examples of chiral separations on cyclodextrin-based columns are given below. Most published work has involved the use of Cyclobond column, produced by Daicel in Japan.

1). β -Cyclodextrin column (Cyclobond I).

Typical compounds resolved on this column include carbohydrates, crown ethers, peptides, pharmaceuticals and chiral molecules with single aromatic rings with α chiral centres, fused rings β to chiral centres, and chiral centres between an aromatic ring and a carbonyl group.

Resolution of phenolic metabolite enantiomers of 5,5-diphenylhydantoin and 5-alkyl-5-phenylhydantoins (Fig. 21) [46] has been a success on β -cyclodextrin. It was concluded that the separation can occur when the chiral centre is sandwiched between 2 π systems, leading to a reasonable degree of rigidity of the structure beneficial to hydrogen bonding at the mouth of the cavity for one of the isomers. Resolution is the result of the phenyl substituents binding in the hydrophobic cavity and the interaction of the carbonyl, imide and amide functionalities of the hydantoin ring with at least two secondary hydroxyl groups. It was observed that bulky alkyl substituents at the 3- position of the hydantoin ring disrupt hydrogen bonding and can prevent chiral recognition.

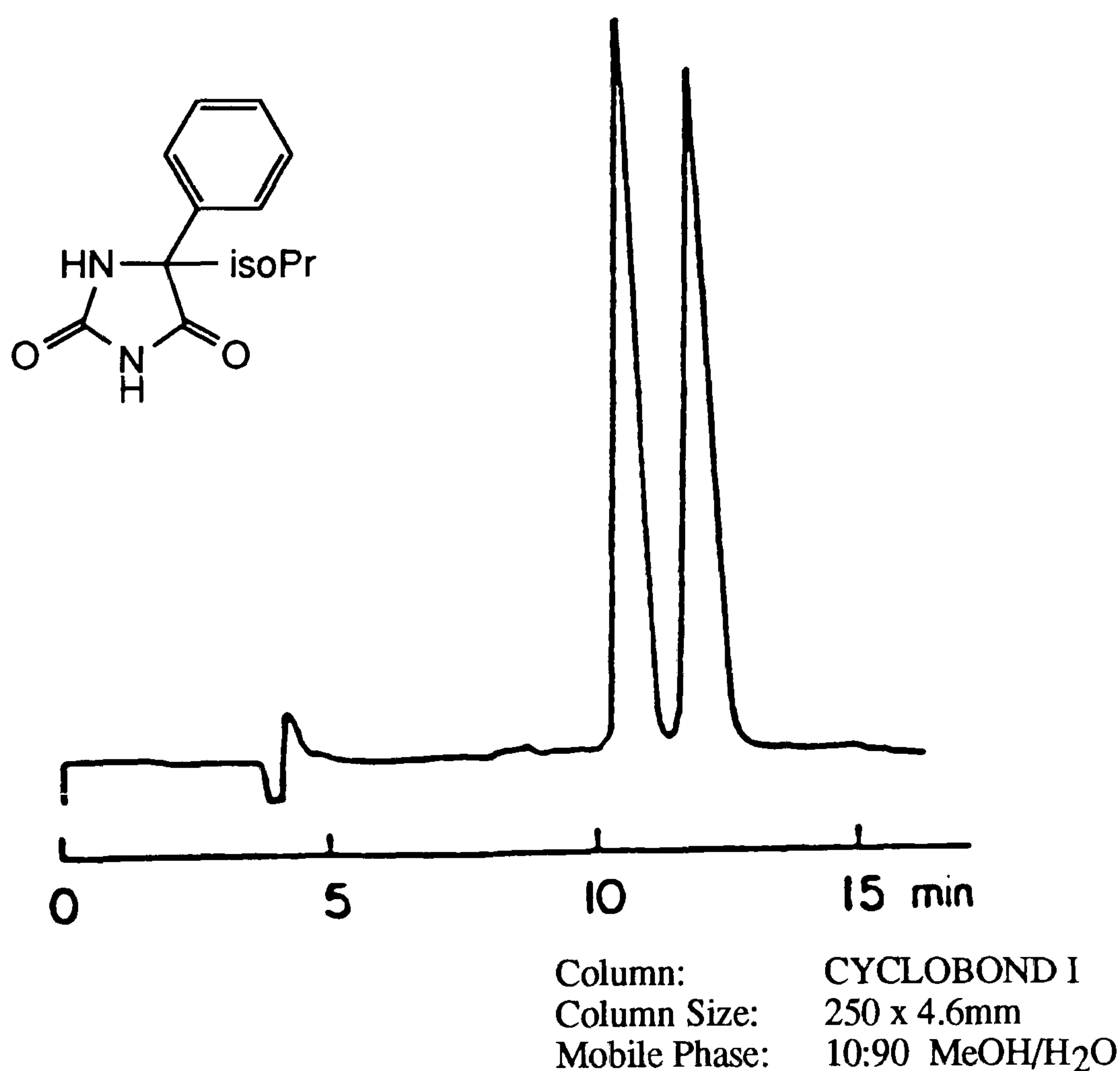


Fig. 21 Resolution of 5-isopropyl-5-phenylhydantoin

2). γ -cyclodextrin (Cyclobond II)

This is useful for isomeric compounds based on anthracene, chrysene and pyrene type ring structures. Typical compound separated include chiral crown ethers, steroids enantiomers (Fig. 22) and steroid epimers [47].

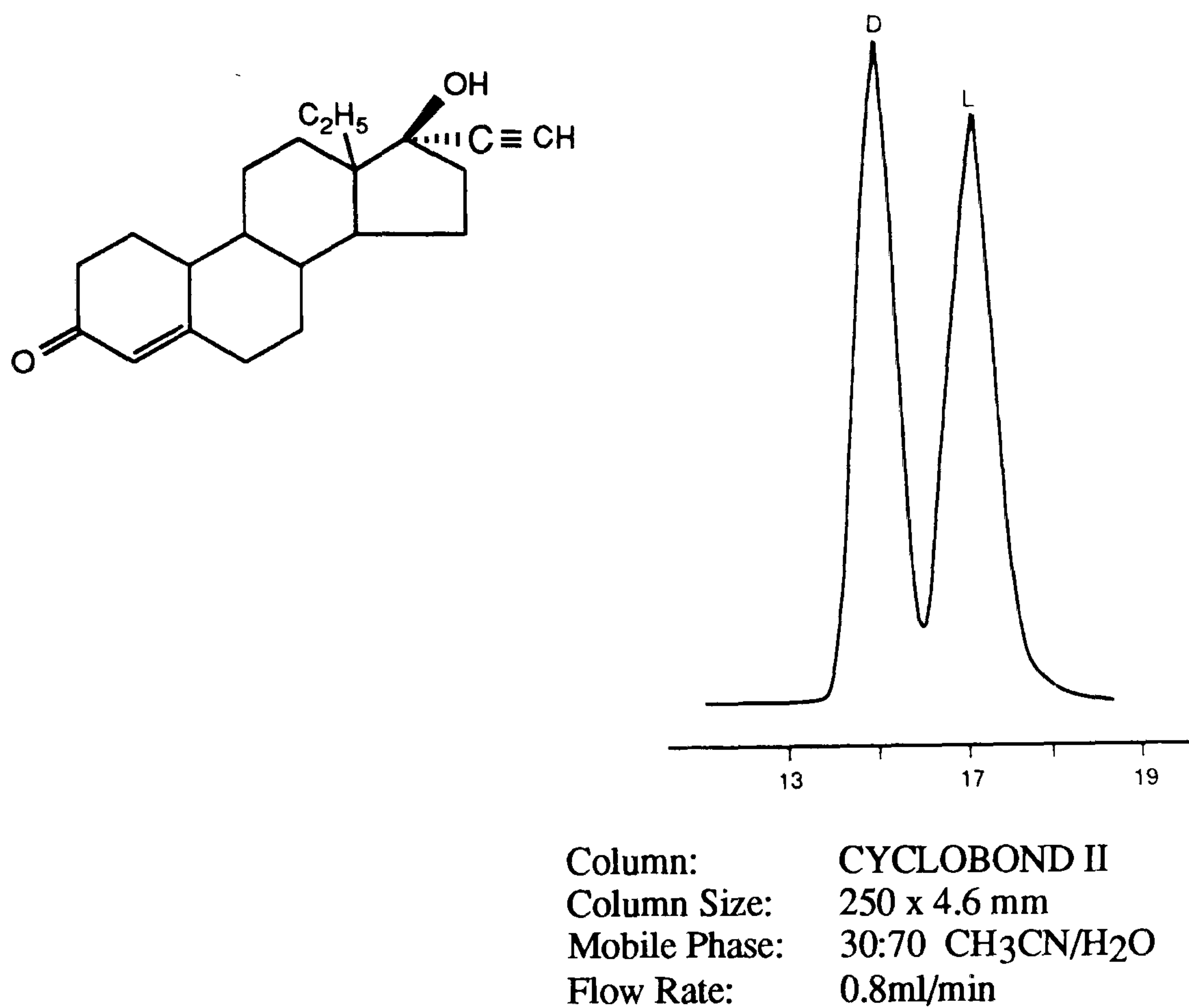


Fig. 22 Separation of norgestrel enantiomers

3). α -Cyclodextrin column (Cyclobond III).

The first reported separations on an α -cyclodextrin column were of the enantiomers of tryptophan, phenylalanine, tyrosine and their analogues [48].

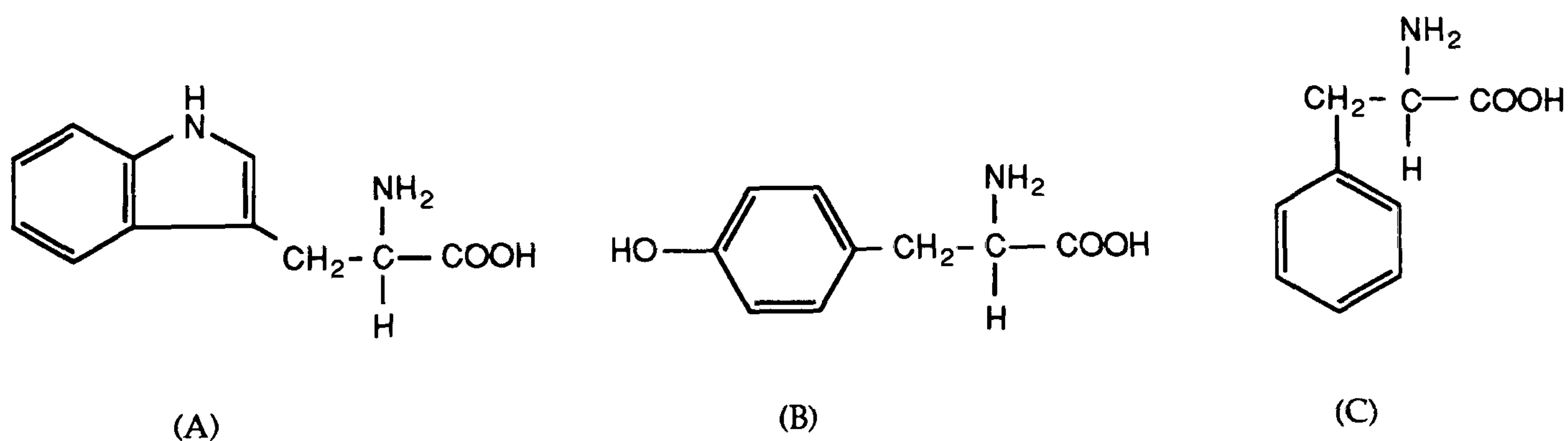
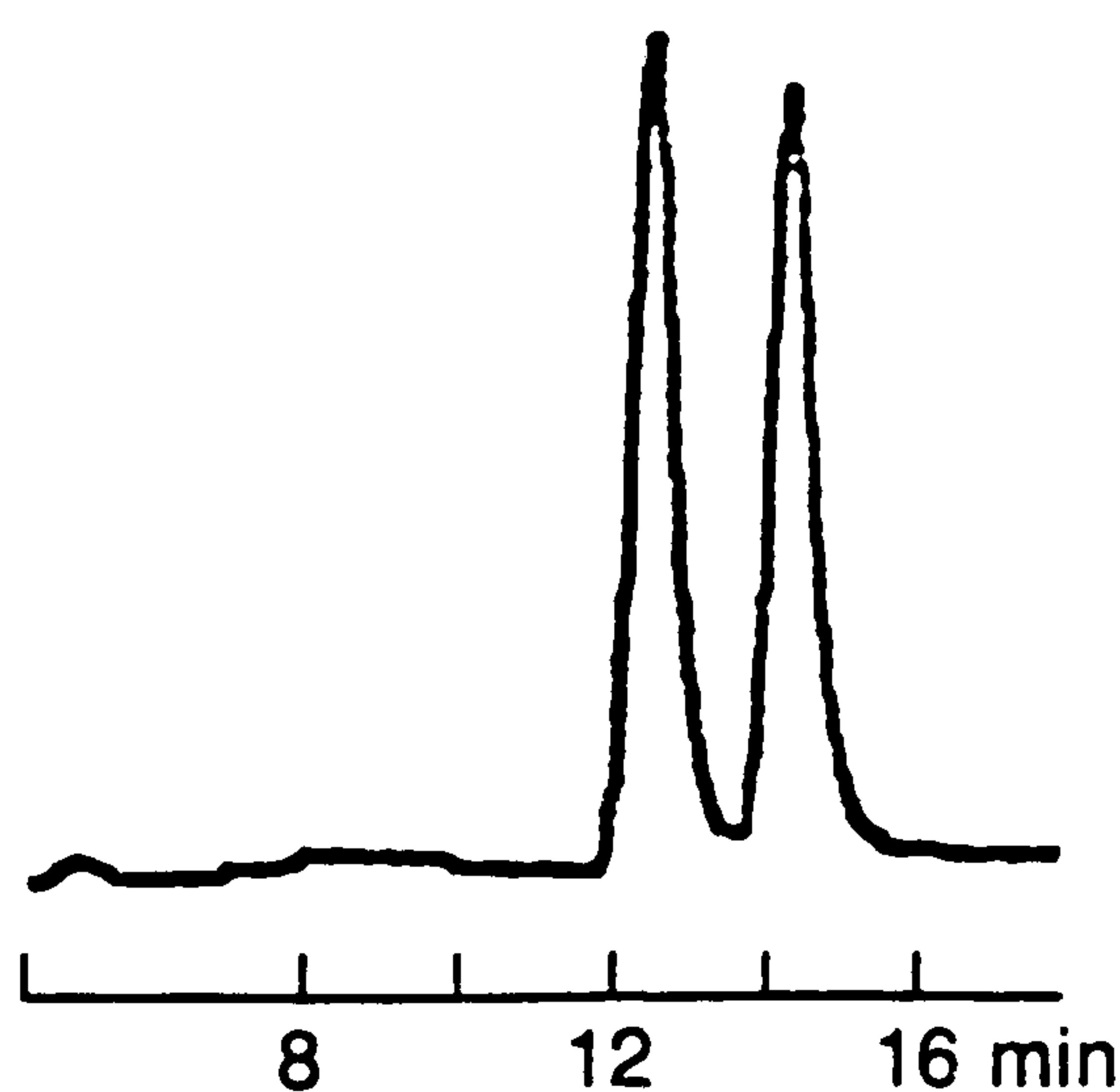
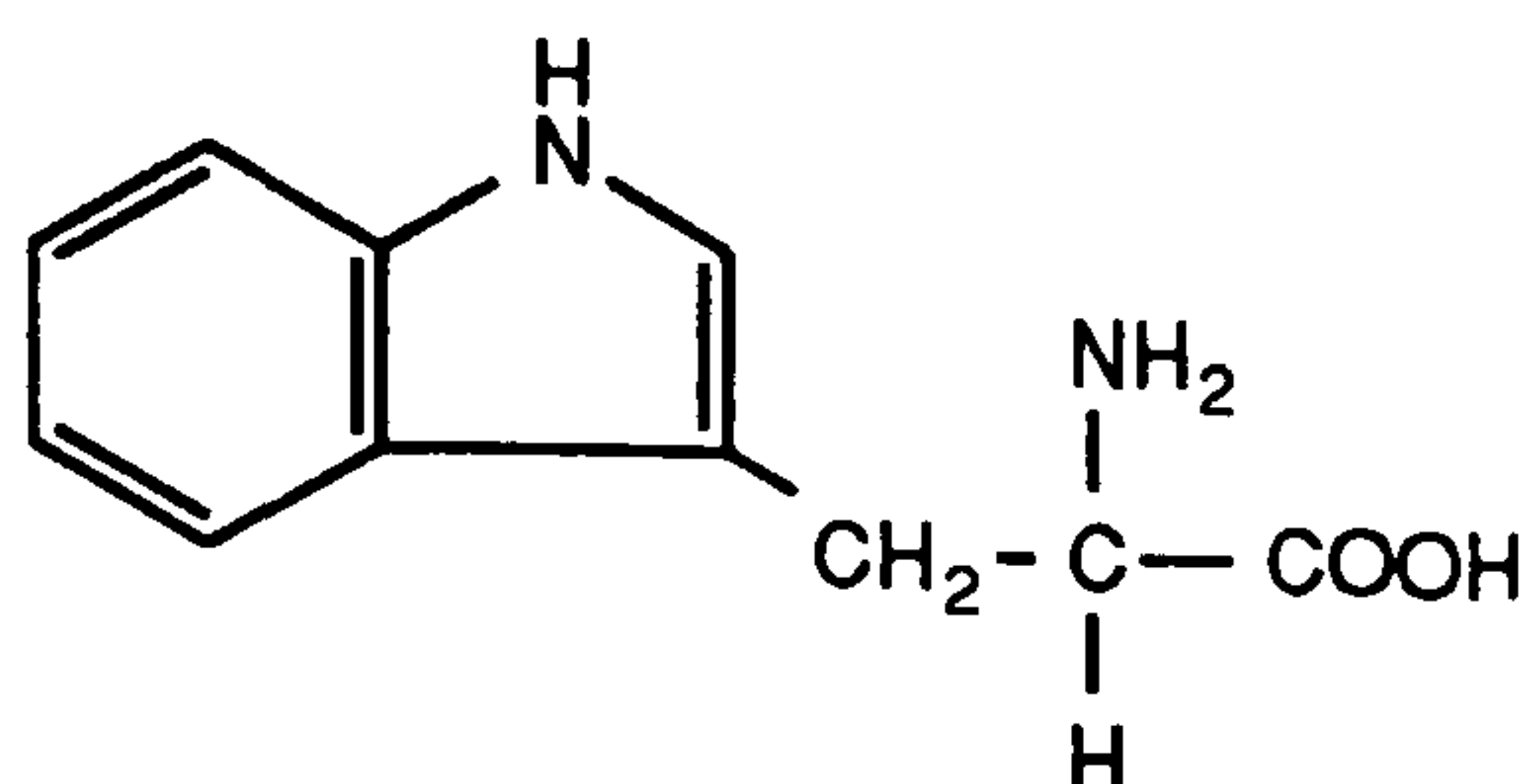


Fig. 23 The structure of (A) tryptophan, (B) phenylalanine, (C) tyrosine

Chiral selectivity as a function of structure indicates that ring substitutions generally decrease enantioselectivity except where the substituent may enhance inclusion complexing, i.e., para substituents that can enter the cavity like NO₂, OH or halogens.

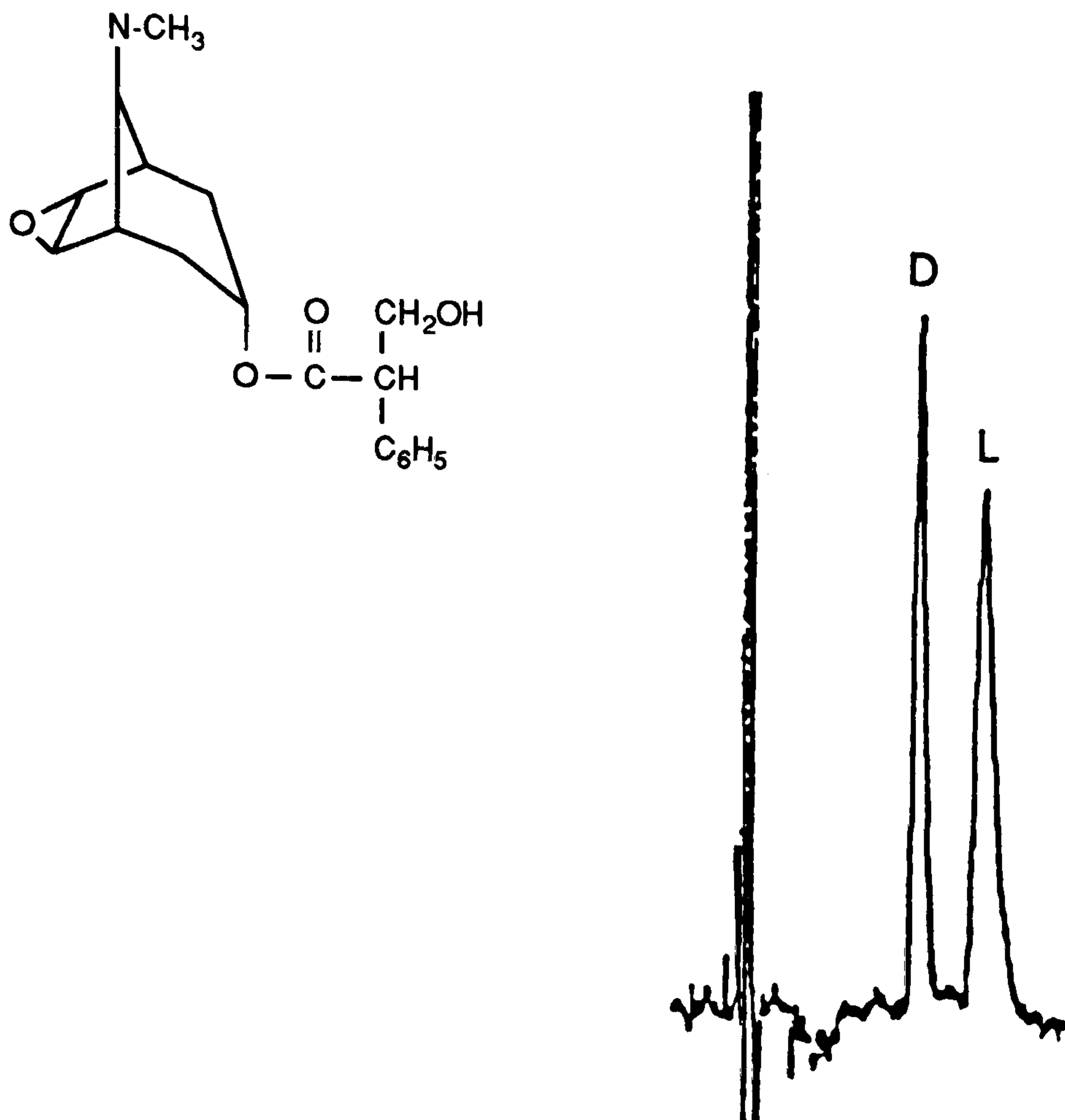


Column: CYCLOBOND III
Column Size: 250 x 4.6 mm astec-PAK
Mobile Phase: 15:85 MeOH/1%TEAA, pH 5.0
Flow Rate: 0.4 ml/min

Fig. 24 Resolution of tryptophan

4). Acetylated- β - cyclodextrin (Cyclobond I Ac).

Derivatisation of β -cyclodextrin, modifying the nature of interactions, allows separations of compounds that are not possible using β -cyclodextrin itself. Cyclobond I Ac has been used successfully in both the reverse phase and normal phase modes. It is prepared by the selective acetylation of only 2- and 3- position secondary hydroxyls. An example of an application of this phase is in the resolution of the enantiomers of scopolamine [49] (Fig. 25).

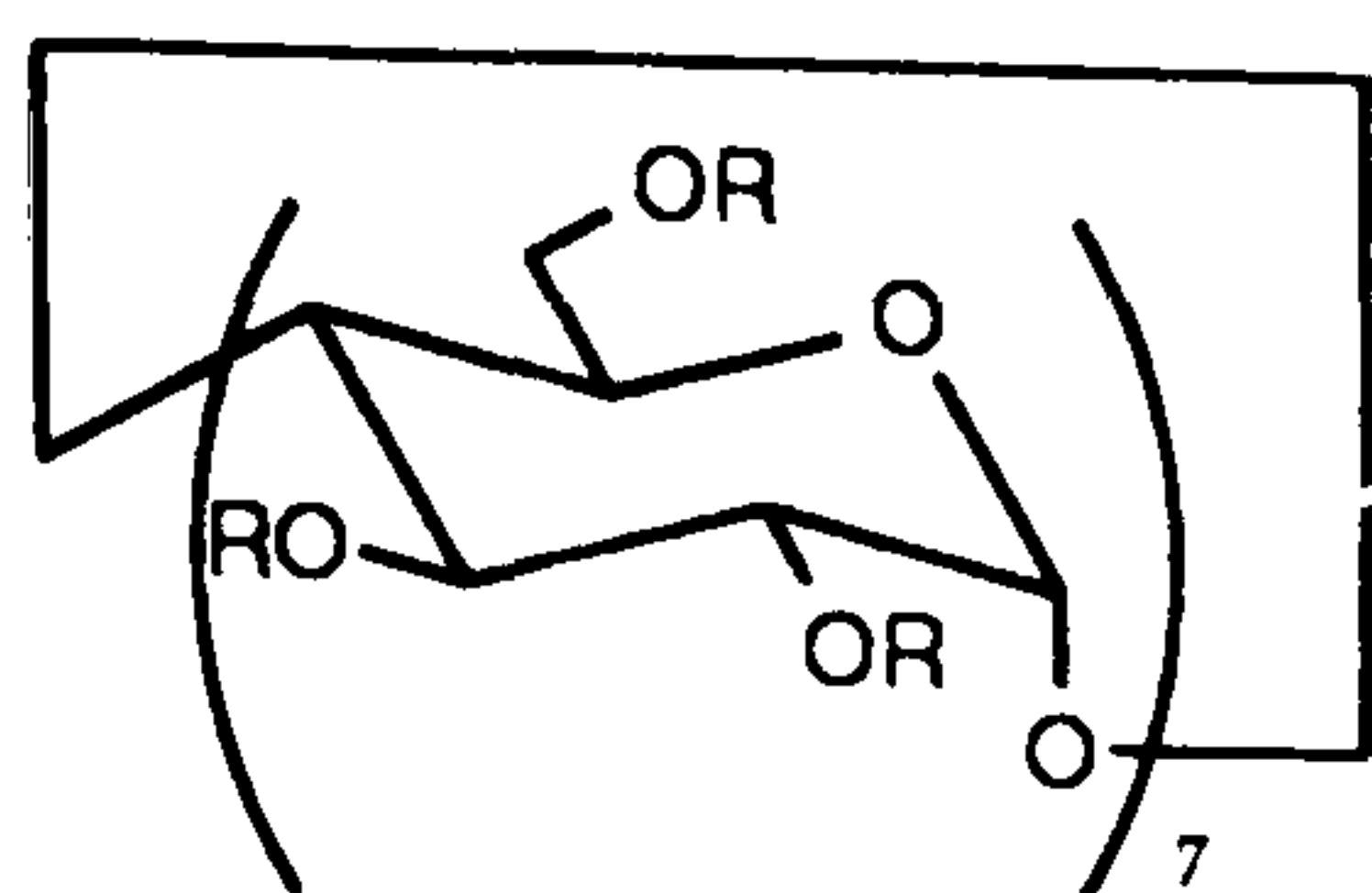


Column: CYCLOBOND I Ac
 Column Size: 250 x 4.6 mm
 Mobile Phase: 2:98 CH₃CN/0.5% TEAA, pH 4.1
 Flow Rate: 1.2 ml/min

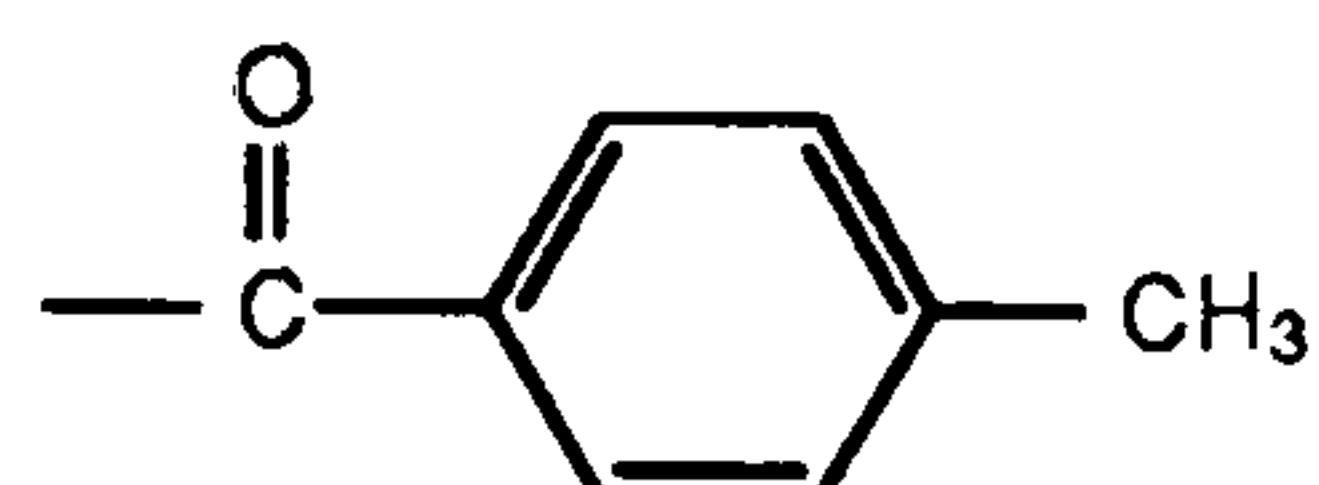
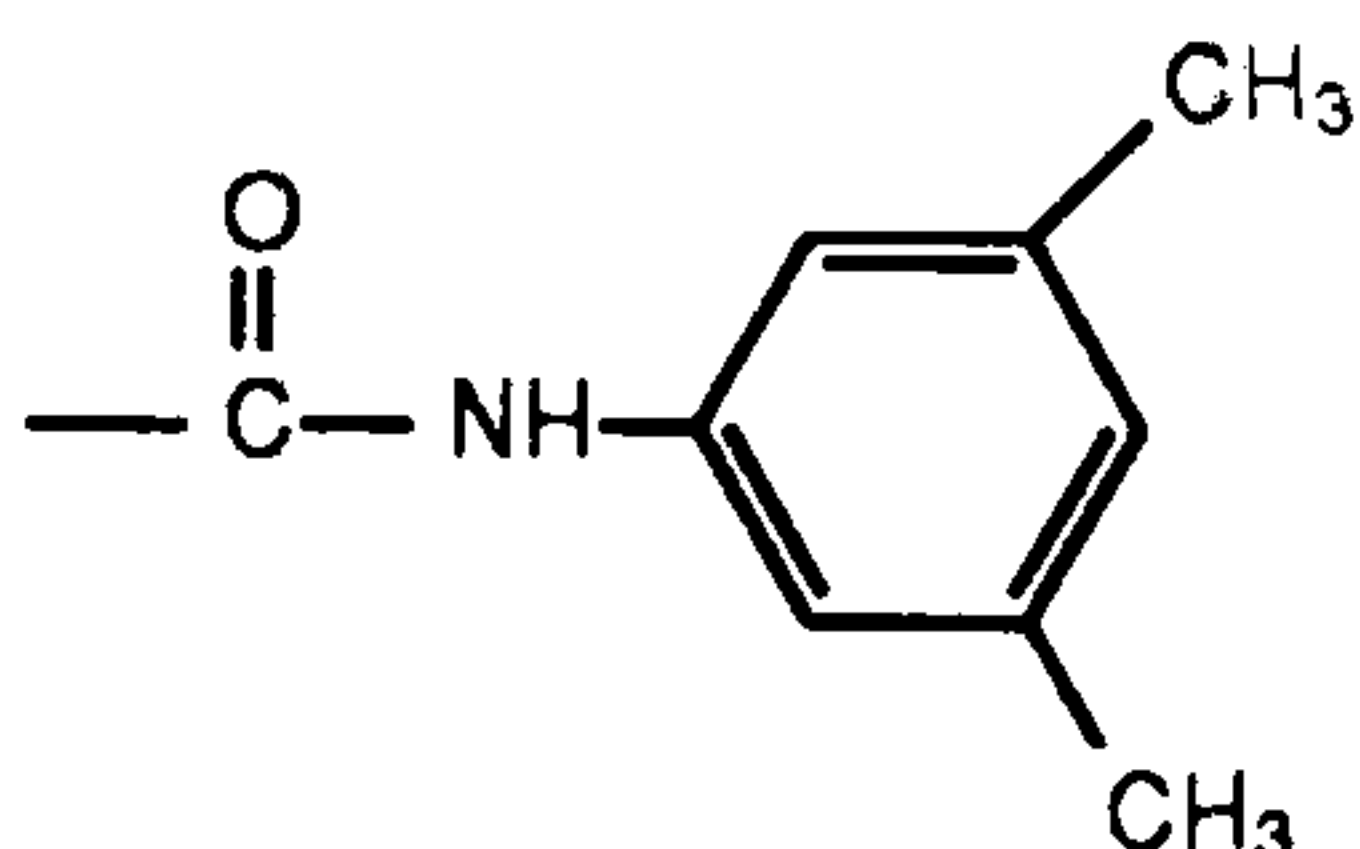
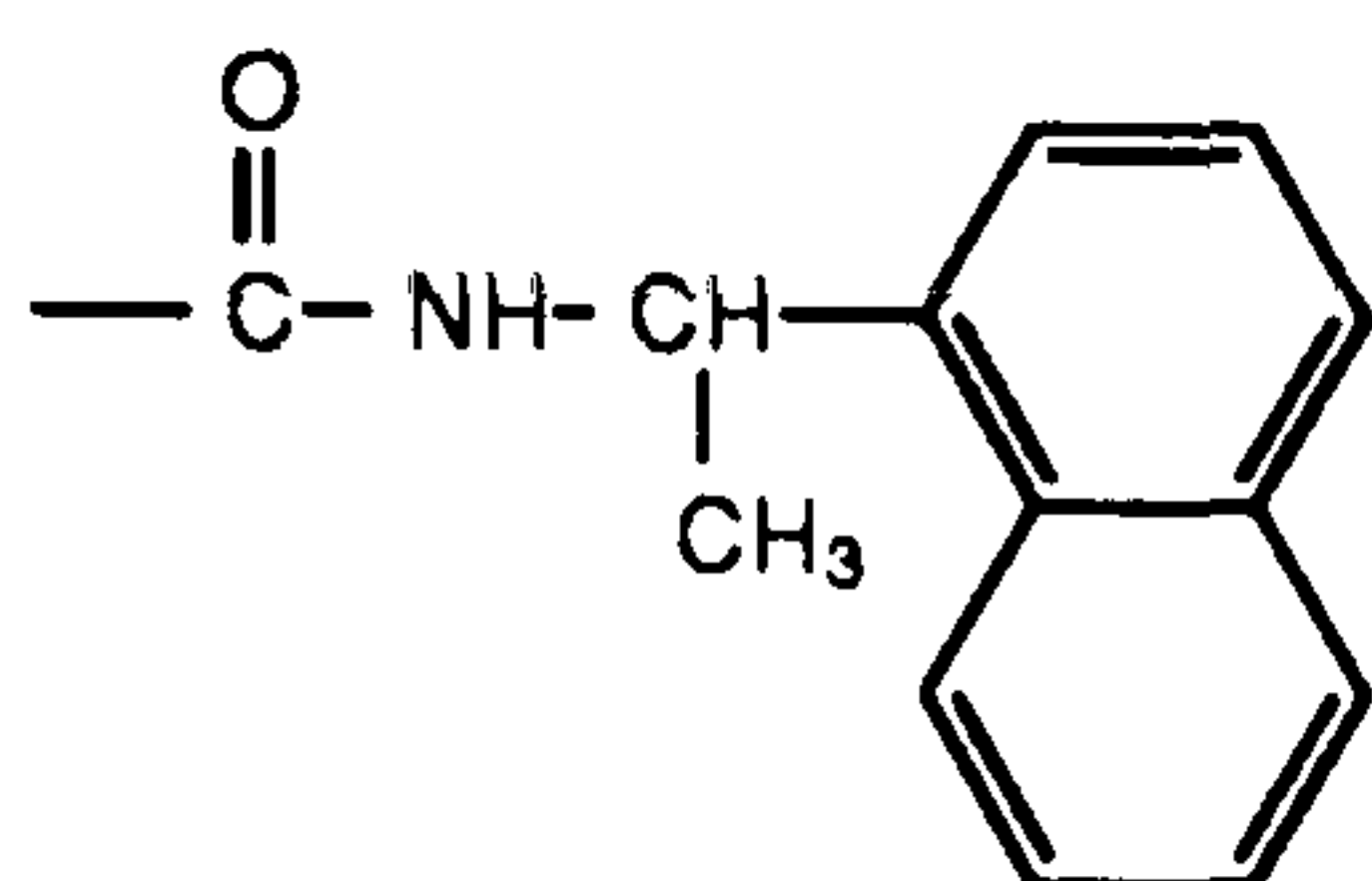
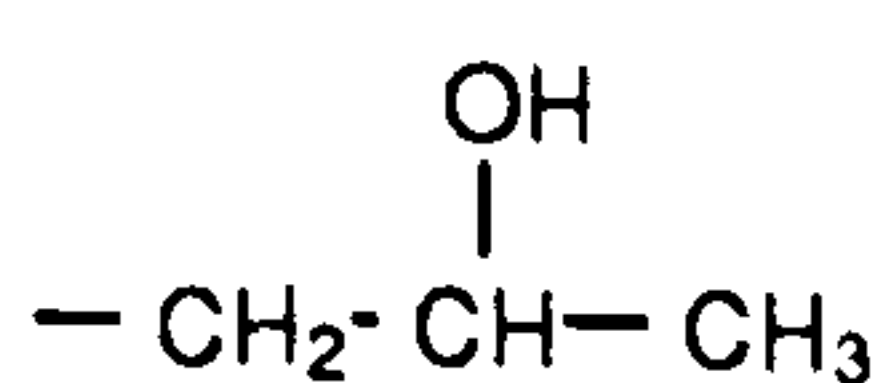
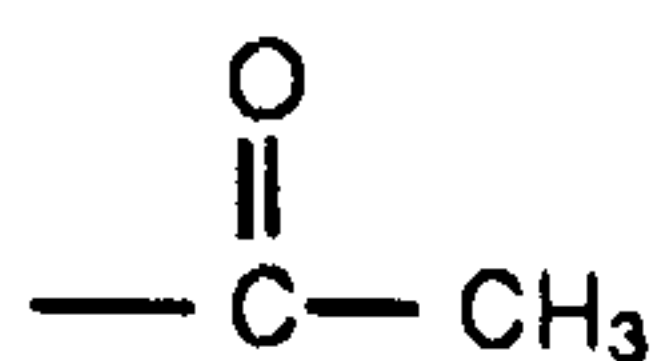
Fig. 25 Resolution of scopolamine

5). Derivatised β -cyclodextrines:

Several different derivatised β -cyclodextrins were synthesised and used as chiral stationary phases in normal phase liquid chromatography [50]. Substituted derivatives were made with acetic acid anhydride (Cyclobond Ac), with (R)- and (S)-1-(1-naphthyl) ethyl isocyanate (Cyclobond I RN and SN, respectively), with 2,6-dimethylphenyl isocyanate (Cyclobond I DMP) and with p-toluoyl chloride (Cyclobond I PT), as shown in Fig. 26.



R = — H



β-cyclodextrin

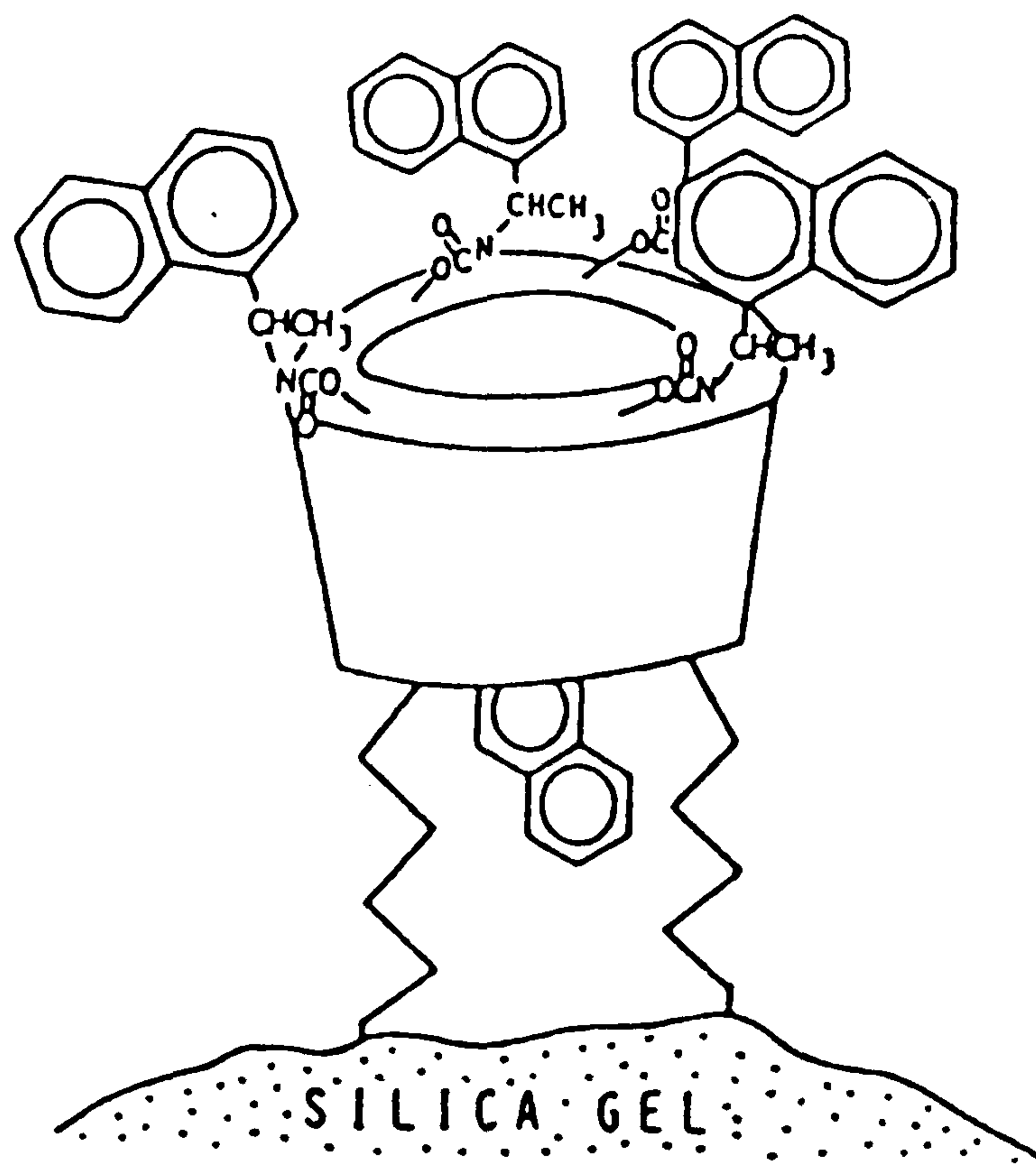
Ac

SP or RSP

RN or SN

DMP

PT



Schematic representation of (Naphthyl)-ethyl isocyanate cyclodextrin derivative.

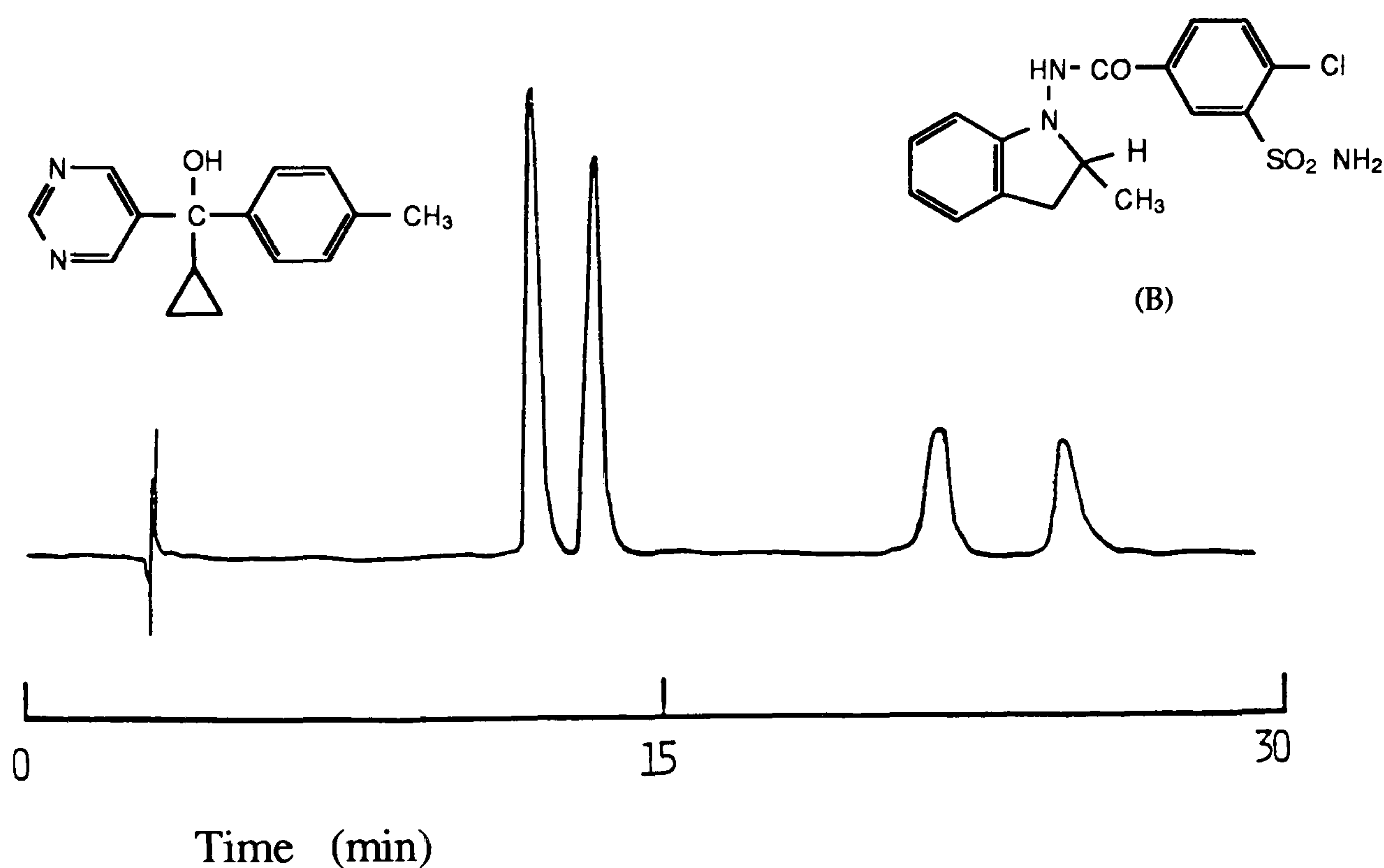
Fig. 26 Commercial names of derivatised cyclodextrin chiral stationary phases.

While native cyclodextrin bonded LC stationary phases have shown little enantioselectivity in the normal phase mode, derivatised CD stationary phases show a definite enantioselectivity for a variety of compounds.

The retention mechanism on these new phases in the normal phase mode is not thought to be dependent on inclusion complexation [51]. The presence of aromatic and carbonyl groups in the modified CDs provide opportunities for π - π interactions that do not exist with native cyclodextrins. This, combined with the hydrogen bonding sites of the residual hydroxyl groups, provides the type of interactions commonly associated with Pirkle-type chiral stationary phases[52]. Furthermore, the

cyclodextrin derivatives are chemically bonded to silica, there is no limit to solvent combinations that can be used in the normal phase mode when compared to the limitations published for the polysaccharide derivatives coated phases.

The (R) and (S)-naphthylethylcarbamate- β -cyclodextrin bonded phases (Cyclobond I SN, I RN) were originally developed for the normal phase separation of enantiomers. Because of its stability, the naphthylethylcarbamate- β -CD stationary phase was utilised in reversed phase separations. It was found to resolve racemic pesticides such as dyfonate, ancymidol and a variety of pharmacologically active compounds such as indapamide that had been resolved previously only by indirect methods [53].



Column: CYCLOBOND I SN
 Column Size: 250 x 4.6 mm
 Mobile Phase: 80:20 CH₃CN/1% TEAA, pH 7.1
 Flow Rate: 1.0 ml/min

Fig. 27 Separation of acymidol (A) and indapamide (B)

Cyclodextrins can also be used as mobile phase additives in the resolution of enantiomers. However, this tends to give lower resolution, is expensive and is unsuited to preparative HPLC. The resolution of enantiomers using this technique is very dependent on the concentration of cyclodextrin in the mobile phase [54]. If the

concentration of cyclodextrin is too low, the formation of the inclusion complex is incomplete and the resolution is poor.

1.4.5- Polysaccharide Phases

Polysaccharides such as cellulose consist of conformationally stable, cyclic sugar residues connected through glycosidic linkages. Cellulose and amylose are the most readily available optically active polymers. Cellulose was chosen for a chiral stationary phase as it is the most abundant organic compound in the biosphere and has a highly ordered structure capable of stereoselectivity. It is a linear polysaccharide containing at least 1500 D(+)-glucose residues joined by 1,4- β -linkages.

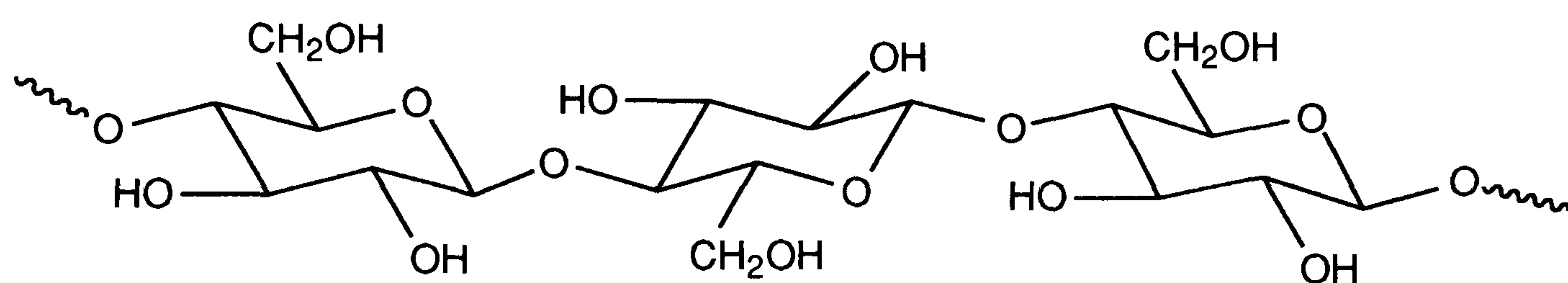
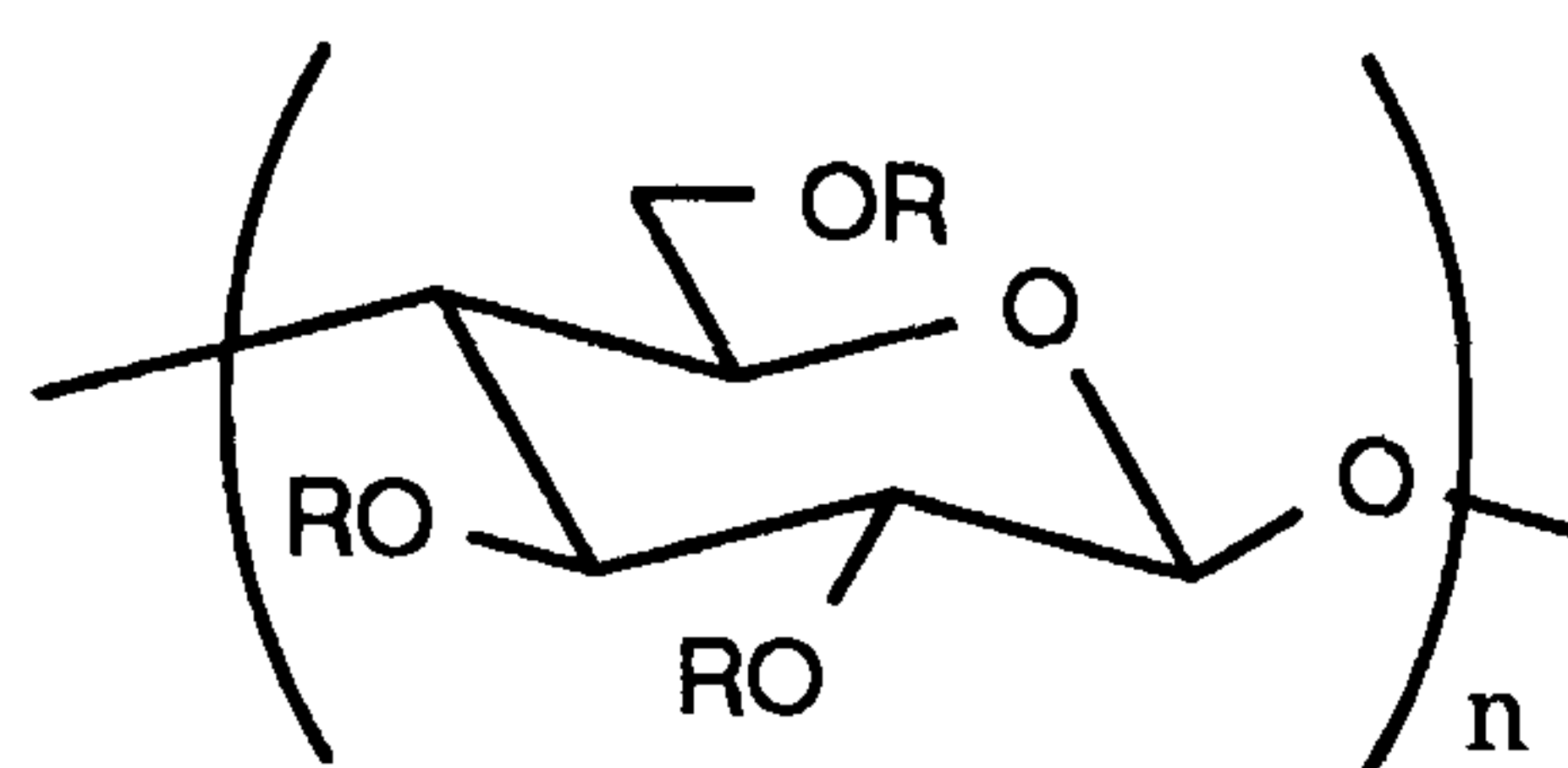


Fig. 28 Structure of cellulose

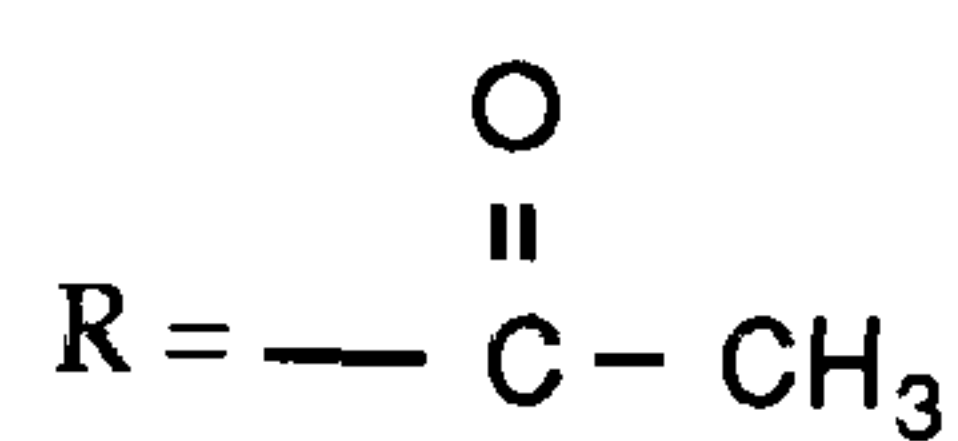
X-ray and electron microscopy indicate that these chains lie side by side in bundles, held together by hydrogen-bonding. The bundles become twisted to form a rope-like helical structure. This microcrystalline structure is thought to be necessary for chiral recognition. Derivatisation of the hydroxyl groups of cellulose was found not to destroy its helical structure [55].

A useful derivative of cellulose is the triacetate described by Hesse and Hagel in 1973 [55] and prepared by heterogeneous acetylation of crystalline cellulose. It was found that the triacetate derivative showed good chiral recognition when used directly in its microcrystalline form (MCT) [55]. Chiral recognition was lost if the material was recrystallised, presumably due to destruction of the chiral cavities in the helical structure of the polysaccharide. Inclusion complexes are considered to be formed between the solute enantiomer and the helical chains of cellulose triacetate. Partial separation of a cyclic allene hydrocarbon was achieved by Lindner and Mannschreck [56] and resolution of racemic compounds carrying aromatic groups was possible [57].

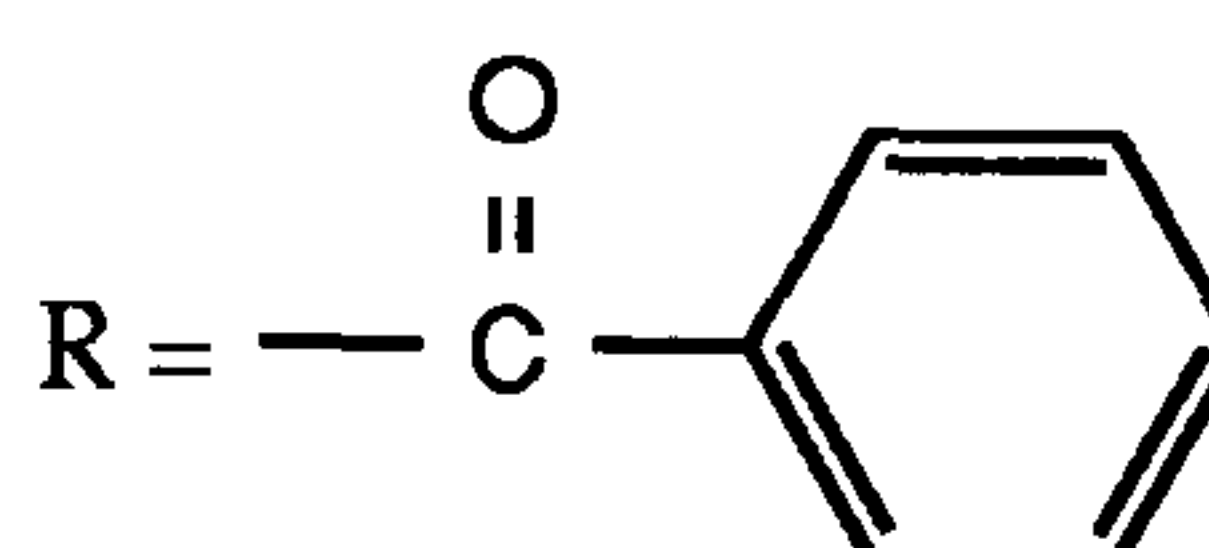
Cellulose derivatives are formed by reaction with reagents such as acid anhydrides and chlorides to form the triester derivative and with isocyanates to form tricarbamate derivatives. In the process developed by Okamoto and commercialised by Diacel, these are coated onto the surface of macroporous silica which has been treated with aminopropylsiloxane or trimethylsilyl chloride. The resulting phases, sold under the name Chiralcel (Fig. 29), have an excellent ability to resolve enantiomers.



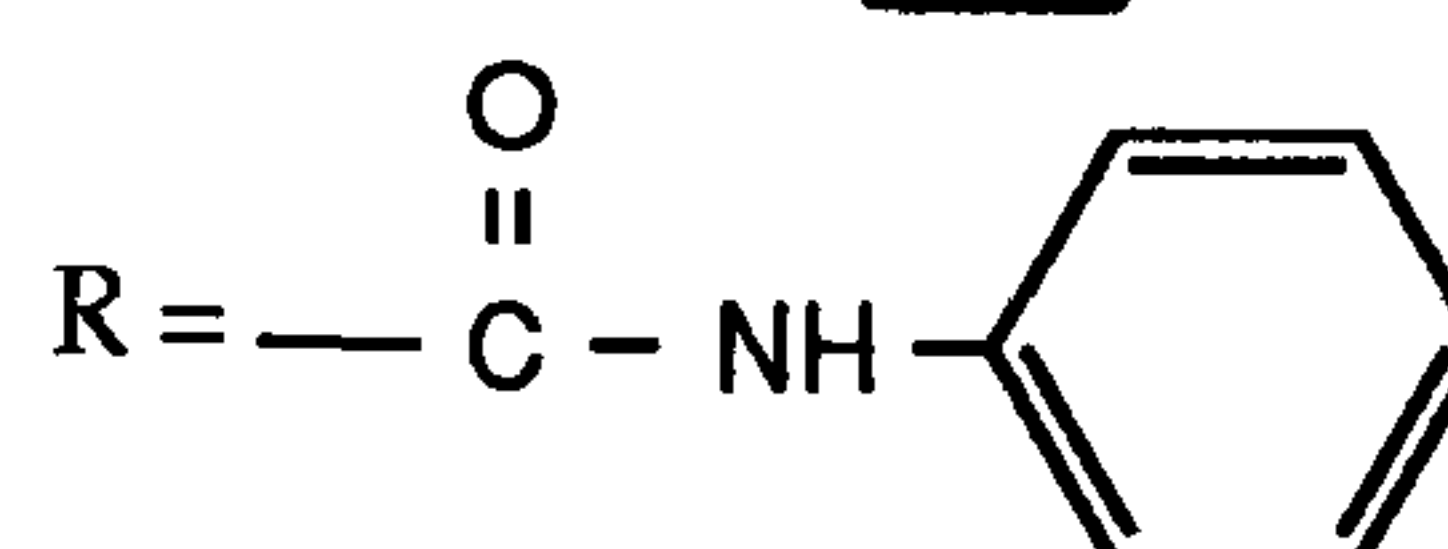
triacetate (OA)



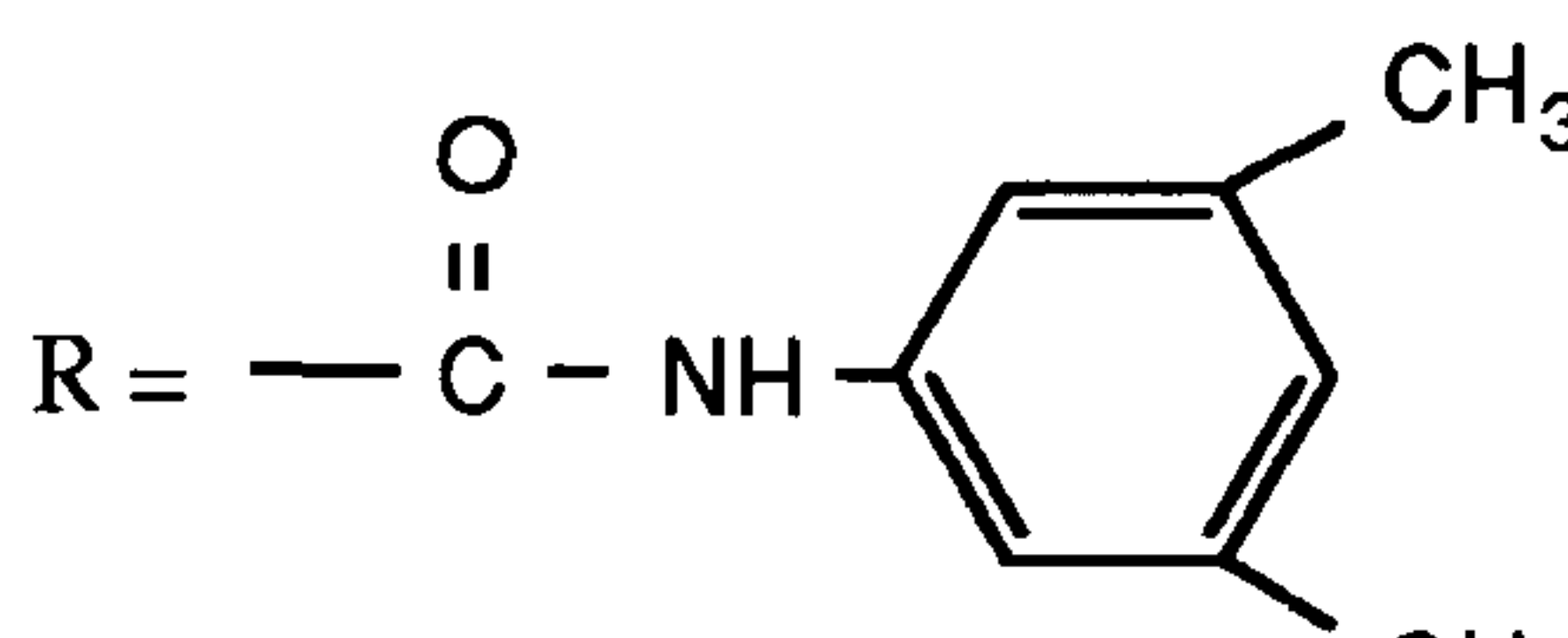
tribenzoate (OB)



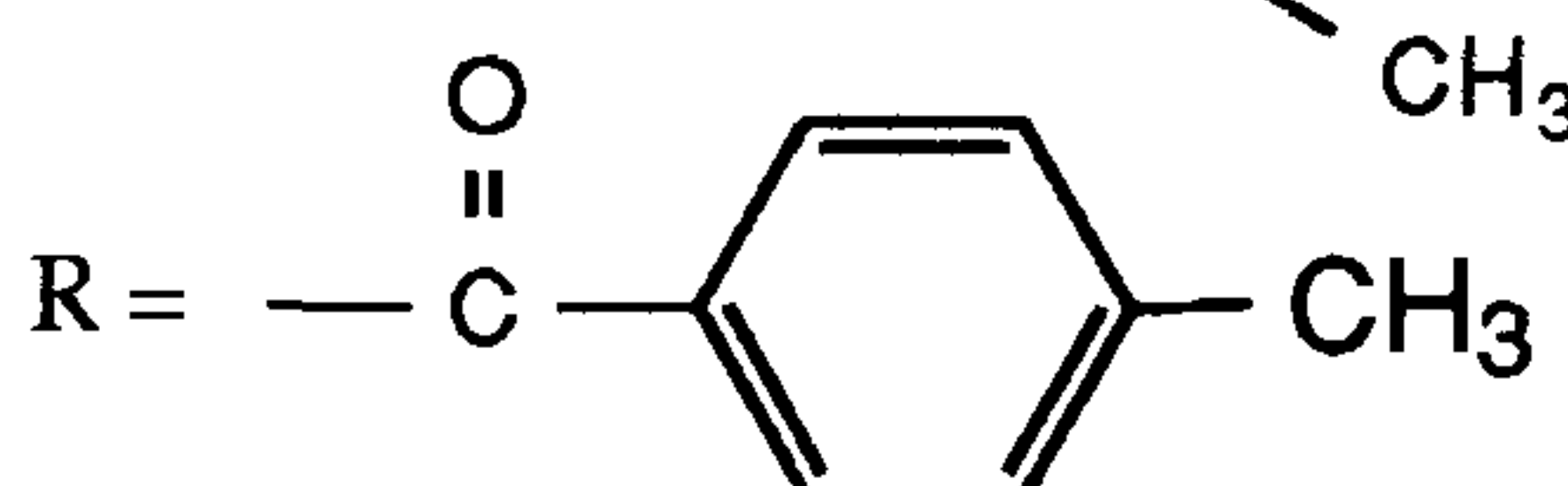
trisphenyl carbamate (OC)



tris-3,5-dimethylphenylcarbamate (OD)



tri-4-methyl benzoate (OJ)



tricinamate (OK)

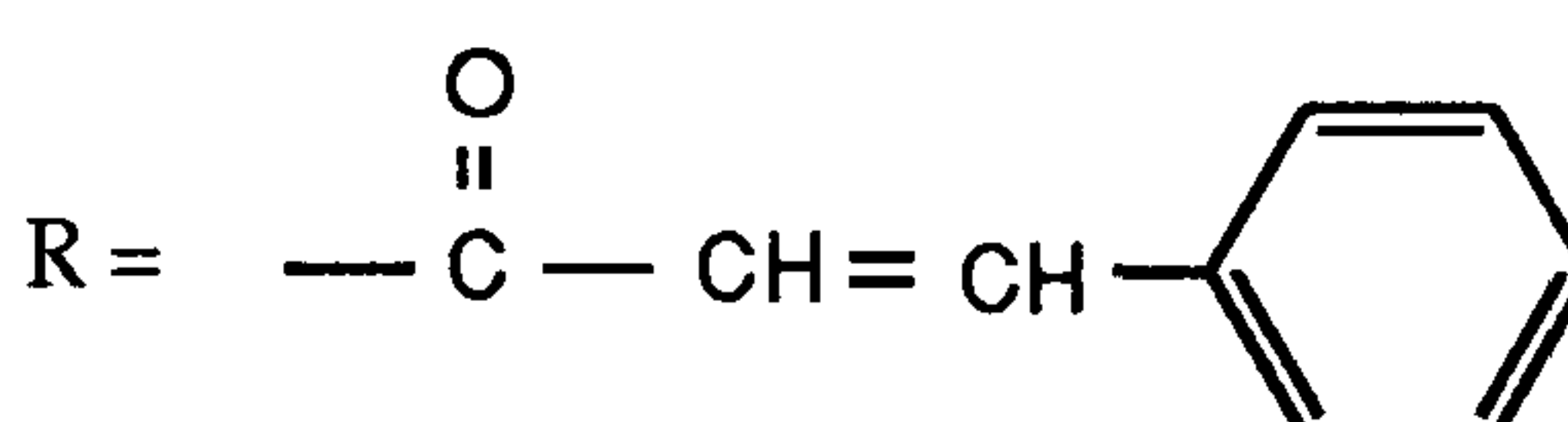


Fig. 29 Cellulose derivatives used in Chiralcel commercial columns.

Chiralcel phases are prepared by coating the derivatised polysaccharide on the surface of spherical silica (7-10 μm with a pore size of 1000 to 4000 \AA) to form a coating of approximately a 25% w/w of the support material. Amongst the complete range of cellulose derivatives, the tribenzoate (OB) and tris(dimethylphenylcarbamate) (OD) exhibit the best selectivity for sulphoxides, whereas the benzyl ether showed no signs of separation [58].

The choice of the mobile phase has an important influence on chiral recognition. In general, suitable solvents are limited to hexane/2-propanol and methanol or ethanol. Chlorinated solvents such dichloromethane will remove the derivatised cellulose from its silica support. Similarly, acetone, tetrahydrofuran, dihydrofuran, methylethyl ketone, dimethyl sulphoxide, toluene or acetonitrile cannot be used. A relatively low flow rate (e.g. 0.5 ml/min) is recommended, since the thick polymeric coating leads to slow mass transfer in the stationary phase.

The enantioseparation mechanism is unknown, although it could be based on a multimode mechanism involving hydrogen-bonding, π - π interactions, dipole stacking and inclusion complexes. The interactive sites are located within the chiral cavities of the helical structure of the cellulose derivatives. The main chiral recognition sites are considered to be the polar carbamate or ester groups, which can interact with a solute via hydrogen bonding or dipole-dipole interactions. Resolution also appears to depend on the "steric fit" into the cavities of the material. It is thought^[55] that the chiral cavities of the stationary phase have a high affinity for aromatic groups and, for those compounds containing such groups, it is the aromatic portion of the molecule which will enter the cavity ^[59]. For resolution to occur, the aromatic moiety needs to fit reasonably tightly into the cavity and at least one of the substituents on the chiral centre needs to be able to interact with functionalities on the periphery of the cavity. For compounds with an active hydrogen such as amines and carboxylic acids, derivatisation of the molecule to a neutral function such as an amide may often be necessary before injection, e.g. α -amino acids are not suitable for these columns.

The solute always competes with the modifier for hydrogen bonding sites on the CSP. This competition takes place at both chiral and achiral sites on the CSP^[60]. This does not preclude interaction between the modifier and the solute, which appears to play a lesser rôle in the determination of the chromatographic parameters.

Cellulose derivative phases are suitable for preparative applications due to the high capacity and good efficiency of these phases. Typical applications include compounds possessing aromatic, carbonyl, nitro, sulphanyl, cyano or hydroxyl groups ^[61].

Derivatives of other polysaccharides

Arylcarbamate derivatives of other polysaccharides have been examined by Okamoto [62]. Chiral packing materials were prepared from arylcarbamates of amylose, chitosan, xylan, curdlan, dextran and inulin, coating on to APS. The chiral discrimination of the stationary phases was highly dependent on the polysaccharides: the xylan phase resolved the same series of compounds 1-(9-anthryl)-2,2,2-trifluoro ethanol, Tröger base and cobalt (III) tris(acetylacetonate) ($\text{Co}(\text{acac})_3$) as did the cellulose phenylcarbamate, whereas inulin resolved only $\text{Co}(\text{acac})_3$ with high efficiency. It was noted that xylan and dextran showed remarkable chiral discrimination against 1-(9-anthryl)-2,2,2-trifluoroethanol and $\text{Co}(\text{acac})_3$.

Two carbamate derivatives of amylose have been coated on silica and are marketed by Daicel as Chiralpak (Fig. 30). They operate in a similar way to the cellulose derivatives phases, but they show different selectivities.

The resolving power of the amylose derivatives was also greatly varied by the introduction of aromatic substituents. Introduction of an electron-donating substituent appears to be more favourable than of an electron-withdrawing substituent.

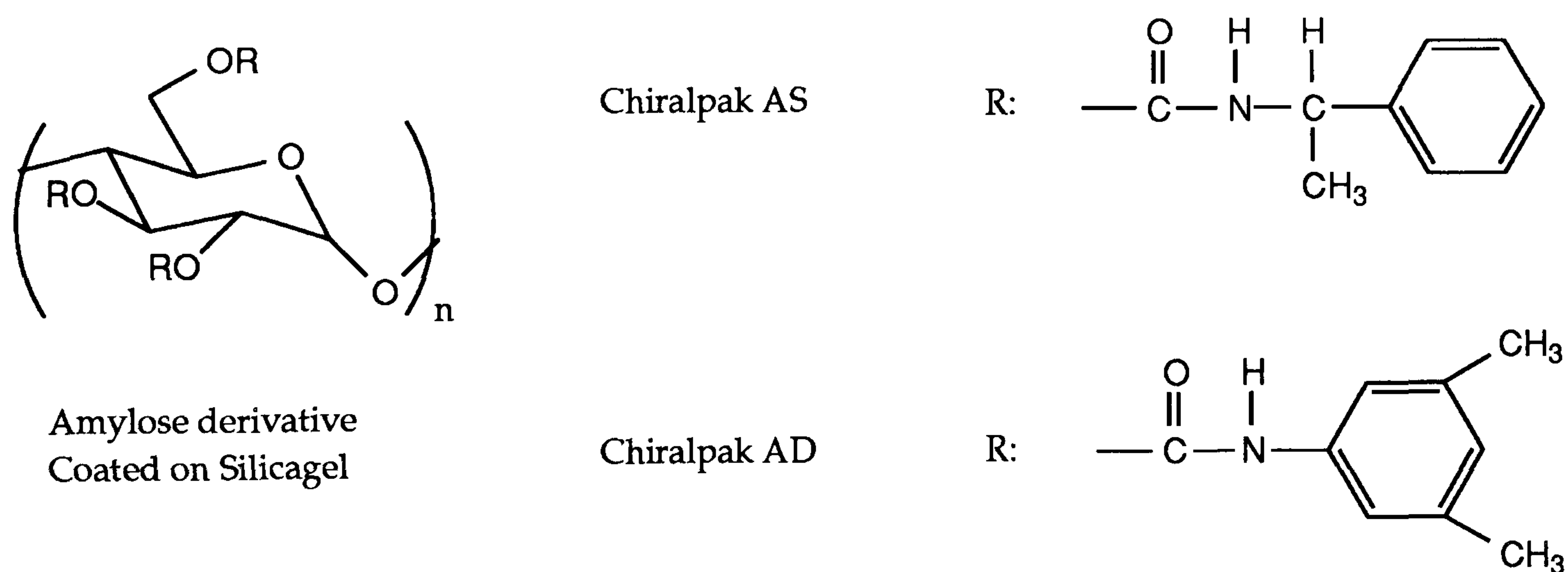


Fig. 30 Commercial columns of amylose derivative

1.4.6- Synthetic Polymer CSPs.

Synthetic polymer chiral stationary phases are amongst the most recent to be made commercially available. They are designed to mimic or improve upon the success of the cellulose type polymers. They are produced by polymerisation of methacrylate esters with a bulky substituent using a chiral catalyst, so that the backbone of the polymer has asymmetrically induced chiral centres and develops a helical (right or left handed) chiral twist. This helical structure contributes to the process of chiral recognition in these synthetic polymers. Enantioselectivity of the analyte is achieved by the formation of inclusion complexes of different stabilities within the chiral ravines of these helical polymers. To maintain the helical structure in solution during the synthesis, a very bulky group must be introduced e.g. triphenylmethyl substituent. To make them suitable for HPLC applications, these helical polymers are also coated onto a modified silica packing stationary phase. The first example of this type of optically active polymer to be developed was poly(triphenylmethylmethacrylate) (PTrMA) [63]. It is a high-molecular weight, highly crystalline polymer which is insoluble in most organic solvents and whose chirality is due to its helical structure. This was found to be useful for the separation of some alcohols, esters, amines and hydrocarbons. Non-polar aromatic compounds are also well resolved. The polymer was prepared using a (-)-sparteine-butyllithium complex as the catalyst in dry toluene [64].

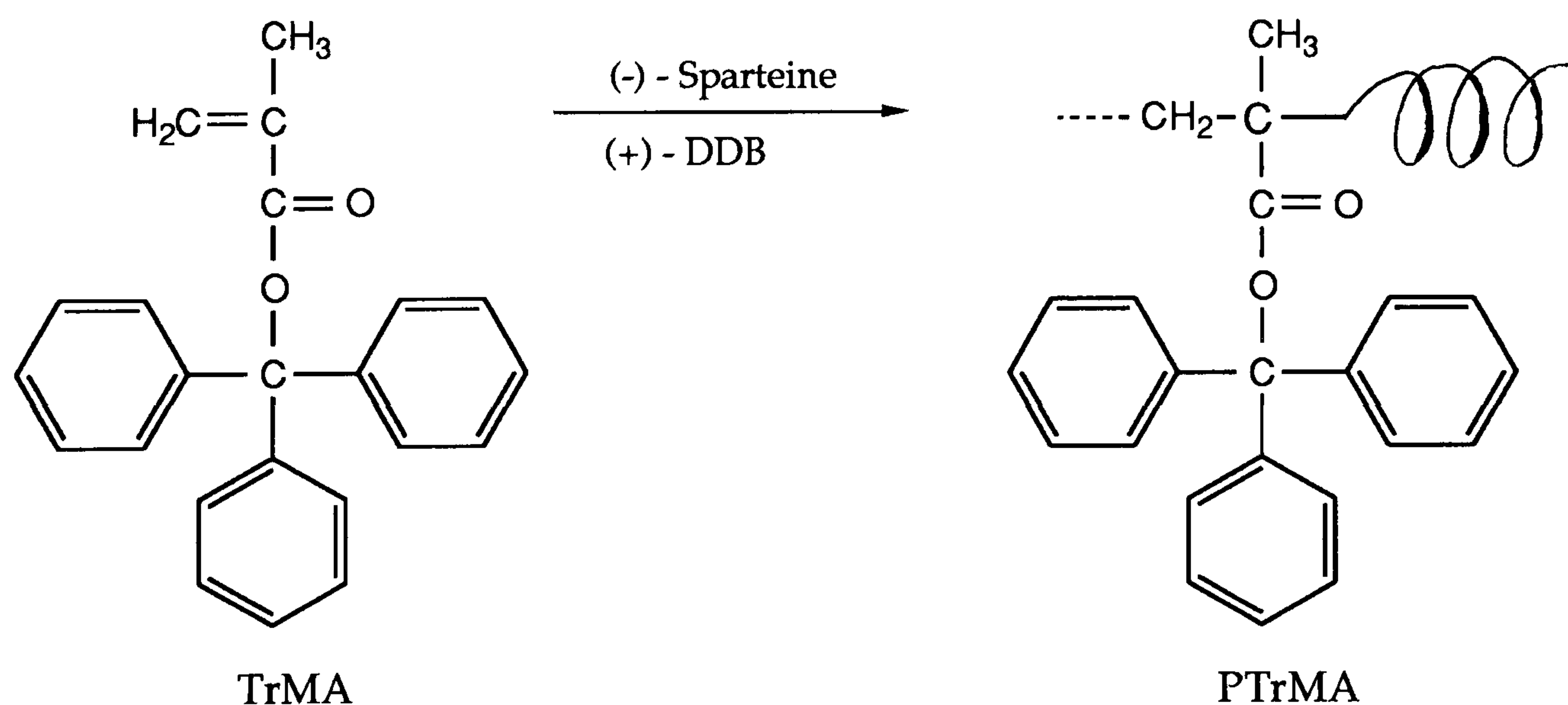


Fig. 31 Asymmetric polymerisation of synthetic chiral polymers

When the degree of polymerisation was 220, the polymer was very insoluble and the stationary phase formed was rather brittle, making column packing difficult. If the degree of polymerisation was 40 or less, the polymer became soluble in many common solvents, maintaining its helical structure in solution at room temperature. This low-molecular-weight, soluble PTrMA was adsorbed on to the support using THF as solvent. The support was prepared by treating 10 μ m, 1000Å pore size silica with a large excess of dichlorodiphenylsilane and triethylamine [65].

One of the novel effects of coating (+)-PTrMA on to silanised silica is that the new packing showed different chiral recognition when compared with high-molecular-weight (+) - PTrMA. In general, this new packing showed more efficient and more rapid separations.

Commercially, (+)-PTrMA coated on to APS silica gel is available from Diacel as Chiralpak OP (+). There is also Chiralpak OT (+) available, in which one of the phenyl rings in each triphenyl group is exchanged for a pyridine ring. The OT phase could separate the four stereoisomers of an insecticide called phenothrin (3-phenoxybenzyl chrysanthemate) (Fig. 32) [66].

Racemic compounds containing a phosphorus or sulfur atom at the chiral centre have also been successfully separated on this stationary phase [67].

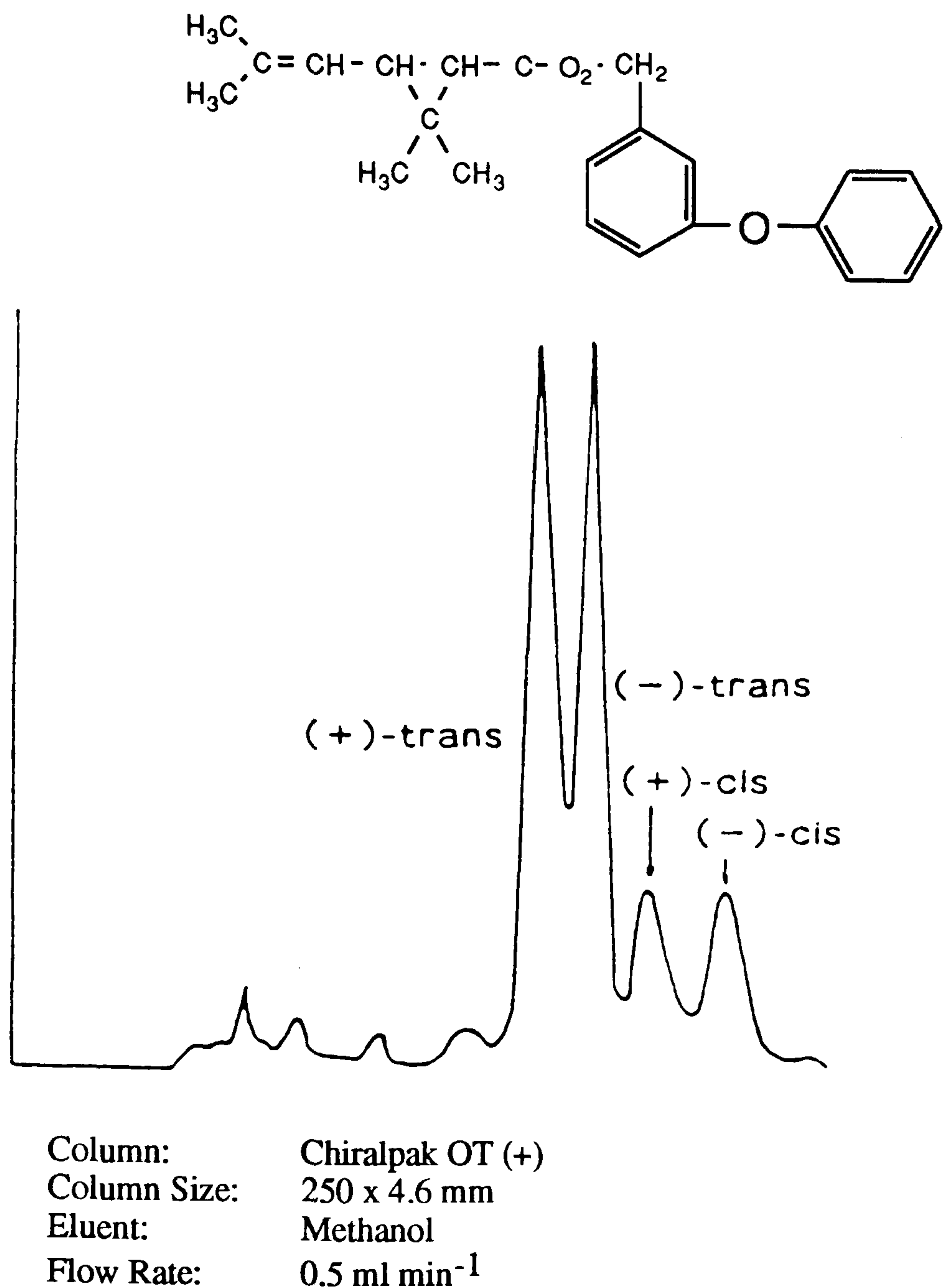


Fig. 32 Separation of the pyrethroid, phenothrin.

These phases resolve compounds containing aromatic groups, near to or at the chiral centre. Best resolution is obtained with non-polar compounds and those which contain an axis of dissymmetry or have inherent helicity.

The recommended mobile phase for the polymethacrylate CSPs is hexane modified with propanol or methanol. Best chiral resolution is obtained with MeOH, suggesting that non polar or hydrophobic interactions between the triaromatic methyl groups of the stationary phase and the non polar groups of the solutes are important for chiral resolution. In the case of Chiralpak PO(+), the addition of up to a maximum of 20% water to the methanol increases chiral resolution and retention time. This is probably due to increased hydrophobic interactions, however, the addition of water to the mobile phase should be restricted to a maximum of 0.05%

when using Chiralpak OT(+) as there is a danger of hydrolysing the ester group in this phase. The use of ethanol and isopropanol gives lower enantioselectivity than methanol with both chiral phases.

The ester bond in PTrMA is also susceptible to methanolysis when this phase is used with methanol at temperatures above 15°C. Thus, for Chiralpak OT (+), the temperature should be below 15°C (down to 0°C) when using methanol as this improves separation and makes the column life longer. However, room temperature is recommended for Chiralpak OP (+) as it is much less susceptible to methanolysis. Optimum flow rates are 0.5 to 1.5 ml/min. Solvents such as chloroform, acetone, tetrahydrofuran, dihydrofuran, dimethylsulphoxide, methylethylketone, toluene and acetonitrile should be avoided as they dissolve the polymer and cause irreversible damage. In general, samples containing strong acid and bases should be avoided when using these columns. Derivatisation is necessary in these cases, and good results are obtained if carboxylic acids are converted into phenyl esters, amines into benzoic amides and alcohols into benzoic acid esters. These synthetic polymer phases are suitable for preparative work, as they have good efficiency and high capacity.

More synthetic polymer based CSPs are commercially available, these include the polyacrylamide-based CSPs [68]. The range of racemates that have been resolved on these phases is very similar to the types of compound that have been resolved on the helical polymethacrylate CSPs and the same types of mobile phase have been used.

1.4.7- Chiral Crown Ethers.

Crown ethers (CEs), first introduced by Pedersen in 1967 [69] are synthetic, macrocyclic polyethers. The name "crown ether" comes from the appearance of its molecular model and its ability to "crown" the cations. Since the nomenclature for these compounds recommended by IUPAC is very cumbersome, trivial names are used to indicate the kind and number of substituent groups, the ring size and the number of oxygen ligands. For example: 2,3,11,12-dibenzo-1,4,7,10,13,16-hexaoxacyclo-octadeca-2,11-diene is called "dibenzo-18-crown-6", where "dibenzo" indicates the substituent groups, "18" means the total number of the atoms in the polyether ring and "6" means the number of oxygen atoms in the ring.

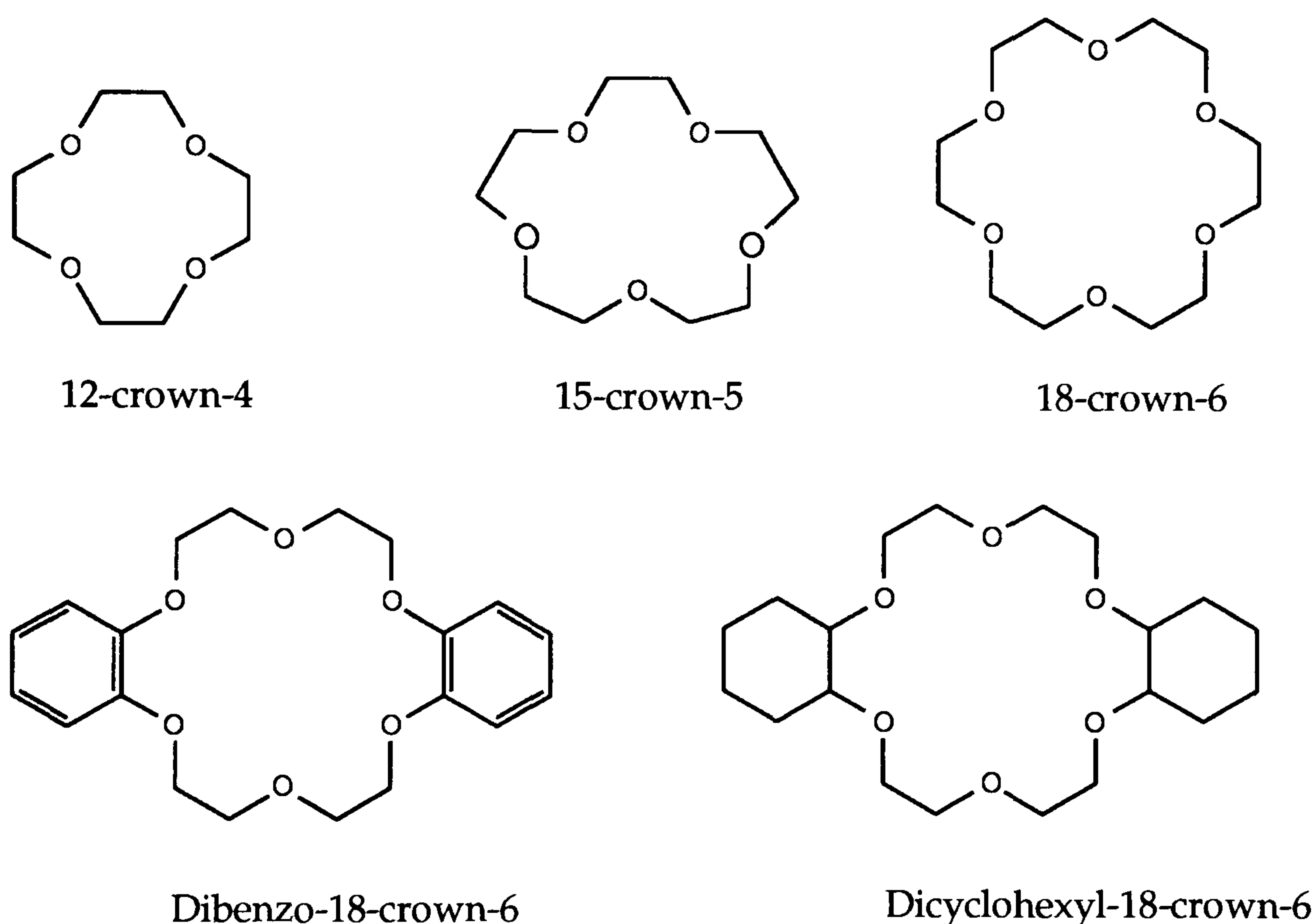


Fig. 33 Structures of crown-ethers

The ether oxygen atoms are placed regularly around the inside wall of the crown cavity, and are surrounded by hydrophobic $-\text{CH}_2-$ groups like a collar. Oxygens in the polyether ring can act as ligand atoms and metal cations are incorporated into the ring cavity based on ion-dipole interactions. CEs have the ability to complex a number of cations, e.g. Na^+ , K^+ , NH_4^+ .

The stability of the crown ether-ion complex depends on the tightness of fit of the ion in the crown cavity. For example, 18-Crown-6 forms its most stable complex with K^+ , which has an ionic diameter that best fits the cavity size [70] and forms a less stable complex with a cation that is smaller or larger in size. CEs form very stable complexes with ammonium cations by forming H-bonds between the ether oxygens of the crown ether and 3 hydrogens of the ammonium cation. They also make stable complexes with several protonated primary amino compounds. Molecular recognition is achieved through a host/guest complex mechanism often referred to as the "key and hole" relation.

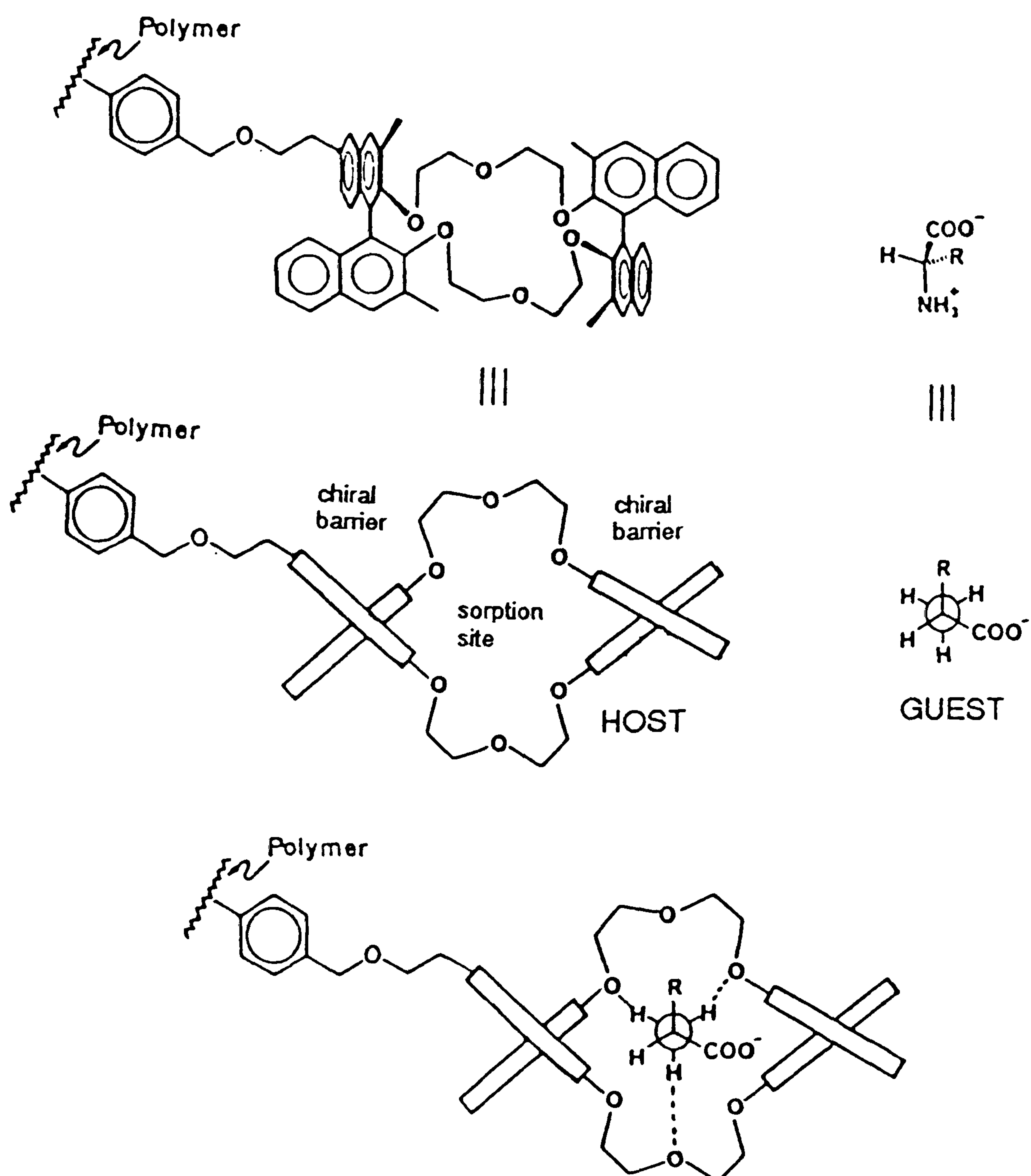


Fig. 34 Chiral separation by chiral crown ether phase

The chiral character of the crown ether used as chiral selector is given by incorporating chiral molecules with a rigid structure, such as hindered binaphthyl residues, into the crown ether structure. In some cases, the chiral crown ether selector is covalently bonded to a resin or to silica matrix to form the stationary phase (Fig. 34).

The most systematic and successful studies of chiral CEs were performed by Cram *et al.* [71]. They synthesised a series of chiral CEs bearing a 1,1'-binaphthyl moiety, such as CE-1 to CE-6 (Fig. 35).

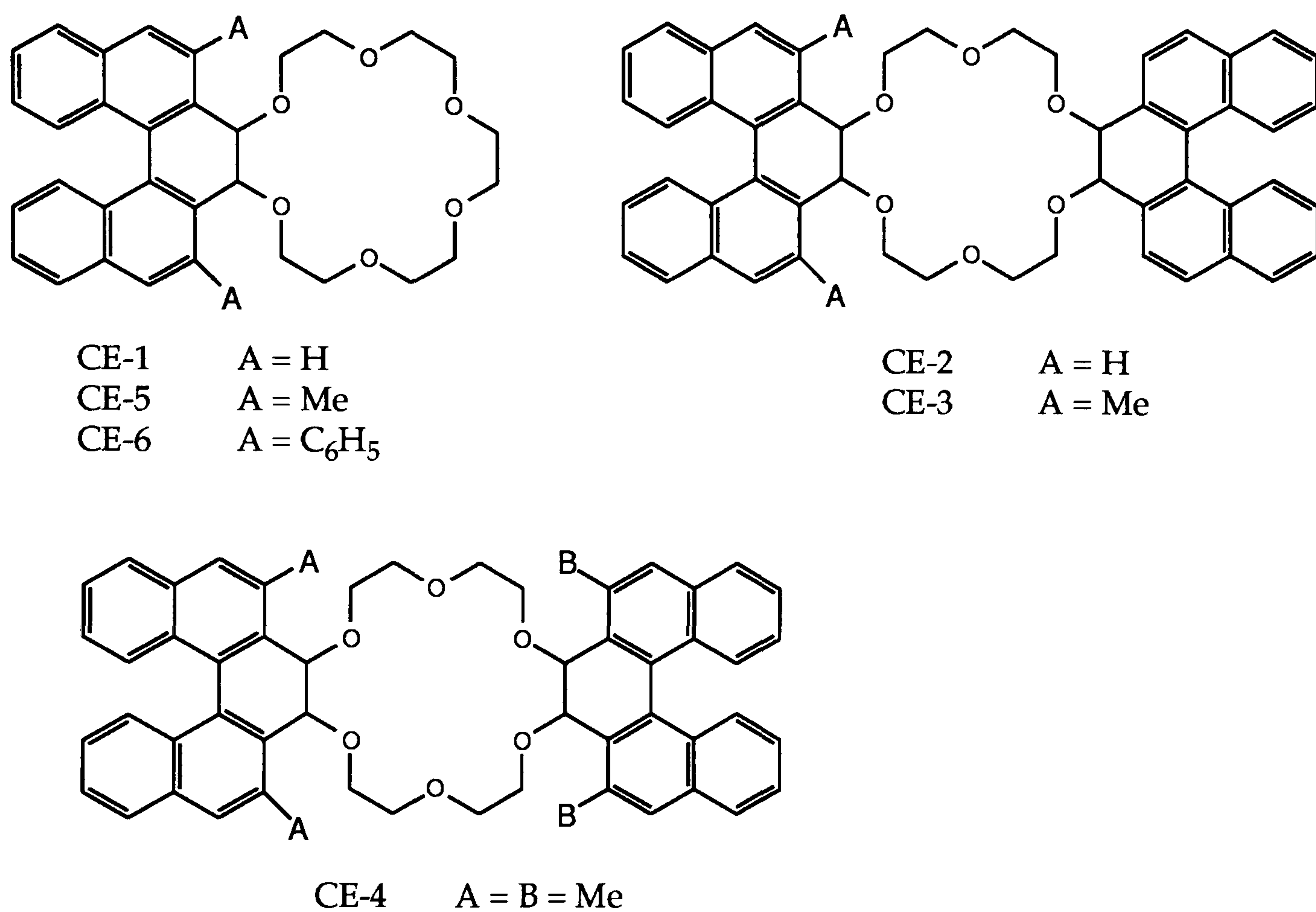
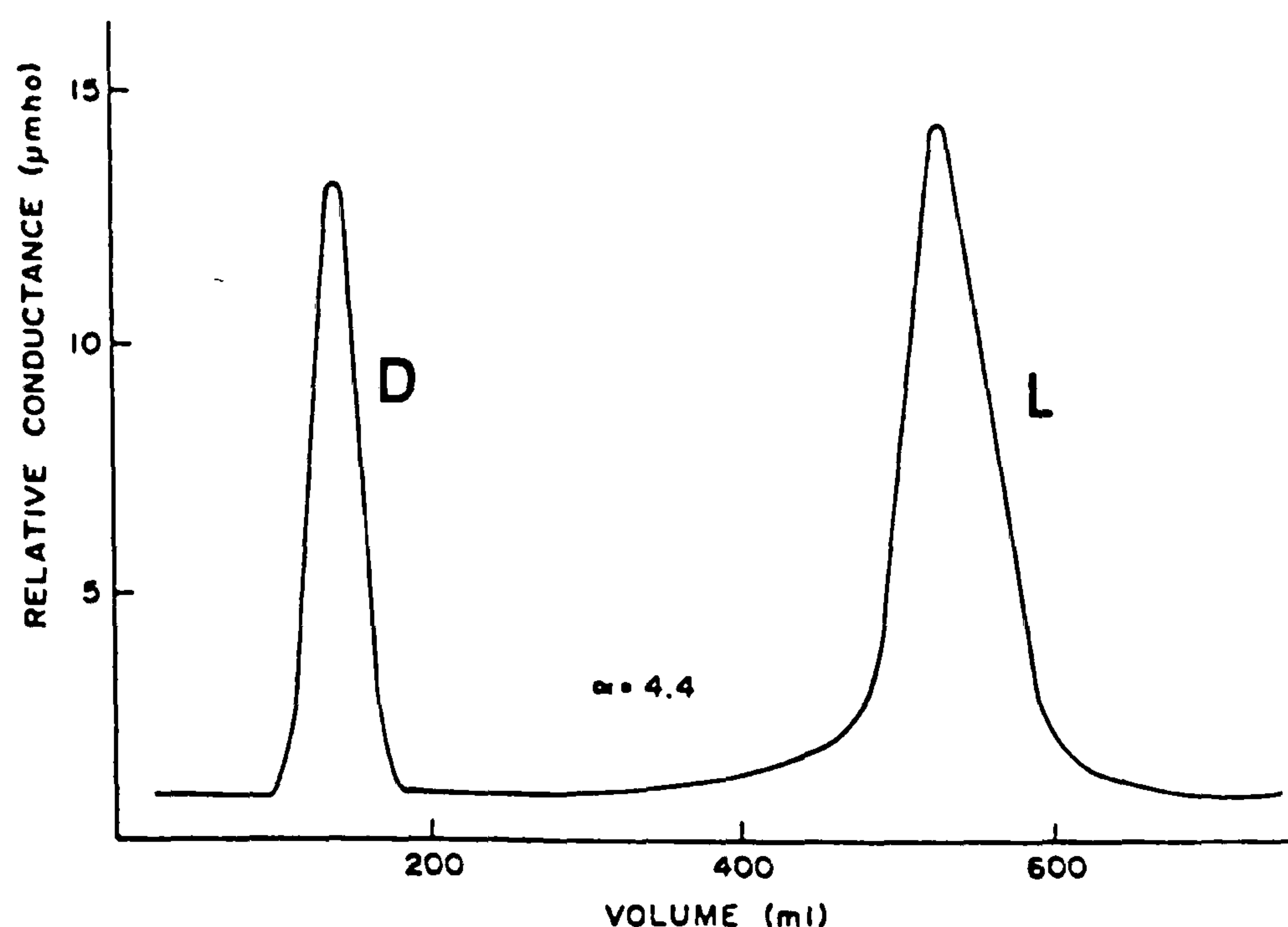


Fig. 35 Structures of chiral crown ethers

Cram's group applied chiral CEs to the optical resolution of racemic amines, amino acids and amino esters through the following three approaches:

- a) Use of CE-2 as mobile phase additive in normal-phase LC.
- b) Immobilisation of CE-2 by covalent bonding to silica gel in reverse phase (Fig. 36).

c) Immobilisation of CE-3 by covalent bonding to the polystyrene resin in reverse phase.



Stationary phase: (RR) CE-2 immobilised on silica gel
Mobile phase: CHCl₃

Fig. 36 Chromatographic optical resolution by immobilised (RR)CE-2, system (b) of phenylalanine methyl ester [72].

The elution order correlates well with the direction of stereochemical bias in complexation. The enantiomer that forms a more stable complex with the chiral CE elutes faster in system a) and slower in systems b) and c) than the other enantiomer.

The degree of resolution depends on the nature of the organic modifier in the mobile phase. In each of these systems, the use of CH₂Cl₂ as mobile phase instead of CHCl₃ caused a decrease in chiral resolution. In the system c), substitution of ethers or alcohols for CHCl₃ eliminated chiral recognition, apparently because these solvents provide enough hydrogen bonding sites to inhibit complexation.

Shinbo *et al.* [73] prepared ODS silica coated with CE-6 to resolve underivatized racemic amino acids and amines in HPLC.

At present only one crown ether phase is commercial available from Diacel, Crownpak CR. It contains a 18-crown-6 type chiral crown ether as a chiral selector which is coated on spherical silica. Its chiral recognition can be achieved when a complex is formed between the crown ether and an ammonium ion derived from a sample. It is possible to resolve not only amino acids but also many compounds bearing a primary amino group near the chiral centre. An aqueous acidic mobile phase is required to form the ammonium ion. The lower pH results in good resolution but a shorter column life. Various kind of acids can be used such a nitric and trifluoroacetic acid. However, perchloric acid is recommended because of better resolution and low UV-absorption.

The absolute configuration of the sample is deduced from the elution order on the column. Using Crownpak CR(+) the D-form of an amino acid always elutes faster than its antipode. Hydrophobic compounds are retained more strongly than hydrophilic ones. Generally, the lower the temperature the better the resolution becomes.

Chapter Two

LITERATURE REVIEW: SOME ASPECTS OF THE CHEMISTRY AND PROPERTIES OF HELICENES

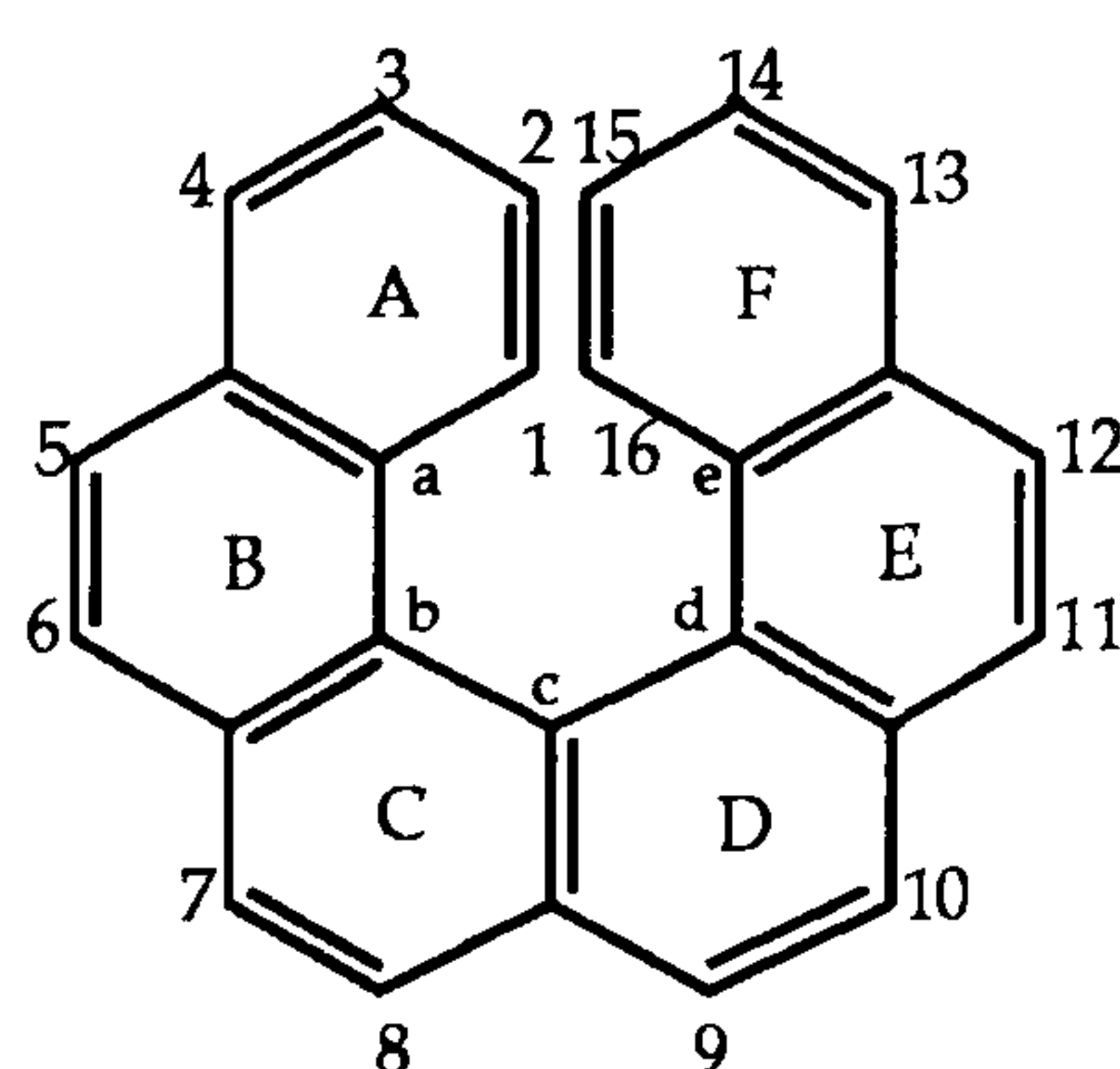
2.1- Helicenes [74]

Molecules which suffer from overcrowding between internal structural components try to minimise their high energy steric interactions, often resulting in a twisted structure lacking in conformational flexibility and possessing chirality. This is the case for helicenes.

"Helicene" is the name introduced by Newman in 1955 [75] to describe the benzologues of phenanthrene in which the extra orthocondensed rings give rise to a regular cylindrical helix. This characteristic helical structure is a consequence of the repulsive steric overlap of the terminal aromatic nuclei. The helicenes are further characterised by the presence of a powerful inherently dissymmetric chromophore exhibiting a very high specific optical rotation and by the possibility of interactions (e.g. electronic interactions) between overlapping aromatic rings.

The scientific interest in helicenes arises from the unique combination of these three properties in a single molecule. Many studies of their chemical and physical properties have been performed on helicenes because these molecules deviate from what aromatic hydrocarbons 'ought' to be: planar and rigid.

Helicenes which contain only carbon atoms in the skeleton are called carbohelicenes or all-benzene helicenes and those containing heteroaromatic rings in the skeleton are called heterohelicenes. The commonly used numbering system (1) is that first proposed by Newman [75].



(1)

The absolute configuration of helicenes is designated according to the "helicity rule". A right handed helix is denoted P (plus) and a left handed helix M (minus). The dextrorotatory hexahelicene has a right handed helix and is P-(+)-hexahelicene.

The main progress in the chemistry of helicenes in the last ten years came from:

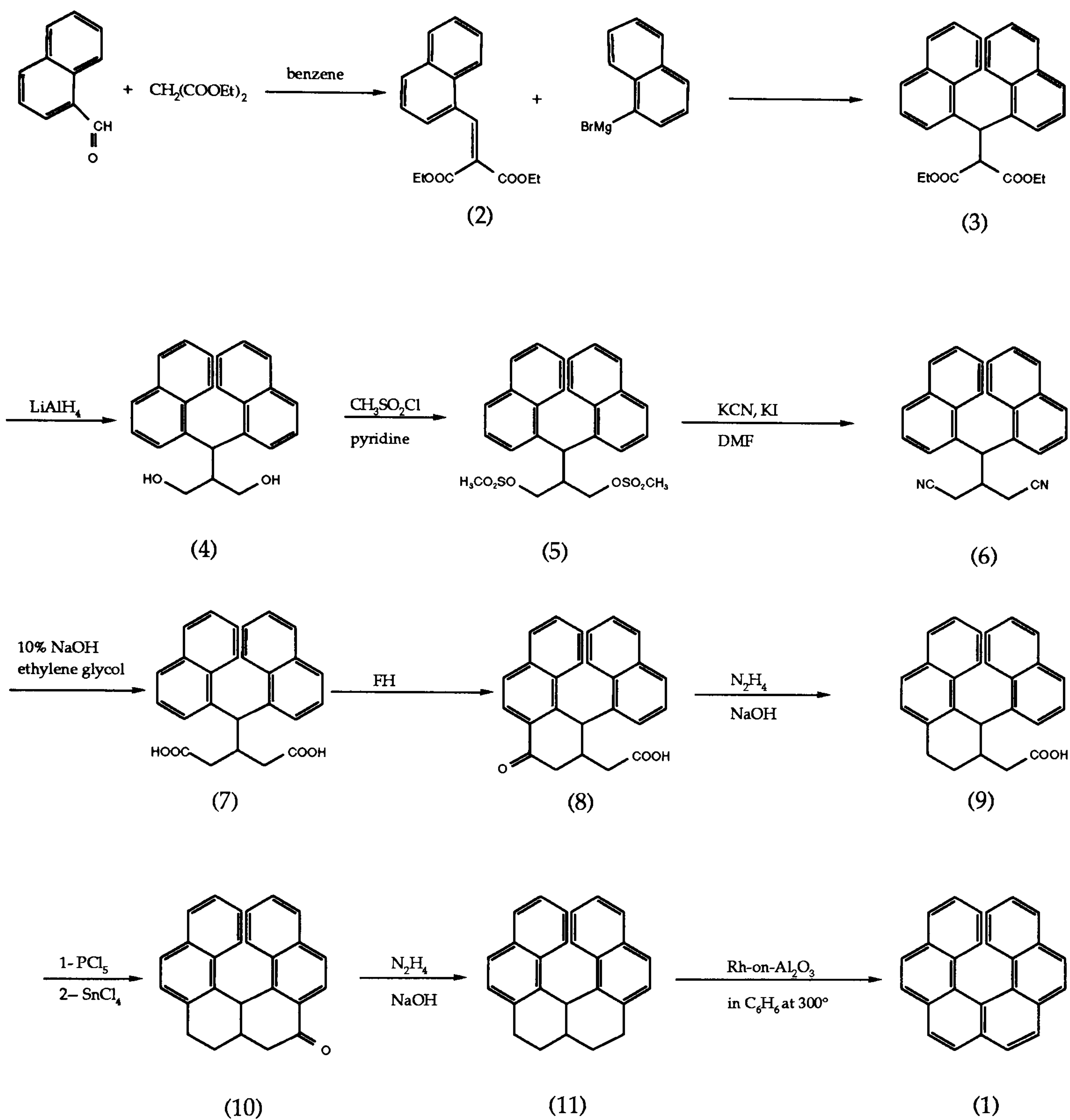
- 1) The results of X-ray analyses of various helicenes.
- 2) The resolution of enantiomers by HPLC techniques, mainly based on charge-transfer complexation.
- 3) Asymmetric syntheses by different methods.
- 4) Use of force-field calculations.
- 5) Refinement of the interpretation of NMR Spectra.

2.2- Syntheses of Hexahelicenes. Methods and Their Problems.

2.2.1- *Non-Photochemical Syntheses.*

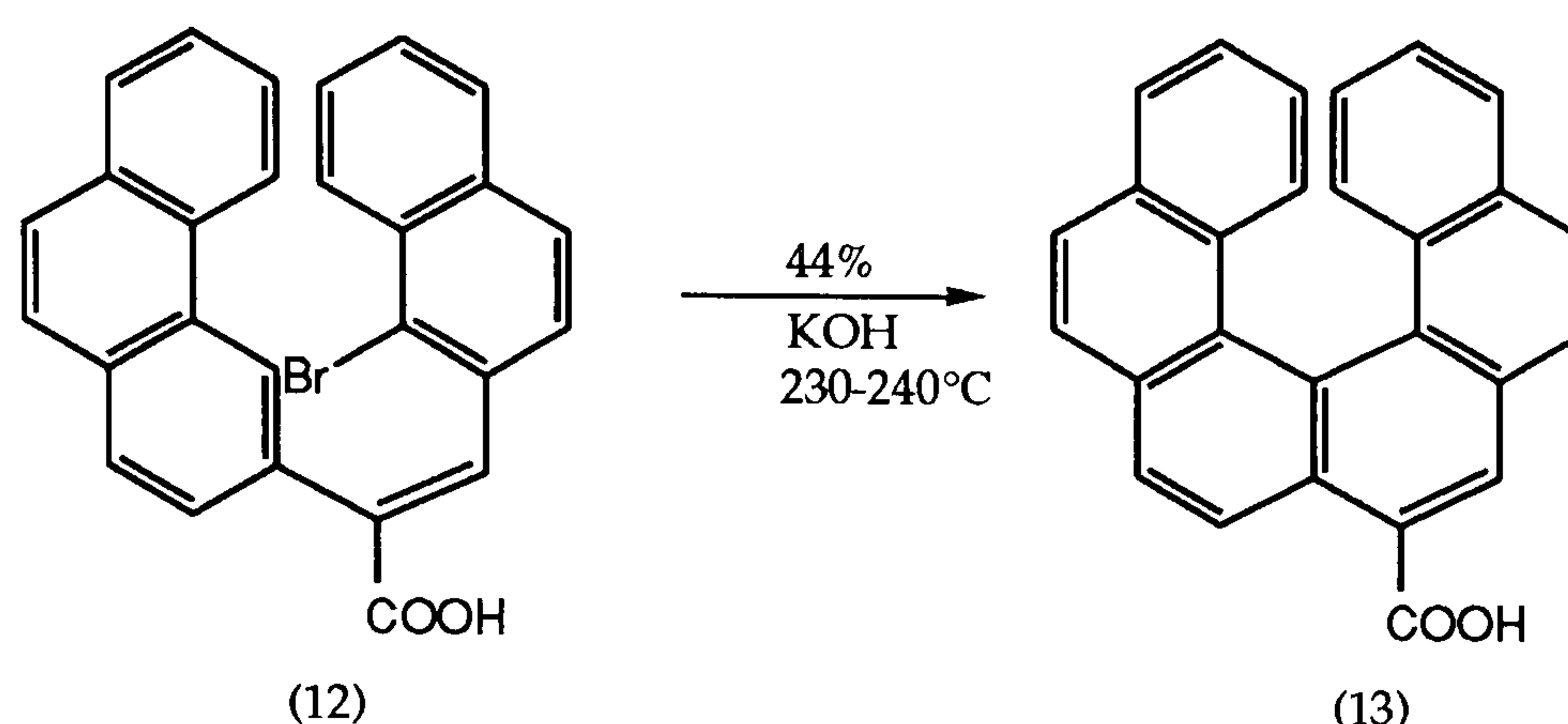
The synthesis of racemic helicenes remains a laborious process involving, at a certain crucial stage, the possibility of isomer formation which cannot be avoided.

The first synthesis of an all-benzene helicene, if we exclude tetra- and pentahelicene, is the well known twelve-step synthesis of hexahelicene described by Newman and Lednicer [75]. The attempt to use this scheme for the preparation of the higher benzologue heptahelicene was unsuccessful.



Scheme 1

The second synthesis of hexahelicene was achieved eleven years later by Bogaert-Verhoogen and Martin [76]. The crucial step of this synthesis is the potash fusion of (12) leading to hexahelicene-8-carboxylic acid (13) in 44% yield [77].



Scheme 2

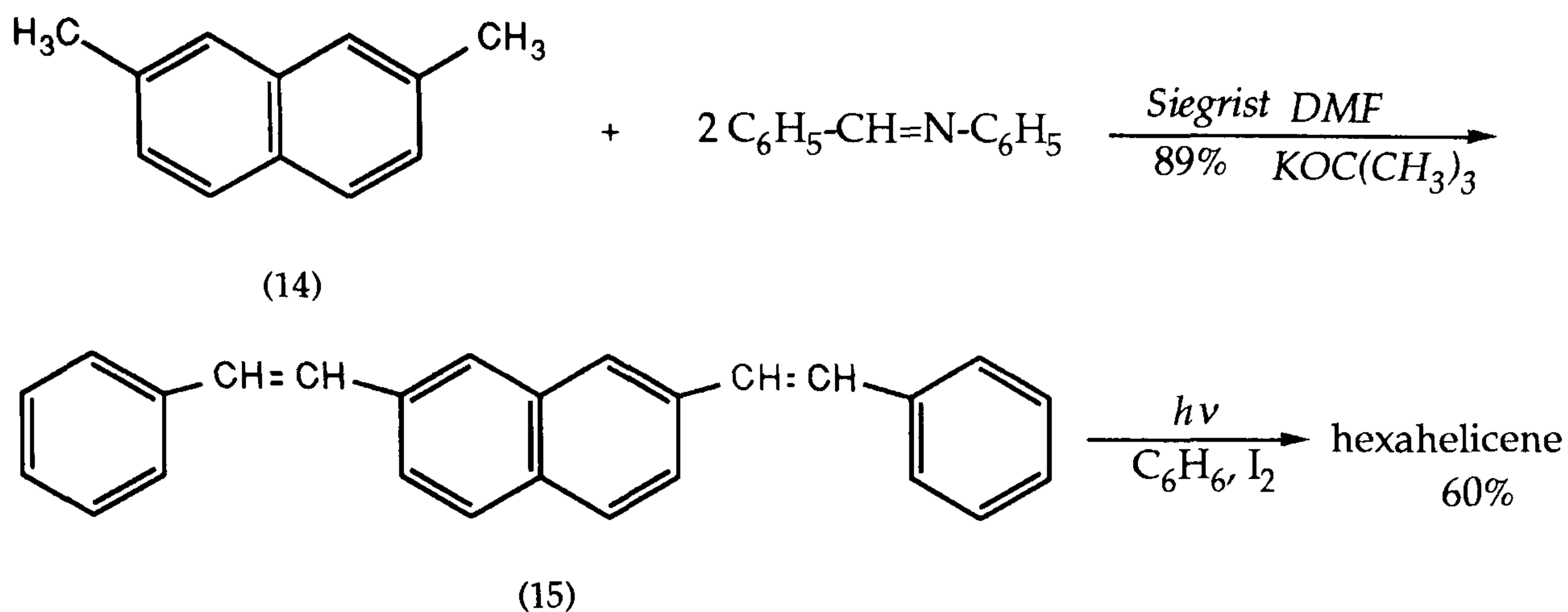
2.2.2- Photochemical Syntheses.

The breakthrough came when it was found that helicenes could be prepared in fair to excellent yields by irradiation of 1,2-diarylethylenes in a dilute solution and in the presence of an oxidising agent. This approach is based on the known photocyclodehydrogenation of stilbene into phenanthrene[78].

Photocyclisation has proven exceptionally rewarding in the helicene field and the process is extremely simple. The required 1,2-diarylethylenes or bis (arylvinyl) arenes can usually be prepared, in excellent yields, either by the Wittig reaction or by the Siegrist reaction. The stereochemistry of the starting material is unimportant because of *cis-trans* photoequilibration.

The two-step synthesis of hexahelicene [79](Scheme 3)

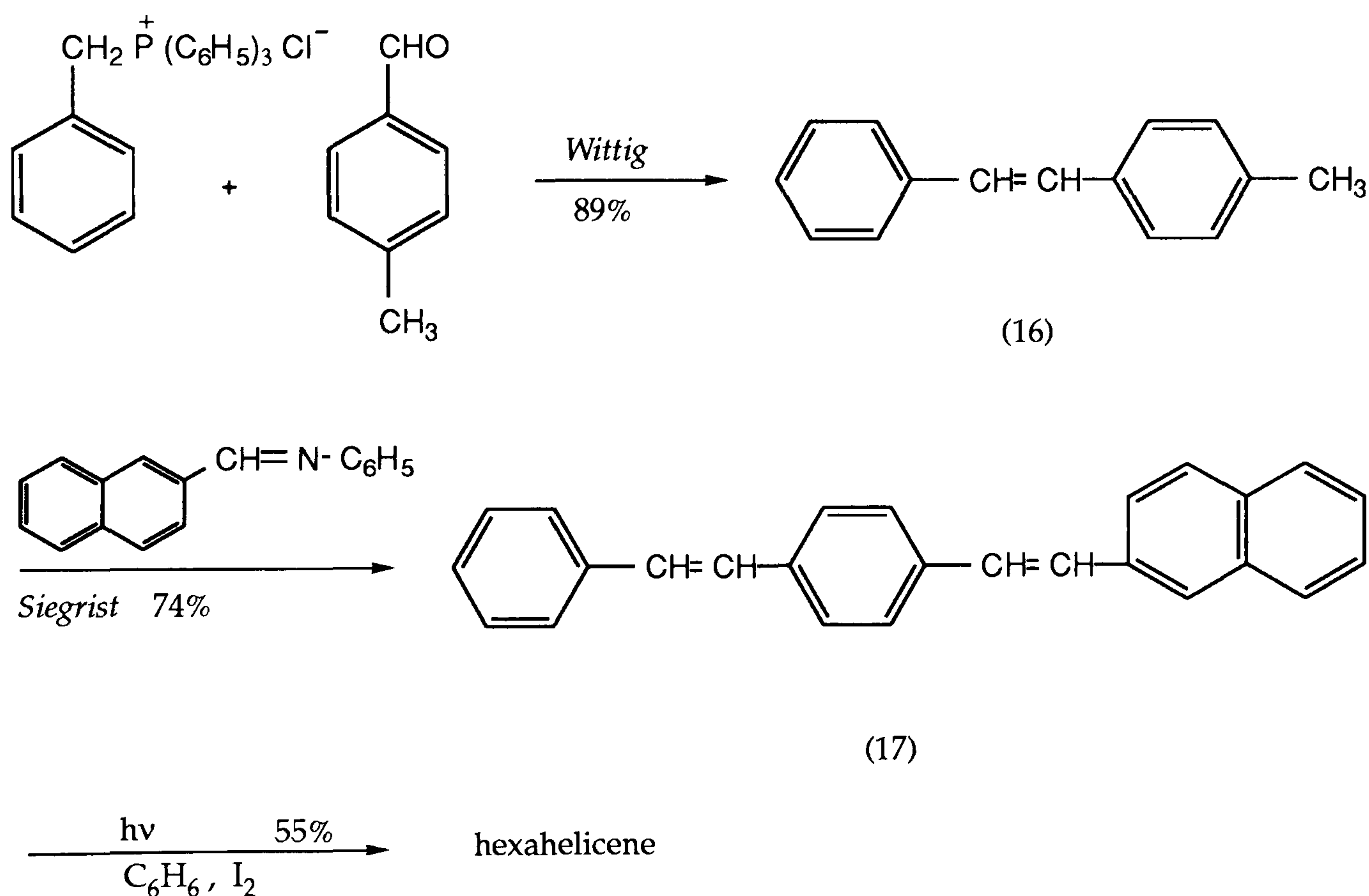
Hexahelicene can be prepared in a few days from 2,7-dimethylnaphthalene (14) via a Siegrist reaction giving 2,7-distyrylnaphthalene (15) (89%) followed by a double photocyclisation of the product (60%), the overall yield being 53%.



Scheme 3

Three-step synthesis of hexahelicene (Scheme 4)

4-Methylstilbene (*cis* + *trans* (16)) prepared by a Wittig reaction (89%) is condensed with N-(2-naphthylmethylene) aniline (Siegrist reaction, 74%) and the reaction product (17) is submitted to a double photocyclisation (55%) to give hexahelicene in 33% over-all yield.

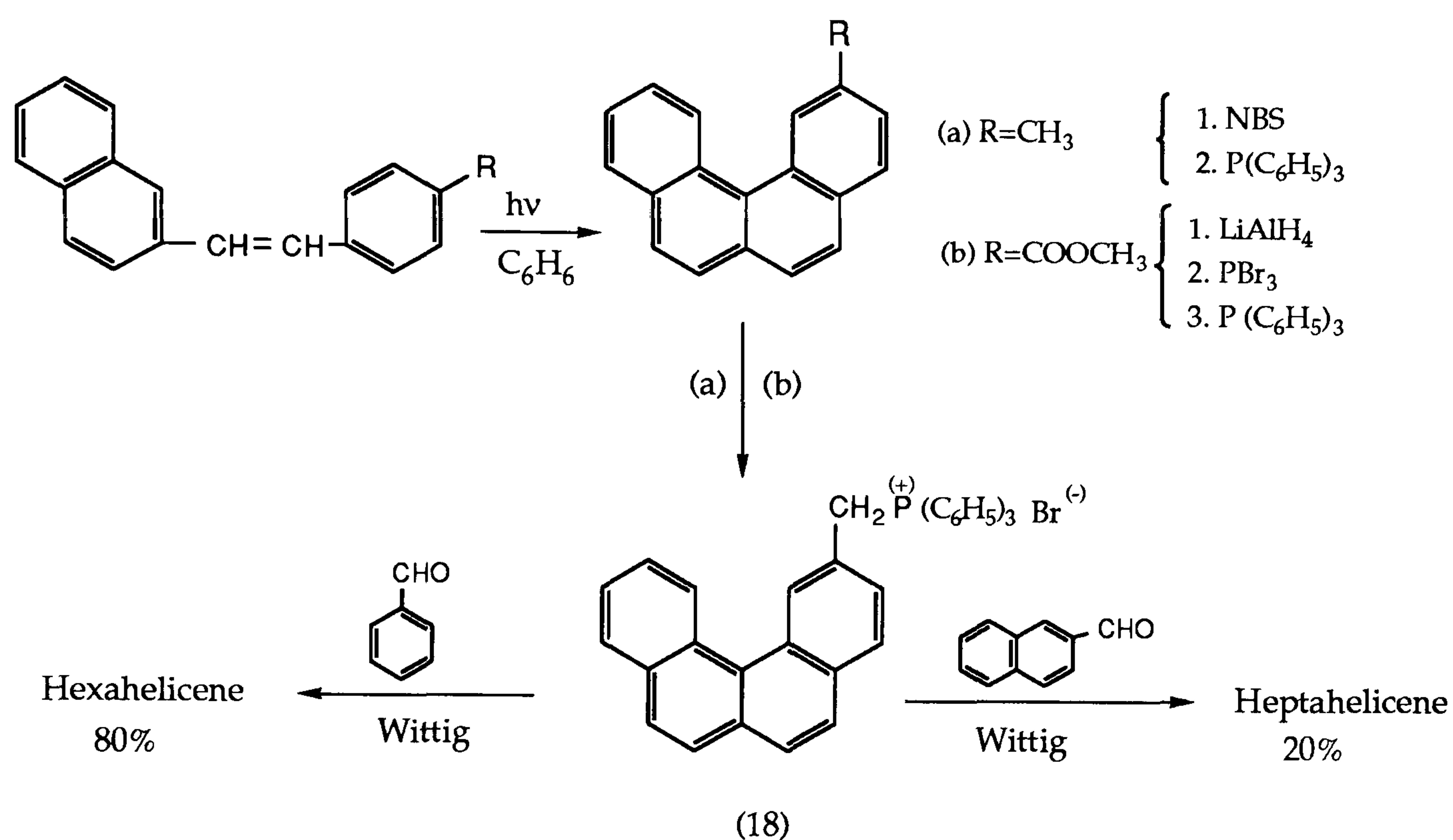


Scheme 4

The photocyclodehydrogenation in solution of *cis*-stilbene and its analogues (17) is a very complex process. The common method employed for

photocyclodehydrogenation reactions to obtain phenanthrene derivatives involves UV irradiation in the presence of air and iodine. Similar reactions also occur with other 1,2- diarylethylenes.

Wittig reactions of (18) (Scheme 5) with benzaldehyde and 2-naphthaldehyde gave the corresponding 1,2-diarylethylenes which were cyclised (1.5 hours irradiation) to give hexahelicene (80%) and (20%) heptahelicene respectively [80].



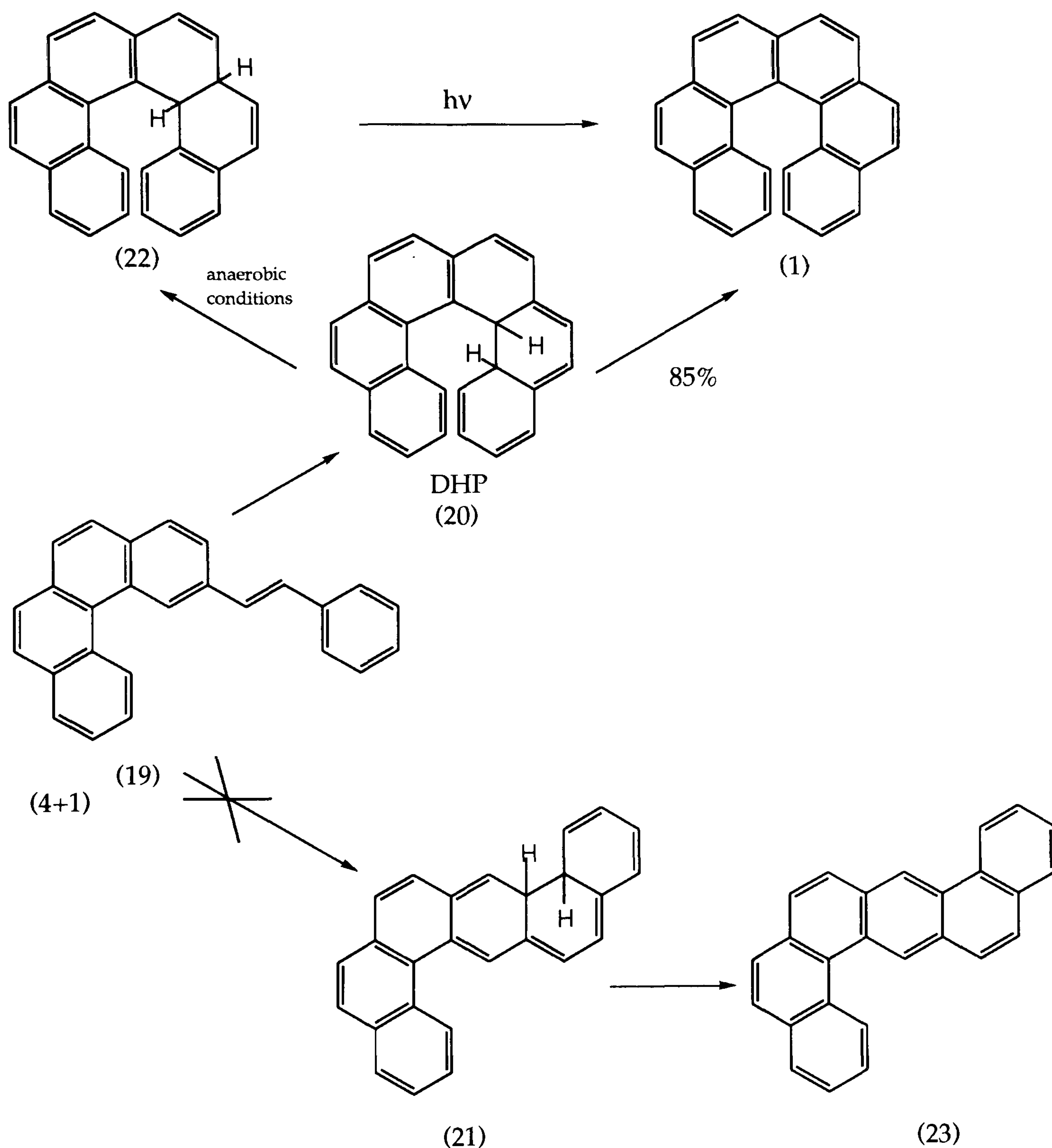
Scheme 5

Ring closure of arylolefins into helicenes can be effected by irradiation of a dilute solution. In principle all solvents that do not absorb at the wavelength of irradiation and do not react with the helicene precursor or the oxidising agent can be used. The most commonly used are methanol, hexane, cyclohexane and benzene.

To prevent dimerization the concentration of the precursors have to be kept low (10^{-3} mol/l). To prevent oxidation of the end product, de-aeration of the solvent is recommended. In most cases the helicenes are easily separated from the irradiation mixture by evaporating the solvent followed by chromatography of the residue.

Depending on the symmetry of the diaryl ethylenes, one to four cyclisation products may be formed. One of the most striking features of the formation of cyclic

aromatics by photocyclisation of 1,2-diarylethylenes is the selectivity of the reaction. For example, on irradiation of 2-styrylbenzo[*c*]phenanthrene (19), combination of a monocyclic aromatic component with a tetracyclic aromatic component, i.e. (4+1)- hexahelicene (1) is obtained in 85% yield, whereas no trace of benzo[*a*]naphtho[1,2-*h*]-anthracene (23) is formed.



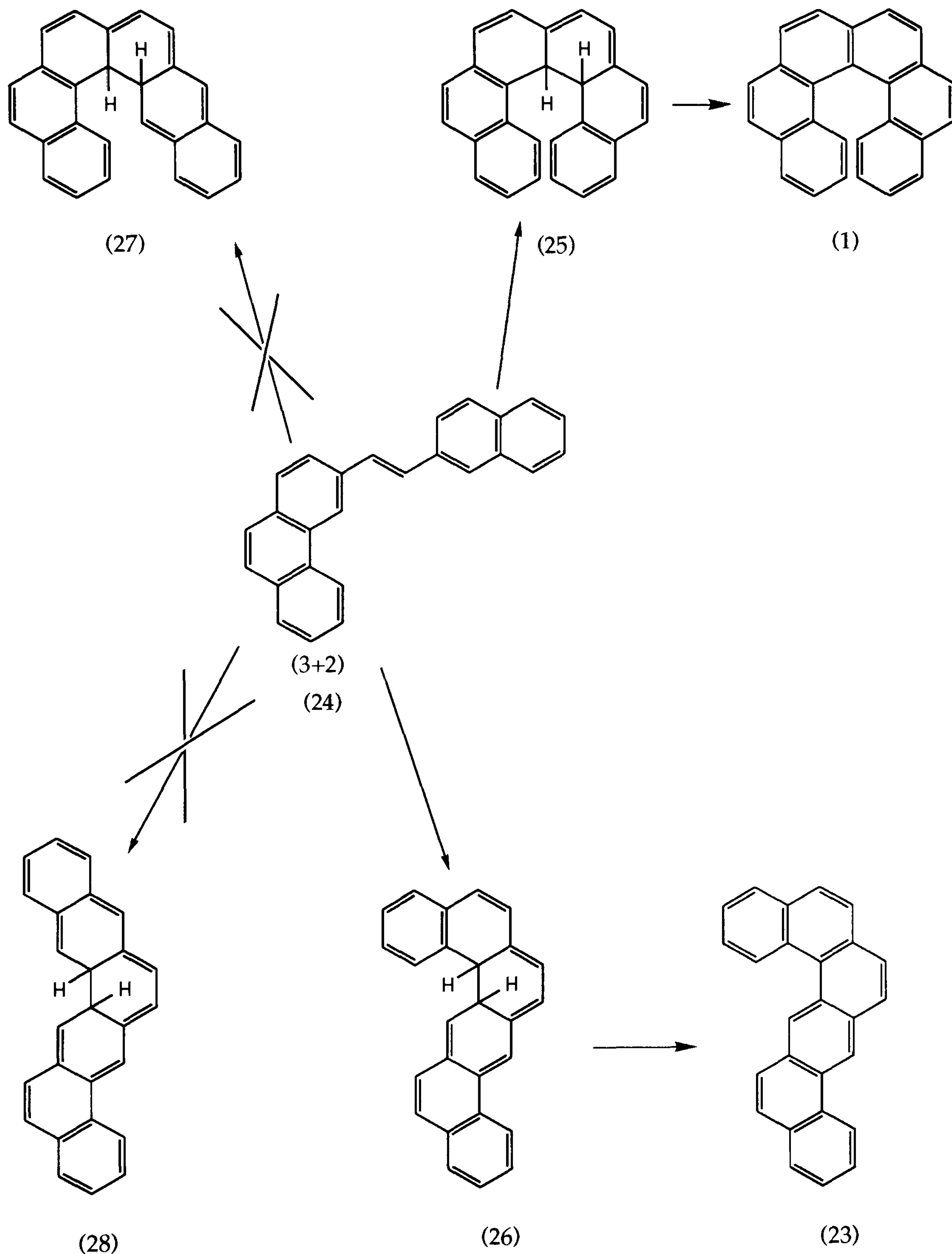
Scheme 6

The selectivity of this reaction is caused by the electron distribution in the excited diarylethylene (19).

When the 2-styrylbenzo[*c*]phenanthrene (19) is photocyclised under anaerobic conditions, a more stable dihydrohexahelicene is formed^[81] via

dihydrophenanthrene (DHP) (20) (Scheme 6) and whose structure was elucidated as trans-6a, 16d-dihydrohexahelicene (22). It is converted into (21), both thermally and photochemically.

However, the cyclisation of a (3+2) combination (24) gives mainly compound (23) (Scheme 7).

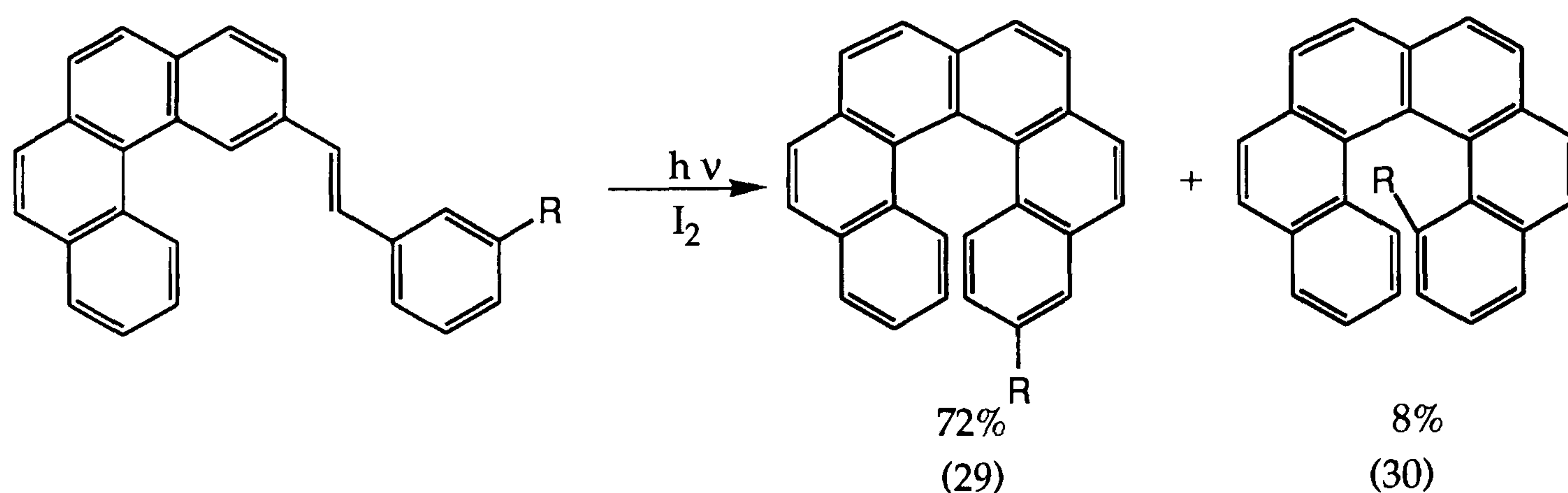


Scheme 7

A simple way to determine the preferred route for the photocyclisation of a diarylethylene is to count the number of benzene rings in the arylated dihydrophenanthrenes resulting from ring closure. From Scheme 7 it can be deduced that (25) with one benzene and one naphthalene ring is preferred to (26) (2 benzenes), to (27) (1 naphthalene) and (28) (1 benzene).

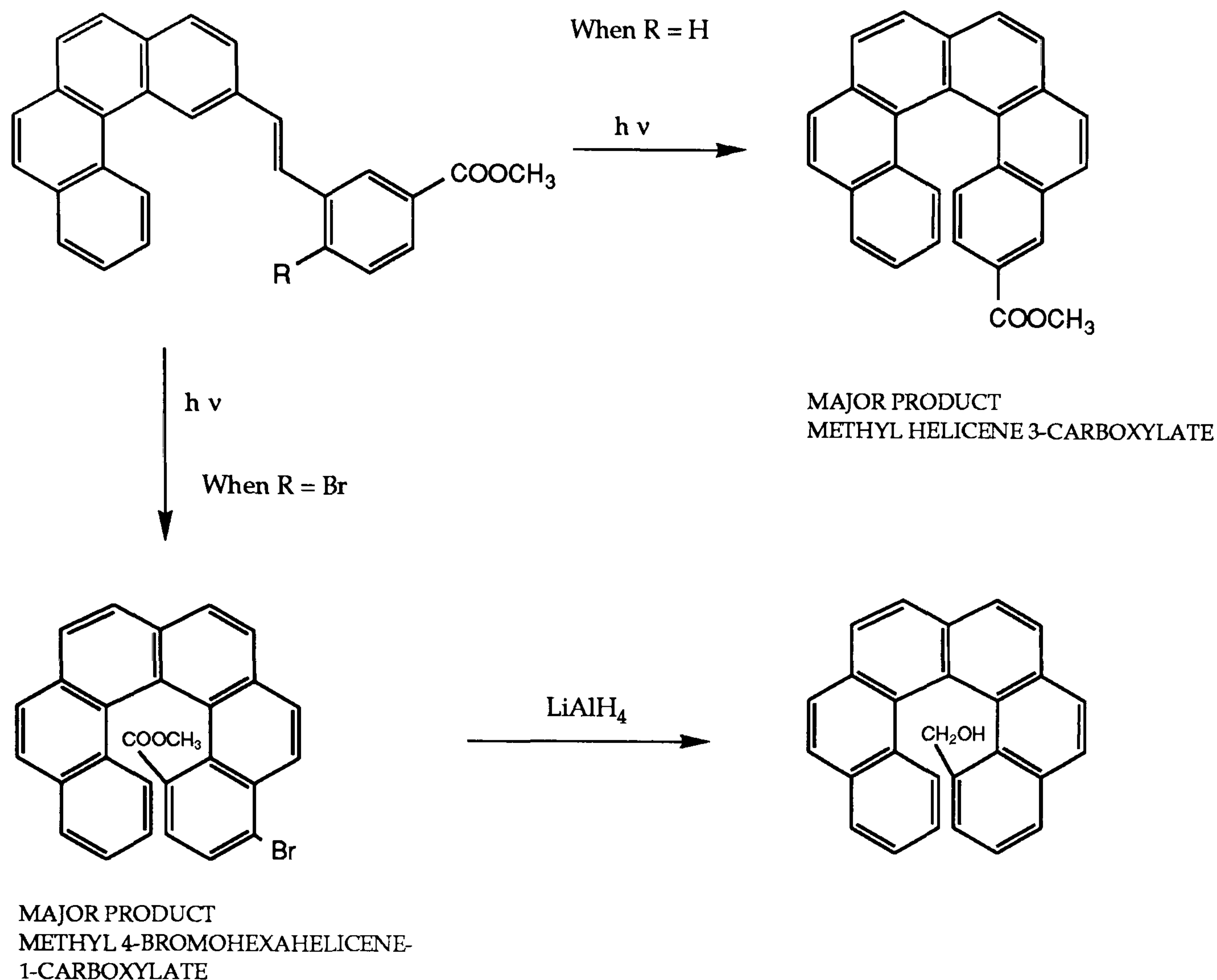
Substituted all-benzene-helicenes can be prepared without difficulty if the substituent is already present in the molecule to be cyclised. Exceptions are acetyl, dimethylamino and nitro groups [82], which enhance intersystem crossing to the triplet state. Addition of an acid, however, cancels the $n-\pi^*$ transition and can lead to normal photocyclisation products.

With a meta substituent in the styryl group, two helicenes are often formed, which are the 1- and 3- substituted isomers [83] (29 and 30) (Scheme 8). The formation of the product (29) can be either fully or largely prevented by the introduction of a bromine atom [75]. The ratio between the isomers depends on the lifetime of the corresponding DHPs and the rate of oxidation [84]. The amount of the 1-substituted product tends to be lower than that of the 3-substituted one because the lifetime of more hindered DHP is usually shorter.



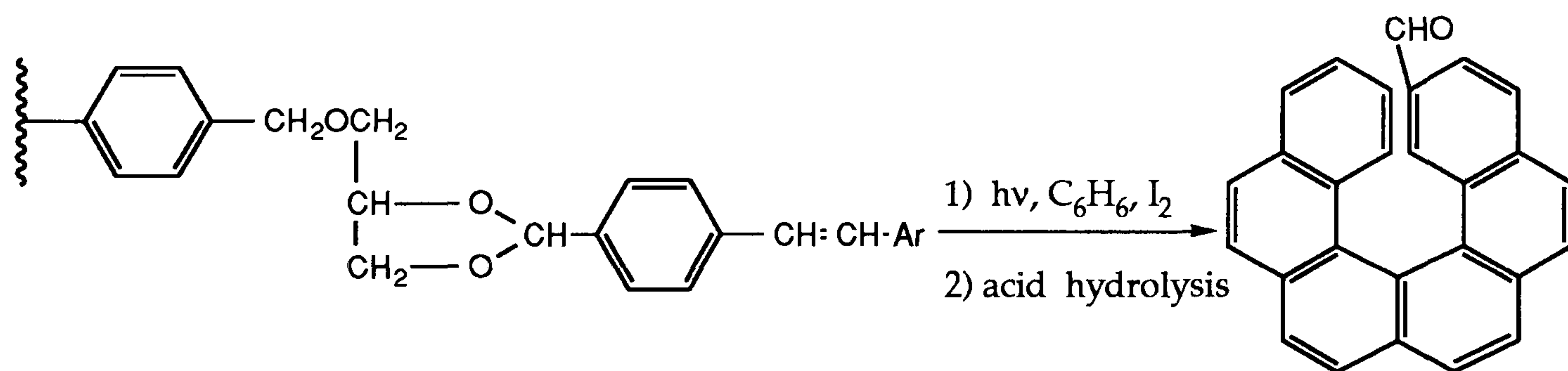
Scheme 8

To enhance the formation of the 1-substituted helicene, Martin [74] introduced an additional ortho-bromo substituent into the meta substituted styrylbenzo[c]phenanthrene (Scheme 9).

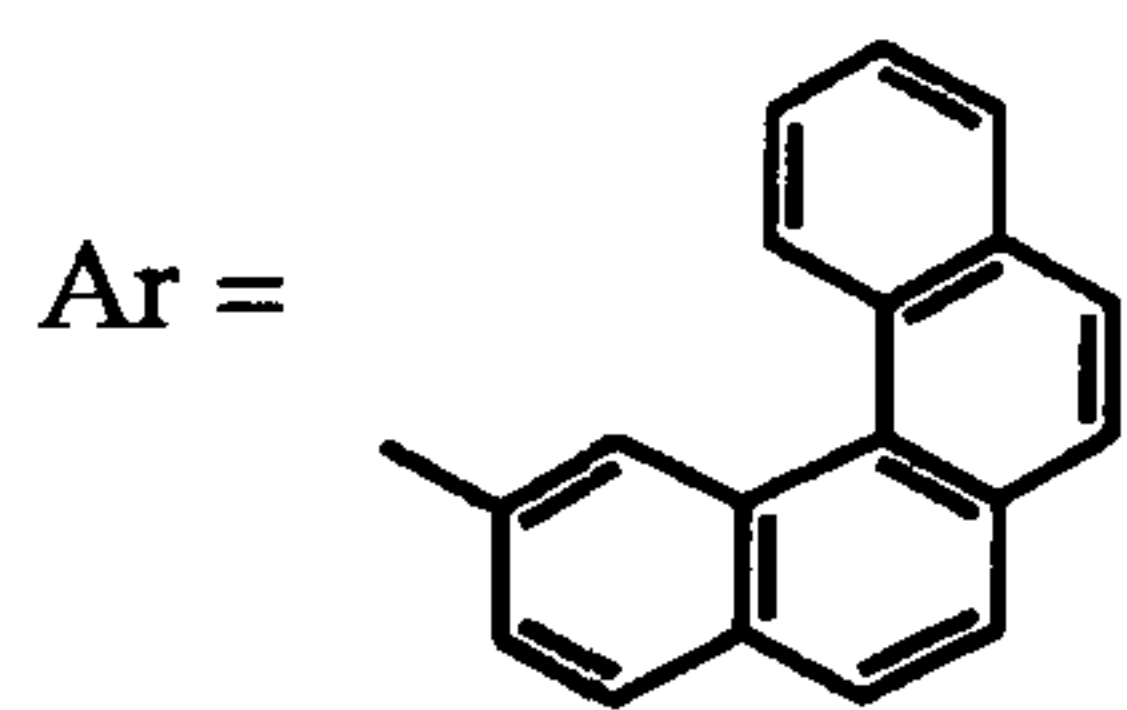


Scheme 9

Martin described the synthesis of hexahelicene-2-carbaldehyde by irradiation of a polymer-supported 1,2-diarylethylene containing a masked aldehyde function suspended in benzene for 4h [84]. After prolonged hydrolysis (60h), the product was obtained in low yield (5%).



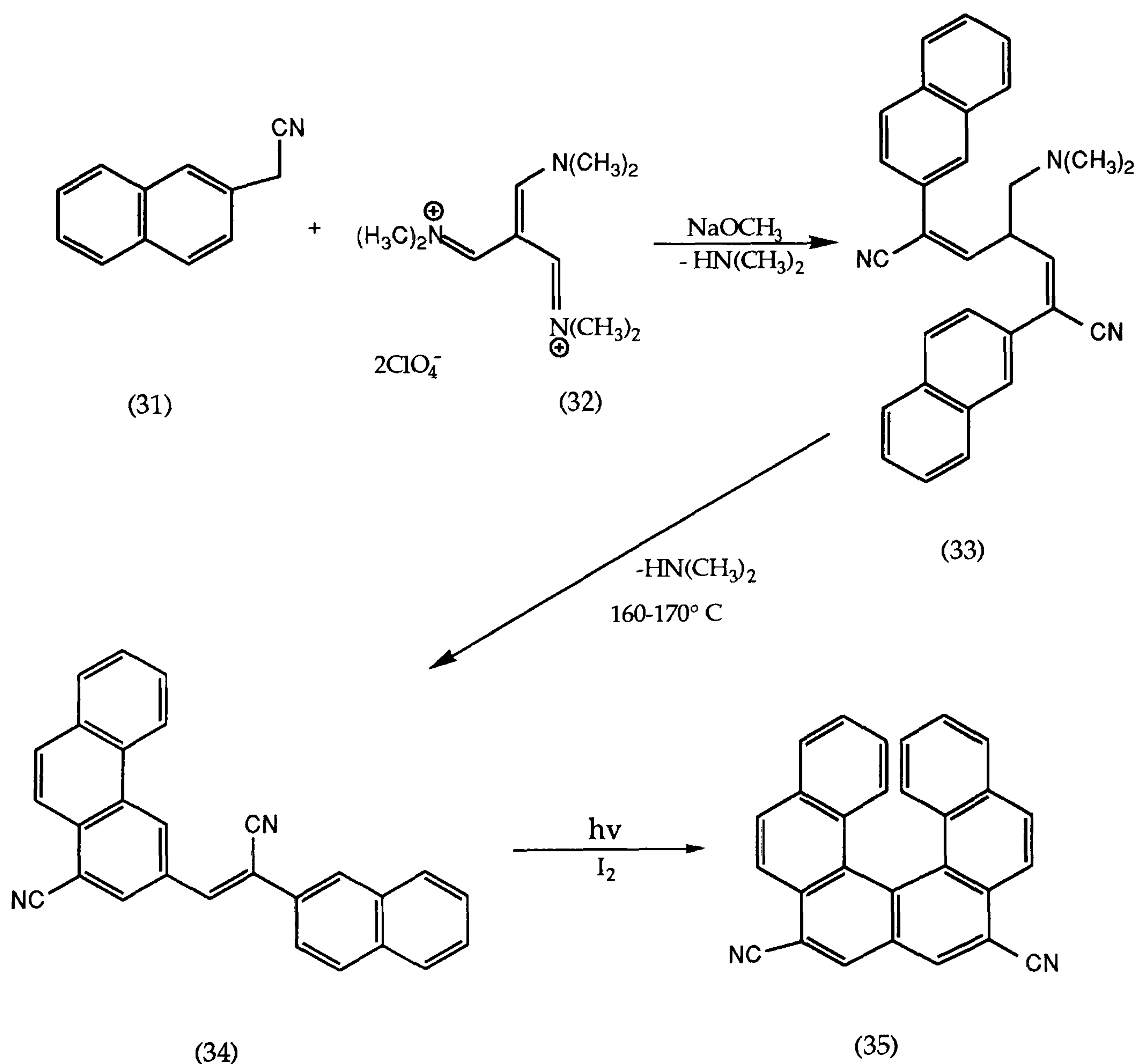
1-(4-formylphenyl)-2-(2-benzo[c]phenanthryl)ethylene



Scheme 10

A novel and simple synthesis of helicenes reported by Jutz^[85] (Scheme 11) started with the condensation of 2-naphthylmethylcyanide (31) and the bis-perchlorate salt of 2-dimethylamino-1,1-bis-(dimethyliminomethyl) ethylene (32) in the presence of a base (sodium methoxide). This gave 1,5-dicyano-1,5-dinaphthyl-3-(dimethylaminomethylene)-penta-1,4 diene (33), which on heating gave 1-(1'-cyanophenanthr-3'-yl)-2-naphthylacrylonitrile (34). Irradiation of a benzene solution of the latter compound in the presence of iodine and atmospheric oxygen for 3 hours gave 7,10-dicyanohexahelicene (35).

This method has a restricted applicability because of the poor availability of substituted 2-naphthylmethylcyanides.



Scheme 11

2.2.3- *Asymmetric Syntheses of Helicenes.*

Several studies have been undertaken to make optically active helicenes by means of an asymmetric photocyclization and can be categorised into four types:

2.2.3.1- *Asymmetric Synthesis with Circularly Polarised Light.*

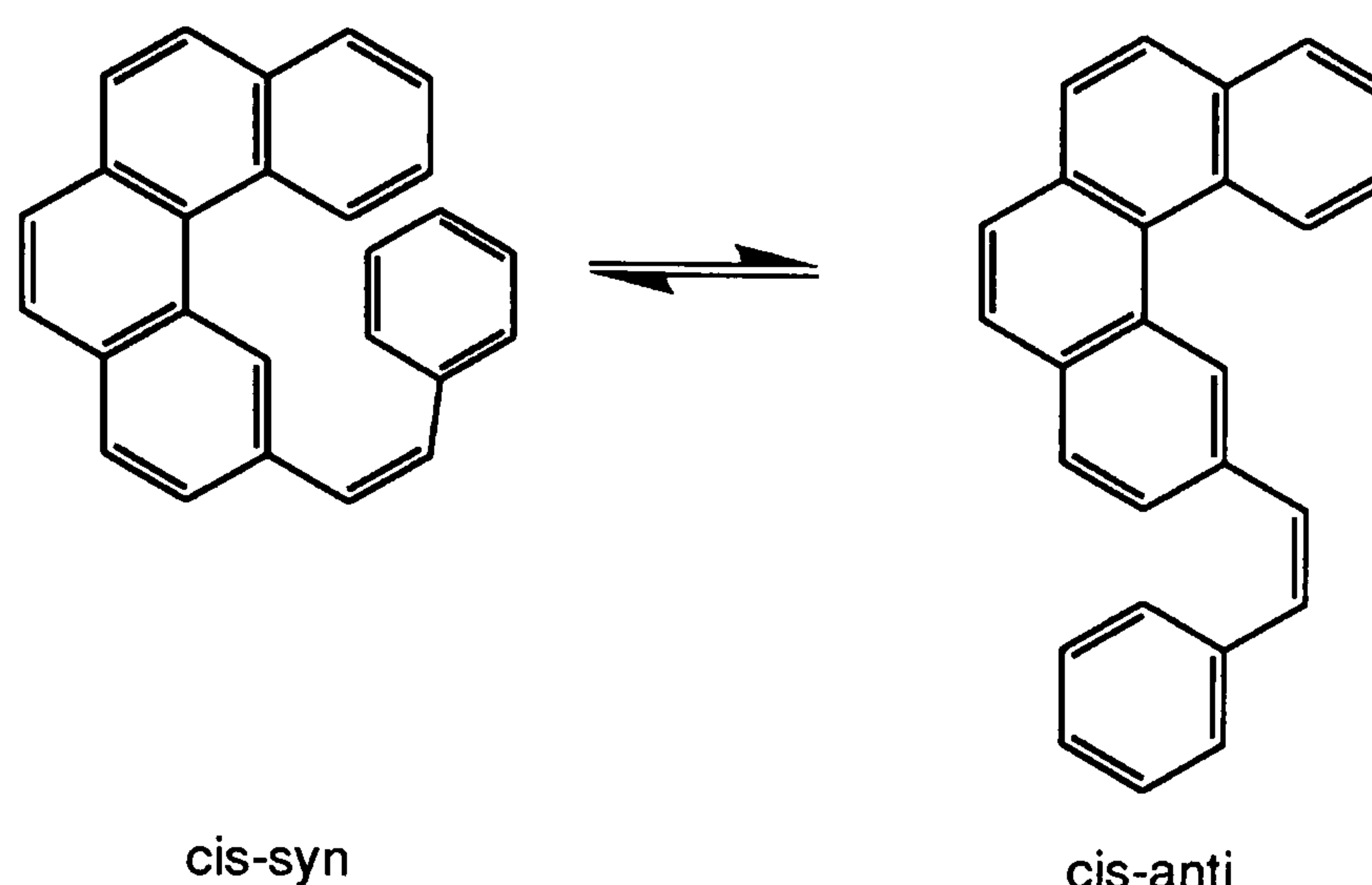
A century ago, Le Bel [86] pointed out that the use of (left or right-handed) circularly polarised light (CPL) must, in principle, induce "absolute asymmetric syntheses". A synthesis of optically active hexahelicene induced by circularly polarised light was achieved by Kagan [87], using the photocyclization of 1,2-diarylethylenes to dihydrohelicenes in the presence of oxidants such as I₂ and O₂. After purification by chromatography on alumina using n-hexane as an eluent, the optical rotations of the hexahelicene were directly measured without recrystallisation. The optical yields obtained (calculated by multiplying $[\alpha]$ of product / $[\alpha]$ of pure enantiomer by a factor 100) were low (0.20%). They depend on the precursor and the wavelength used. In all cases studied, right-handed CPL forms M-helicenes and left-handed CPL affords P-helicenes [88].

The most probable mechanism is that the diarylethelene precursors, which exist as a mixture of P- and M-forms in rapid equilibrium at room temperature, give rise when irradiated with circular light to different amounts of excited P- and M-forms.

2.2.3.2- *Asymmetric Synthesis in Chiral Solvents.*

Laarhoven *et al.* [89] have studied the influence of chiral solvents ((+)- α -pinene, (S)-(-)-ethyl lactate, etc.) on the optical yield of hexahelicene, synthesised by the photodehydrocyclisation of various precursors. Irradiation of 2-styrylbenzo[c]phenanthrene (19) in eleven different chiral solvents gave rise to the formation of non-racemic hexahelicene with optical yields ranging from 0.2-2.0%. It was not necessary to use the pure *cis*-isomer as the starting compound because it is known that *trans-cis*-isomerisation is a much more efficient process than photodehydrocyclisation. The *cis*-2-styrylbenzo[c]phenanthrene, which contains a

large aryl residue, has a preference for the cis-syn-conformation over the cis-anti-conformation (Scheme 12).



Scheme 12

This conformational equilibrium depends not only on the nature of the aryl residue but also on the temperature and the solvent used. As the cis-syn conformation exists in two enantiomeric forms (P and M) [90] it was anticipated that the equilibria between the cis-anti-form and the P and M conformations of the cis-syn-form should be influenced differently by a chiral solvent, leading to a higher equilibrium concentration for one of the enantiomeric forms.

Experiments with chiral solvents showed that dilution leads to a proportional decrease of the specific rotation of the isolated product. Experiments at higher temperatures revealed that the optical yield under these circumstances is lower than at room temperature. This can be explained by the lower preference of cis-2-styrylbenzo [c]phenanthrene at higher temperatures for the cis-syn conformation [91] which results in diminished stereospecific interaction of the substrate with the solvent molecules.

The magnitude and sign of the optical rotation is not directly related to the absolute configuration of the solvent used but seems to depend on the size and position of the largest apolar hydrocarbon residues at the chiral centre in the solvent molecules. It was concluded that the solvent behaves as a matrix in which the photoreaction takes places [92].

2.2.3.3- *Asymmetric Synthesis in Cholesteric Liquid Crystals.*

It is well established that a cholesteric liquid crystal used as a solvent for a chemical reaction can induce asymmetric synthesis [93].

Macroscopic helical structures formed by cholesteric liquid crystals such as cholesteryl benzoate and cholesteryl chloride have been used as chiral helical media for the asymmetric synthesis of helicenes. Several cholesteric mesophases were studied and the experiments indicated that a cholesteric mesophase with a right-handed helix gave an excess of (+)-hexahelicene, which is known to have also a right-handed helix, whereas a left-handed helix yielded mainly (-)-hexahelicene. Optical yields were low [94].

2.2.3.4- *Chemically Induced Asymmetric Synthesis .*

There is a stereoselectivity in the formation of helicene skeletons by photocyclodehydrogenation of 1,2-diarylethylenes carrying a chiral group [95].

In order to study the influence of a given chiral group at different positions in the precursor styrylbenzo[c]phenanthrenes (19), several carboxy styrylbenzo[c]phenanthrenes were synthesised and esterified with chiral alcohols and the products were used to prepare hexahelicenes substituted at various positions. The results showed an appreciable enantiomeric excess for 1-substituted helicene, a lower excess for 5-substituted and low selectivity of 2-3-or 4- substituted products.

The chemically induced asymmetric photocyclisation of the 1-(-)-menthyl ester showed a striking temperature dependence [96], a reversed ratio being obtained at low temperature $-78^{\circ} = (+) 98 : (-) 2$ compared with the result at higher temperature $+80^{\circ} = (+) 20 : (-) 80$.

2.2.4- Problems in the Synthesis of Helicenes.

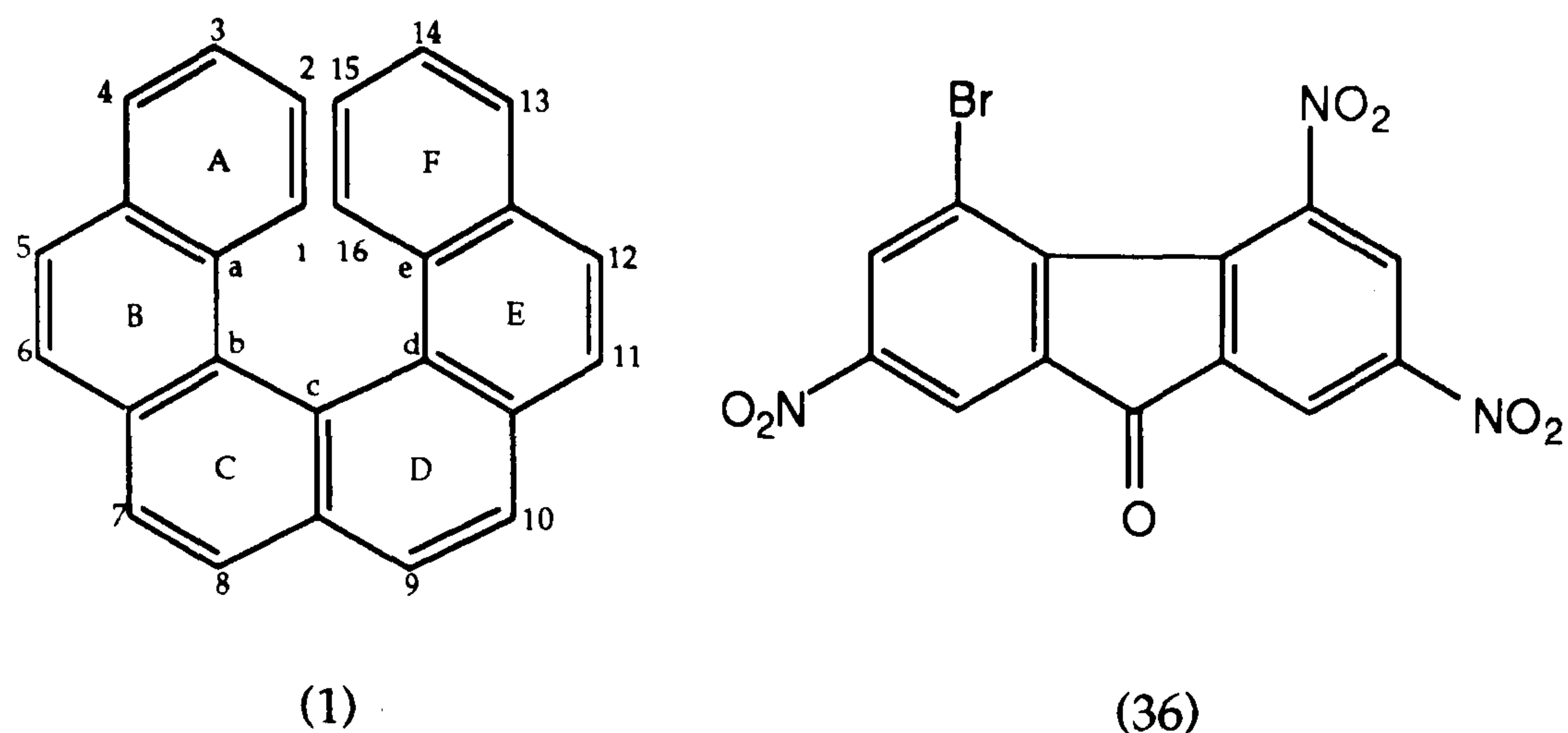
The original Newman synthesis is long and laborious, but has the advantage that it can be carried out on a large scale.

Later, photochemical syntheses appear to be much more attractive, being generally short and high-yielding. However, this advantage is deceptive because, in practice, the photochemical routes all involve intermediates with low solubilities and must be carried out at dilutions of a few milligrams per litre. Consequently, they are totally unsuitable to the preparation of multi-gram quantities of helicenes under laboratory conditions. They are also more restricted in the range of functionalities that can be tolerated for the construction of substituted helicenes once the latter may be formed as mixtures of regioisomers which are difficult to separate.

2.3- Structural Determinations.

The structural determination of the all-benzene-helicenes is of crucial importance because the photocyclisation of 1,2-diarylethylenes and bis (arylviny) arenes leading to the helicenes can give rise to isomeric polycyclic aromatic systems. In most cases, the chemical structure of the cyclised products can be deduced from the study of their UV, NMR and mass spectra. Other techniques have been applied with success, e.g. X-ray diffraction, independent syntheses, specific deuterium labelling [97], gas liquid chromatography (GLC)[98] and the study of chiroptical properties.

When hexahelicene (1) became known in 1955 its structure determination by X-ray was impossible because of the absence of a heavy atom, necessary to determine phases. The first X-ray structure determination of hexahelicene was reported in 1969 for a complex of the compound with 4-bromo-2,5,7-trinitrofluorenone (36) [99] and in 1973 the structure of hexahelicene itself and some derivatives of helicene were reported [100].



The crystals of the complex 1:1 hexahelicene:4-bromo-2,5,7-trinitrofluorenone are triclinic with four molecules of $C_{26}H_{16} : C_{13}H_4O_7N_3Br$ in a unit where two of the four hexahelicene molecules are the left-handed enantiomer and two are right-handed. Each 4-bromo-2,5,7-trinitrofluorenone molecule (36) is sandwiched between two helicene molecules with the three rings of the former compound lying nearly parallel to rings A,B,C and rings D,E,F of the two adjacent hexahelicene molecules at approximately Van der Waals separation distances.

Helicenes have a two-fold symmetric axis, C_2 , perpendicular to the cylindrical helix. Hexahelicene can be roughly depicted by three planes (Fig. 37).

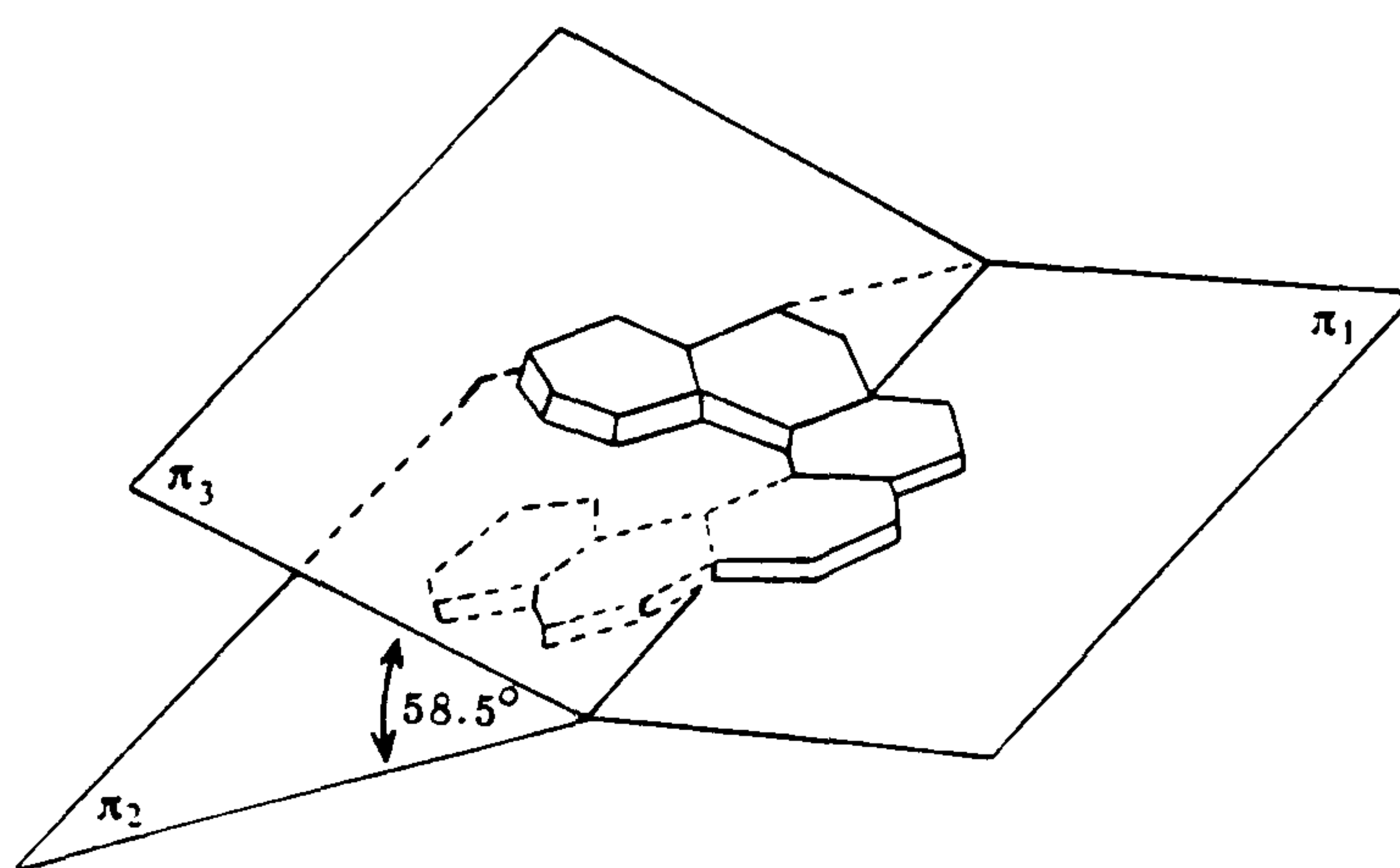


Fig. 37

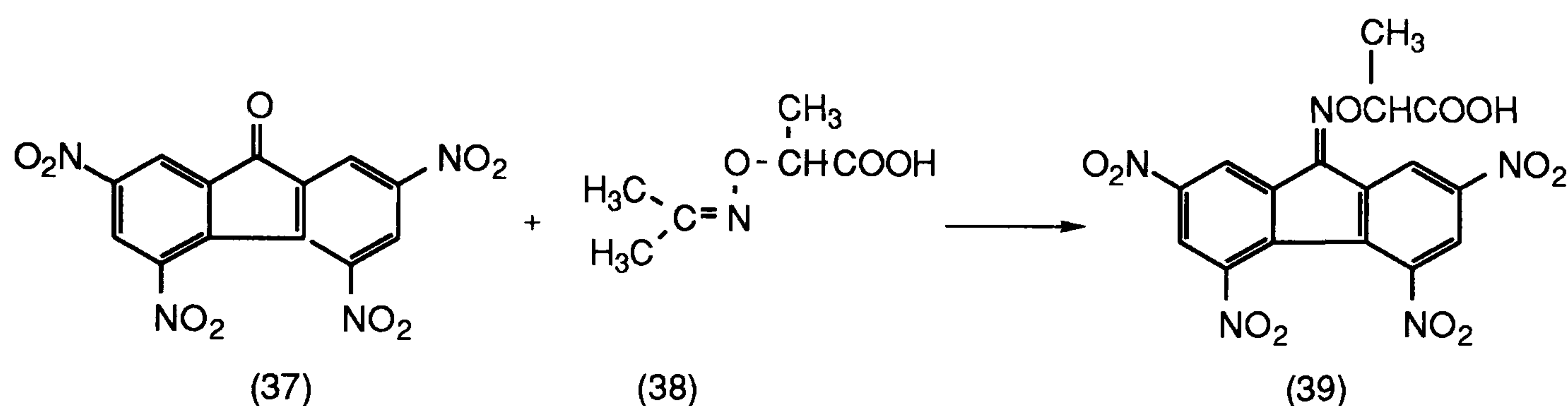
The interplanar angle between the terminal rings is 58.5° . The distance between the closest pair of non-bonded carbon atoms (1 and 16) is 3.05\AA [101]. The outer bonds $C_{(13)}-C_{(14)}$, $C_{(1)}-C_{(2)}$, $C_{(3)}-C_{(4)}$, $C_{(11)}-C_{(12)}$ and $C_{(9)}-C_{(10)}$ are larger than the average benzene length (1.746, 1.764, 1.764, 1.710, 1.710) respectively compared with benzene 1.667 [102].

2.4- Chiroptical Properties

The helicenes are chiral molecules with an inherently dissymmetric chromophore (i.e. the chromophore itself contains the asymmetry).

2.4.1- Optical Resolution.

a) The first experimental proof of the chirality of a helicene was given by Newman, Lutz and Lednicer in 1955 [103]. They resolved hexahelicene by crystallisation with an optically active π -acid complexing agent, 2-(2,4,5,7-tetranitro-9-fluorenylideneaminoxy) propionic acid (TAPA) (39) prepared from 2,4,5,7-tetranitrofluorenone (37) and (-) + (+) (isopropylideneamino-oxy) propionic acid (38), in the presence of p-toluenesulfonic acid.



Scheme 13

TAPA is a chiral charge-transfer complexing agent (CT) in which the strong electron-accepting tetranitrofluorenylidene moiety provides the binding power and the oxypropionic acid moiety provides the chiral selectivity for electron-donating helicenes.

(R)- and (S)- TAPA form diastereomeric charge-transfer complexes with the enantiomers of hexahelicene. d,l-Hexahelicene and the π -acid TAPA, both pale yellow, form a deep red complex in benzene solution. When a hot concentrated benzene solution of hexahelicene with one-half equivalents of (-)-TAPA was treated with a controlled amount of ethanol, yellow plates of partially resolved (-) hexahelicene separated.

Repetition of the process and recrystallisation afforded pure (-)-hexahelicene $[\alpha]_{24}^{\circ} = -3640^{\circ}$ and with (+)-TAPA afforded (+)-hexahelicene $[\alpha]_{24}^{\circ} = +3707^{\circ}$. Several other helicenes could also be resolved using this reagent.

Resolution of helicenes has also been performed by the laborious method of picking single crystals until no more variations in optical rotation occur. The crystal picking method is not always applicable because some helicenes crystallise into racemic crystals by lamellar intergrowth of pure M and P forms.

b) Conventional chromatography on a silica gel column coated with a chemically bonded layer containing optically active TAPA (Fig. 38) results in partial resolution of some helicenes, but this technique is slow and inefficient [104].

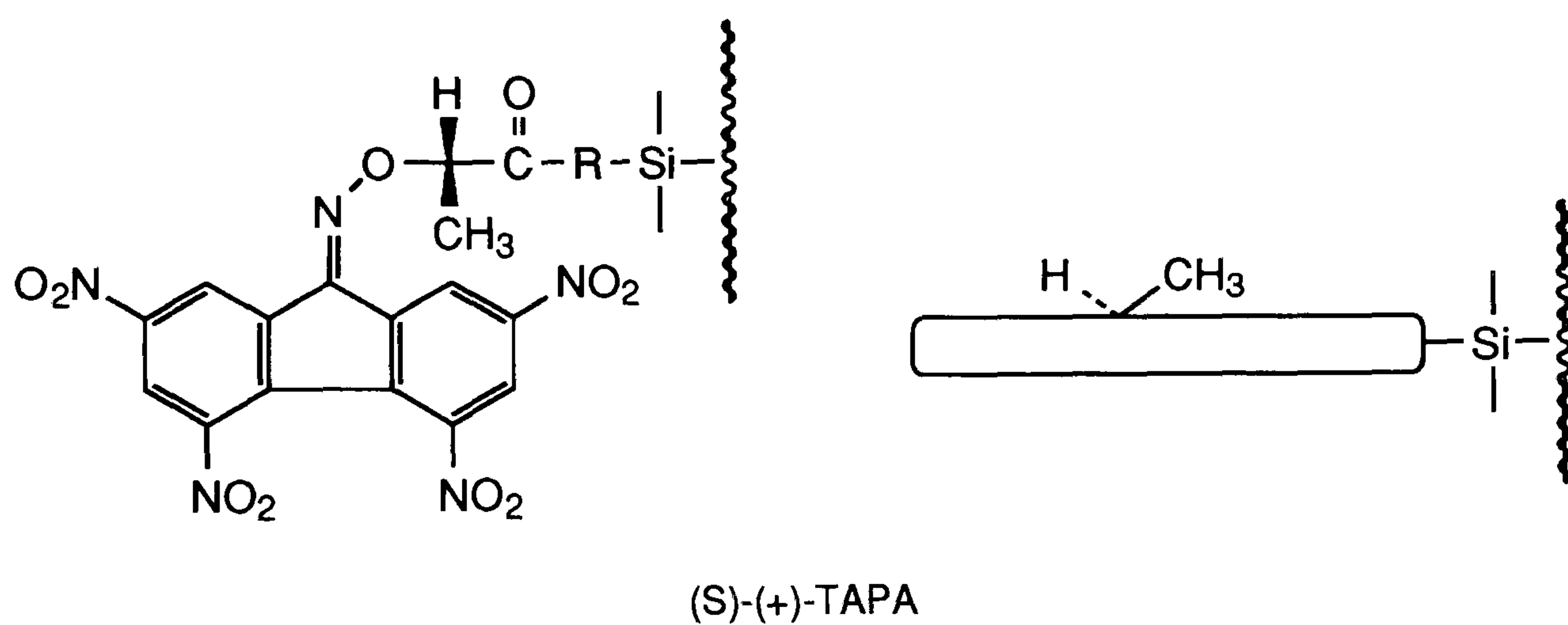
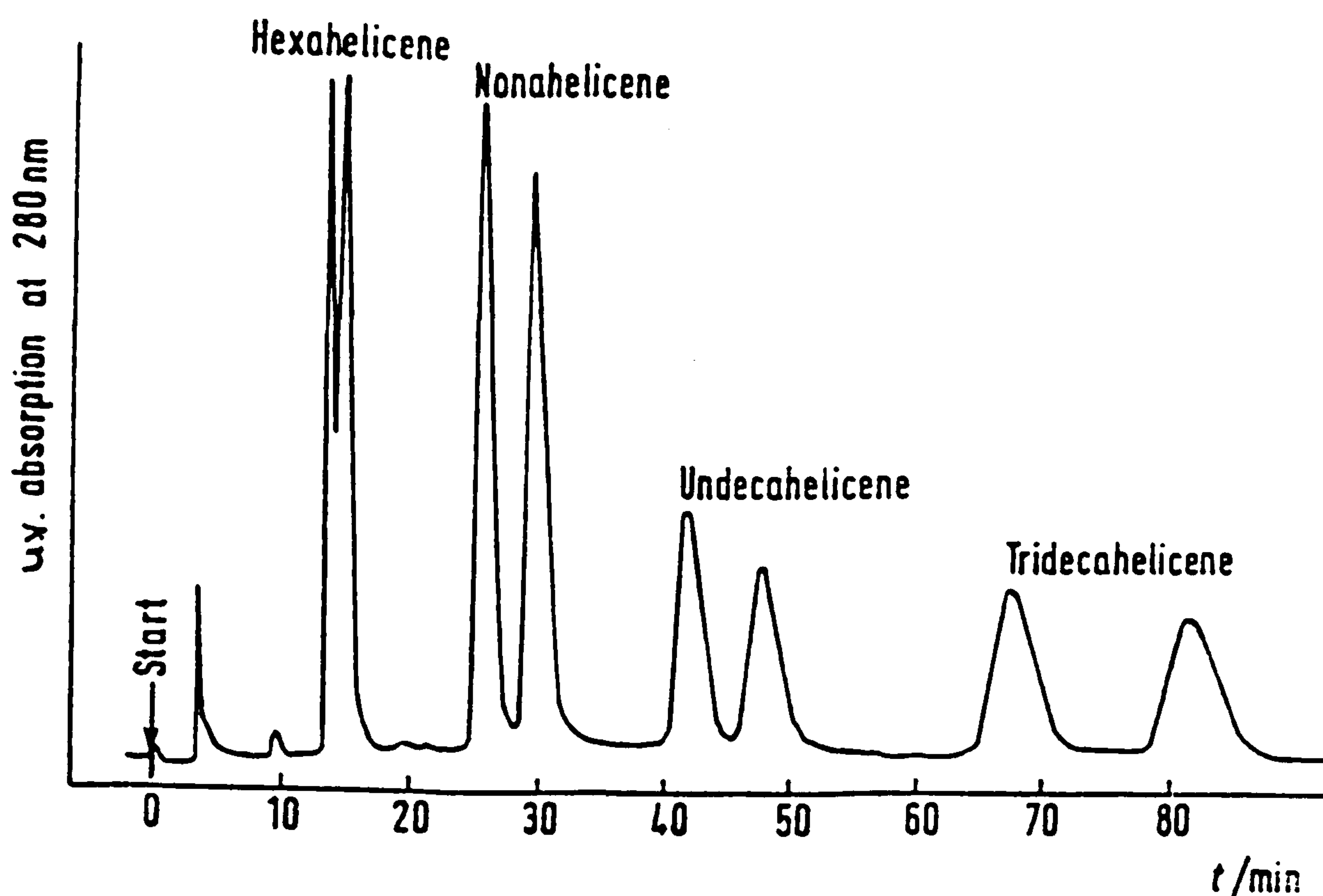


Fig. 38

Resolution has also been achieved by employing columns of optically active stationary phases as such triacetylcellulose [105] and (1)-poly-(triphenyl methyl methacrylate [106] coated on APS-silica.

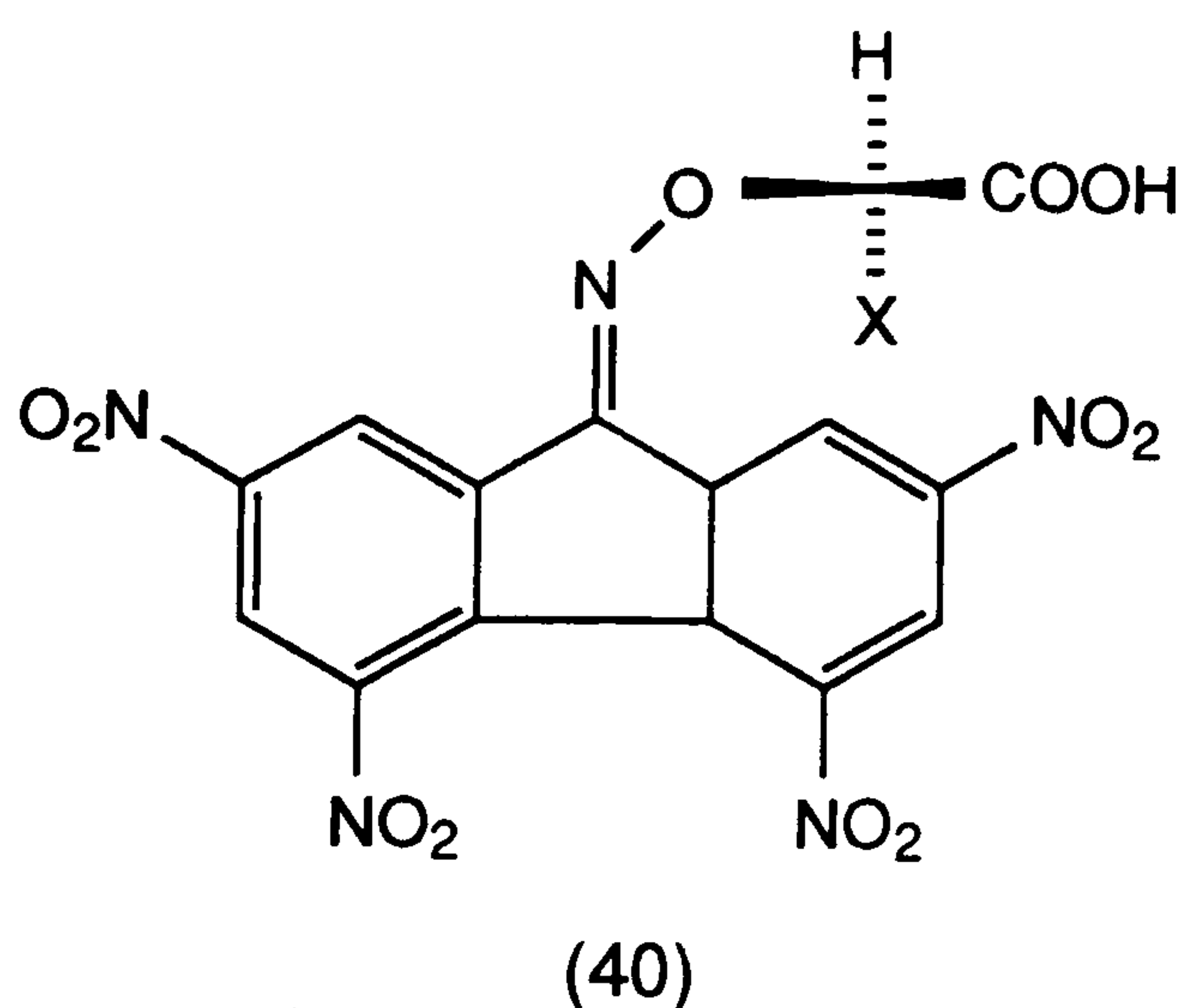
c) High Performance Liquid Chromatography (HPLC) on columns coated with, or bonded to, TAPA can resolve helicenes completely. For example hexa-, nona-, undeca- and tridecahelicenes are resolved with cyclohexane- dichloromethane as the mobile phase (Fig. 39) [107]. The method is not only more efficient but also less time consuming than those given above.



Column: 7 μ m silica coated "in situ" with 10-25% (+)-TAPA
 Mobile phase: cyclohexane-dichloromethane

Fig. 39

TAPA has a better resolving capacity than derivatives containing larger alkyl groups at the chiral center ((40): TABA, TAIVA, TAHA).

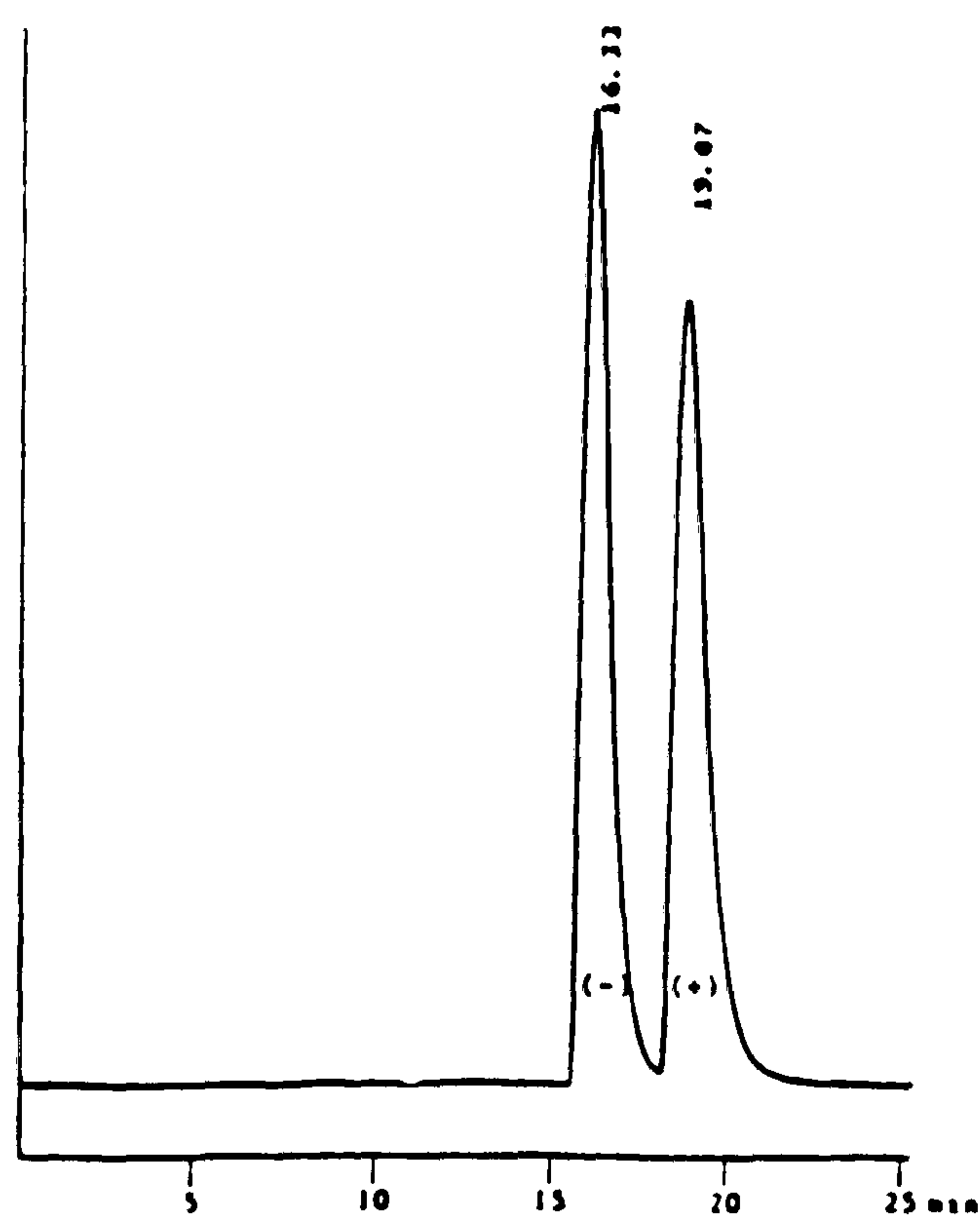


- | | | |
|--------|---|---------------|
| X=Me | : | (R)-(-)-TAPA |
| X=Et | : | (R)-(-)-TABA |
| X=i-Pr | : | (R)-(-)-TAIVA |
| X=n-Bu | : | (R)-(-)-TAHA |

The bulkiness of the group of the charge-transfer- π -acceptor is crucial for the ease of resolution of the helicenes [108]. It was supposed that the alkyl group should be small enough to fit in the central hole of helicene, but large enough to discriminate between M- and P- helicene. The larger the alkyl group the more

difficult it would be to be accommodated. This model was considered to explain the observed decreased of resolution factors with increasing bulk of the alkyl substituent in the HPLC phase.

The charge-transfer-complex of P(+)-hexahelicene and (R)-(-)-TAPA is more stable than the complex of M(-)-hexahelicene and (R)-(-)-TAPA which agrees with the observation that P(+)-helicenes are retained longer on columns with (R)-(-)-TAPA than M(-)-helicenes (Fig. 40). Decreasing the temperature was found to improve resolution [109].



Column: 15mg of R(-)-TAPA coated on 1g of LiChrosorb Si 250 x 4.6 mm
Mobile phase: 5% diethyl ether-light petroleum (bp, 60-80°C)

Fig. 40

Some of the helicenes have been resolved with stationary phases containing a variety of charge transfer materials [110] such as:

Binaphthyl-2,2'-diyl Hydrogen Phosphate (BPA) [111]

The cyclic atropisomeric selector binaphthyl-2,2'-diyl hydrogen phosphate was able to resolve helicenes, especially those containing heteroatoms or electron-donating substituents.

The binaphthyl unit of BPA together with its 7-membered phosphadioxepin ring (which helps to fix the spatial conformation of its binaphthyl planes) form a pentahelicene-like configuration (Fig. 41).

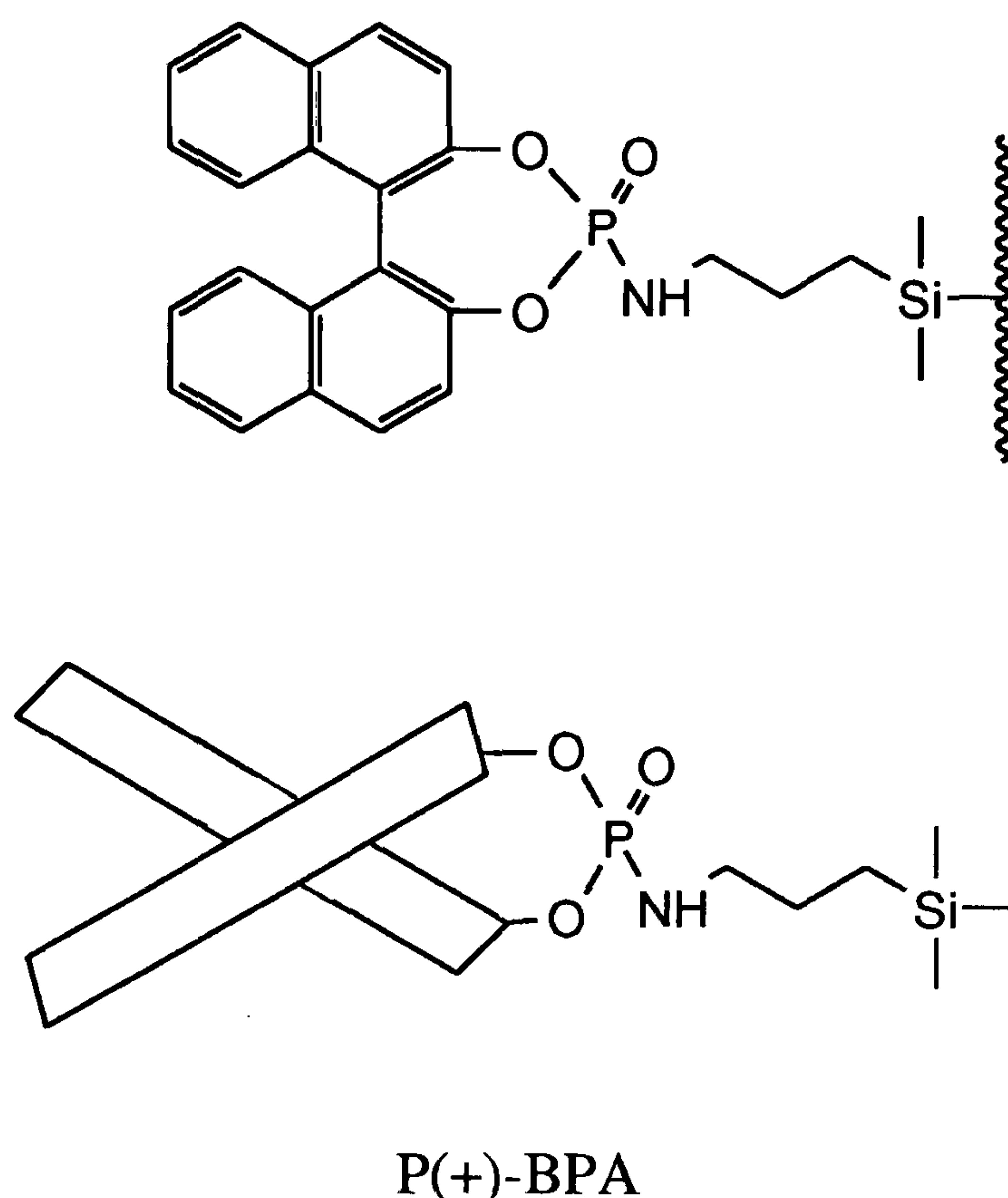


Fig. 41

A silica column physically-coated with BPA gave slightly higher separation factors for diaza and brominated helicenes than did a BPA bonded phase linked through an aminopropyl spacer. However using longer spacer, $\text{NH}(\text{CH}_2)_2\text{NH}(\text{CH}_2)_3\text{Si}$, between the silica gel and BPA gave worse resolution.

BPA covalently bonded to silica gel showed poor resolution for carbohelicenes, only the hepta-, trideca- and tetradeca-carbohelicenes showed a small degree of resolution at room temperature. By decreasing the column temperature to 2°C , resolutions were observed for the hexa-, octa- and nonacarbohelicenes and also an increase in the resolution factors for those previously resolved at higher temperatures. The poor resolution of carbohelicenes indicates that this selector forms only a weak charge-transfer complex.

Better resolutions were obtained with 8,20-dibromodiphenanthro [4,3-a;4',3'-j] chrysene (41) and 9,10-diaza [7,8;11,12]dibenzoheptahelicene (42) [112]. These observations suggested that increasing the electron-donating

capacity of the helicenes results in a stronger interaction with BPA and would lead to their better resolution. Hexahelicene was therefore brominated to give the dibromo hexahelicene^[113] (43) (the position of the bromine atoms has not been determined). While capacity factors increased with stronger charge transfer complexation, separation factors were lower (Fig. 42).

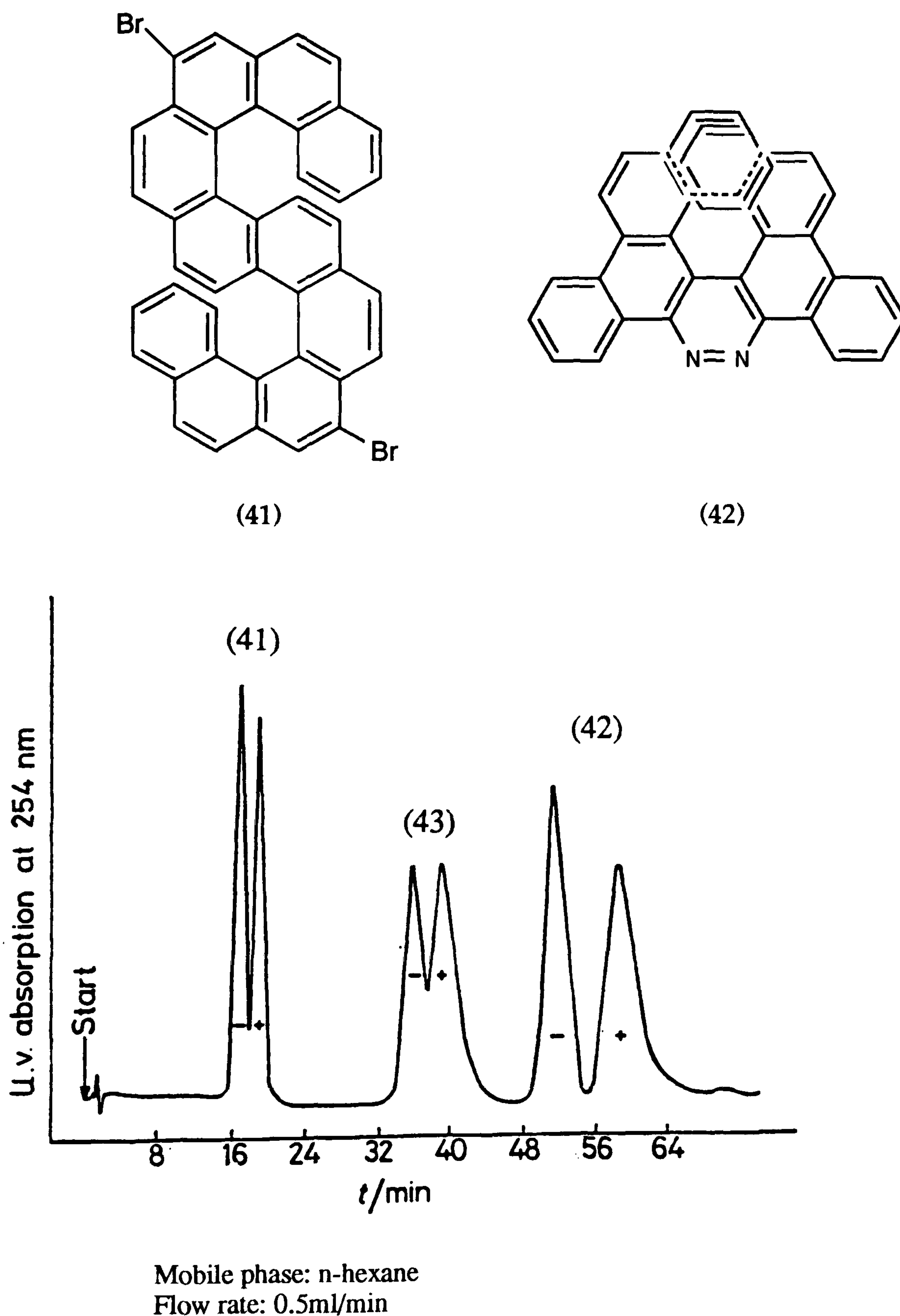


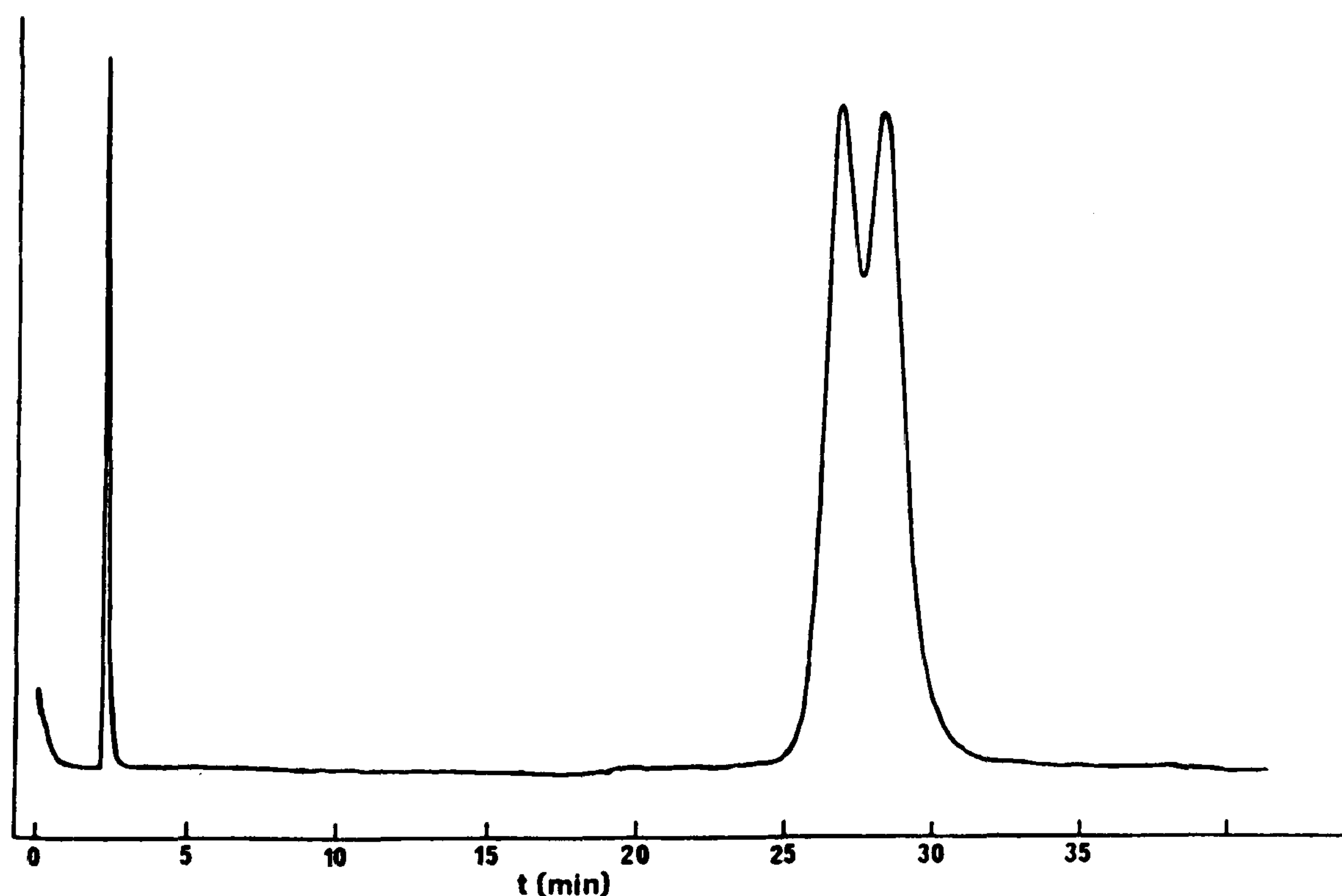
Fig. 42 Resolution of helicenes containing heteroatoms or electron donating substituents on a linked P(+) BPA column

The M(-) helicene enantiomer always precedes the P (+) isomer on a P(+)-BPA column.

Dinitrophenyl- α -aminoamide [114]

The aminopropyl function has been used as a linkage unit for the preparation of surface-modified silicas. When N-2,4-dinitrophenyl-L-phenylalanine was bonded to APS, the resulting chiral phase was shown to resolve 7,10-dicyanohexahelicene[115].

Lochmuller and Ryall [116] reported the synthesis of a chiral bonded phase based on alanine. The phase was prepared by linkage of N-tert-butyloxycarbonyl-L-alanine to aminopropyl silica using 1-ethyl-3,3-dimethylaminopropyl-carbodiimide, removal of the protecting group with trifluoroacetic acid and derivatisation of the amino group with 2,4-dinitrobenzene. The phase was successfully applied to the resolution of heptahelicene and of 1-aza-hexahelicene (Fig. 43). Lochmuller and Ryall suggested that such enantiomeric recognition and resolution may need only one significantly strong interaction (such as the site of charge-transfer) near a center of asymmetry.

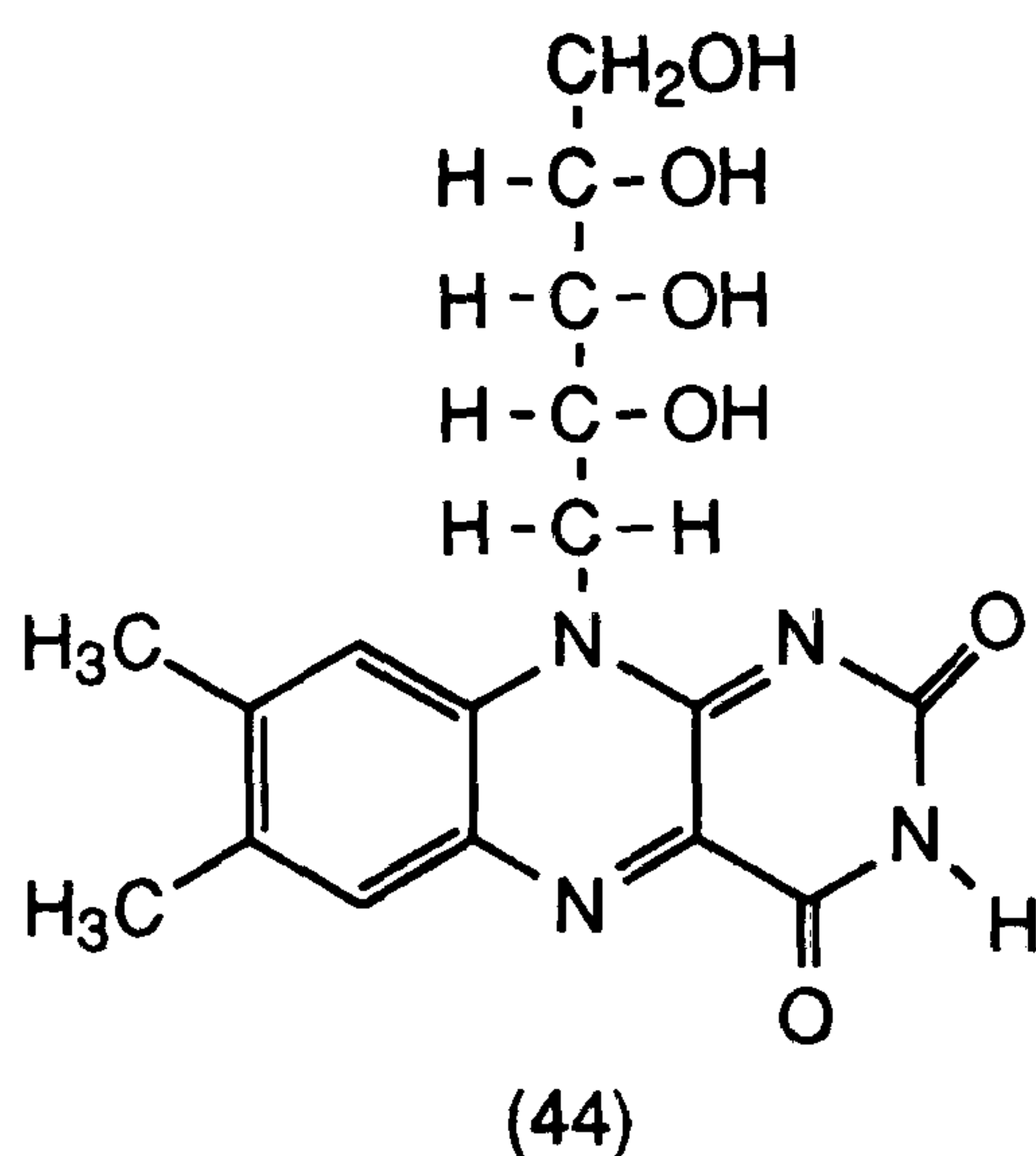


Mobile phase: 1.5% acetonitrile in isooctane
Flow rate: 0.90 ml/min
Detector: UV at 254nm, 0.02 a.u.f.s

Fig. 43 Resolution of 1-aza-hexahelicene ion using two 240 x 2.1mm columns connected in series.

Riboflavin [117]

This was chosen, in part, because of its general structural similarities to TAPA. Both compounds have a tricyclic system known to form charge-transfer complexes with various compounds and both have a side chain attached to the central ring and contain at least one asymmetric centre (44).



Dichloromethane/n-hexane was used in various proportions as the eluent and two different percentage coatings of riboflavin on the silica gel were tested. The order of emergence was the reverse of that found on (R)-(-)-TAPA [108] as P(+) precedes the M(-)-isomer. There was a tendency for the retention times and resolution factors of helicenes to increase with the number of rings, as shown in Fig. 44.

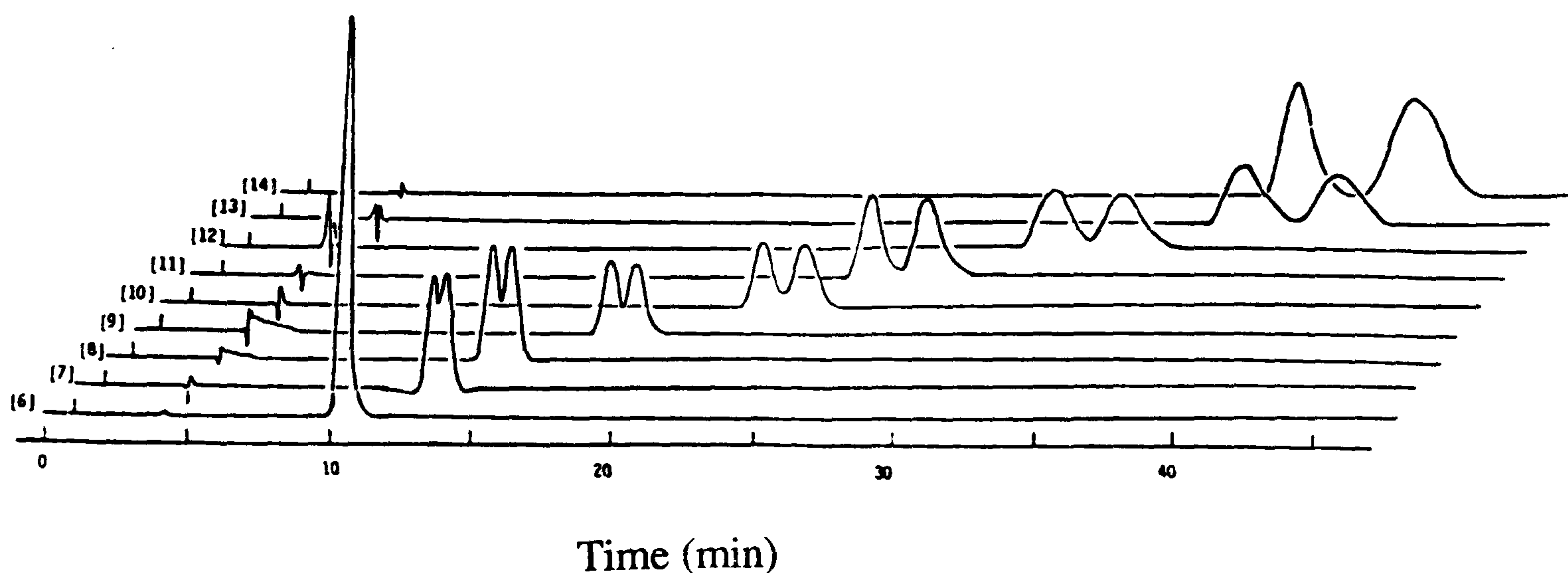
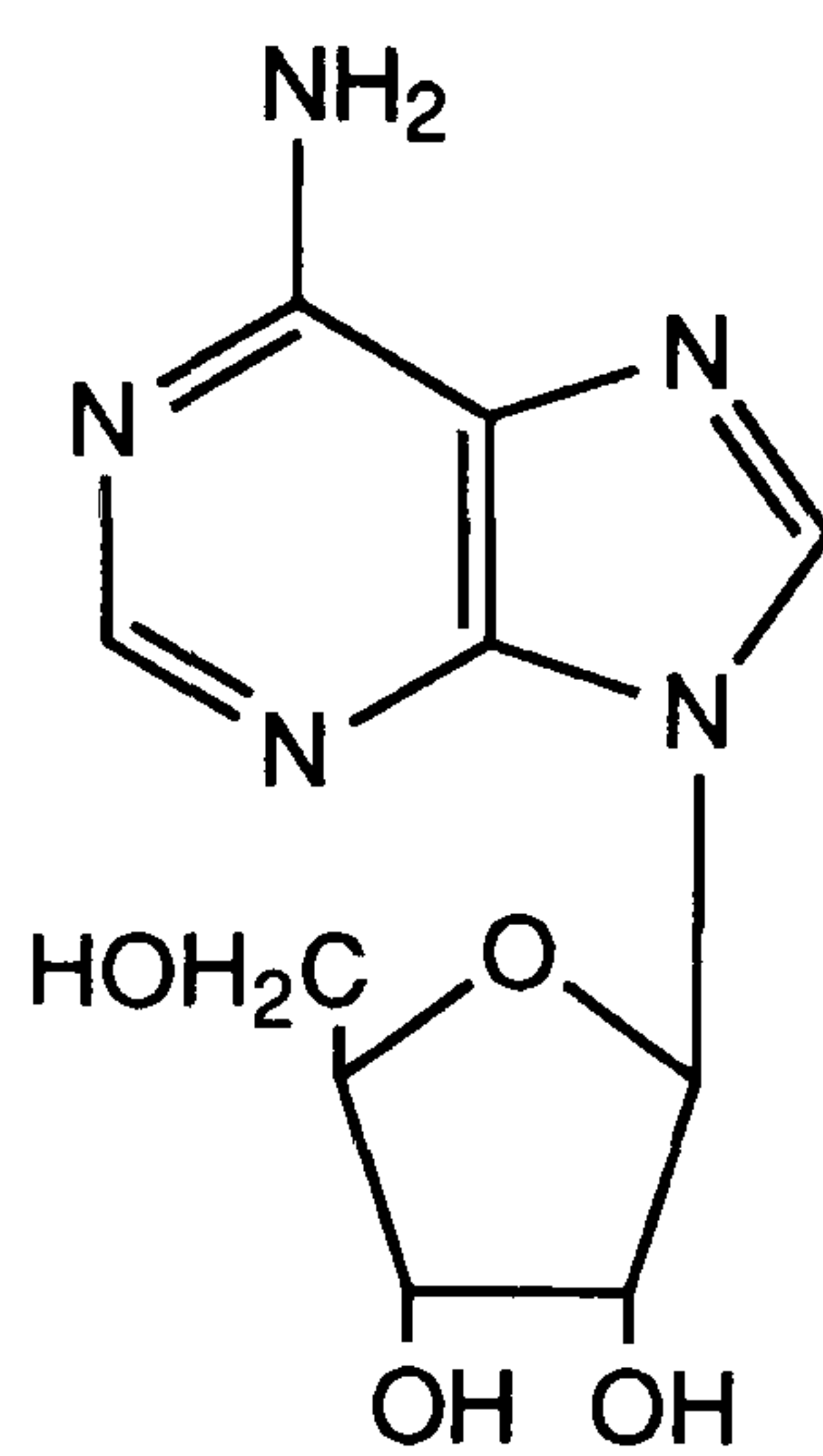


Fig. 44 Resolution of carbohelicenes by high-pressure liquid chromatography on silica gel coated with riboflavin.

Nucleosides and nucleotides [118]

Purines and pyrimidines form complexes with polyaromatic hydrocarbons. Combination of a purine or pyrimidine with a chiral substituent such as ribose could lead to chiral differentiation of optically active polyaromatic hydrocarbons. Kim and co-workers [118] have studied chiral recognition of helicenes by nucleosides and nucleotides coated on silica gel HPLC columns.



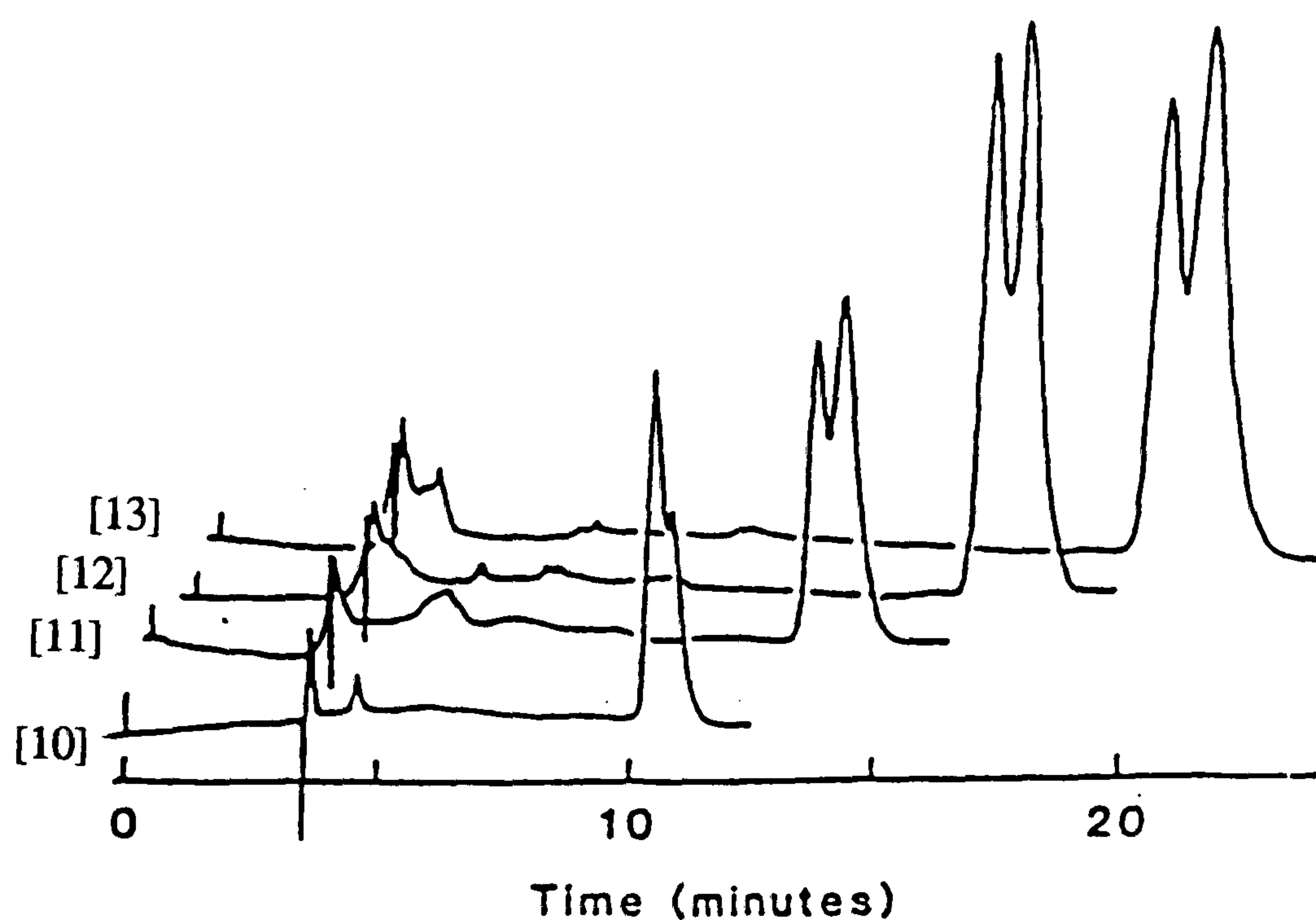
(45)

Stereoselectivity was detected when the nucleobase was a purine, as in adenosine (45), deoxyadenosine, adenosine 3'-monophosphate, adenosine 5'-monophosphate, adenosine 3',5'-monophosphate and guanosine, but was not detected with the pyrimidine derivative uridine. In all cases the M(-)-isomers were found to emerge first (Fig. 45).

The molecular mechanism for the chiral differentiation is not known. Some structural features such as possible effects of the silica gel support, puckering of a sugar moiety and its relative orientation to the nucleobase and interaction of the helicenes with the respective nucleobases probably influenced stereoselectivity.

The lack of chiral differentiation by uridine is in keeping with the lower stability of complexes of pyrimidines with polyaromatic hydrocarbons, as compared with purines [119].

For a given nucleobase (adenine), all changes in the ribose moiety affected the resolution factors. The resolution factors are somewhat reduced with respect to those of adenosine when the C'2 carbon acquires a symmetric configuration as in deoxyadenosine. Molecular rigidity can be favourable for chiral recognition and there is less rotational freedom in 5'-nucleotides than in 3'-nucleotides and in nucleosides [120] i.e. adenosine 5'-monophosphate shows the highest resolution factor among the all adenine derivatives studied.



Column: 200 x 4.6 cm i.d
 mobile phase: 1:9 CH₂Cl₂/n-hexane
 Flow rate= 1ml/min
 λ= 254nm

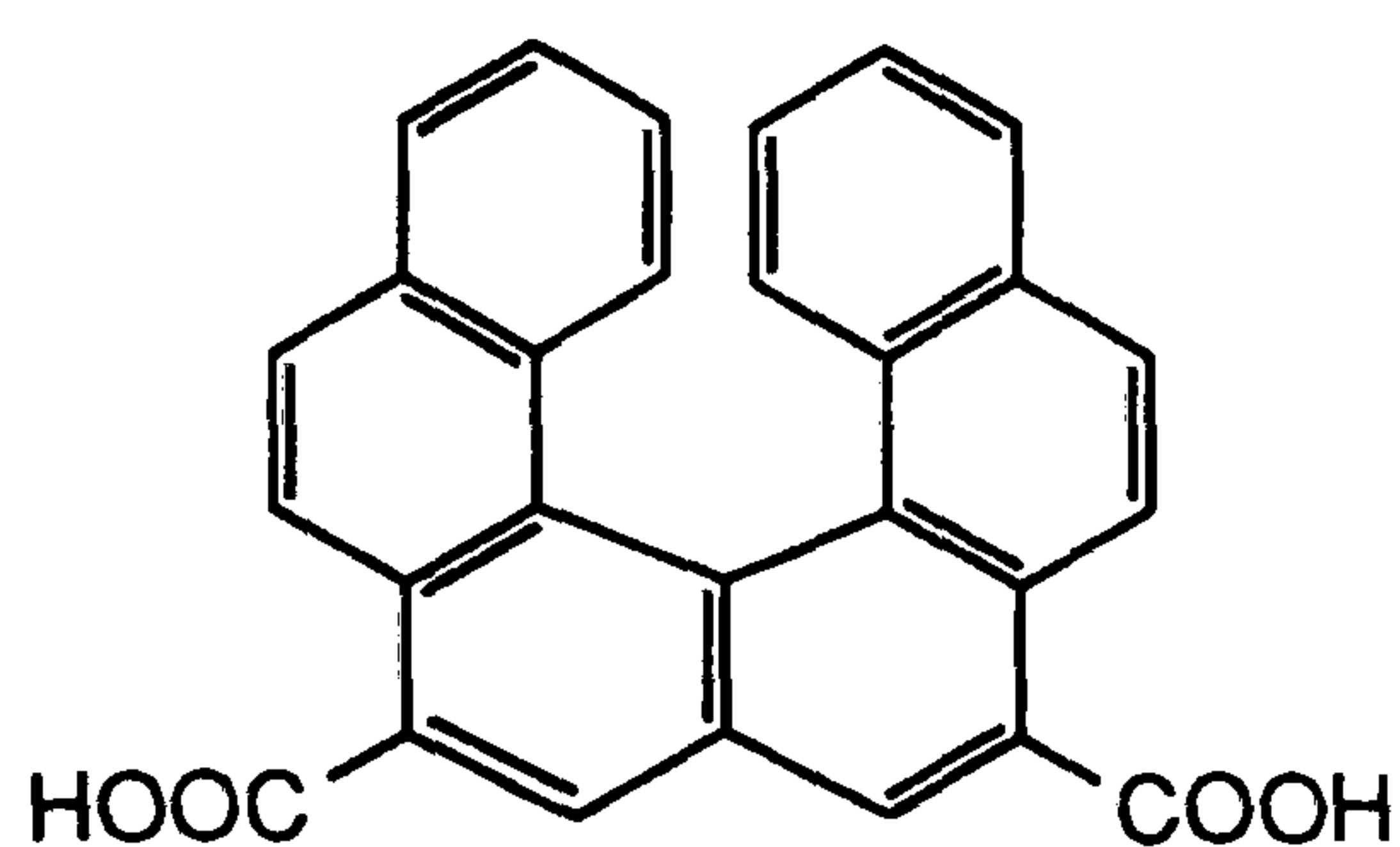
Fig. 45 Resolution of the optical isomers of [10] to [13] carbohelicenes on adenosine coated with silica gel

Summary

The above results show that several chiral HPLC phases containing a wide range of structural classes of enantiomers and usually including an electron-deficient centre (e.g. nitroaryl ring) are capable of resolving helicene enantiomers.

These observations led to the consideration of a possible reversal of roles according to the "Principle of Reciprocity" introduced by Pirkle [9].

As predicted, when a helicene enantiomer was included in a stationary phase, it was able to resolve several classes of enantiomers (Fig. 46). The disodium salt of P(+)-7,10-dicarboxyhexahelicene, physically coated on silica gel, was successfully used in resolving N-2,4-dinitrophenyl-amino acid esters by HPLC [115]. Good resolutions were found for alanine, isoleucine, valine, phenylalanine and phenylglycine.



P(+)-hexahelicene-7,7'-dicarboxylic acid

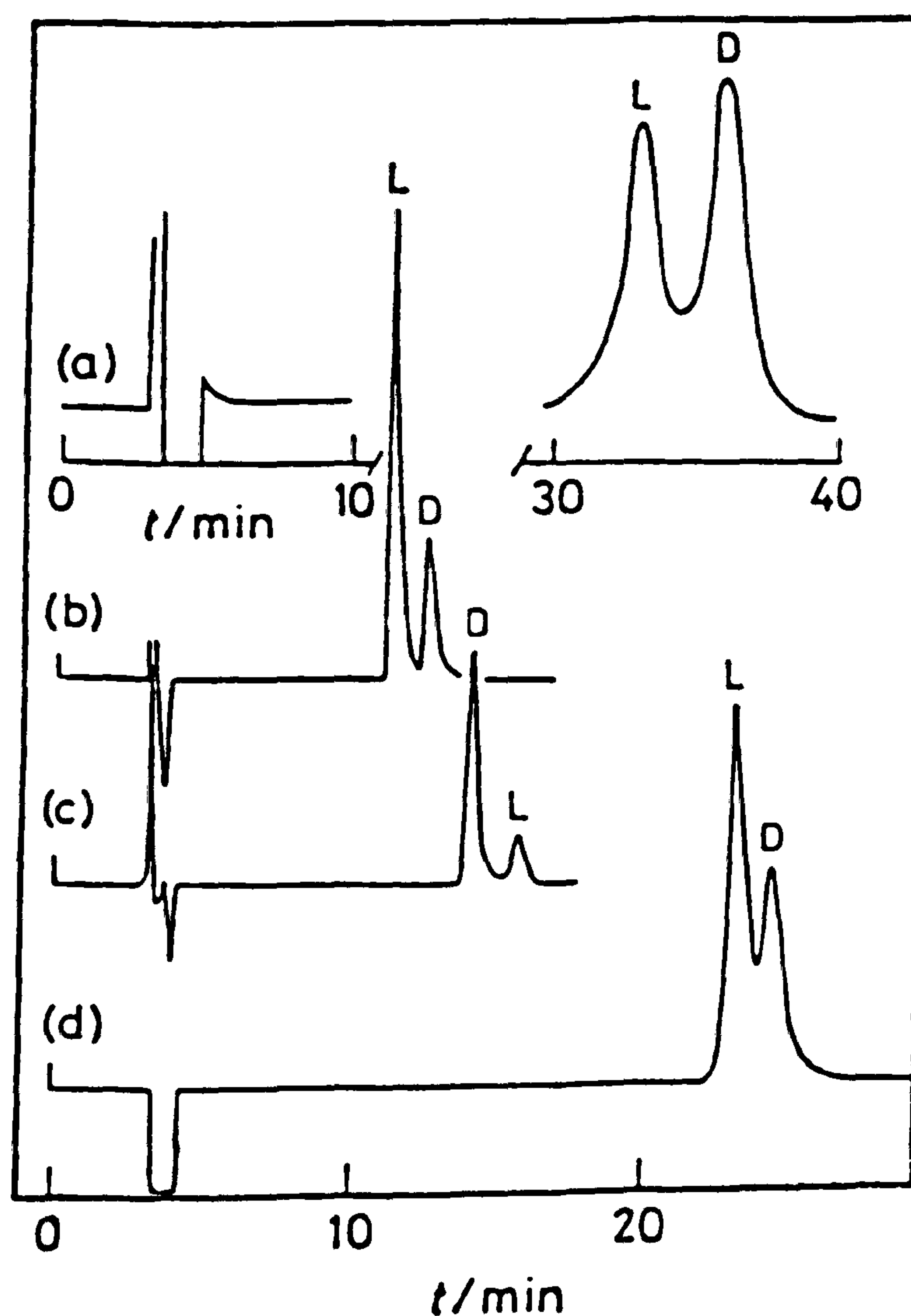


Fig. 46 Chromatograms of dinitrophenylamino acid methyl esters on the P(+)-hexahelicene-7,7'-dicarboxylic acid disodium salt, coated on silica gel. Derivatives of: (a) alanine; (b) valine (L/D=3/1; (c) phenylglycine (L/D=1/3); (d) phenylalanine (L/D=3/1)

For the ester derivatives of alanine and valine, the L-isomer emerged first. There is an inversion in the order of emergence for the enantiomers of phenylglycine with respect to the phenylalanine esters, possibly due to the fact that the intra- and inter-molecular interactions of the phenyl group are markedly different in the crowded α -position compared with the β -position.

2.4.2- Thermal Racemisation.

A very intriguing observation on helicenes is the unexpected ease with which these compounds racemise thermally. A barrier for the racemisation of pentahelicene is low due to the relatively small steric interactions of the terminal benzene rings. The barrier of hexahelicene is higher. Further annellation has a strong influence. The helicenes are recovered in over 90% yields after the thermal racemisations. Partial racemisation of hexahelicene occurred during the melting point determination [75].

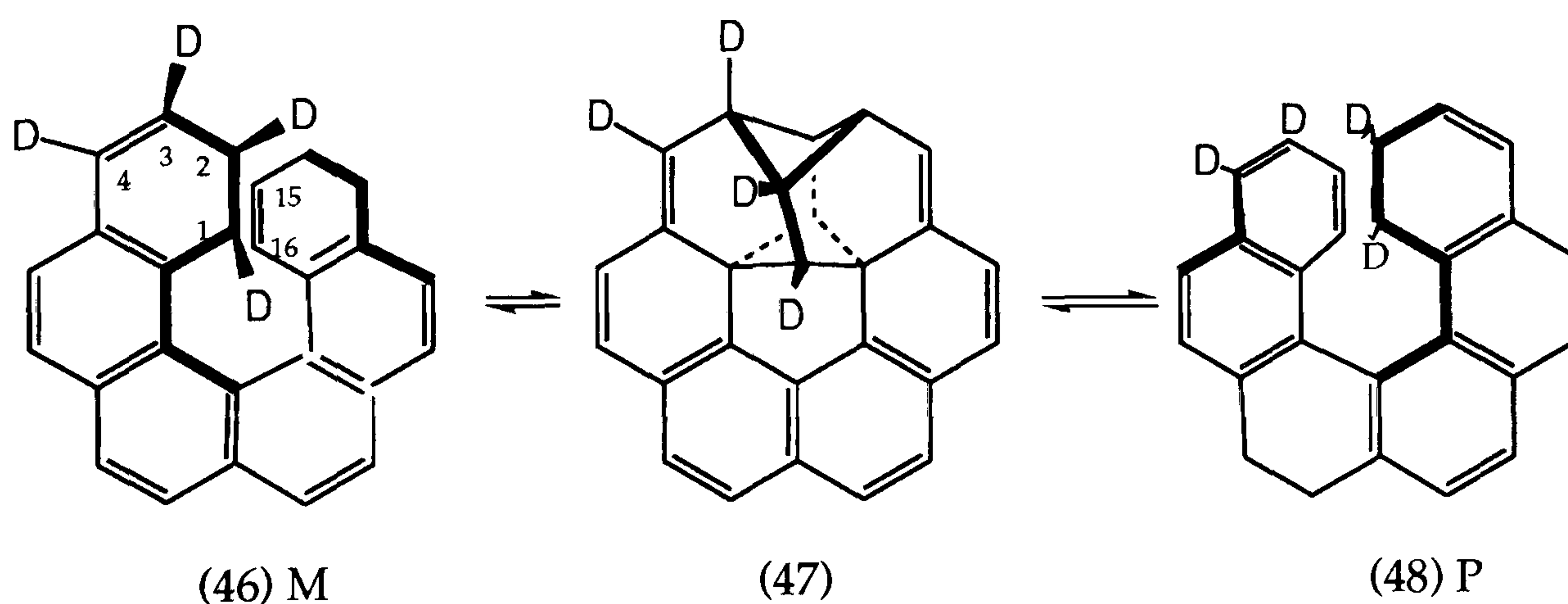
According to Martin [121] three pathways for the thermal racemisation can be considered:

1)- Via bond breaking:

The complete rupture of a C-C bond with formation of non-stabilised diradical is hardly compatible with kinetic results.

2)- Via an internal double Diels-Alder adduct (47):

This was excluded by using substituted derivatives: hexahelicene-1,2,3,4-D₄[122].

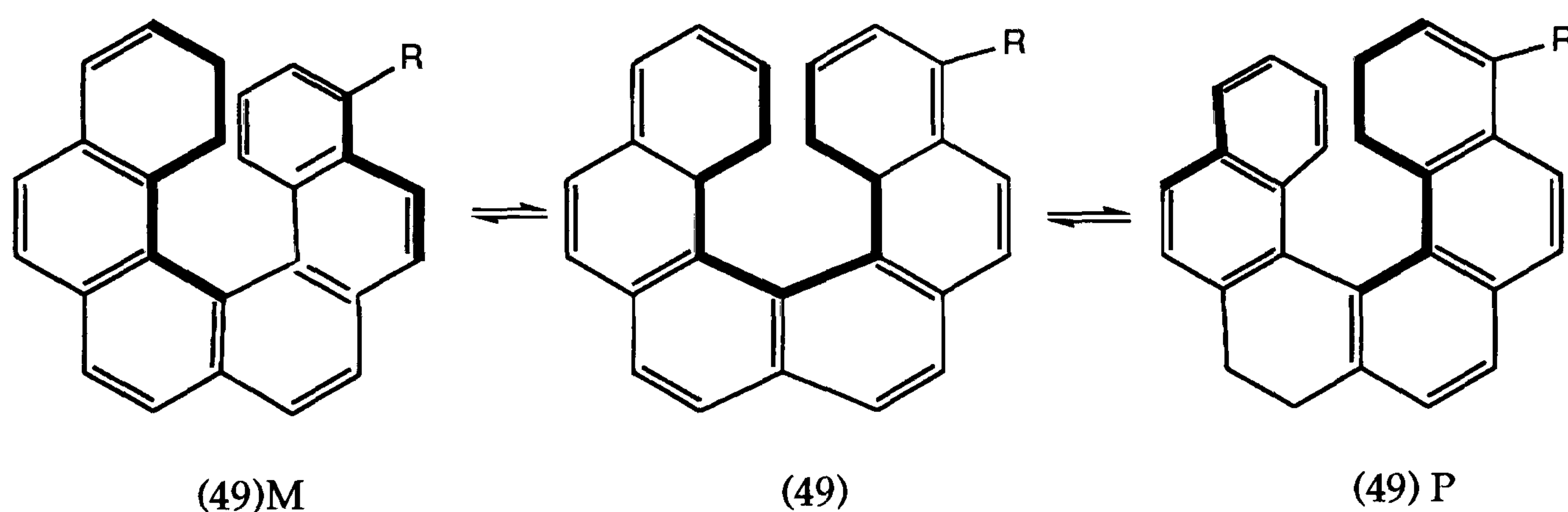


Scheme 14

After treatment of degassed naphthalene solutions at 286°C, ¹H NMR spectroscopy conclusively showed the absence of (48P) and hence ruled out this mechanism.

3)- Via a direct inversion: reversible inversion of the helical structure (M = P) by a "conformational pathway" (Scheme 15): [122]

This hypothesis seems to be the most reasonable one. The "relatively low" potential barriers for these racemisations can be justified in the "conformational process" by the fact that the necessary molecular deformations (bond torsion, bond bending, bond stretching) are spread over a large number of bonds. The racemisation of unsubstituted hexahelicene was supposed to occur via an asymmetrical intermediate state in which the terminal rings are parallel to each other (49) [123].



Scheme 15

Rates of racemisation of several methyl substituted hexahelicenes have been measured [124]. It appeared that the introduction of methyl groups at the positions 3,4,13 and 14, which do not change the helical conformation, have no significant influence on racemisation parameters; at position 2 and 15 the effect is small but significant. A large effect is observed on introduction of a methyl group at position 1. This seems to be due to a larger entropy loss in the course of racemisation of the methyl derivative, because the rotation of the methyl groups is hindered [123]. It is only slightly enhanced when a second methyl substituent is present at C(16).

Although all-benzene-helicenes are photolabile, they do not racemise photochemically.

2.5- Spectral Properties.

2.5.1- ^1H NMR-Spectroscopy.

^1H NMR-spectroscopy is a powerful method for the recognition of helicenes and for the analysis of their conformations in solution. The structure of practically every all-benzene-helicene synthesised by the photocyclization process has been deduced from its ^1H NMR spectrum.

The ^1H NMR-spectrum of hexahelicene at 500MHz is shown in Fig. 47

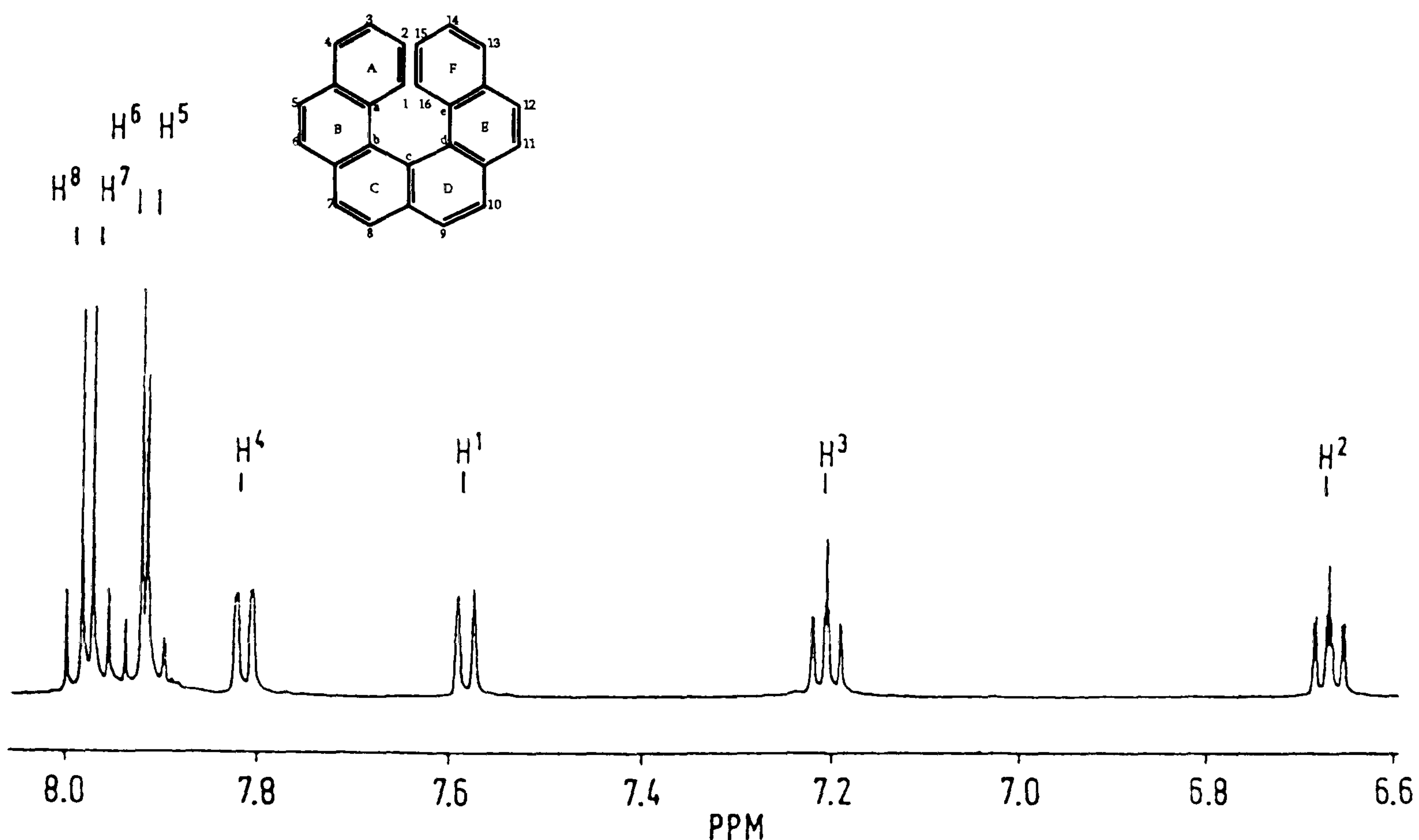


Fig. 47 NMR spectra of hexahelicene at 500 Hz

The spectrum consists of two AB systems (H_5 and H_6 and of H_7 and H_8) and a ABCD system (H_1 to H_4). The overcrowded proton 1 is no longer the lowest field proton observed and H_2 absorbed to quite unusually high field for an unsubstituted condensed benzenoid hydrocarbon.

In interpreting the spectra, Haigh and Mallion [125] argued that the chemical shift of a strongly overcrowded proton like H₁ of hexahelicene (7.55 ppm in CDCl₃) depends on three different effects:

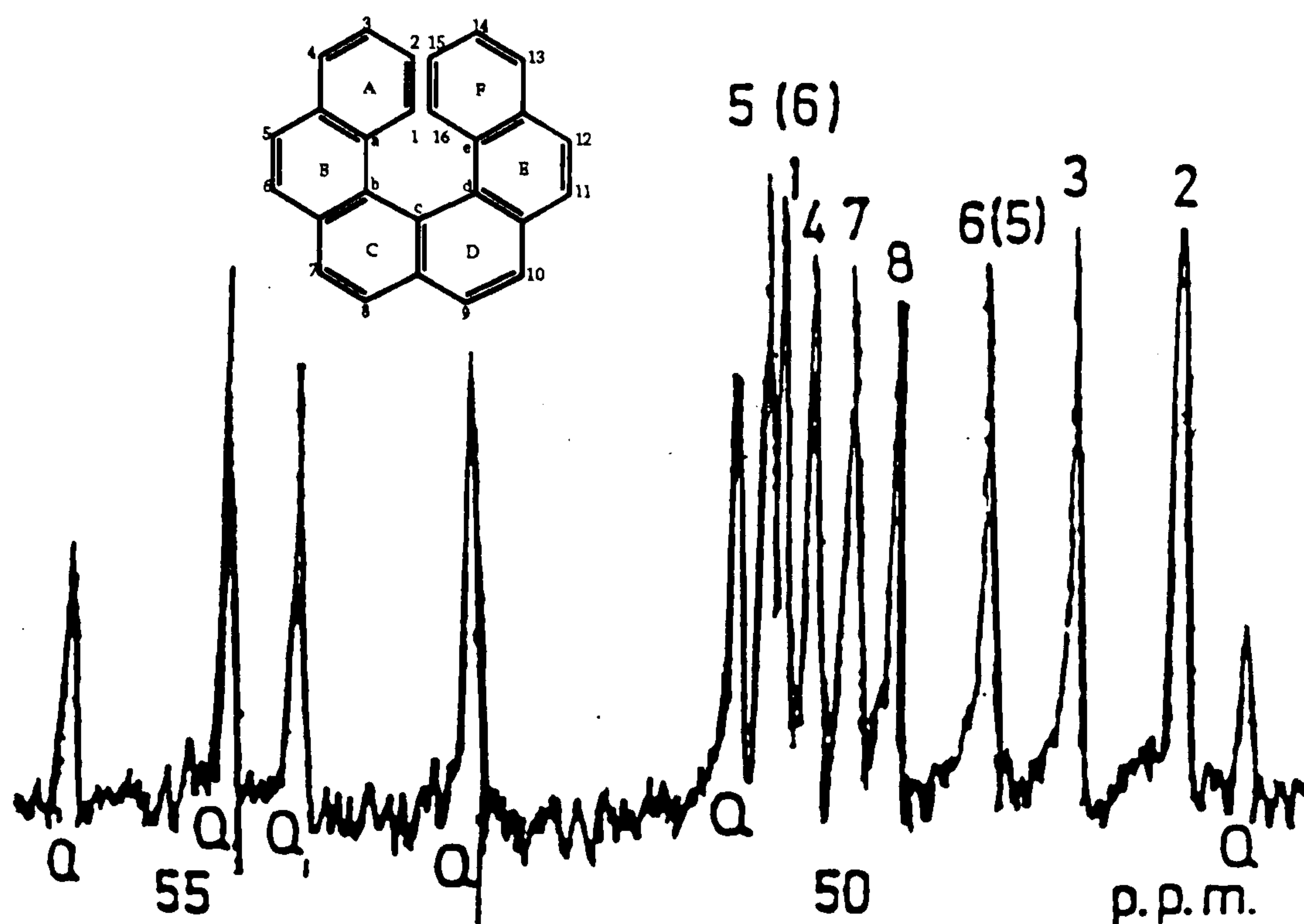
- 1) Deshielding due to the ring currents of the benzene ring to which the proton belongs and the neighbouring rings.
- 2) Shielding due to the ring current of overlapping rings.
- 3) Deshielding due to Van der Waals interactions with nearby H and especially C-atoms. Depending on the contribution of each of these factors the chemical shift of H(1) and H(16) of a helicene derivative can vary between 6.00 and 8.00ppm. The introduction of a substituent at C(1) of hexahelicene will result in an enlargement of the pitch of the helix and in a reduction of the deshielding Van der Waals interaction. The δ -value of a 1-methyl substituent is always at higher field than that of the methyl groups at other positions.

Some of the protons of the all-benzene-helicenes from hexahelicene onwards, show specific solvent effects (C₆D₆ versus CDCl₃) which distinguish them from the less distorted isomers which can be formed in the photocyclisations. Also concentration effects are observed, especially leading to upfield shifts of the non-overcrowded protons at higher concentrations.

2.5.2- ¹³C NMR Spectroscopy.

¹³C NMR spectroscopy is less suitable than ¹H NMR spectroscopy as a probe for the magnetic properties of the cyclic π -system of the helicenes. Besides ¹³C-spectra of penta-, hexa- (Fig. 48) and heptahelicene in which all the protonated carbon atoms could be assigned [126] no other reports on ¹³C NMR analysis of helicene have been published as far as the author is aware.

The noise off-resonance decoupling technique (NORD), was used to distinguish the non-protonated carbons from the other carbon atoms. The assignment of individual protonated carbon atoms was done by selective double resonance.



Q = non protonated carbon atom

Fig. 48 Fully decoupled ^{13}C -NMR spectrum of hexahelicene (145 scans)

2.5.3- Photoelectron and Ultraviolet Spectra.

Photoelectron spectra (PES) of helicenes have been measured to trace whether interactions between the orbitals of overlapping benzene rings occur, as observed in cyclophanes [127].

The effect of progressive annelation in the helicene series is a suppression of the intensities of all π -bands relative to the σ -bond system. The π -bands become more symmetrical and the vibrational structure is blurred out. This indicates that the geometry changes upon ionisation [128]. The values of π -ionisation potentials show that transannular effects are not present. The absence of such effects in the helicene is due to the greater flexibility of these molecules compared with cyclophanes. The PES have proved of great value in the interpretation of U.V spectra.

In the U.V spectra (Fig. 49) the longest wavelength absorption band of carbohelicenes shifts with increasing number of benzene rings to higher wavelengths. The structure of the absorption curve becomes more diffuse, and differentiation between the α , ρ and β -bands cannot be made for helicenes higher than octahelicene [129].

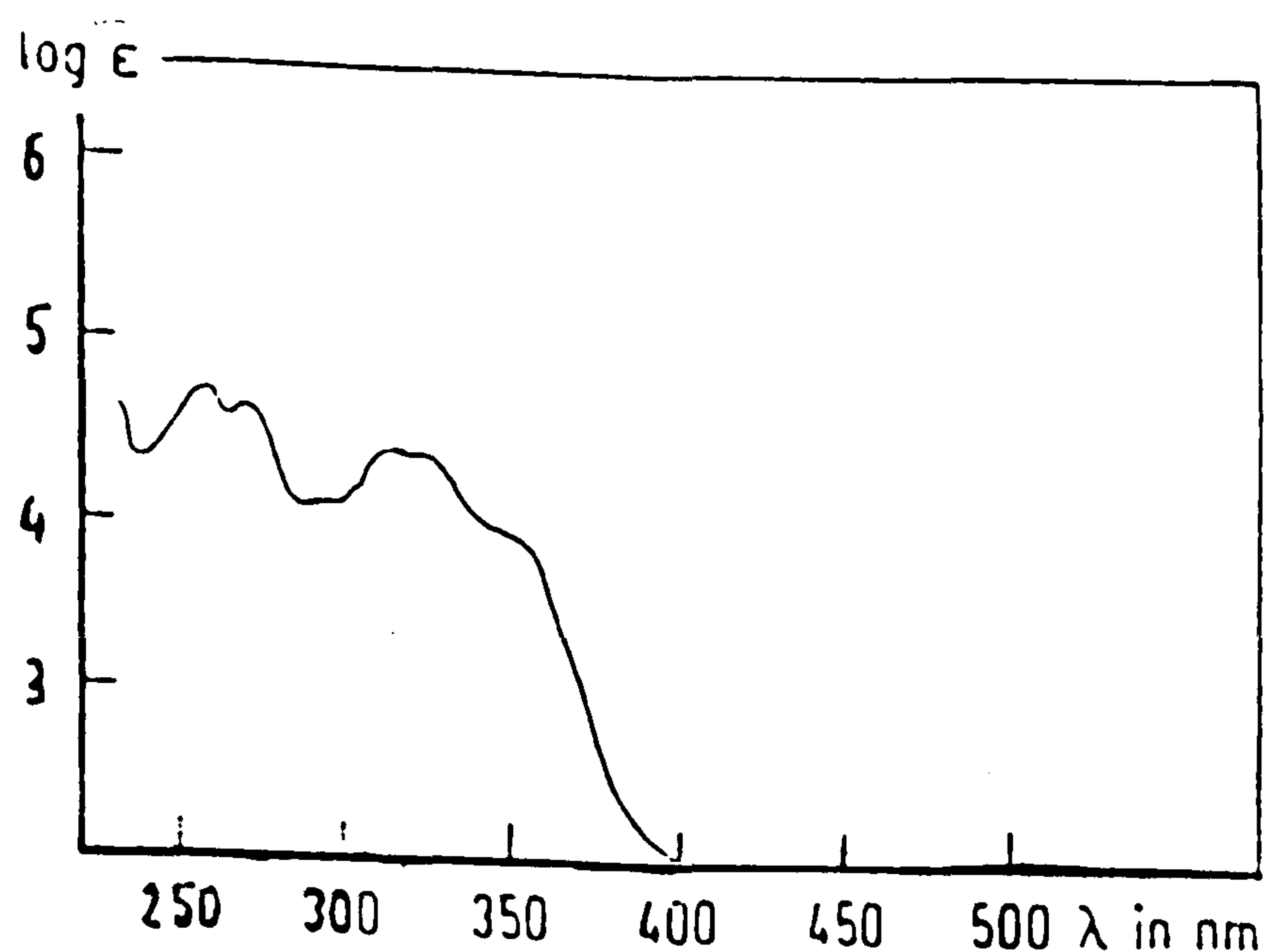
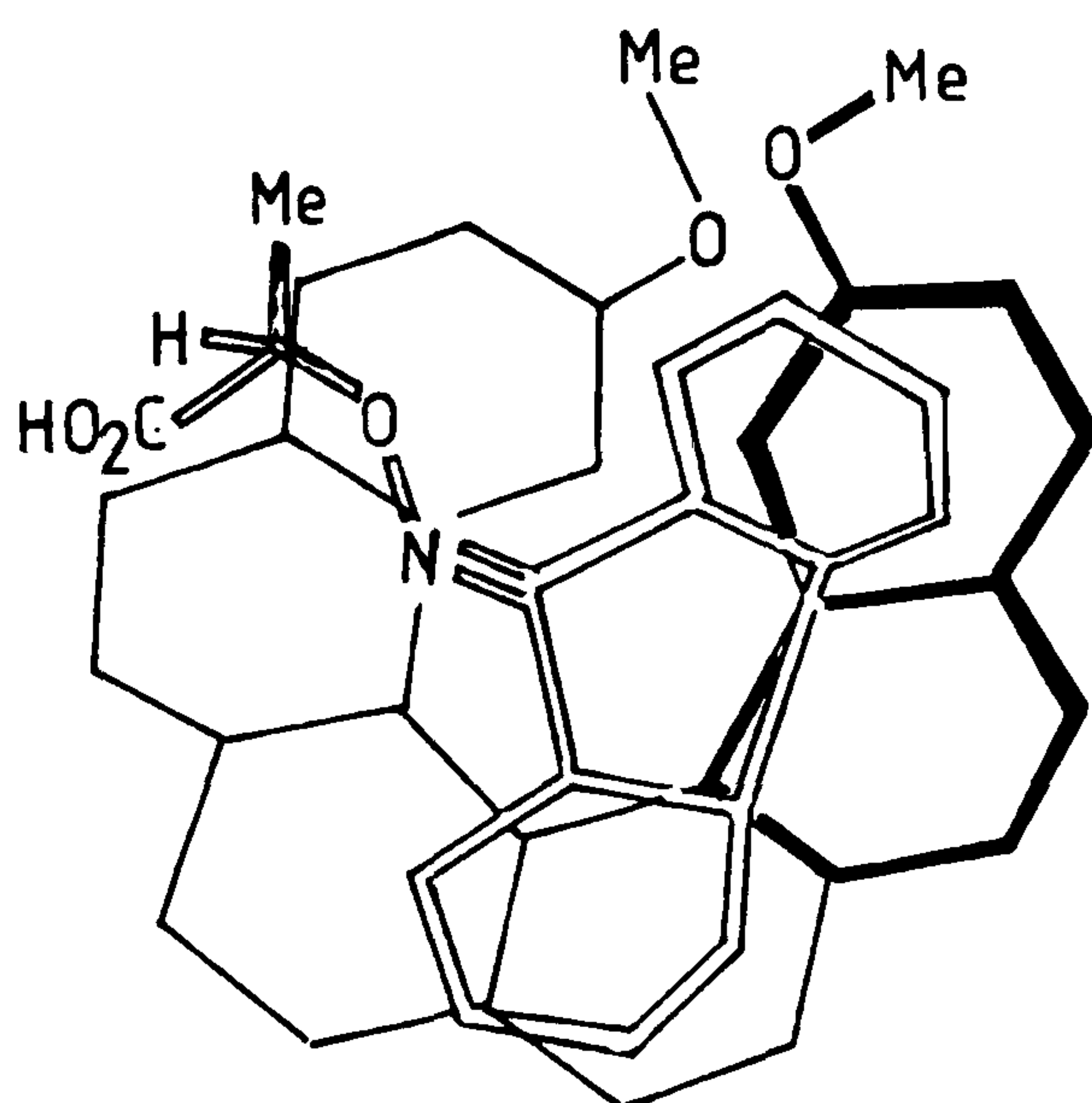


Fig. 49 Ultra violet spectrum of hexahelicene.

2.5.4- Charge-Transfer Complexation.

Newman developed tetranitrofluorenylideneaminoxypropionic acid (TAPA) (39) as a new agent for the resolution of hexahelicene. Its molecular structure, including a large moiety with strong electron acceptor properties, promised good complexing properties towards aromatic compounds like hexahelicene.

The complexation of TAPA with hexahelicene was studied in more detail by Brown *et al.* [130] using NMR-spectroscopy. The results suggested that the R(-)-isomer of TAPA which binds slightly more strongly to P(+)-2,15-dimethoxy hexahelicene than does the (S)-isomer, forms a complex with the geometry shown in Fig. 50. This is consistent with the geometry observed in the X-ray analysis of the complex found between hexahelicene and 4-bromo-2,5,7-trinitrofluorenone (36).



R(-) P(+)

Fig. 50

2.5.5- Photophysical Properties [131]

All carbohelicenes fluoresce and show a well define fluorescence band which shifts progressively to longer wavelengths from hexahelicene to tetradecahelicene. The spectrum of hexahelicene includes two bands of approximately similar intensity (Fig. 51)

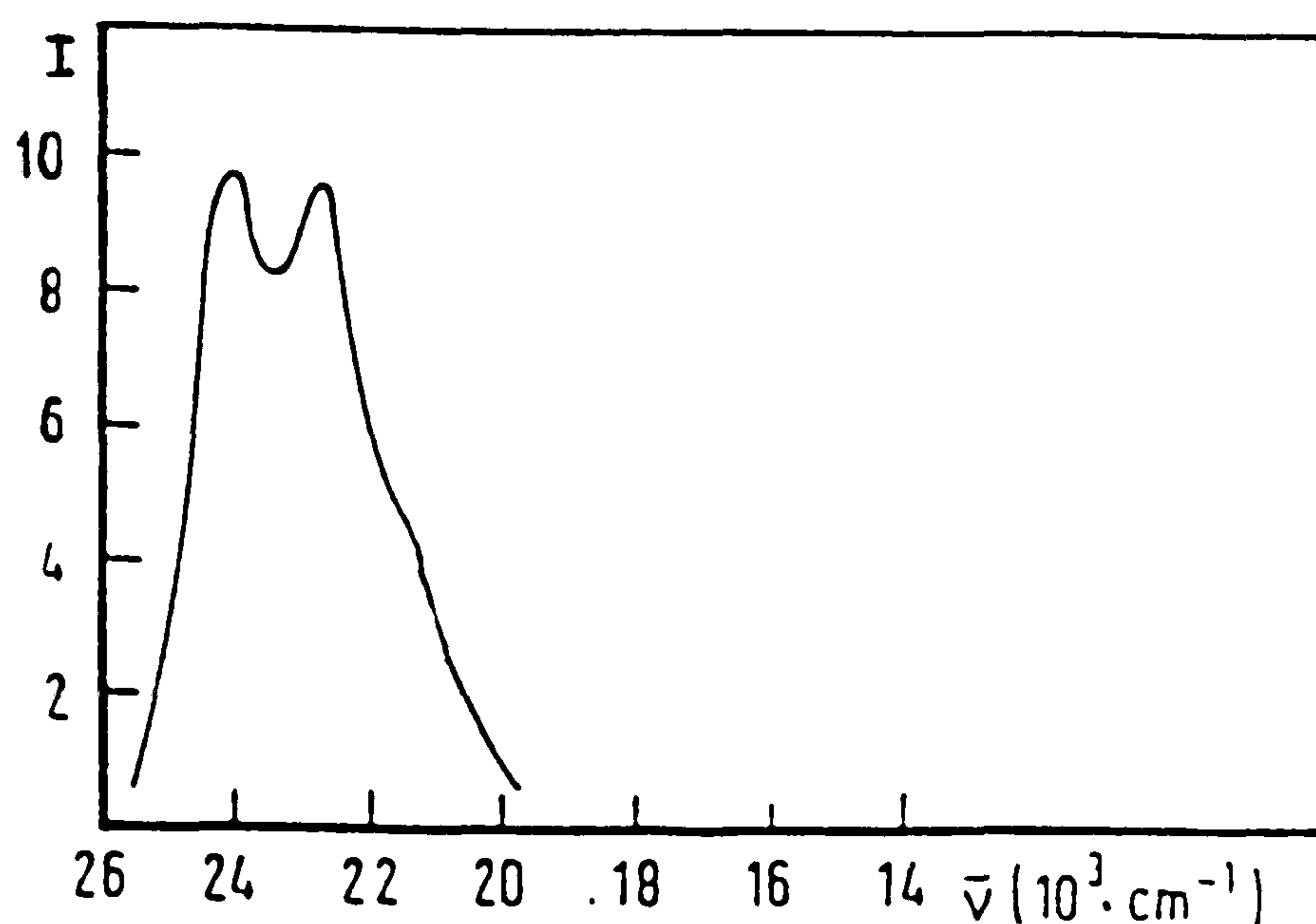
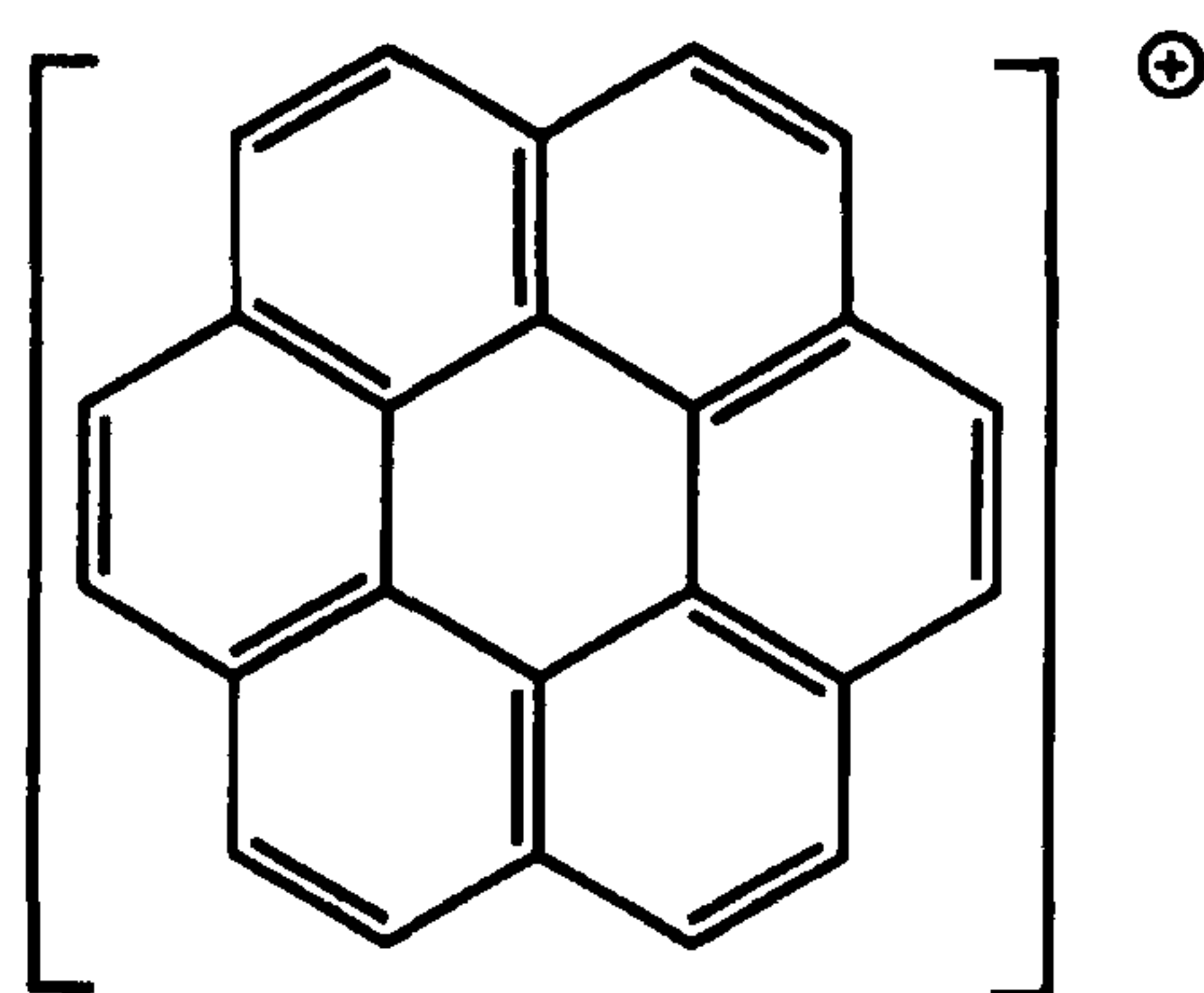


Fig. 51 Fluorescence spectrum of hexahelicene in dioxane.

The fluorescence rate, (K_f), which is the ratio of the quantum yield to the lifetime, of helicenes with an odd number of benzene rings decreases more slowly than that of helicenes with an even number of rings. This is explained by the difference in molecular rigidity of the two groups. The "even" ring helicenes are symmetrical about a naphthalene nucleus and more rigid than the odd ring helicenes, which are symmetrical about a benzene nucleus [132].

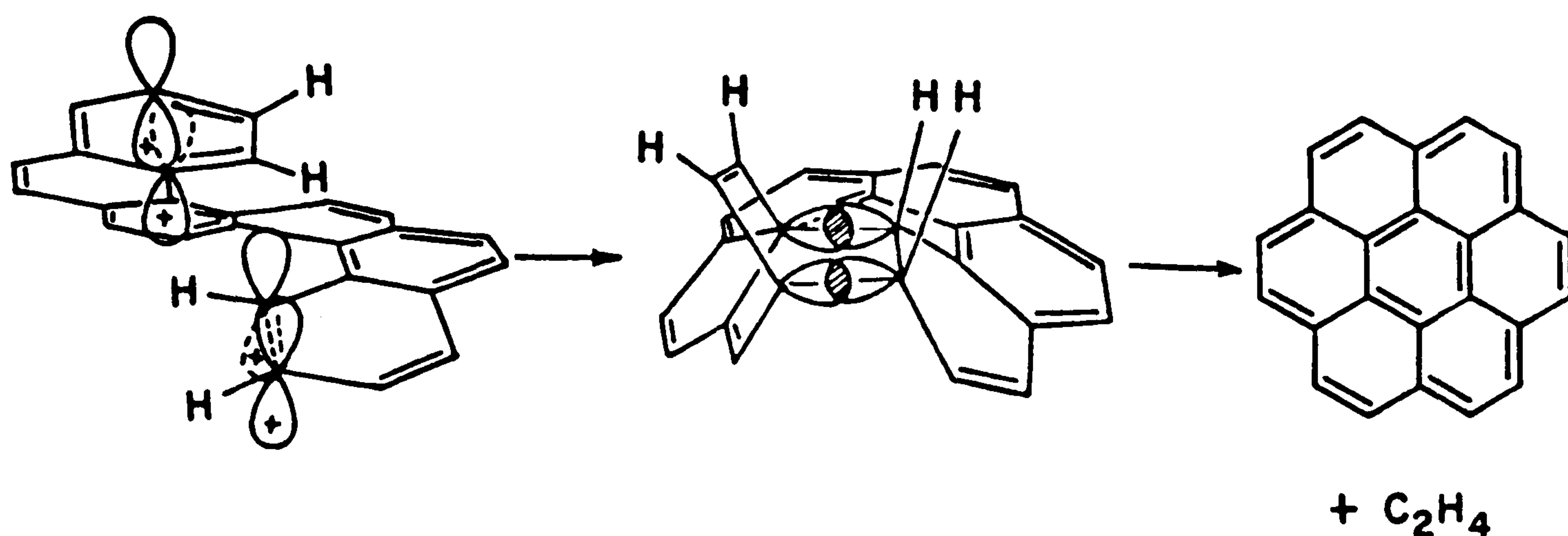
2.5.6- Mass Spectrometry .

The mass spectrum of hexahelicene [75] shows a very intense peak at $m/z = 300$ ($M - C_2H_4$) and intense metastable ion corresponding to the m/z 328 to 300 transition. It also shows peaks for doubly charged ions. There is evidence that the ion with $m/z=300$ is the coronene ion (50):



(50)

In order to explain the formation of (coronene)⁺ from (hexahelicene)⁺, Dougherty has postulated the formation of an internal Diels-Alder adduct, followed by the loss of ethylene [133] (Scheme 16).

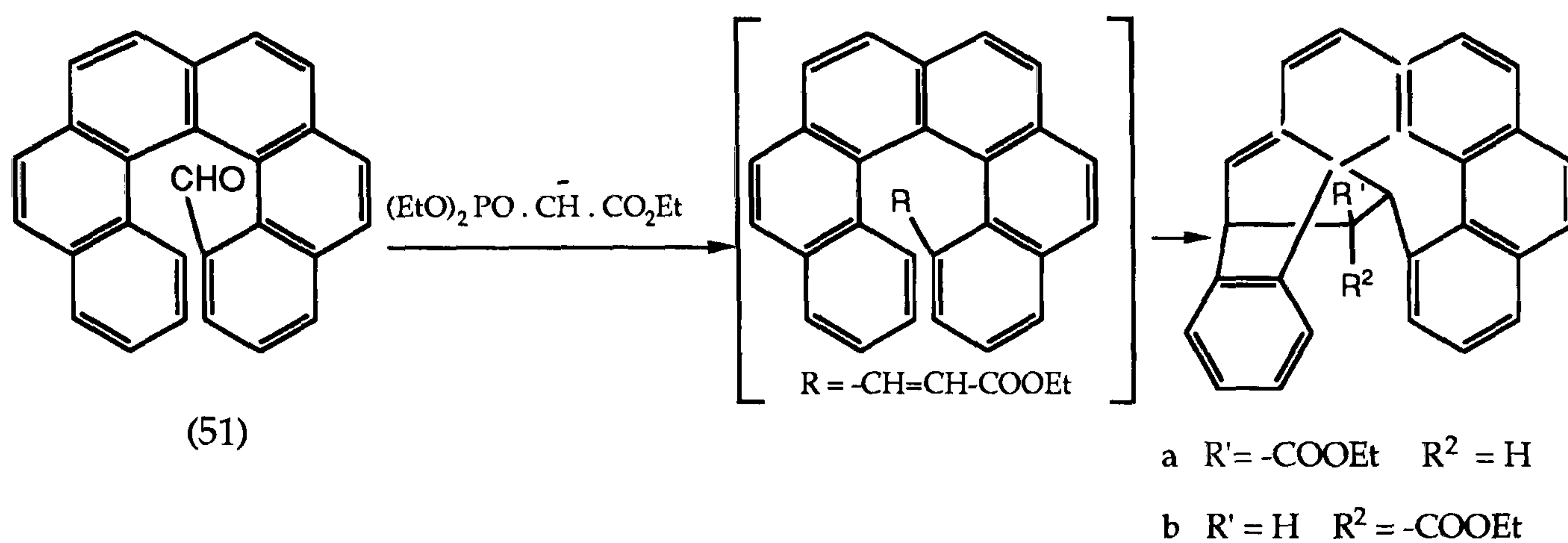


Scheme 16

2.6- Chemical Reactions.

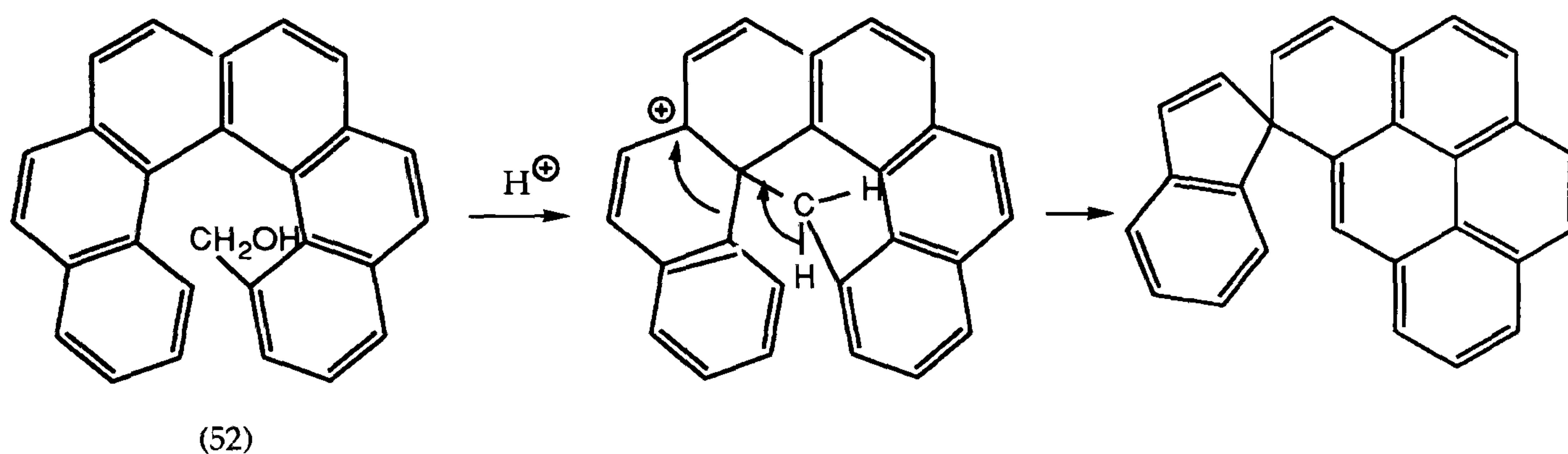
2.6.1- Intramolecular reactions.

Different intramolecular reactions in hexahelicenes have been reported to occur in the mass spectrometer. As noted above, hexahelicene formed the ion coronene $[C_{24}H_{12}]^+$ by an internal Diels-Alder reaction [133]. Another (4+2) cycloaddition was observed by Martin *et al.* [134] when 1-formylhexahelicene (51) was treated with the ylid of $(EtO)_2POCH_2COOEt$ in boiling benzene for 12 h (Scheme 17).



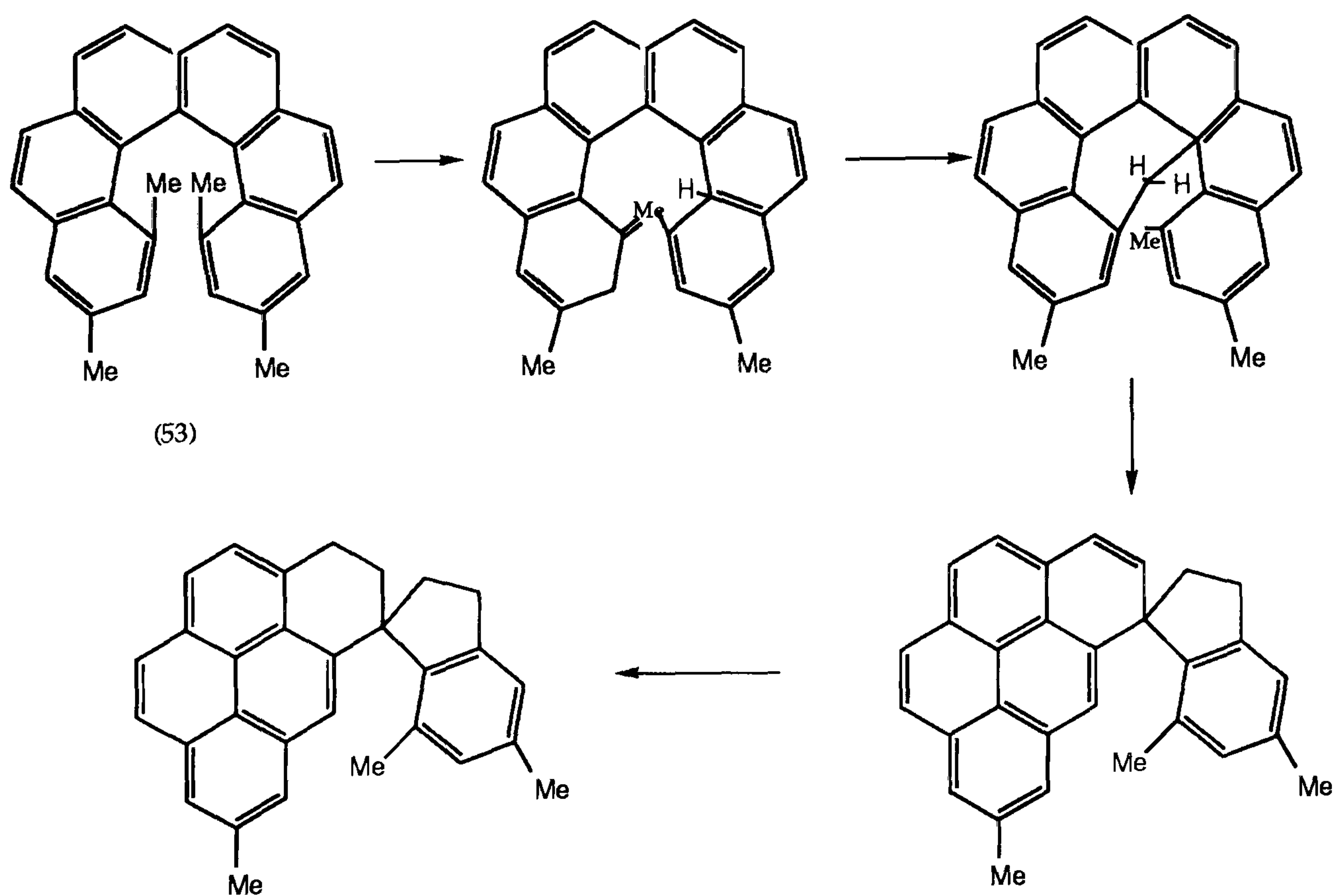
Scheme 17

A methane bridge is also formed when 1-hydroxymethylhexahelicene (52) is treated with an acid [135] (Scheme 18).



Scheme 18

On heating 1,3,14,15-tetramethylhexahelicene (53) above 180°C, two spiro compounds are formed [136] (Scheme 19).



Scheme 19

The same reaction is observed with 1,16-dimethylhexahelicene but not with 1,3 and 1,14-dimethylhexahelicene, showing the strict steric requirement for this reaction.

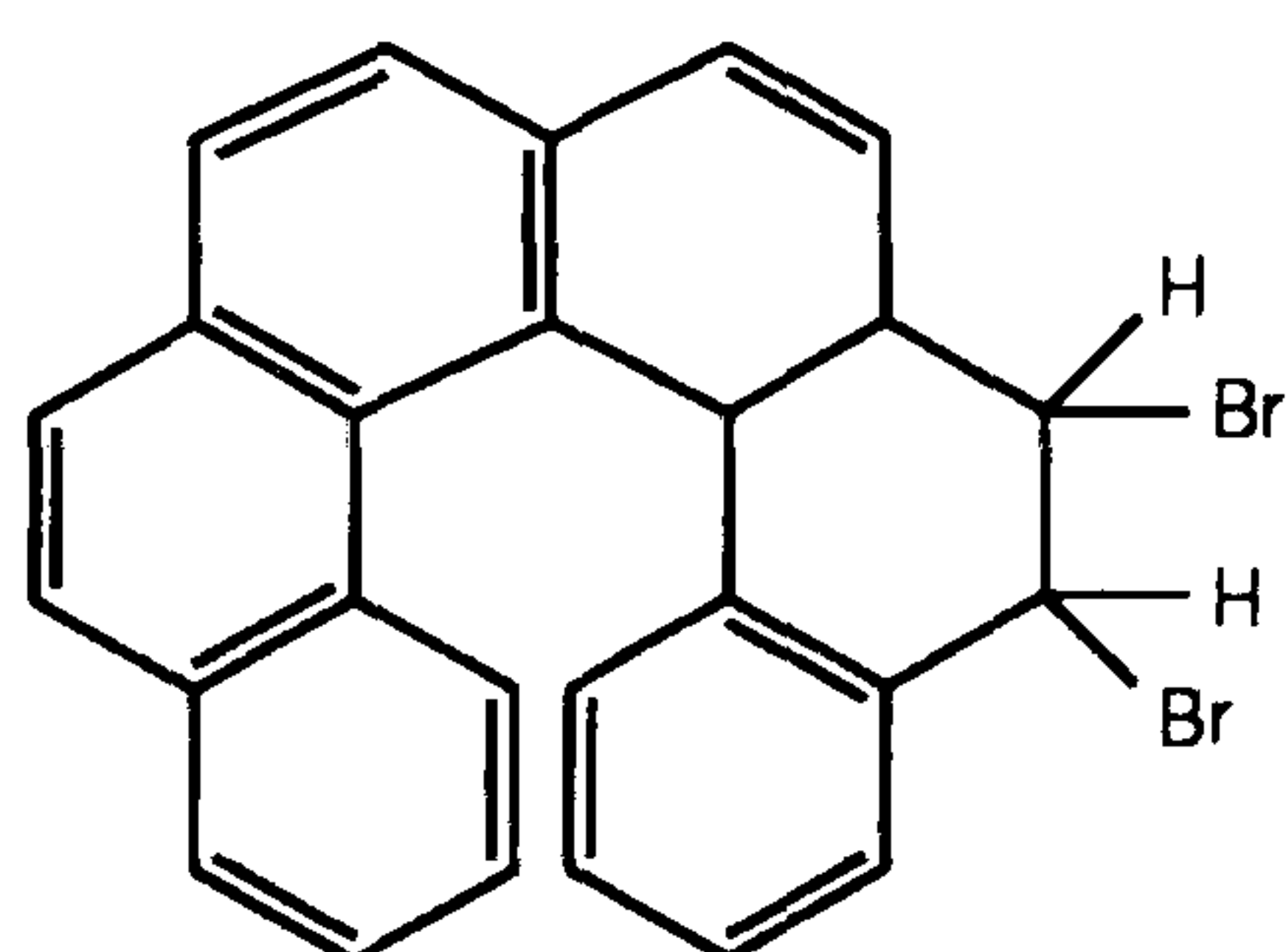
2.6.2- Intermolecular Reactions.

2.6.2.1- Electrophilic Reactions.

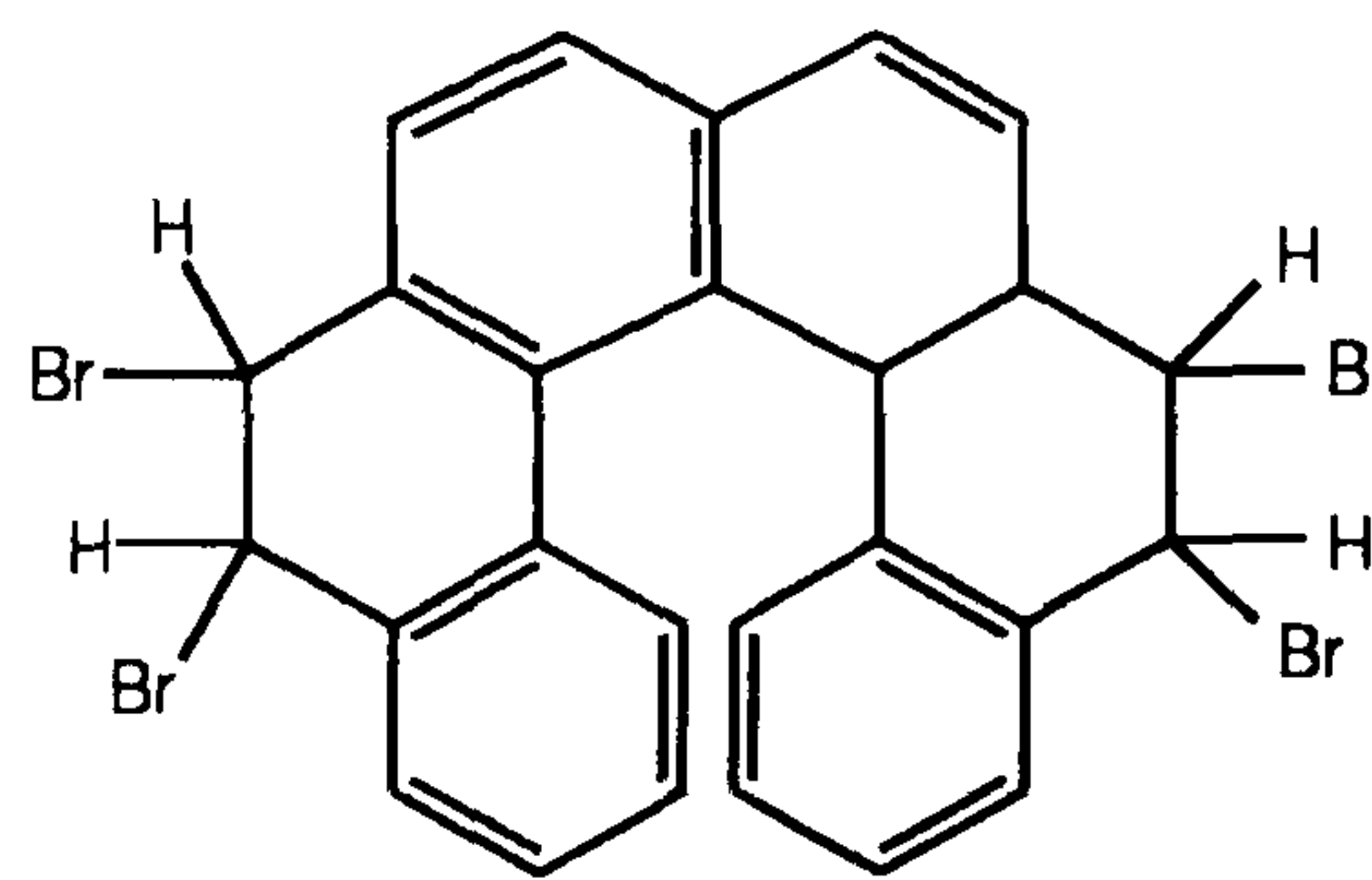
Several reactivity parameters, the simple Hückel indices: N_r (Dewar number), F_r (Free valence number), L_r (localization energy) and the Mulliken overlap population (μ_r), adopted from theoretical chemistry have been used to predict the position at which electrophilic reagents will attack higher aromatics [137].

The applicability of such parameters to non-planar hexahelicene was studied qualitatively in bromination, nitration and acetylation reactions [138] and in a quantitative way in the protiodetritiation of eight monotrityohexahelicenes [139].

Bromination of hexahelicene in CCl_4 leads to two addition products (54) (55), which eliminate HBr only at higher temperature (boiling toluene) affording 5-bromo- and 5,12-dibromohexahelicene, respectively.



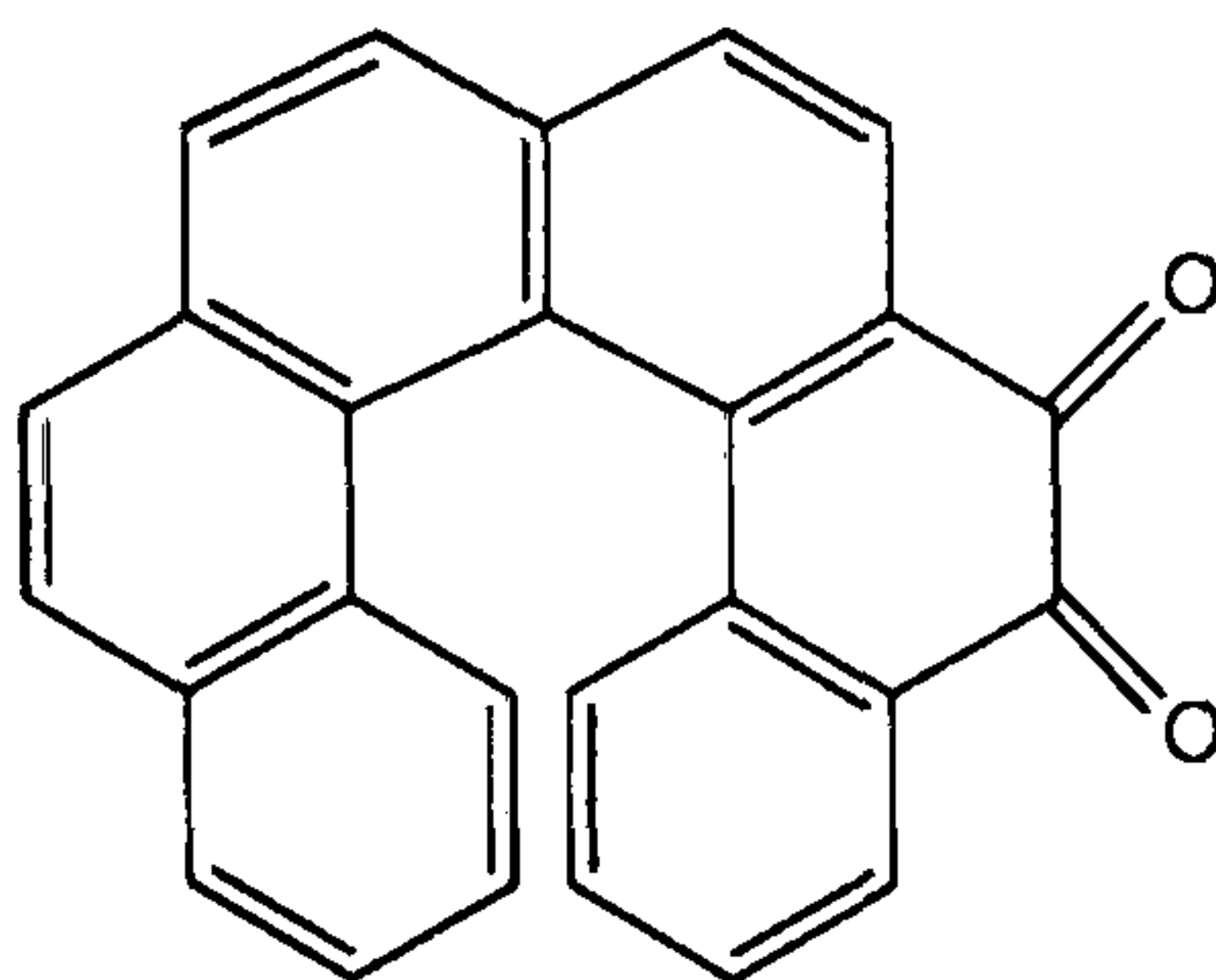
(54)



(55)

2.6.2.2- Oxidation.

Hexahelicene is oxidized by chromic acid under mild conditions giving hexahelicene 5,6-quinone in 70% yield [140] (56).



(56)

2.7- Photoreactions.

No photoreactions, except photodestruction, are known for hexa- and higher helicenes.

2.8- Conclusions.

The most important conclusion from the extensive investigations of helicenes in the past is that the benzene rings are much more flexible than previously thought. In hexahelicenes, this flexibility comes to light in the strikingly easy thermal racemisation of these compounds. The flexibility of the aromatic rings in helicenes is however, not a consequence of their special structural characteristics like the helical form or the occurrence of overcrowded regions. None of the physical properties of helicenes reflects the presence of any unusual effect. The relation between UV and PES spectra is quite similar to that found for planar aromatic compounds. All chemical shifts in NMR spectra can be explained by normal ring-current effects and Van der Waals interactions. Polarographic data do not deviate from those for planar compounds. Though the optical rotation of a number of helicenes is known and the regular increase of the optical rotation with increasing number of benzene rings has been shown, the precise mathematical dependence of the rotation on the helicity is still unknown.

Chapter Three

LITERATURE REVIEW: CHROMATOGRAPHIC SEPARATION OF ENANTIOMERS ON HEXAHELICENE BONDED PHASE

3.1- Resolution of enantiomers of electron-deficient compounds.

The observation that several chiral HPLC phases, containing a wide range of structural classes of enantiomers and usually including an electron-deficient centre such as a nitroaryl ring, were capable of resolving helicene enantiomers encouraged Matlin *et al* . [141] to consider a reversal of roles.

In previous studies carried out [141] in this laboratory, the chiral stationary phase based on the immobilisation of (+)-hexahelicen-7-ylacetic acid was prepared. The (+)-hexahelicen-7-ylacetic acid was coupled to aminopropyl silica using a water-soluble carbodiimide reagent to form the amide bond. Microanalysis showed that one third of the available aminopropyl groups had reacted and it was believed that essentially all of the hexahelicenyl material was present as the amide [142]. The bonded phase was packed into a conventional HPLC column and also a microbore column. It was used for the resolution of a variety of compounds containing one or more chiral centres and an electron deficient aromatic ring.

The 2,4-dinitrophenyl ether of 1-phenylethanol was synthesised and resolved on the hexahelicenyl bonded phase column (250 x 4.5mm). It gave almost baseline separation within 7 min when eluted with 90:10 hexane/IPA (Fig. 52).

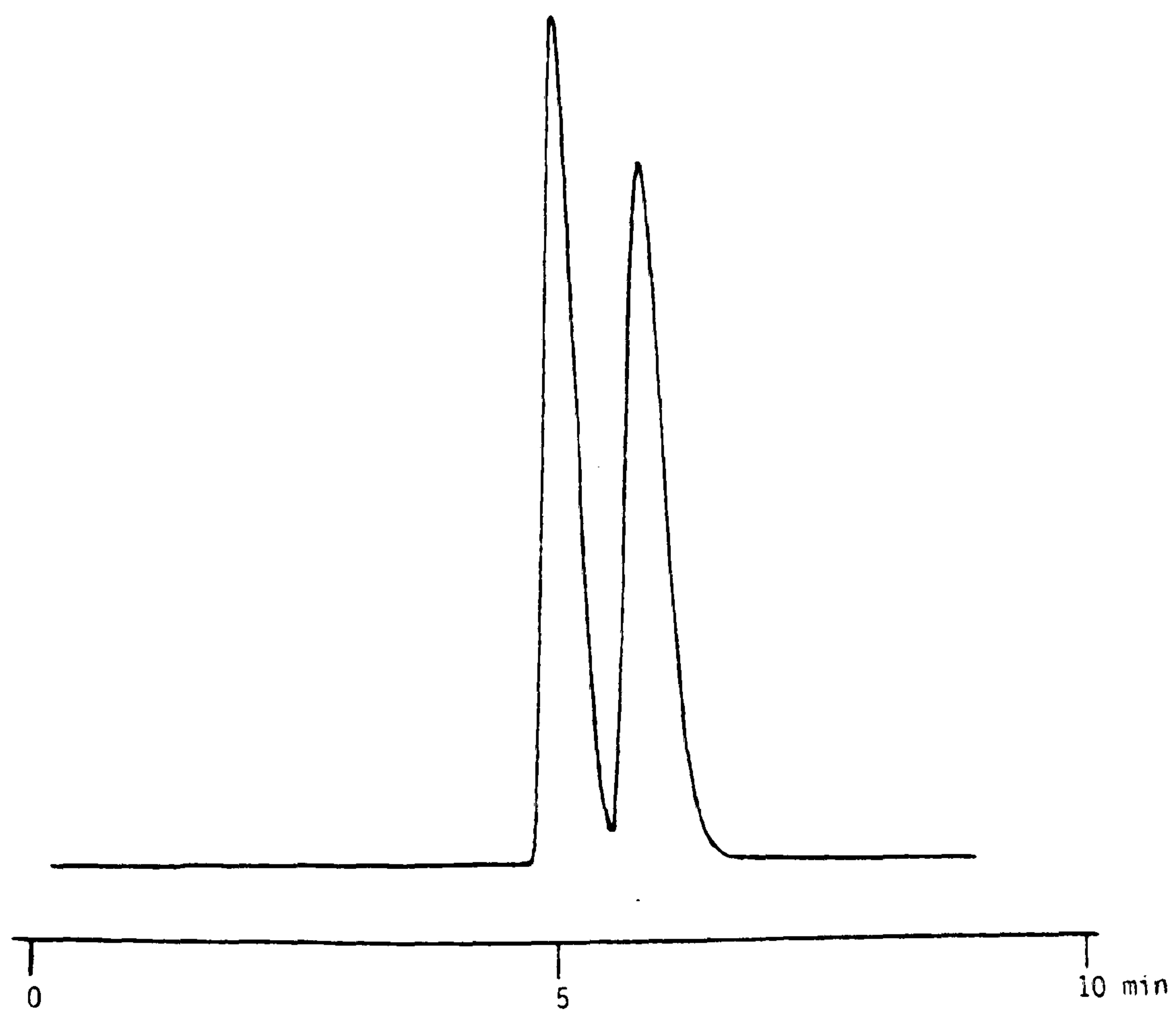


Fig. 52 HPLC resolution of enantiomers of 2,4-dinitrophenyl ether of 1-phenylethanol.

Under the same conditions, the 3,5-dinitrobenzoate of 1-phenylethanol eluted with a similar retention time as a pair of partially resolved peaks (Fig. 53).

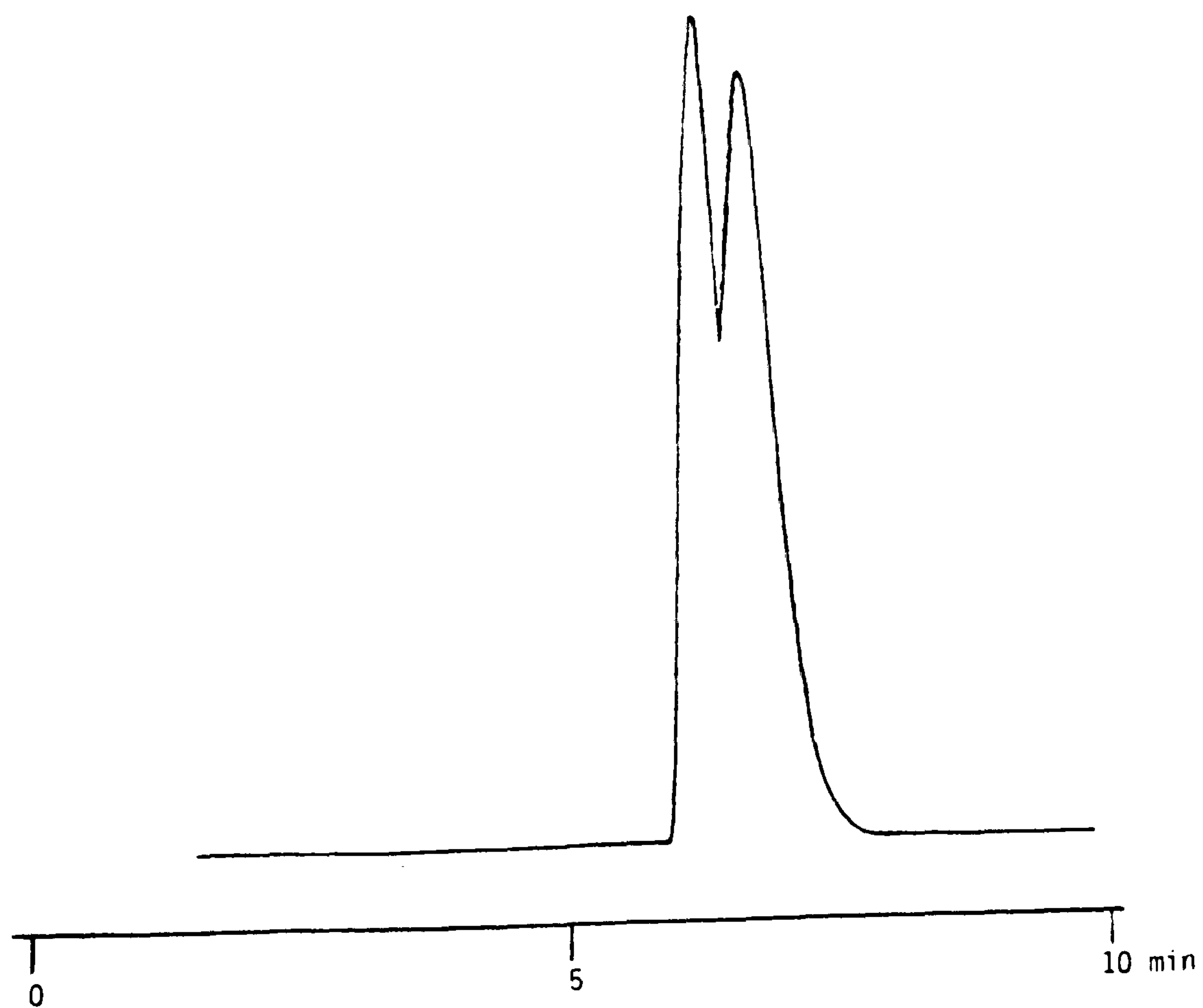


Fig. 53 HPLC resolution of enantiomers of 3,5-dinitrobenzoate ester of 1-phenylethanol

The new phase was also used for the direct enantiomeric resolution of a series of substituted trans-3,4-dihydro-2,2-dimethyl-2H-1-benzopyrans (Fig. 54) which possess two asymmetric carbon atoms, C (3) and C (4).

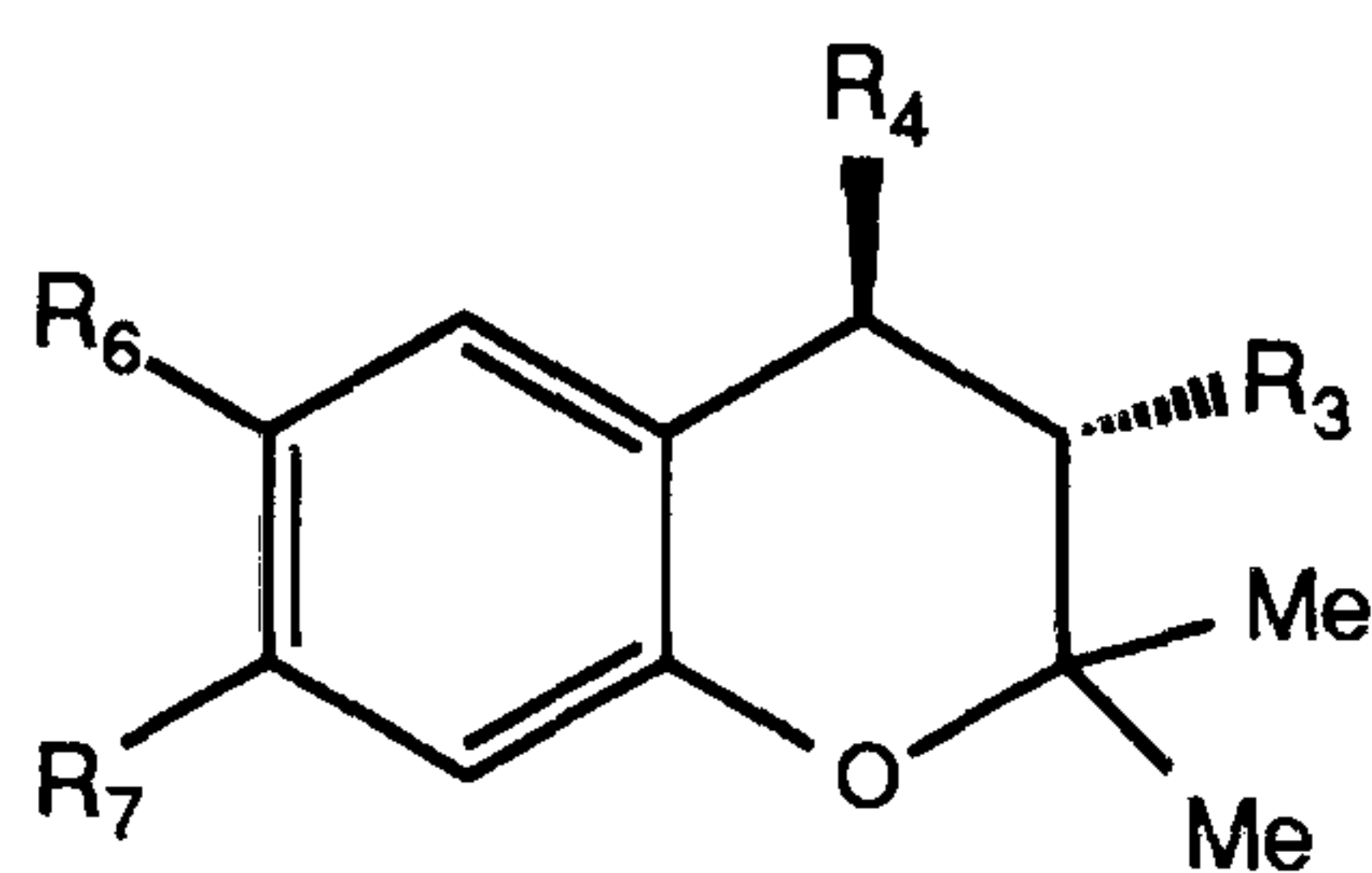
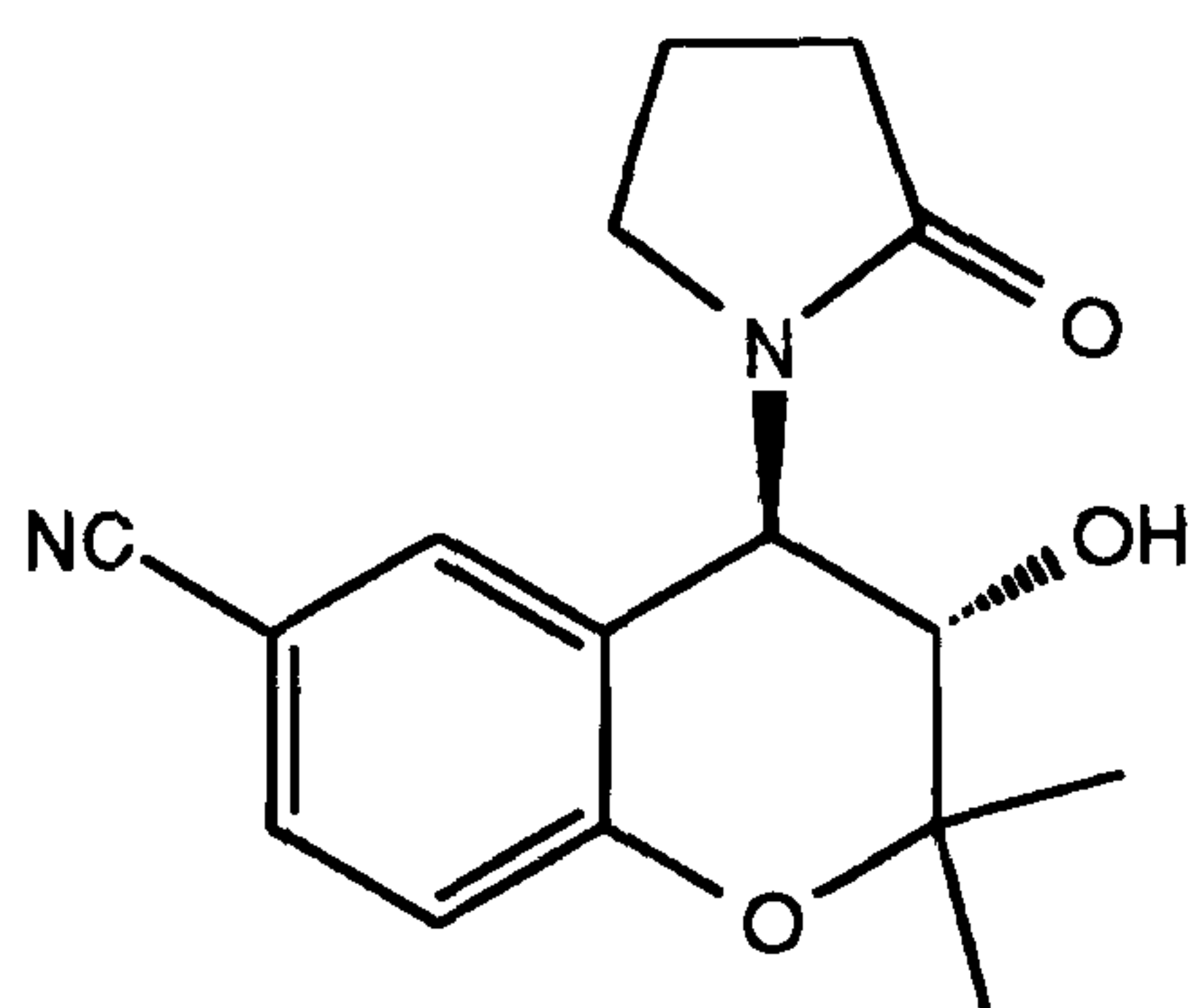


Fig. 54

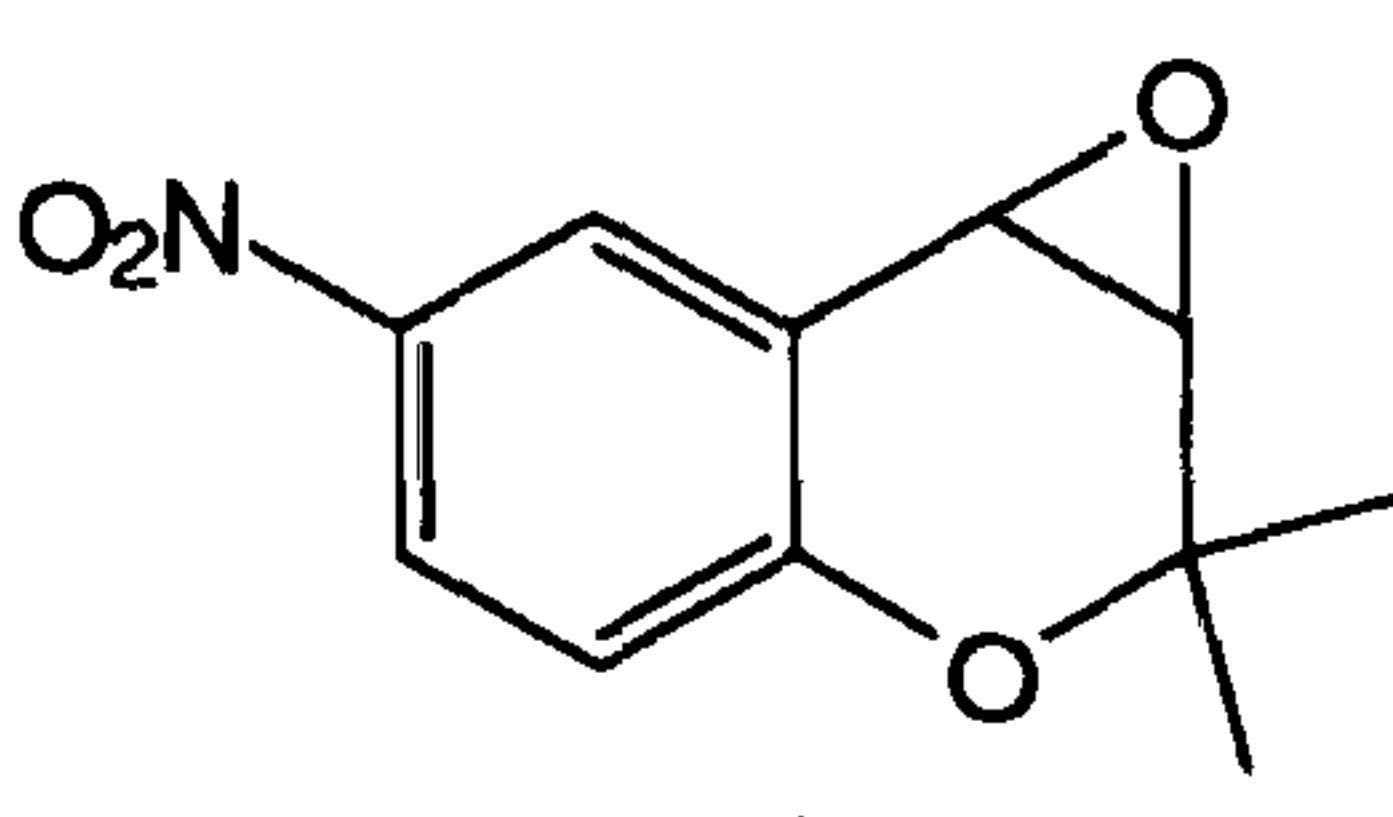
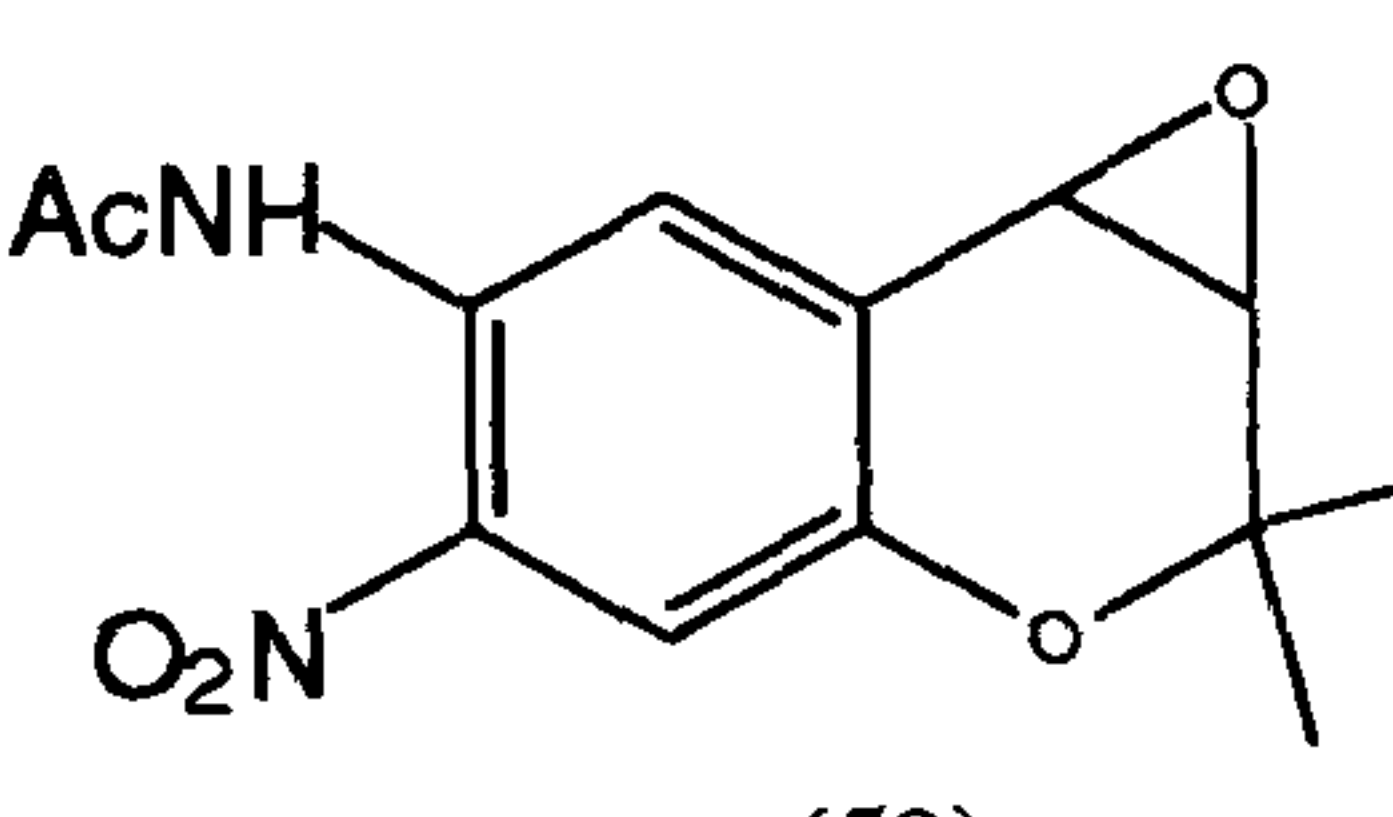
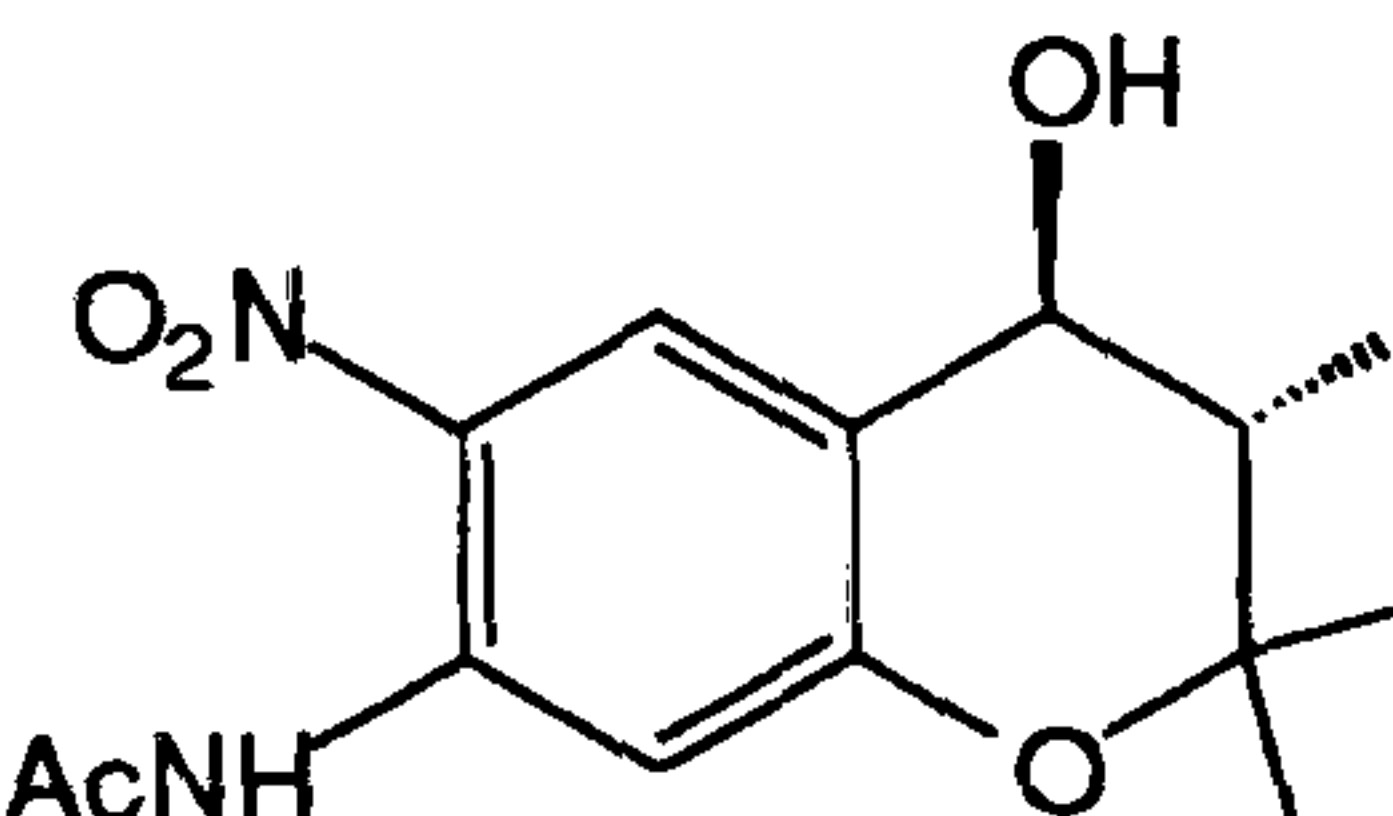
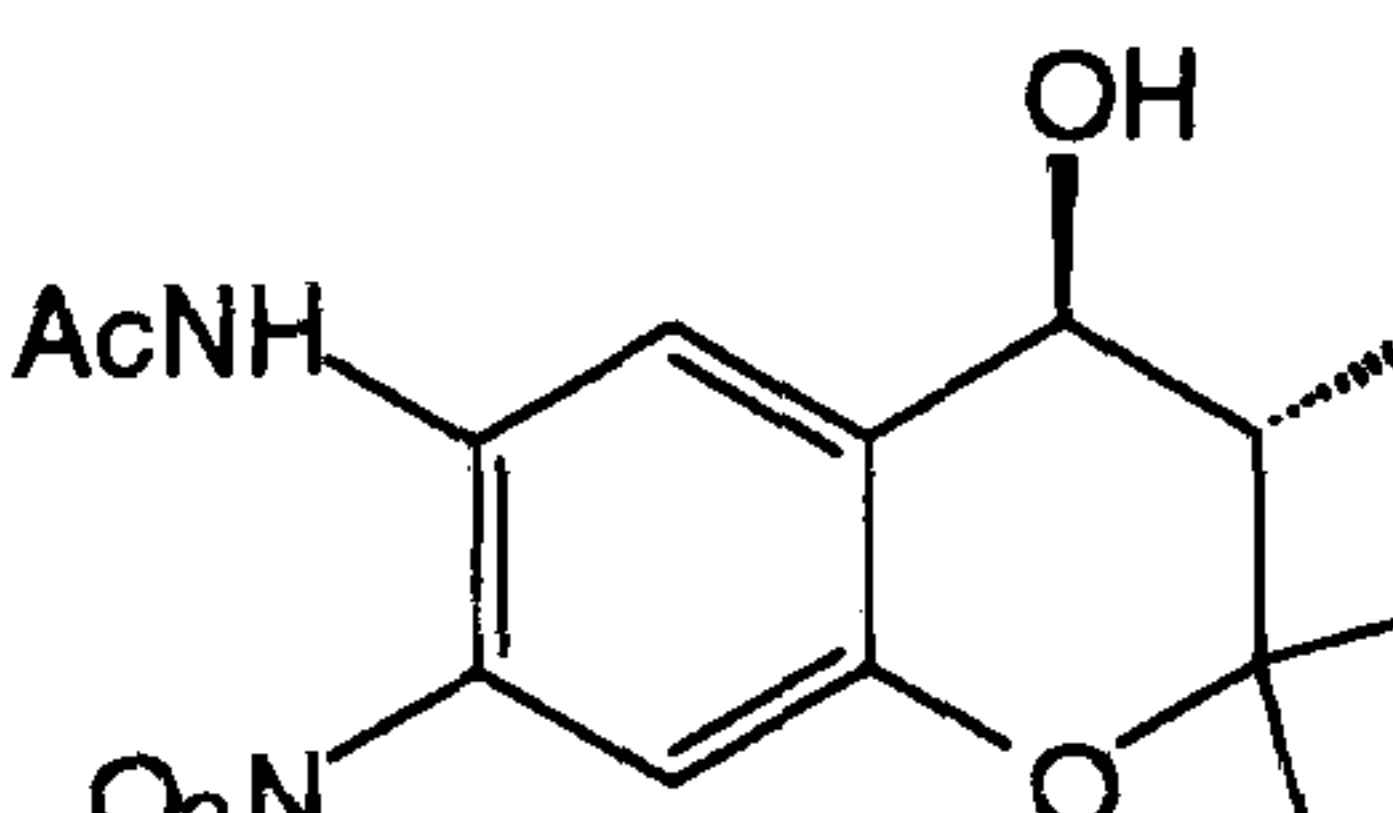
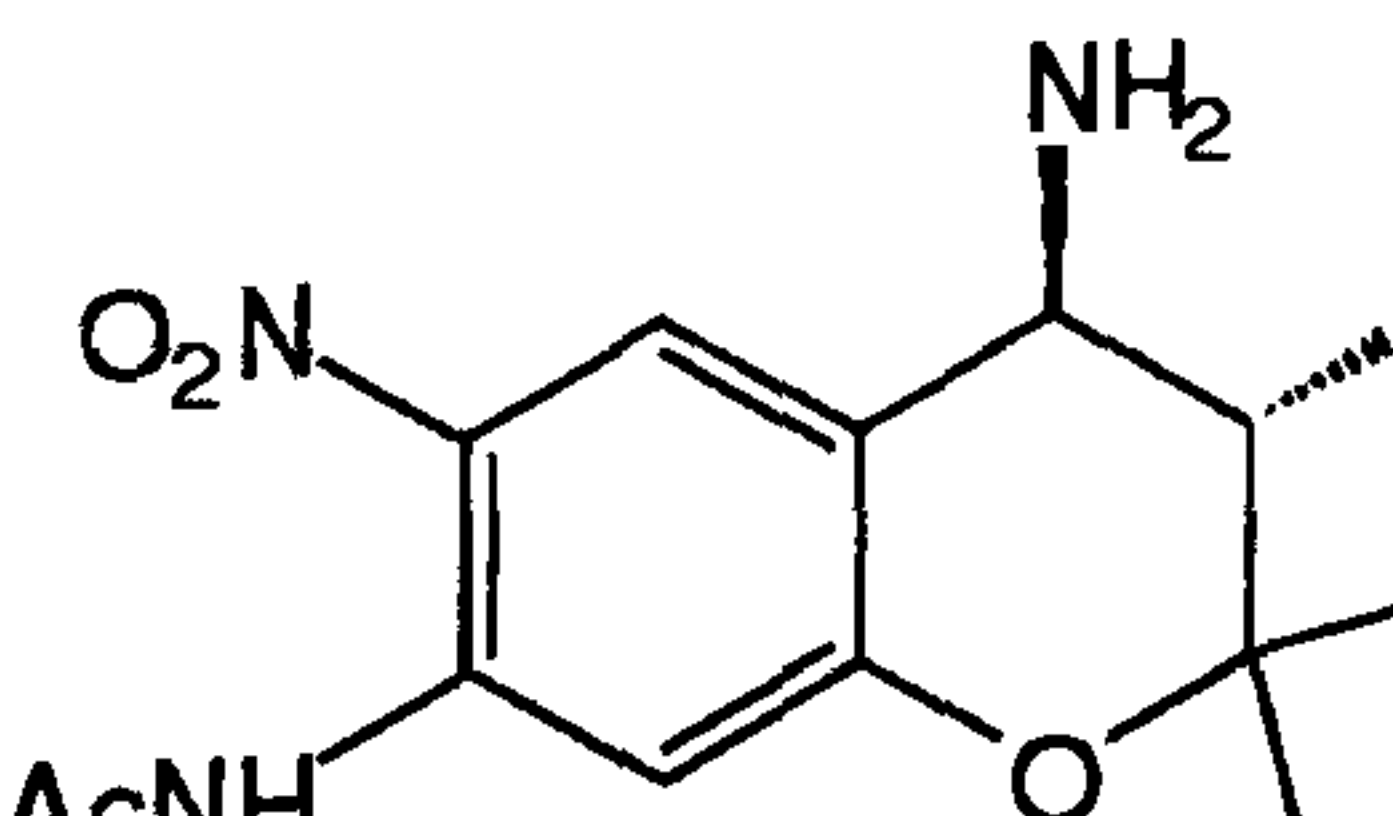
These compounds are related to the drug Cromakalim (57), which exerts its physiological effect by potassium channel activation and has been developed for use as an antihypertensive agent [142]. Their resolution has become very important since it has been demonstrated that, for Cromakalim, there is a marked difference in biological activity between the individual enantiomers [143], with the antihypertensive activity being found to reside primarily in the (-) enantiomer [143].

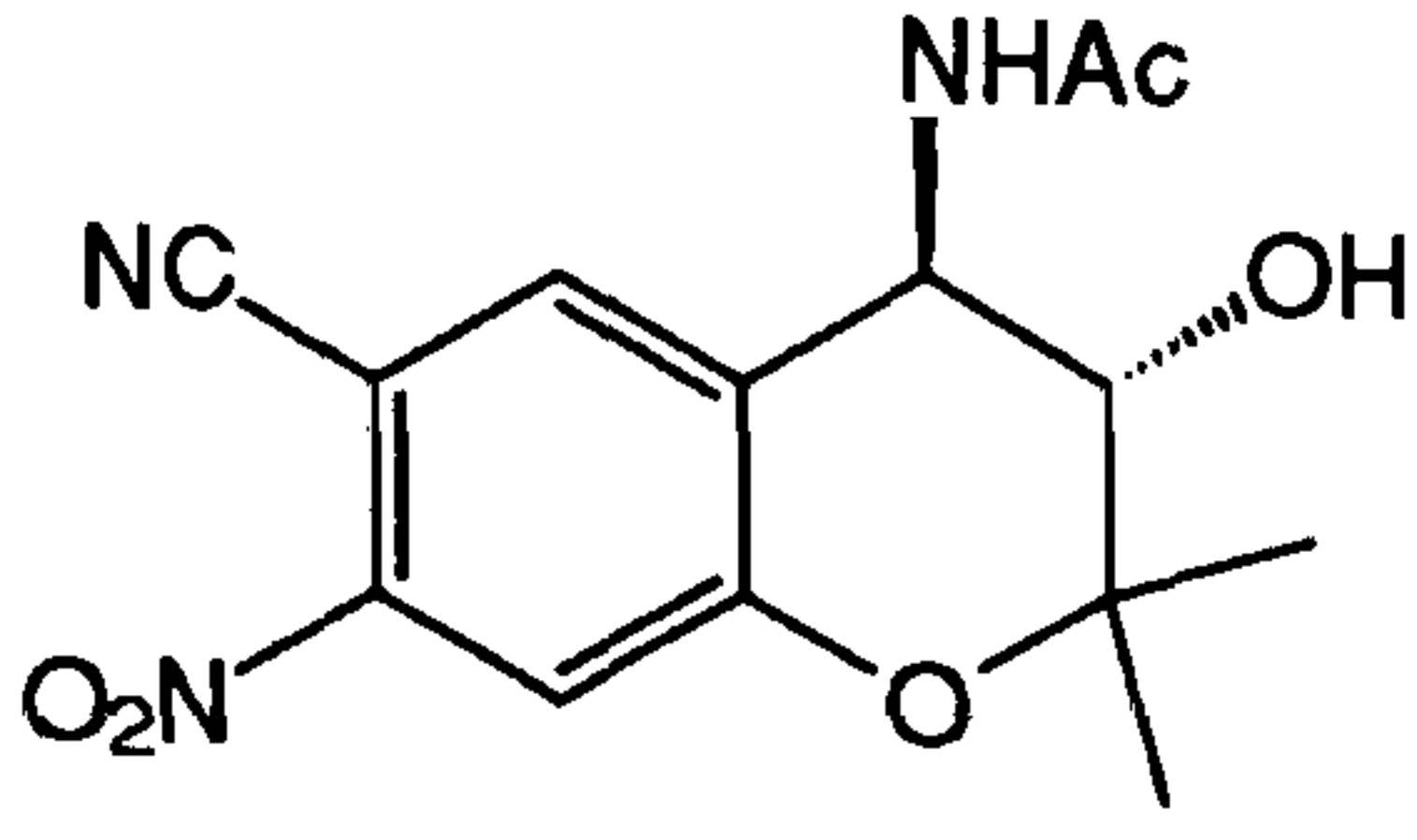
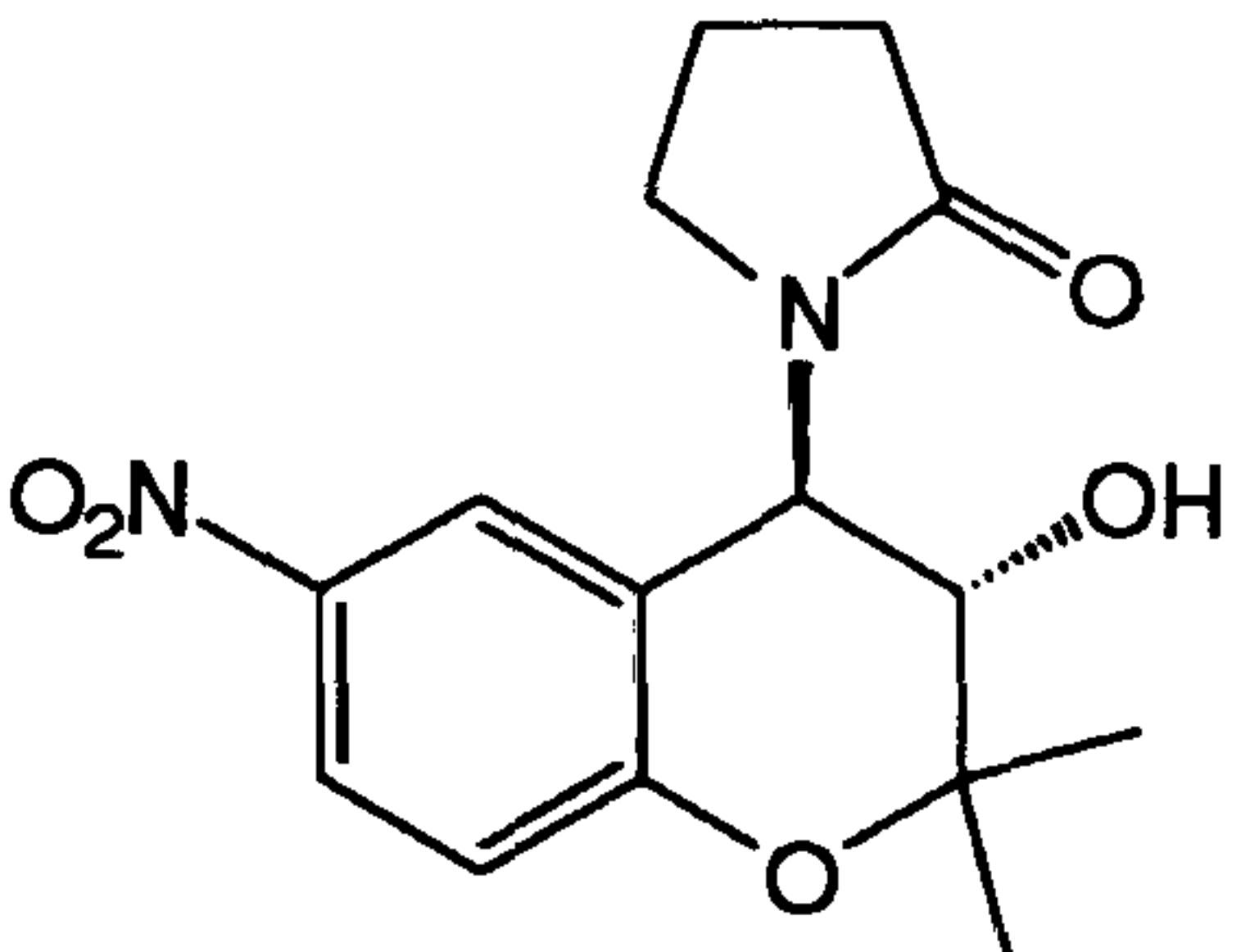
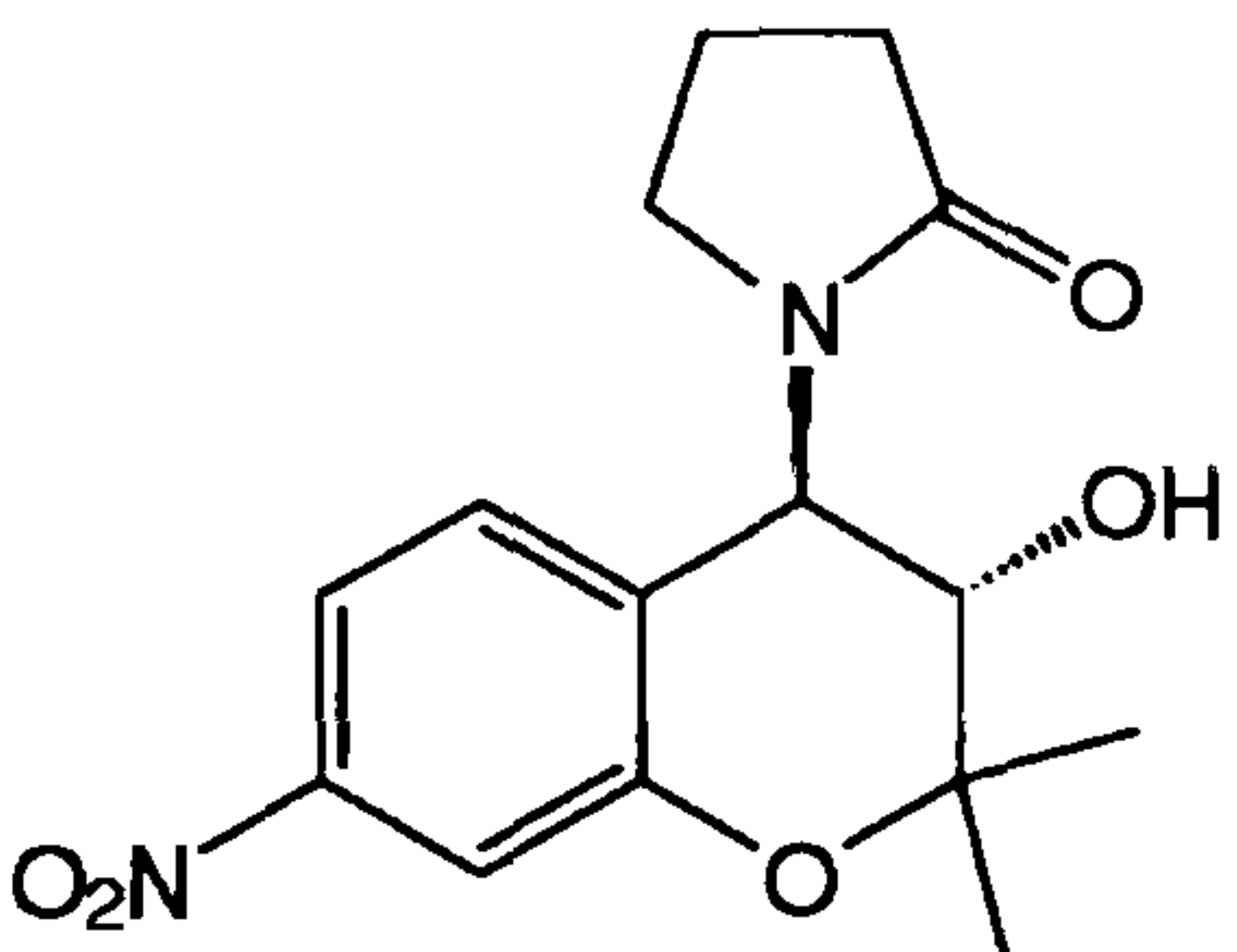
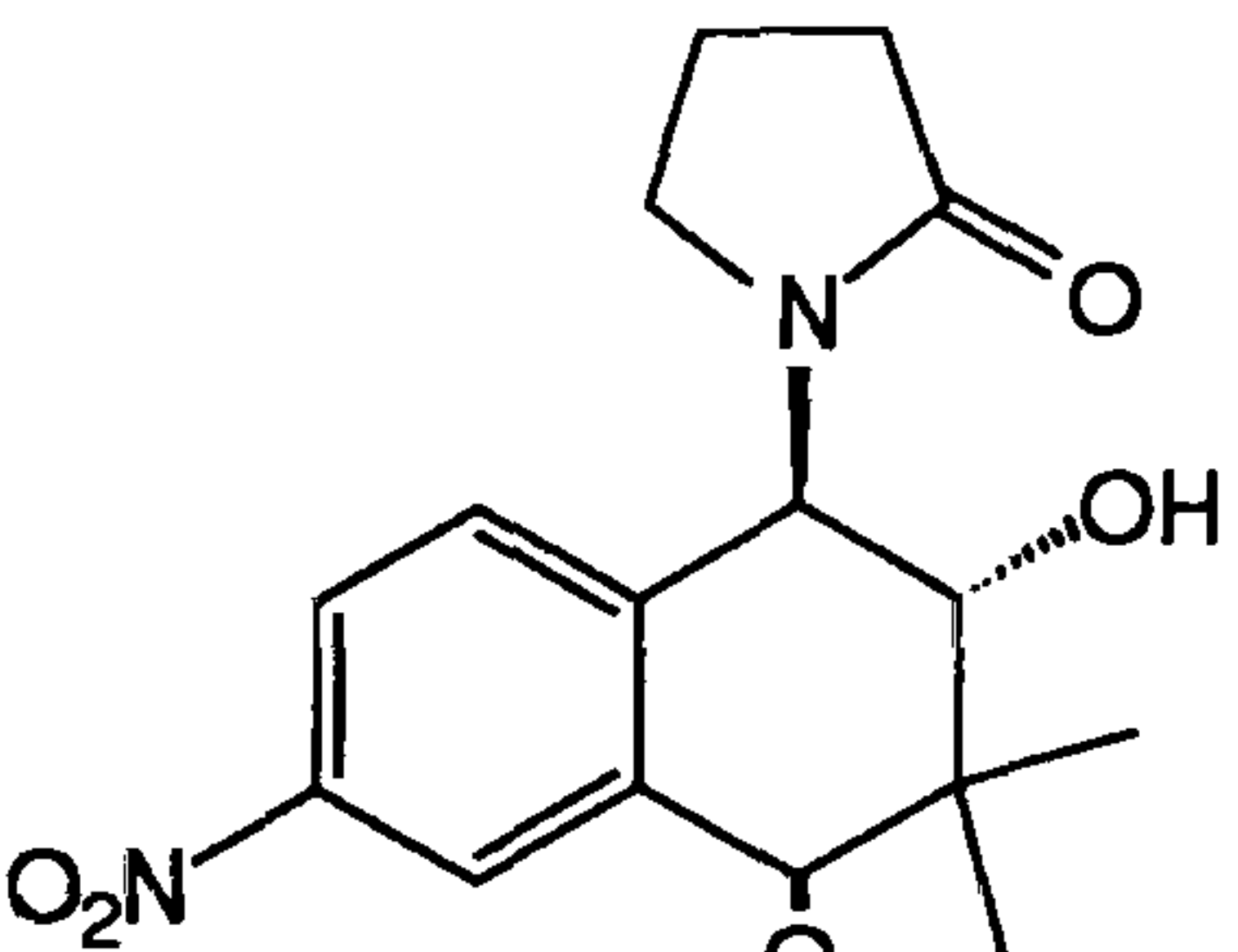


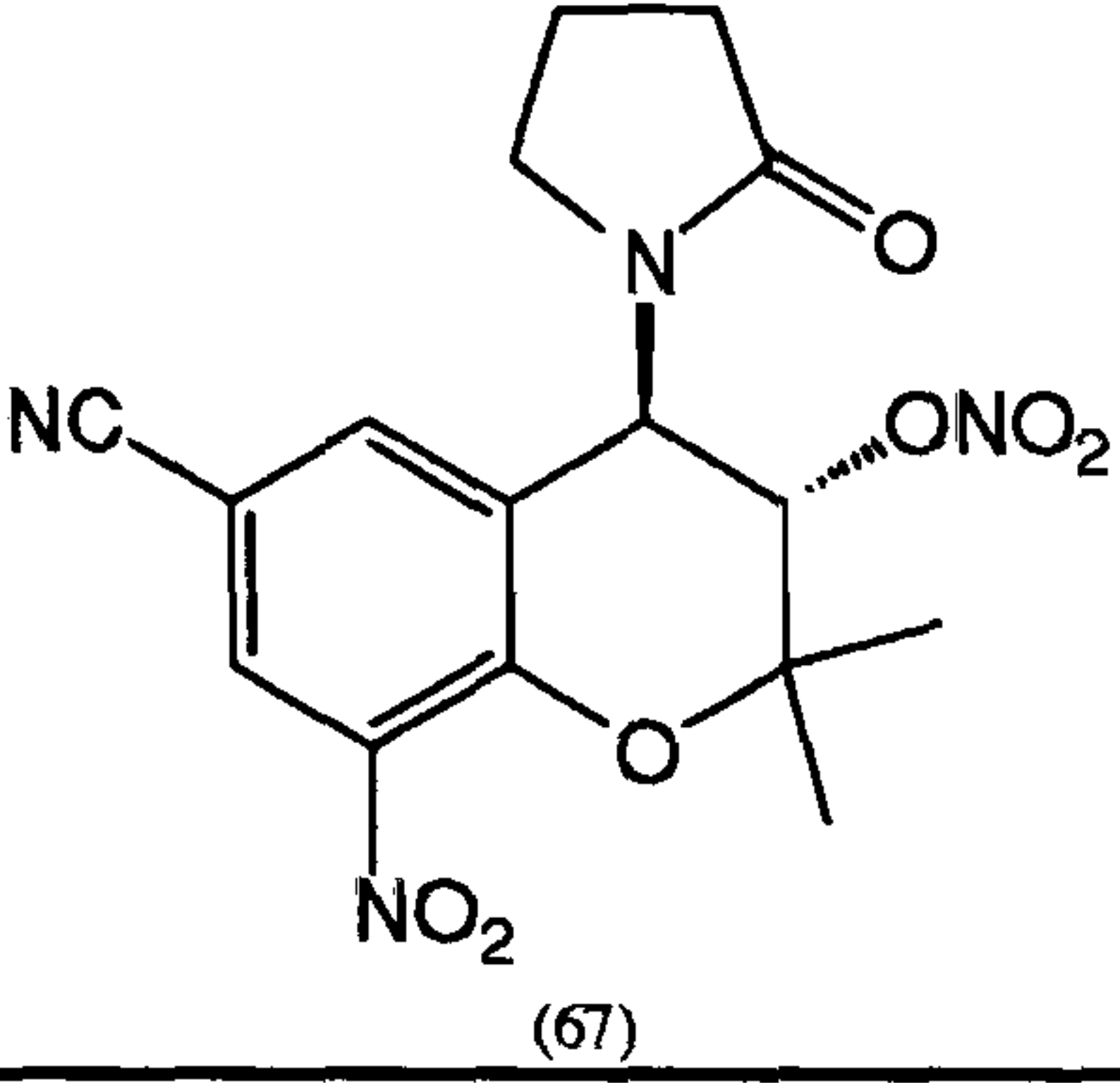
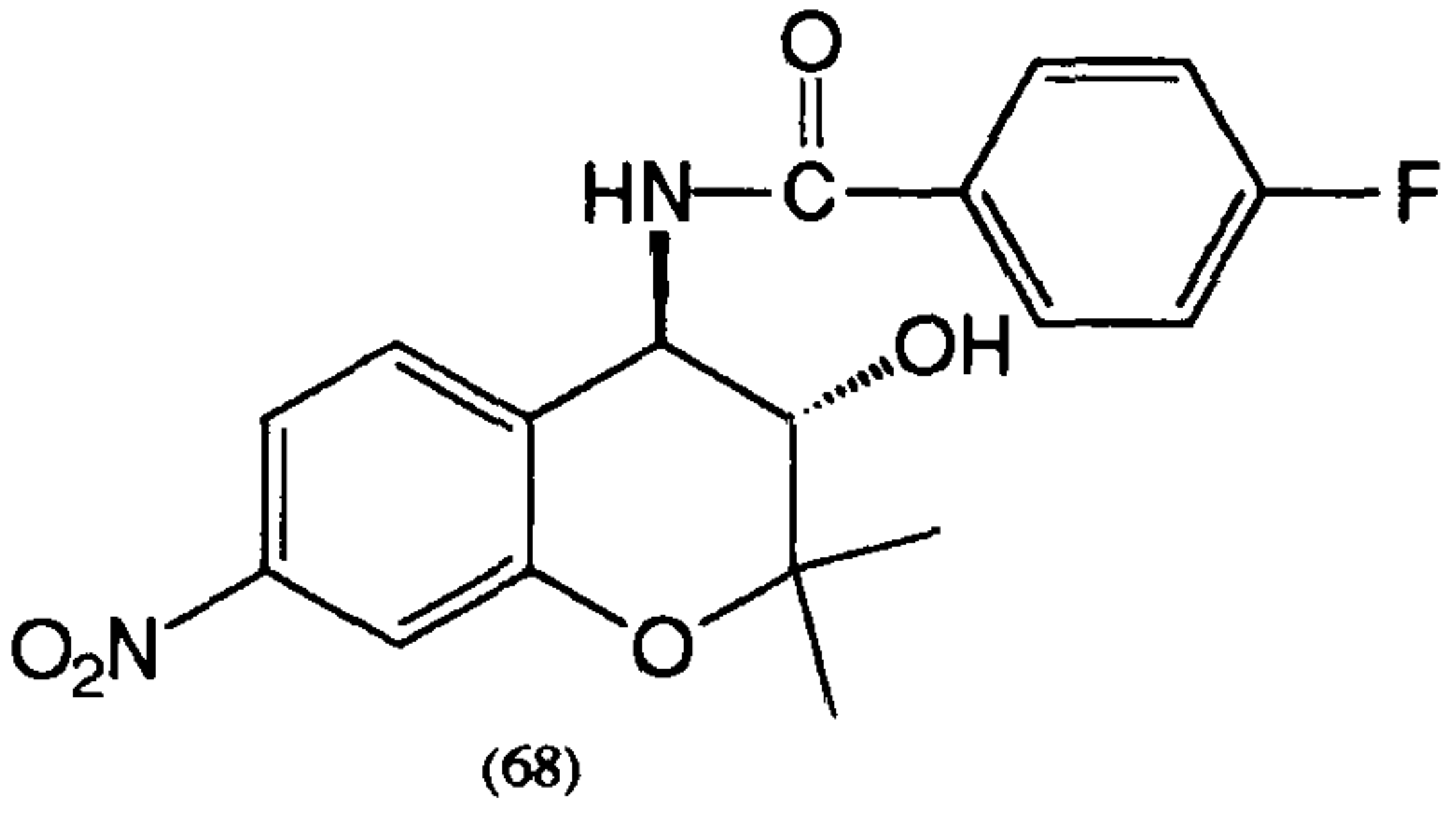
(57)

The enantiomeric resolution of these compounds is based on a charge-transfer interaction between an electron-deficient (π -donor) aryl moiety and an electron-deficient (π -acid) aryl moiety. The following examples (Table 1) of substituted 3,4-dihydro-2,2-dimethyl-2H-1-benzopyrans were chromatographed on a (+)-hexahelicen-7ylacetic acid CSP column (250 x 1mm) using 10:90 IPA/n-hexane mobile phase, flow rate = 2.0ml/min, detection 254nm. All of them showed enantiomeric resolution.

Table 1 Resolution of benzochromans on (+)-hexahelicenyl CSP

Compound	Peak retention times (min)	k'1	k'2	α	R _S
 <p>(58)</p>	3.39 3.59	0.70	0.80	1.14	0.40
 <p>(59)</p>	8.19 10.90	3.10	4.45	1.44	1.38
 <p>(60)</p>	11.12 13.30	4.55	5.65	1.24	0.66
 <p>(61)</p>	8.11 10.76	3.06	4.38	1.43	1.78
 <p>(62)</p>	13.53 18.00	6.77	8.00	1.18	0.56

Compound	Peak retention times (min)	k'1	k'2	α	R _s
 <p>(63)</p>	10.68 11.92	4.34	4.96	1.14	0.72
 <p>(64)</p>	9.82 11.19	3.91	4.60	1.18	0.83
 <p>(65)</p>	19.50 21.72	8.75	9.86	1.13	0.42
 <p>(66)</p>				1.58	

Compound	Peak retention times (min)	k'1	k'2	α	R _S
 (67)	9.03 11.43	3.52	4.72	1.34	1.45
 (68)	6.12 6.47	2.06	2.24	1.08	0.32

Some of the compounds were chromatographed using two different mobile phases (5:95 IPA/n-hexane and 10:90 IPA/n-hexane). There was no marked change in enantioselectivity with a change in mobile phase.

Table 2 Composition of selectivities for the resolution of benzochromans on (+)-hexaheliceny CSP using two mobile phases

Compound	α 5/95 IPA/n-hexane	α 10/90 IPA/n-hexane
58	1.14	1.14
59	1.16	1.44
60	1.56	1.24
62	1.36	1.18
64	1.16	1.18
67	1.31	1.34
68	1.09	1.08

In this series the presence of a nitro group on the aryl ring of the solute molecule appears to be necessary for enantioselectivity. For those 3,4-dihydro-2,2-dimethyl-2H-1-benzopyrans containing substituents on the aryl ring which were less electron-withdrawing than the nitro-group^[144], no successful resolutions of enantiomers were observed. Presumably this is because the aryl ring with substituents less electron-withdrawing than the nitro-group is not a sufficiently strong π -acid to give a suitable charge-transfer interaction with the electron-rich (+)-hexahelicene chiral stationary phase.

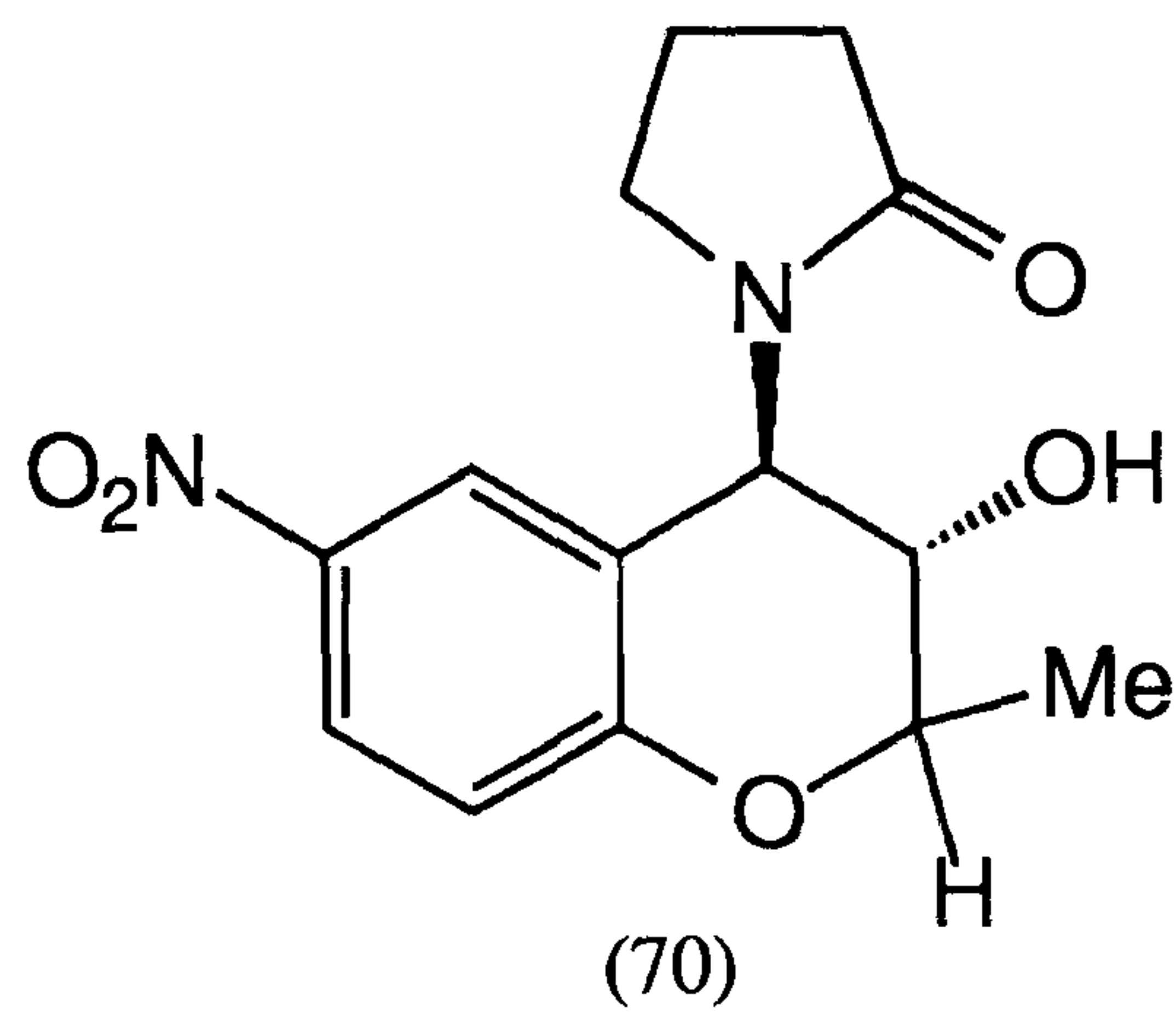
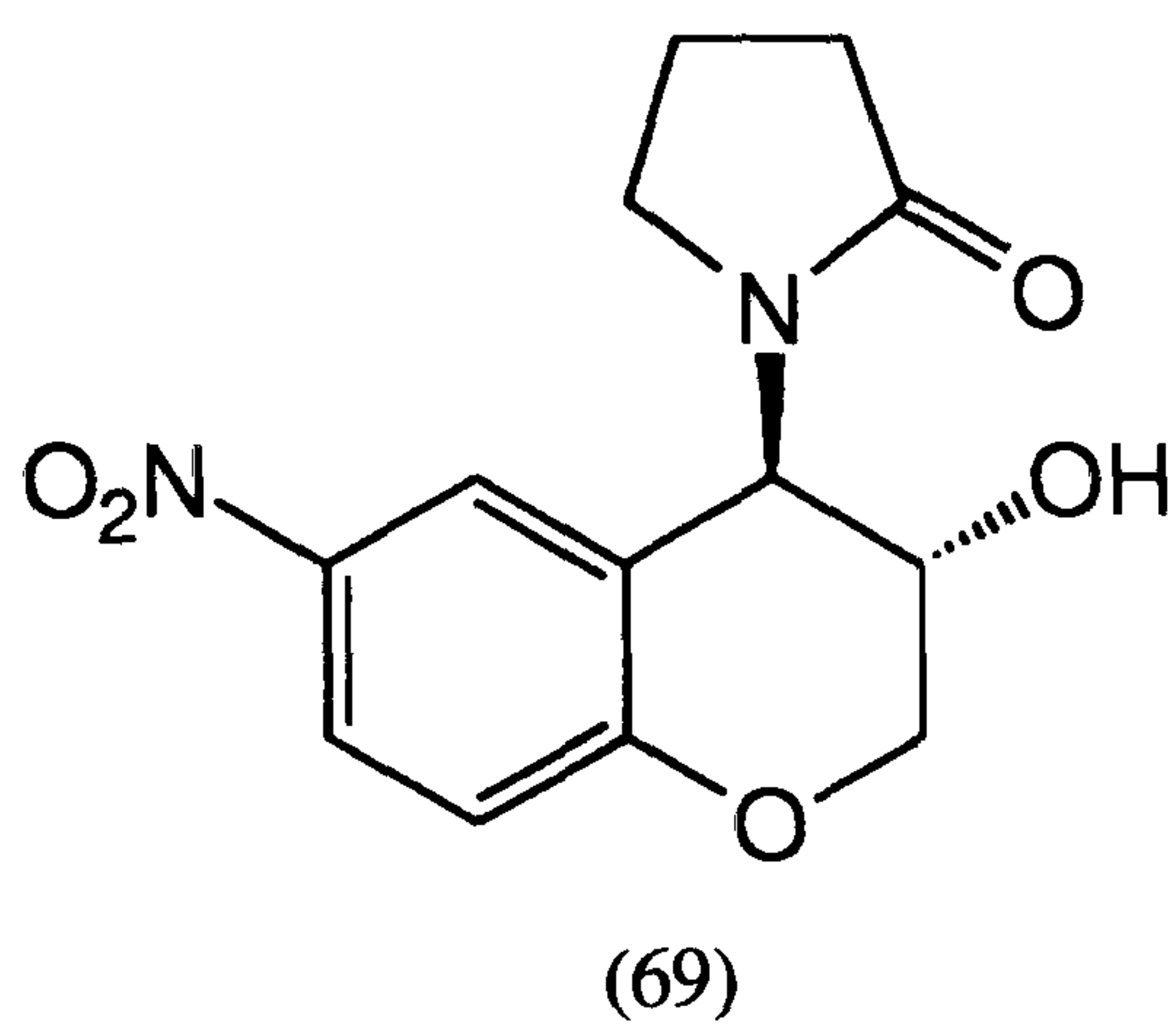
The position of the nitro-group on the aryl ring influenced the degree of enantioselectivity obtained. For the bromohydrins (60) and (61), the 7-nitro isomer was better resolved than the 6-nitro isomer. However, comparing compounds (64) and (65), where the 4-substituent was fixed as pyrrolidine and the substitution pattern of the aromatic ring of the benzopyran structure was varied, the order of enantioselectivity obtained was 6-NO₂ > 7-NO₂. Compound (67), which possesses a nitro-group in 8 position, had better resolution than (64) and (57). This might be because the 6 position is occupied by another good electron-withdrawing group (-CN), and the nitro-substituent on the aryl ring has a strong effect on retention. Compounds containing a nitroaryl group elute in the k' range 2-6 for 10/90 IPA/n-hexane. Compounds containing dinitro-substituents on the aryl ring are more strongly retained (20:80 IPA/n-hexane required for a similar range of k')^[145].

High enantioselectivity ($\alpha = 1.58$) was observed for compound (66), whose structure is related to that of the benzopyrans.

Enantioselectivity was not observed in examples where nitro-aryl substituents were linked to the 3- or 4- position.

The methyl groups on the pyran ring were also found to have an influence on enantioselectivity. For the dihydro-equivalent of compound (64) (i.e. gem-dimethyl at C(2) removed) (69), it was found that the enantioselectivity ($\alpha = 1.09$) was lower than for (64) ($\alpha = 1.18$). Surprisingly this was a marked increase in retention

(average $k' = 12.56$, c.f. 4.25 for (64)). For the compound (70) with one methyl group at C(2), the four possible peaks (two diastereomeric pairs of enantiomers) were merged into a broad envelope which was only slightly more retained ($k' = 5.48$) than compound (69).



Chapter Four

OBJECTIVES AND STRATEGY

4.1- Introduction.

In previous work in Professor Matlin's research group, Vivian Stacey had carried out the synthesis of racemic hexahelicen-7-ylacetic acid methyl ester [141]. This was resolved using a preparative (250 x 22 mm i.d.) Pirkle-type column of TAPA on APS, but the resolution was extremely tedious, as only small amounts could be injected due to the limited resolving power and low sample capacity of this column. Only the first-eluting (+) enantiomer was obtained optically pure in sufficient quantity for the synthesis of the bonded phase. After hydrolysis of the (+)-isomer of the methyl ester, the corresponding (+)-isomer of the free acid was linked to APS using 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-4-toluenesulphonate. Both this carbodiimide and its urea by-product are water-soluble and this reagent had been selected in order to facilitate the clean-up of the resulting bonded phase. In Stacey's work, a conversion of 34% of the available amino group in the APS phase to amide groups was observed. Thorough washings of the phase was carried out, to ensure that only bonded (as opposed to salt-linked) helicenyl groups remained in the phase [141].

Although portions of Stacey's work were published and formed the basis of a UK Patent Application [146] filed by the company sponsoring her work (Beecham Pharmaceuticals), unfortunately Stacey was unable to write up her complete research work as a PhD thesis owing to personal problems. Consequently, only partial and fragmentary details of the synthesis were available and much of the essential data on exact synthesis methodologies and the spectroscopic and microanalytical results on intermediates were lost to our laboratory.

4.2- Objectives.

In the light of the above background, the specific objectives of this research project were:

- 1- To repeat the synthesis of hexahelicen-7-ylacetic acid, where necessary re-developing or optimising the procedures reported by Newman [75] and adapted by Stacey [141]. All intermediates, products and relevant by-products were to be thoroughly characterised by appropriate spectroscopic methods.
- 2- To improve the procedure for the resolution of the enantiomers of the helicene derivative so that, if possible, a method could be found which would be suitable for larger scale work.
- 3- To investigate alternative bonding procedures in order to ensure that the maximum loading of chiral phase was achieved and then obtain the largest possible degree of enantioselectivity.
- 4- To prepare the bonded phases from both the (+) and (-) isomers of the hexahelicen-7-ylacetic acid. This would enable checks to be carried out on the order of elution of test compounds and also to offer an explanation of which enantiomer could be eluted first.
- 5- To establish a model consistent with the observed enantioselectivity of the bonded phase and to examine the utility of this model to describe the behaviour of a series of chiral analytes.
- 6- If time permitted, to prepare a sufficiently large quantities of chiral hexahelicenyl phase for construction of a preparative (22 cm i.d) HPLC column and to evaluate the application of this column for a variety of interesting separations such as the resolution of analogues of chromakalin (see chapter 3) for biological testing.

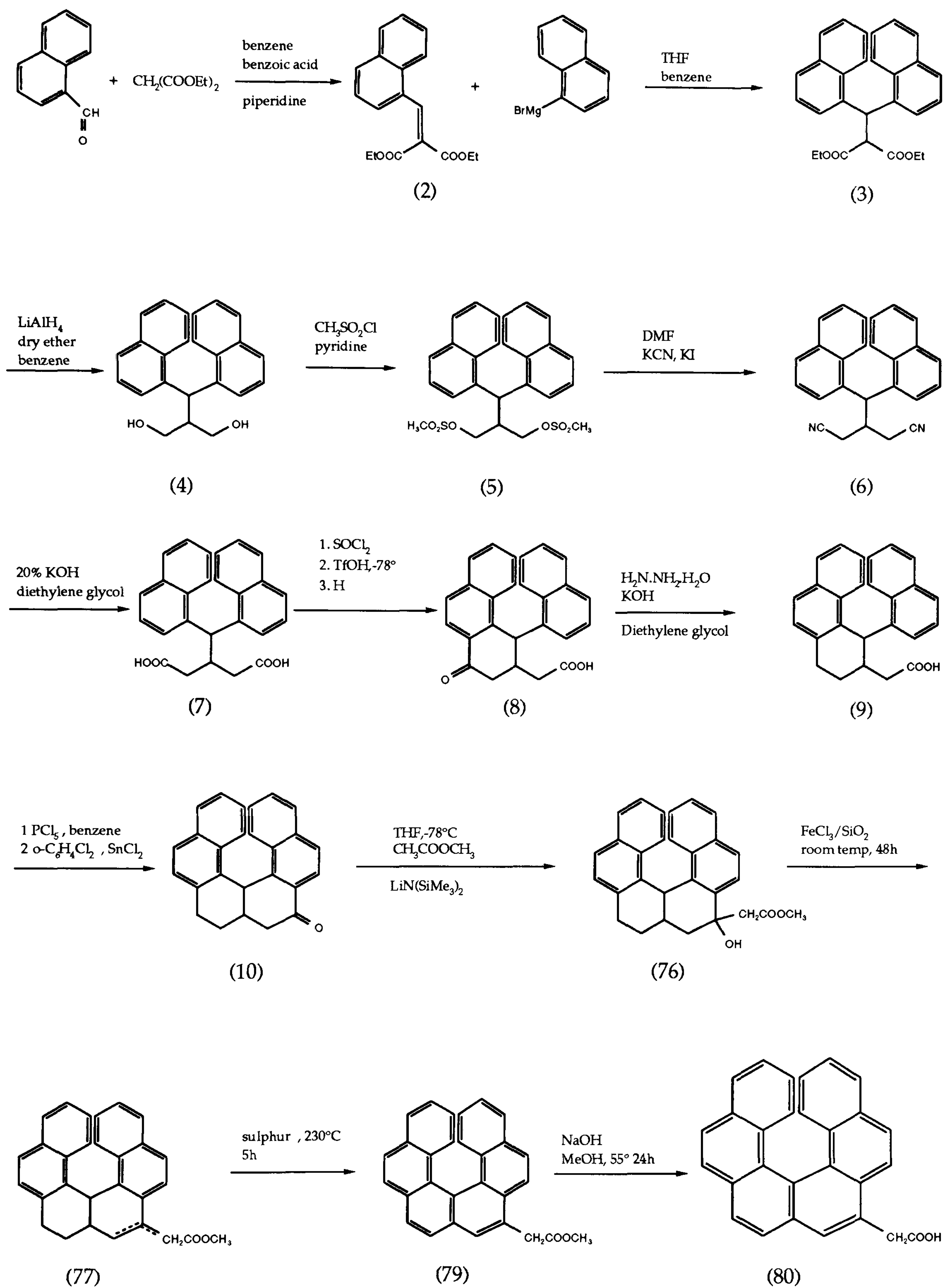
4.3- Strategy

The synthetic route used by Stacey and followed in this research project had been chosen to allow multigram quantities to be prepared in good yield. The reactions involved in this route were selected to minimise the side reactions and to go to completion. At every stage of the synthesis, intermediates would be fully characterised by a combination of microanalysis, infrared, ultraviolet and nuclear magnetic resonance spectroscopy (^1H and ^{13}C) and mass spectrometry.

The strategy did not involve a stereoselective synthesis. The original Newman synthesis [75] of hexahelicene itself (Scheme 1, p47) was adapted in the present work for the formation of hexahelicen-7-ylacetic acid (Scheme 20). A modification was introduced after the formation of the ketone (10) to allow us to attach a chain needed for the bonding to aminopropyl silica. In order to do that, the ketone (10) was reacted with the anion derived from methyl acetate to form the β -hydroxyester. The resulting product (76) was to be dehydrated and then dehydrogenated to give racemic hexahelicen-7-ylacetic acid methyl ester (79). Different columns were to be examined in order to find the most efficient enantiomeric resolution of the racemic ester (79). Once the two enantiomers were resolved, they would be separately hydrolysed to their free acids.

The resulting, resolved enantiomers of the acid would then be separately bonded to aminopropyl silica using carbodiimide or related coupling techniques which have previously proved to be very effective for this kind of linkage [147].

It was intended that the bonded chiral phase would be characterised by techniques such as microanalysis and surface spectroscopy and that both analytical and preparative HPLC columns would be constructed from this material. The scope of the new phase for the resolution of enantiomers could then be investigated in detail, using a wide range of electron-deficient racemic substances. Where useful analytical HPLC separations were found for important drug substances, the method may be scaled up for application to a preparative column. Molecular models would be used to make correlations between structure and resolution.



Scheme 20

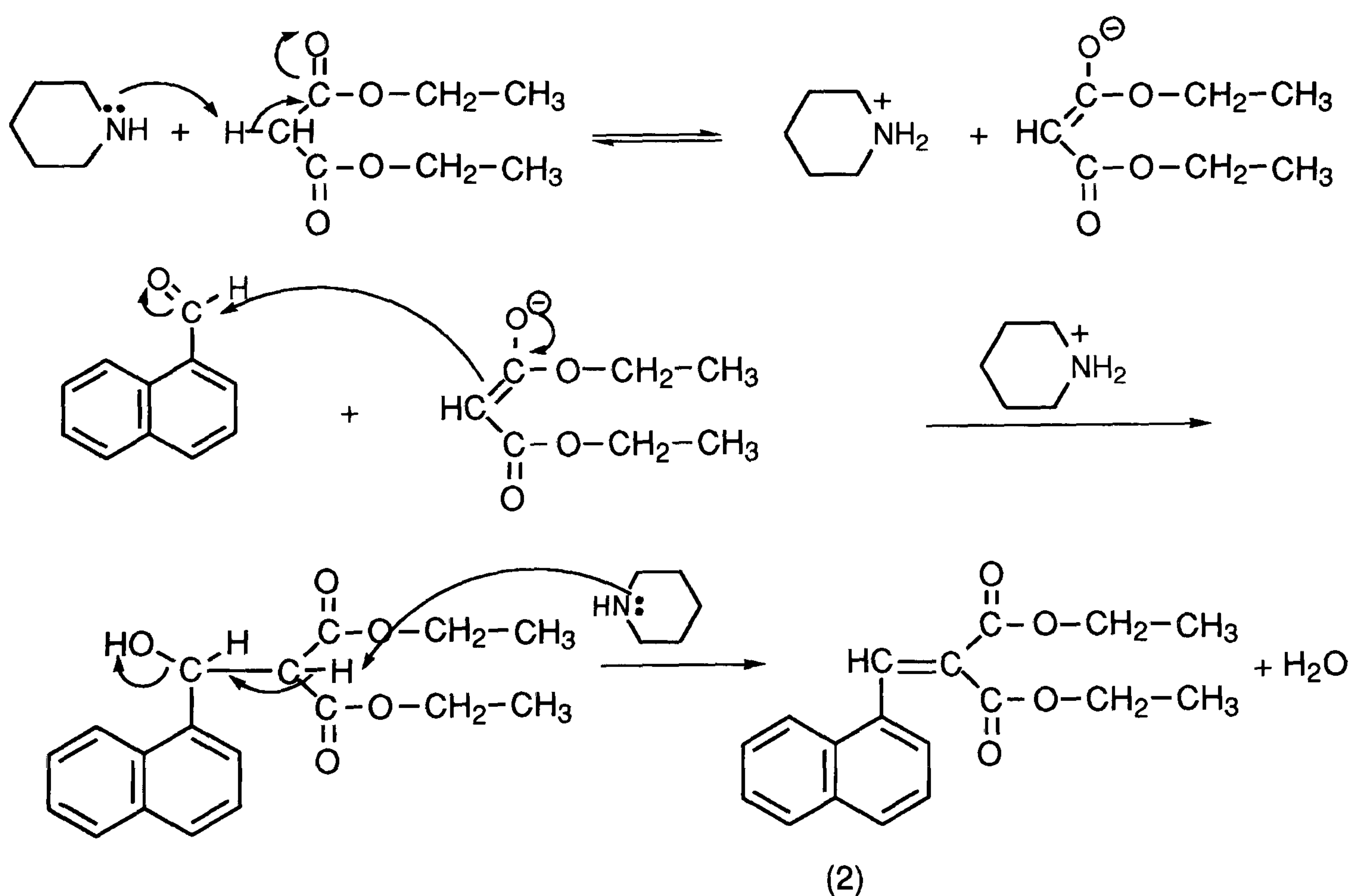
Chapter Five

RESULTS AND DISCUSSION

5.1- Synthesis of hexahelicen-7-ylacetic acid methyl ester.

The synthesis of hexahelicene reported by Newman and Lednicer [75] was adapted for the preparation of hexahelicen-7-ylacetic acid. This route was chosen because, of all the routes to hexahelicenes described in the literature, this one appeared to be the best for the synthesis of the large quantities required for this project and also presented a good opportunity to attach the acetic acid side chain at a well defined site. Although photosynthetic methods are more direct, they require working at high dilution at the irradiation stage. On scaling up, the volume of the solvent would be unmanageable.

The literature method [75] was published in 1955 and pre-dates modern spectroscopic techniques such as ^1H and ^{13}C NMR and MS, and also modern high resolution chromatographic methods, so care was taken to fully analyse and characterise all compounds on the synthetic pathway.



Scheme 21

The Knoevenagel condensation of 1-naphthaldehyde with diethyl malonate (Scheme 21) afforded diethyl 2-(1'-naphthylmethylene)malonate (2) in 80% yield, compared with 70% in the literature [75]. A major factor in improving the yield was the use of freshly distilled 1-naphthaldehyde.

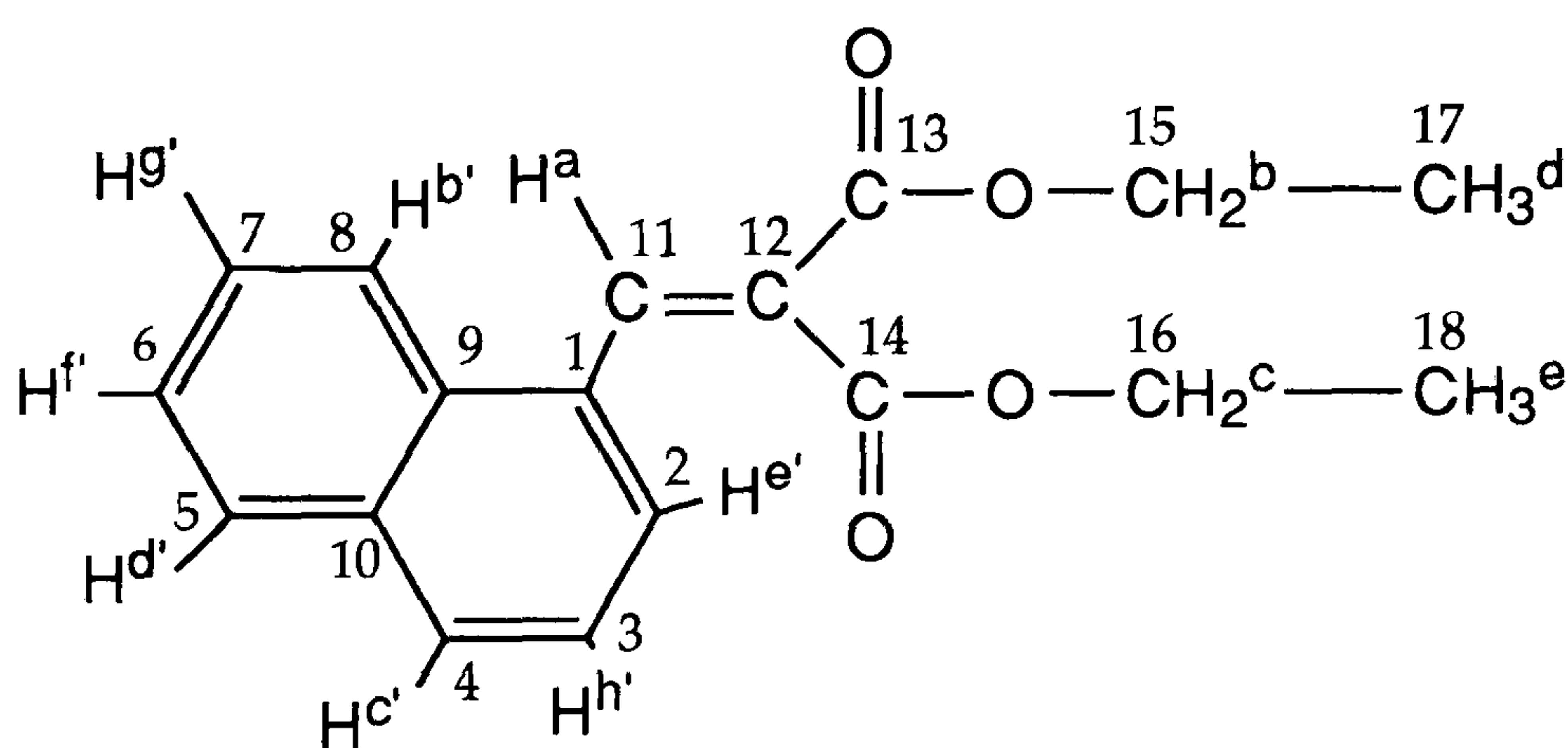
This reaction is a base-catalysed condensation between the acidic methylene group of the diethyl malonate and the carbonyl group of the 1-naphthaldehyde. The absence of an α -H on the naphthaldehyde ensures a clean reaction. Piperidine is typically used as a base to catalyse Knoevenagel reactions, its function being to remove a proton from the active methylene component forming a carbanion [148]. This resonance-stabilised anion adds to the carbonyl component giving an intermediate hydroxyl compound which then eliminates water. The protonated amine is the active catalyst in the dehydration. Weak amines are used to minimise competing reactions. Piperidine has a strong catalytic effect, but addition of benzoic acid to the piperidine-catalysed mixture depresses the rate [149]. The progress of the reaction was observed using a Dean-Stark water separator, and only one spot on the TLC (silica, 50:50 hexane/EtOAc) was shown at the end of the reaction.

Product (2) was obtained as a solid, contrary to the literature [75], which described it as an oil. Recrystallisation from ethanol afforded a pale yellow solid. Spectroscopic analysis of this material gave results consistent with the assigned structure (2).

The IR spectrum shows strong bands at 1212 (C-O, st), 1633 (C=C, st) and 1713 cm^{-1} (C=O, st), all characteristic of the conjugated, unsaturated ester. The presence of the aromatic structure is mainly evident in the low-wavenumber region between 900 and 675 cm^{-1} . The two strong bands shown at 800 and 775 cm^{-1} are characteristic of a 1-substituted naphthalene and result from the out-of-plane bending of the ring C-H bonds. The first band corresponds to the three adjacent hydrogen atoms on one ring and the second band is due to the four adjacent hydrogen atoms on the fused ring. Four characteristic C=C skeletal in-plane

vibrations involving carbon-carbon stretching within the ring, which often occur in pairs, can also be seen in the 1600-1585 and 1500-1400 cm^{-1} regions. The first band $\text{C}=\text{C}$ skeletal aromatic is overlapped with the $\text{C}=\text{C}$ olefinic stretch, which is very intense in this case, due to conjugation with the aromatic ring and with the ester group, and appears at 1633cm^{-1} .

The UV spectrum of compound (2) gives three electronic transitions of the $\pi-\pi^*$ type. The first band at 220nm is of the high intensity and is an allowed transition. The second (244nm) and third band (323nm) are forbidden and are less intense. This molecule has an extended resonance through the double bond attached directly to a naphthalene group and to two carbonyl groups. As resonance is not severely inhibited until the interplanar angle becomes quite large [150], there is an effective overlap of π orbitals resulting in the creation of new energy levels. More $\pi-\pi^*$ transitions are possible, giving an additional bathochromic shift accompanied by an increase in absorption intensity. The $n-\pi^*$ transition of the each of the two lone pairs of the oxygen of the carbonyl groups (forbidden transition) appears at long wavelength and coincides with the third band of the extended π -system.



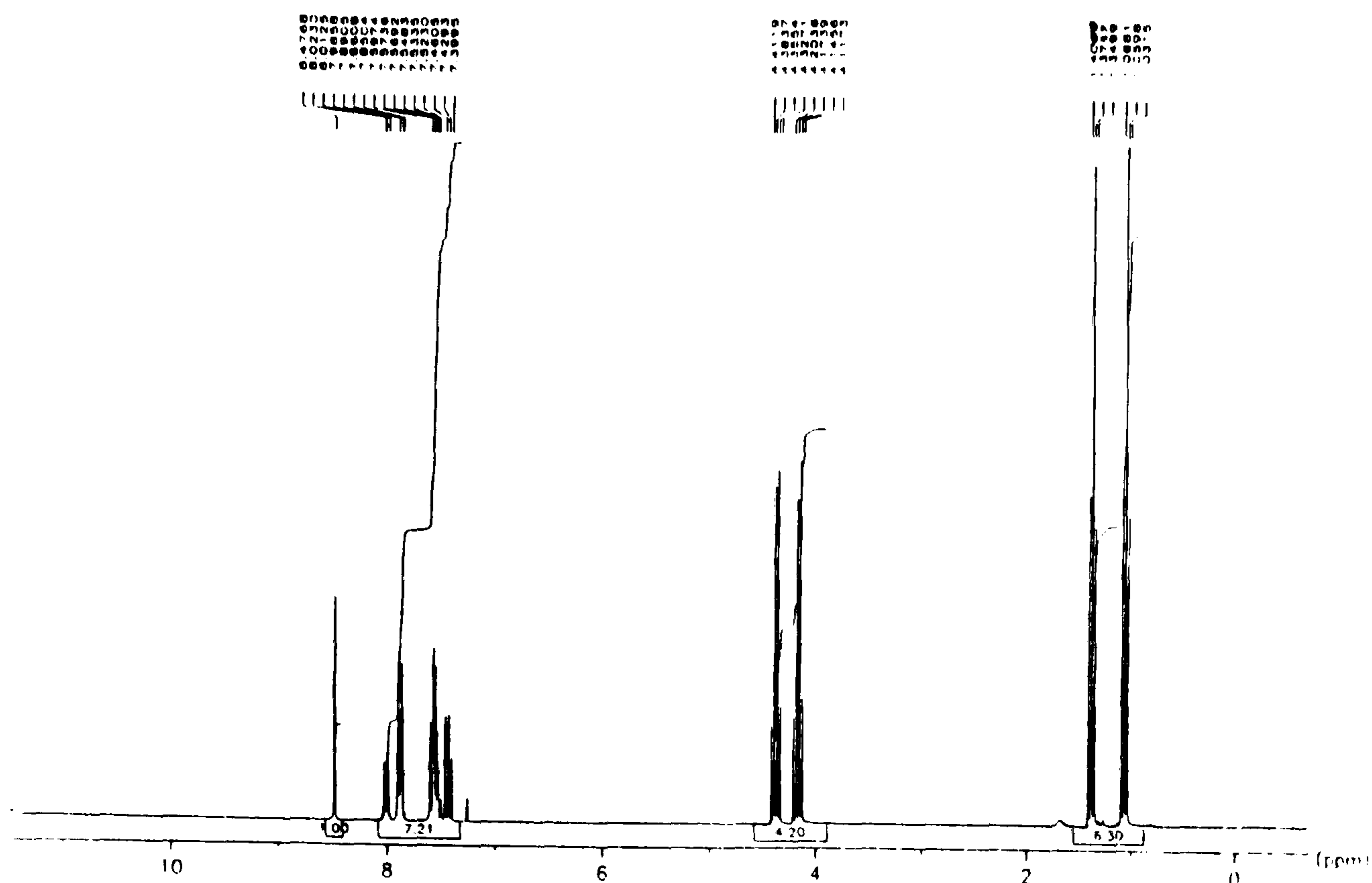
(2a)

The ^1H NMR spectrum (CDCl_3) (Fig. 55a) shows a multiplet between 7.4 and 8.0 δ corresponding to the seven naphthalene protons. All the positions of the naphthalene protons have been assigned with help of COSY and NOE's experiments (Fig. 55b). The most deshielded proton is at the *peri* position $\text{H}^{\text{b}'}$ (8.0 δ , bd, $J = 8.0\text{Hz}$) which couples to $\text{H}^{\text{g}'}$. The most shielded is $\text{H}^{\text{h}'}$ (7.4 δ , t, $J = 8.0\text{Hz}$), which is ortho coupled to $\text{H}^{\text{e}'}$ and $\text{H}^{\text{c}'}$. Protons $\text{H}^{\text{c}'}$ and $\text{H}^{\text{d}'}$ appear together with the same type of couplings at 7.85 δ and the other three protons $\text{H}^{\text{e}'}$, $\text{H}^{\text{f}'}$, $\text{H}^{\text{g}'}$ appear at higher field. There was no evidence for the aldehyde proton of the starting material (1-naphthaldehyde) showing that the reaction was complete. The olefinic proton H^{a} is subject to the anisotropic effects of the naphthalene ring and to the cis-alkoxycarbonyl group and consequently appears at very low field (8.5 δ)

The protons of the methylenes (CH_2^{b} and CH_2^{c}) are chemically and magnetically non-equivalent (one group is *cis* to the naphthalene ring, whereas the other is *trans*) and resonate at different shifts, appearing as two quartets at 4.15 and 4.36 δ with coupling constants both equal to 7.1 Hz. Similarly, the two methyl groups (CH_3^{d} and e) appear as two triplets at 1.05 and 1.37 δ respectively ($J = 7.1\text{ Hz}$).

In the ^{13}C NMR spectrum there are two sets of lines at high field, centred at 13.87 and 61.50 ppm, which are due to the two non-equivalent methyl and methylene groups, respectively; and a group of ten lines, centred at 127.6 ppm, which correspond to the expected pattern of the naphthalene carbons. These ten lines have been assigned using C-H correlations and assuming that the quaternary C atoms are responsible for the weak peaks. There are also two lines at low field (166.02 and 163.84 ppm) due to the two non-equivalent carbonyl carbons. The C-11 appears at very downfield (141.11) as it is next to an olefinic carbon and C-12, the quaternary carbon appears at 133.25 ppm.

(a)



(b)

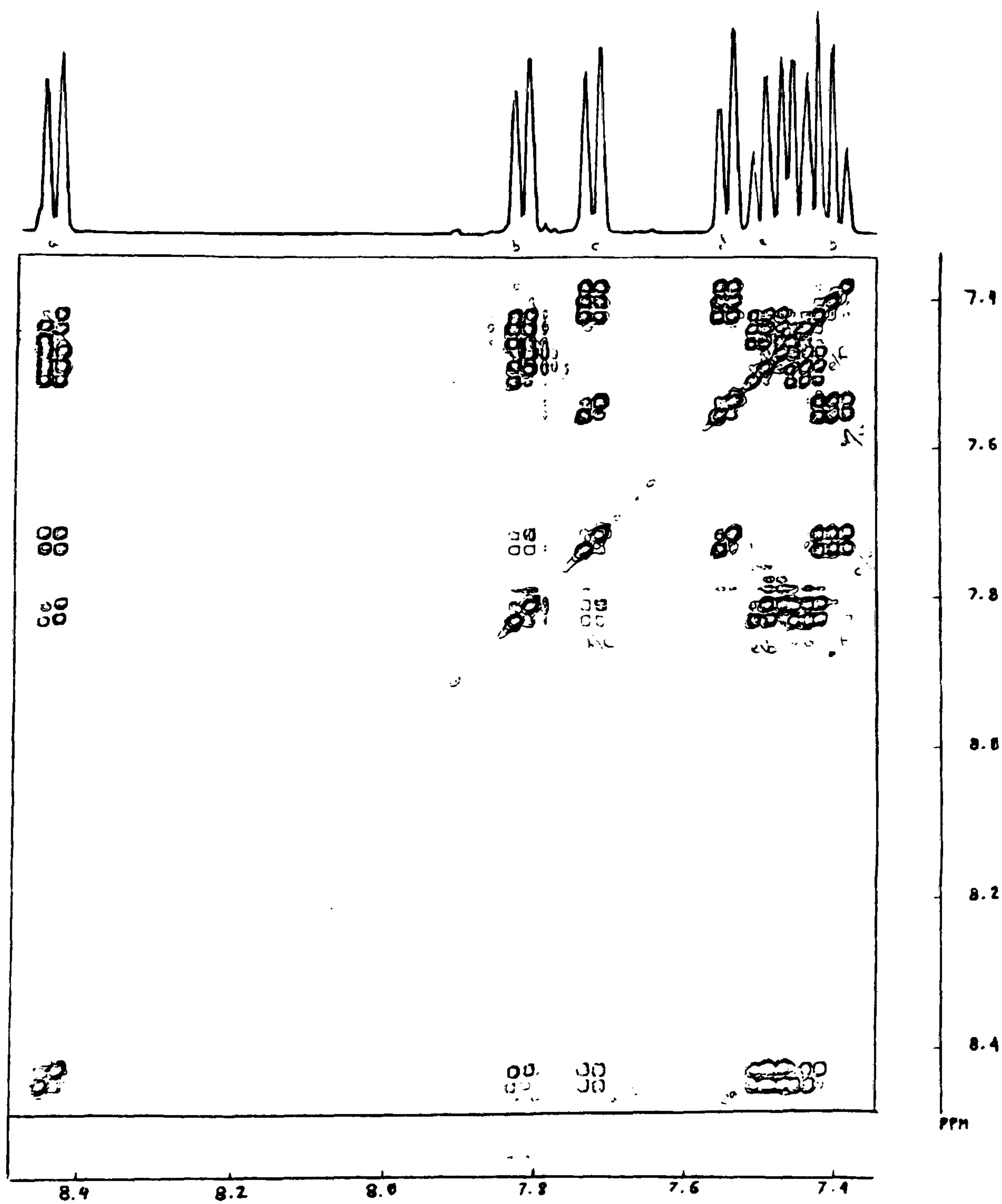
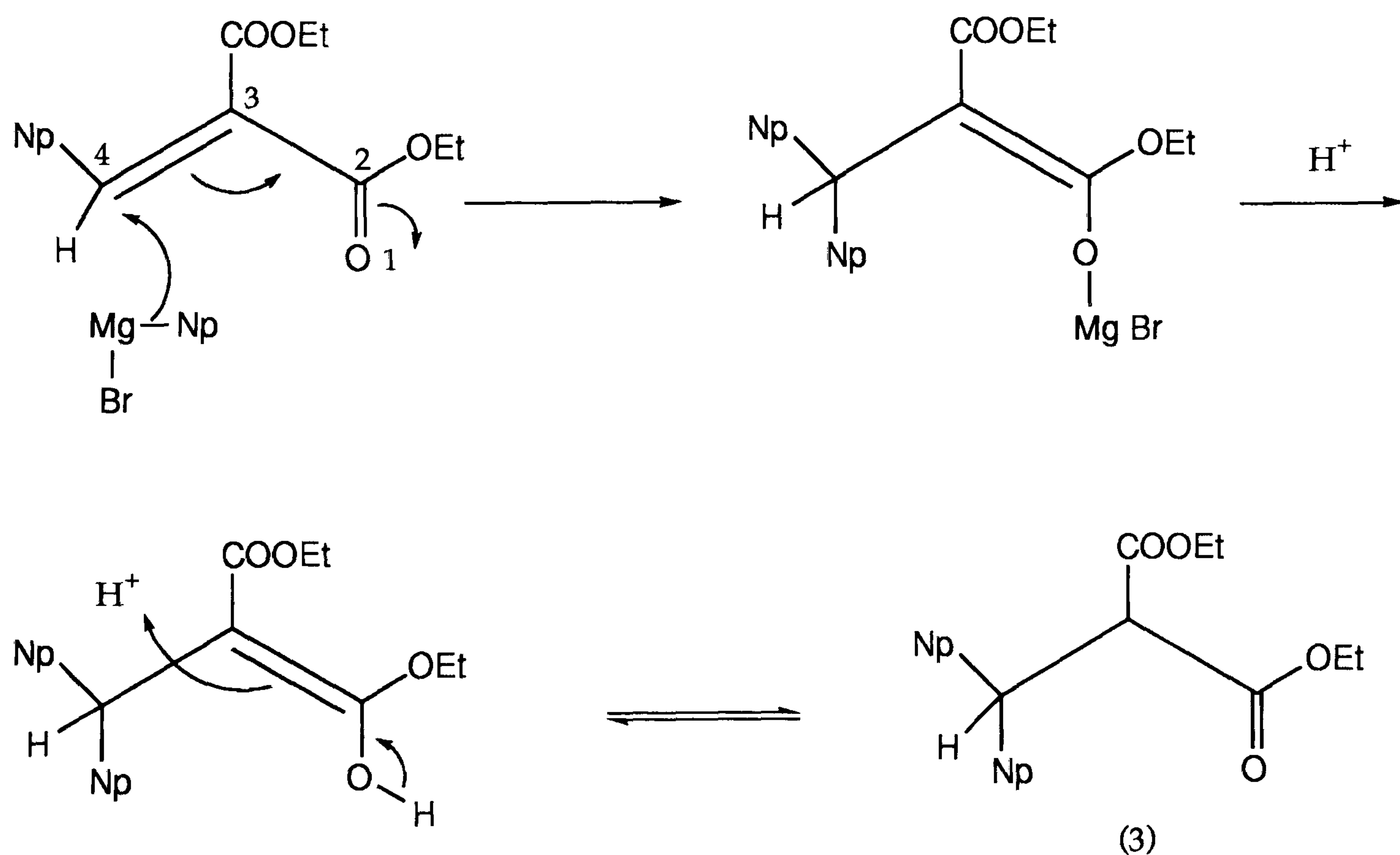


Fig. 55 a)- ^1H NMR in CDCl_3 of diethyl 2-(1'-naphthylmethylene)malonate (2).
b)- COSY of compound (2).

The molecular ion peak in the EI-MS appears at m/z 298 (intensity = 51.3%), and this ion undergoes fragmentation to give the base peak at m/z 152. The latter peak corresponds to a $C_{12}H_8$ fragment resulting from loss of the two $-COOCH_2CH_3$ groups by M^+ . The peak at m/z 224 (77.1%) is due to the loss of just one of these groups.

The synthesis of diethyl 2-(1,1'-dinaphthylmethyl)malonate (3) was achieved in 86% yield by 1,4-addition of 1-naphthylmagnesium bromide to ene diester (2). Previous studies reported that the yields were variable (35-56%) and that using a molar excess of the Grignard reagent reduced the yield further [75].

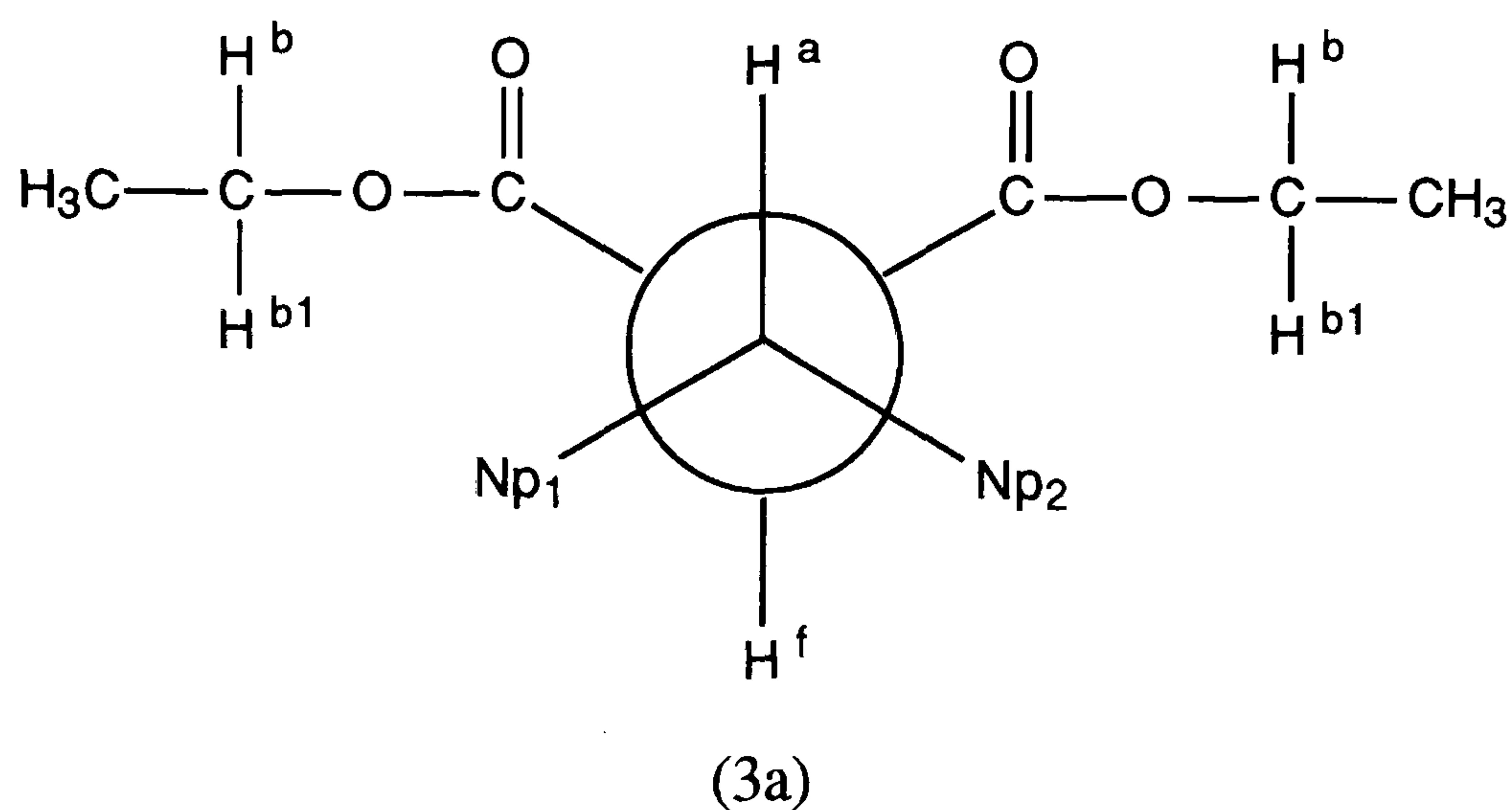
The addition of the naphthyl group to the α,β -unsaturated ester (2) is a Michael-type reaction (Scheme 22). 1,4-Addition is the primary reaction of α,β -unsaturated carboxylic esters with Grignard reagents and ketone or carbinol formation are secondary reactions [151]. However, the reaction may vary with the Grignard reagent and the reaction conditions employed. Large groups at the carbonyl carbon decrease the extent to which 1,2-addition to the carbonyl group takes place [152]. In the present case, no 1,2-addition to the carbonyl groups was observed.



Scheme 22

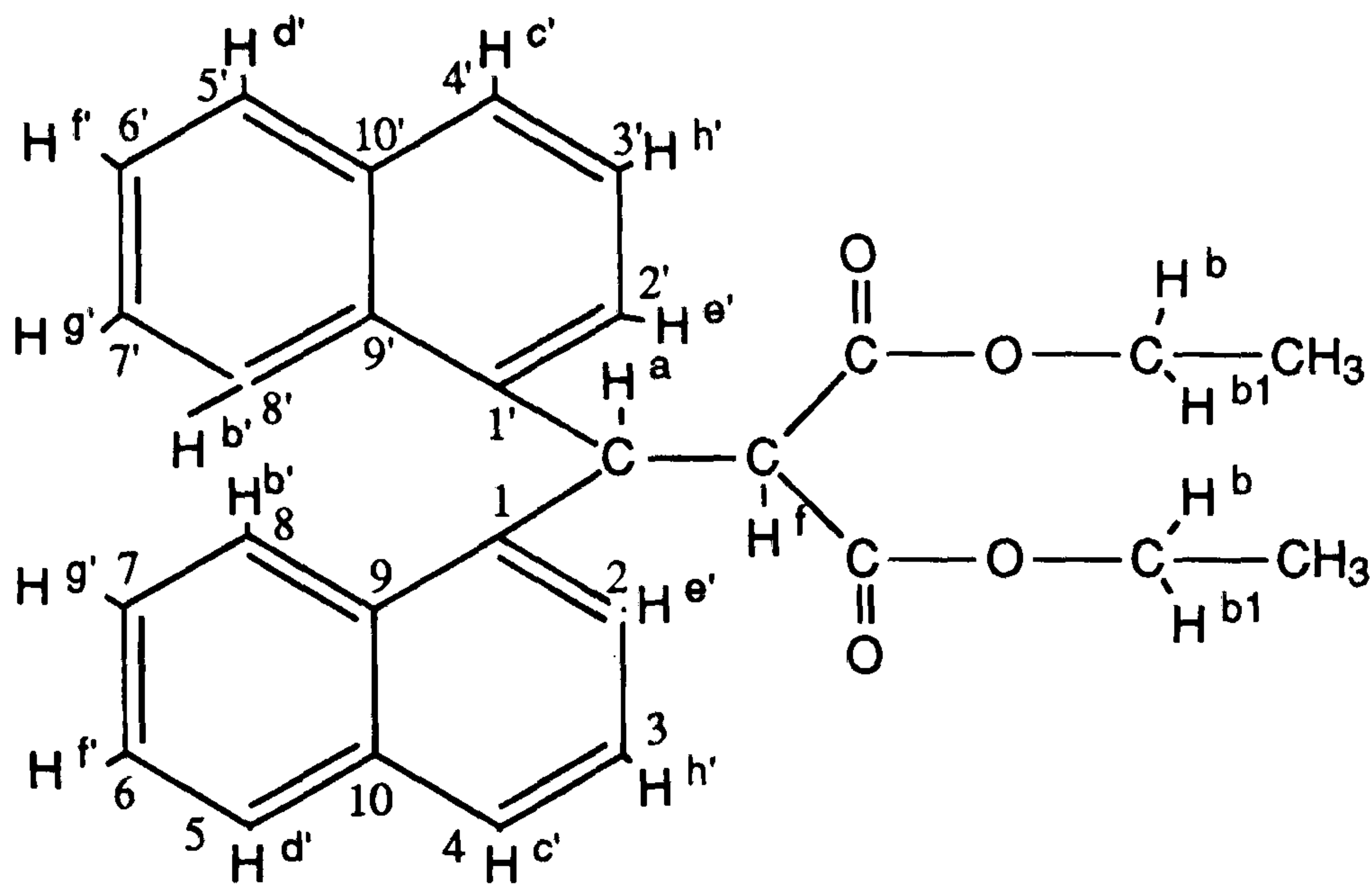
The strong IR band due to the C=O st in (2) shifted to a higher wavenumber (1756cm⁻¹) in the product (3) owing to the loss of conjugation, accompanied by the disappearance of the non-aromatic C=C st at 1633 cm⁻¹.

Two bands are present in the UV spectrum. The introduction of a naphthalene group in the molecule has resulted in a bathochromic shift of the second band and an increase in the intensity of the both bands.



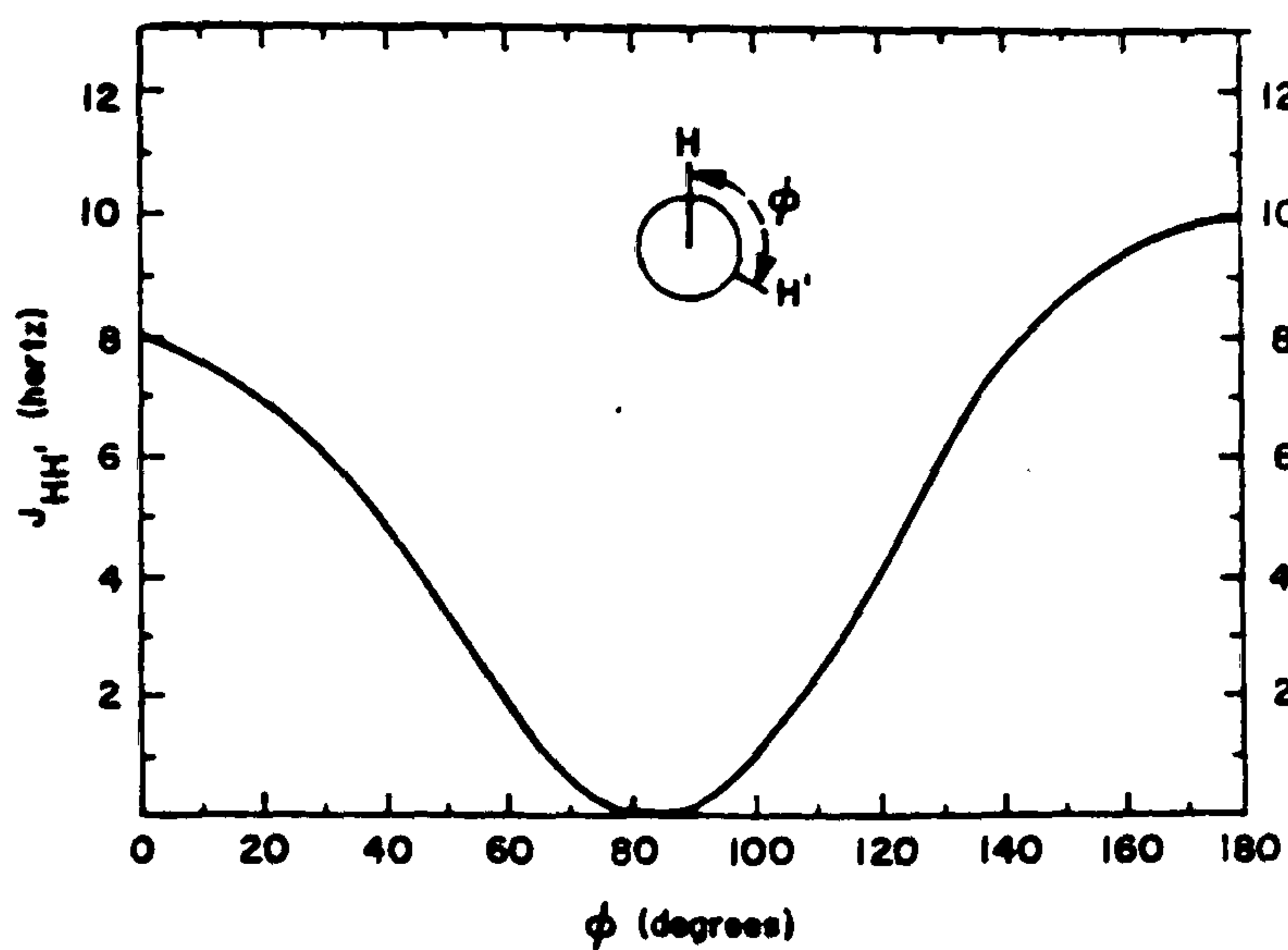
Unlike compound (2), this product has no stereoisomerism and in fact possesses a plane of symmetry which is illustrated in (3a). In the conformation shown, this plane contains the CH^a and CH^f atoms and the bond joining them.

The ¹H NMR spectrum of (3) shows fourteen aromatic protons between 8.5 and 7.38δ. The more deshielded protons are situated at the *peri* position (8.43δ) of each naphthalene. Another three doublets appear, integrating for two protons each. They are due to H^{d'}, H^{c'}, H^{e'}. The more shielded protons are H^{f'}, H^{g'}, H^{h'} which give three triplets (7.50-7.38δ).

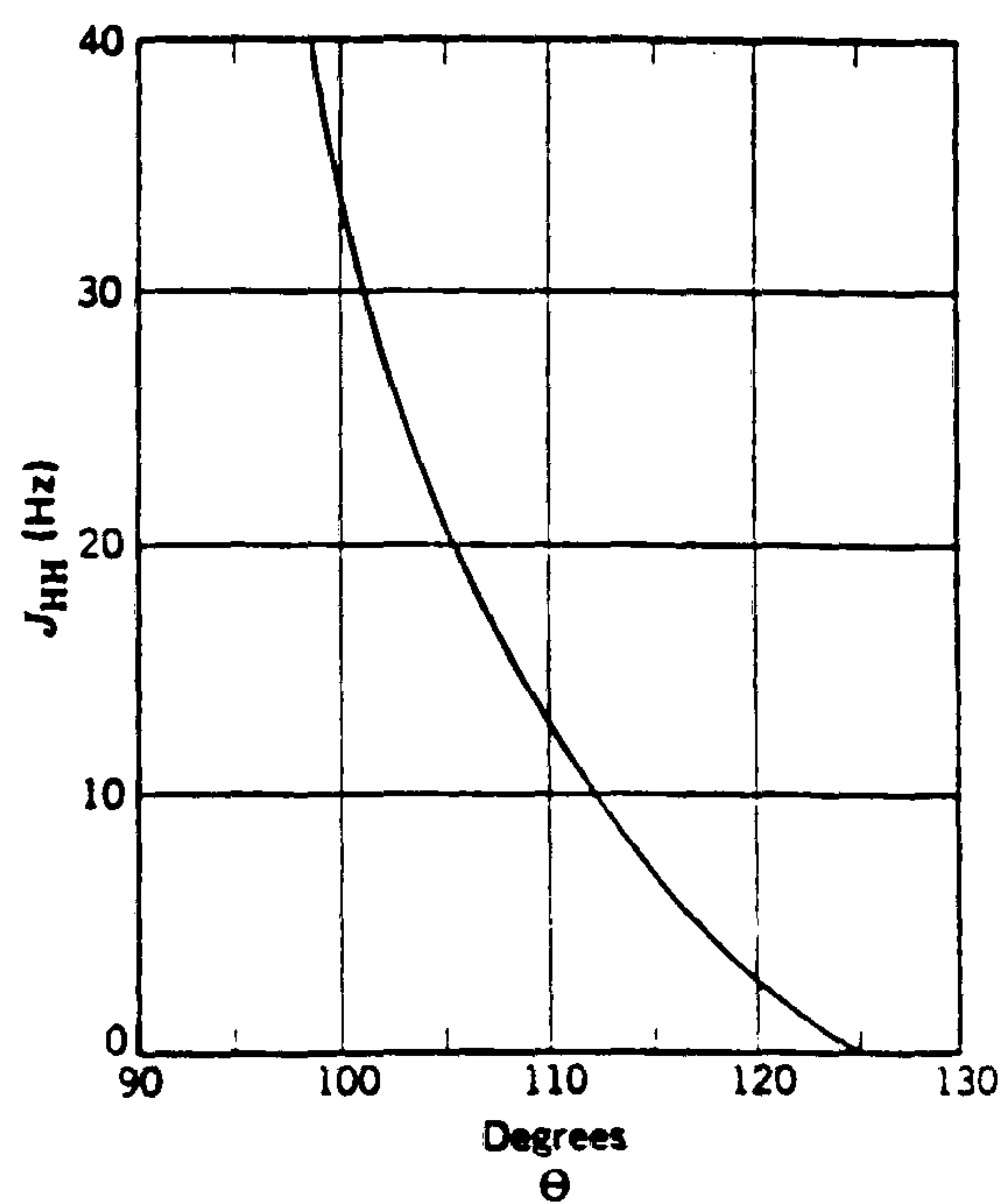


(3b)

H^a and H^f appear as two doublets at 6.55 and 4.55 δ , respectively, with a coupling constant of 11.8 Hz. The Karplus equation for 1,2-disubstituted ethanes is: $J = 4.22 - 0.5 \cos \phi + 4.5 \cos 2\phi$ with variations occurring in the coefficients according to the substituents attached (Fig. 56).



(a)



(b)

Fig. 56 (a) The vicinal H-C-C-H coupling constant and (b) The geminal Karplus correlation for CH_2 groups, as a function of the dihedral angle (ϕ).

The dihedral angle between H^a and H^f (ϕ) in (3) is therefore 180°. This corresponds to what is probably the most stable conformation. Although all conformations will be in dynamic equilibrium with one another at room temperature,

the most stable ones will persist and, if the equilibria are not too rapid, will be seen to predominate in the ^1H NMR spectrum.

The C-12 carbon is prochiral: replacement of each of the $\text{COOCH}_2\text{CH}_3$ groups in turn gives a pair of enantiomers, and these groups are therefore enantiotopic. In an achiral environment, the behaviour of these groups is identical. However, in a chiral environment the differences between them become manifest. In ^1H NMR spectroscopy, enantiotopic groups are generally indistinguishable unless a chiral solvent or shift reagent is employed. However, in the spectrum of compound (3) in CDCl_3 , the methylene signals show splitting indicative of an ABX_3 system. Each proton couples to its geminal partner and also to the adjacent protons of the methyl group. Therefore doublet of doublets of quartets i.e. sixteen lines should be seen. Due to overlap, only fourteen of the sixteen lines are discernible in the experimental spectrum, which are centred at 3.8δ , with two coupling constants of 7.1 and 3.6 Hz. The overlapping is because the difference of chemical shift ($\Delta\nu$) between protons H^b and H^{b1} is small compared to the magnitude of the coupling constant $J_{\text{H}^b/\text{H}^{b1}}$ which links them, resulting then in a second order spectrum. The intensity of the outer peaks decreases with a corresponding increase in the intensity of the inner peaks (Fig. 57).

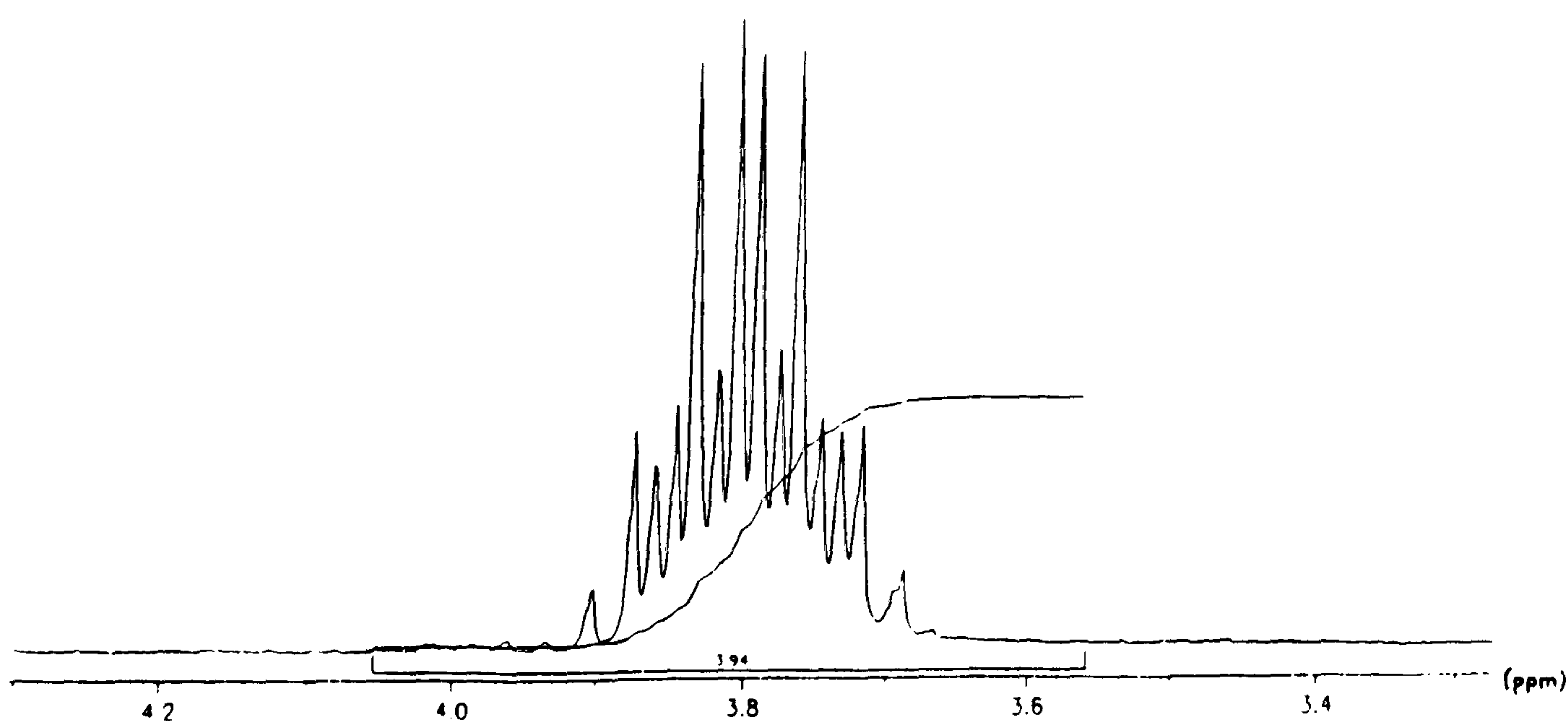
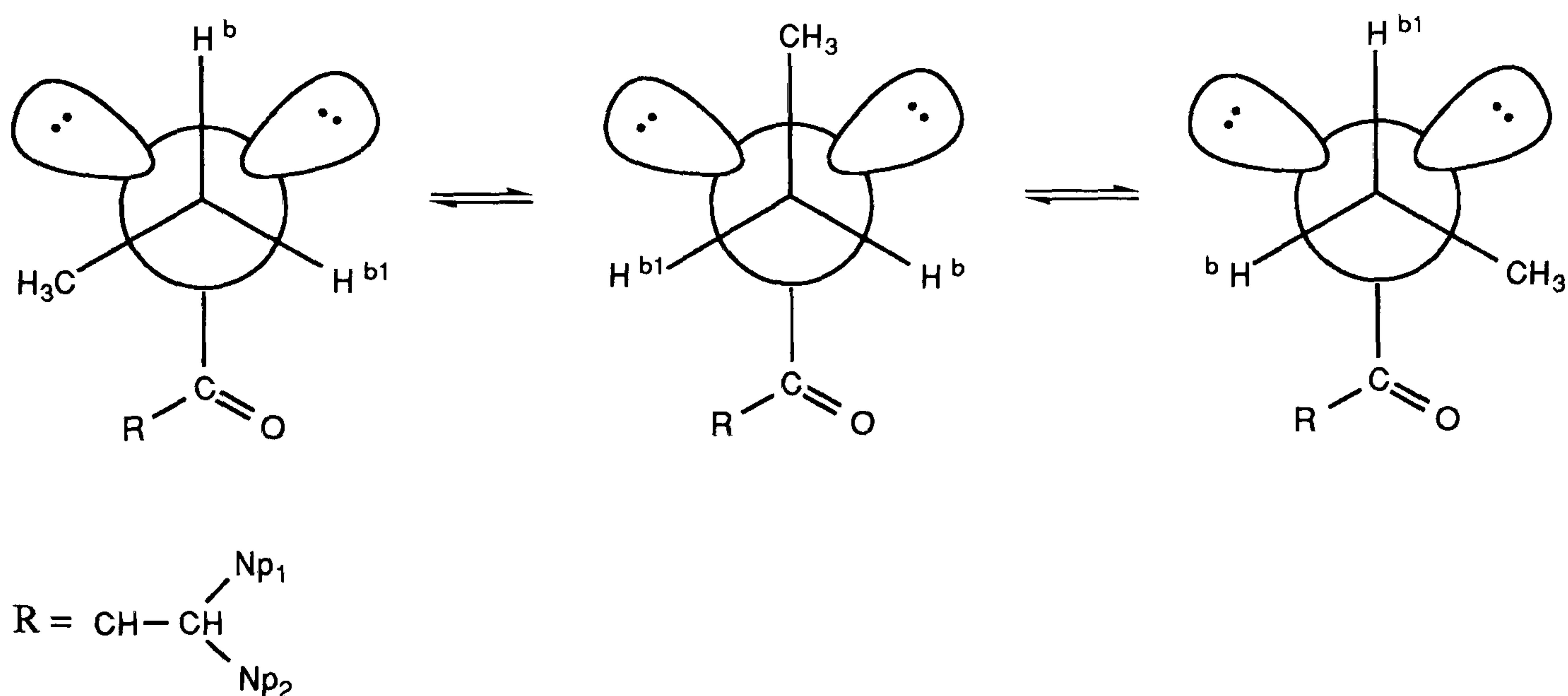


Fig. 57

From structure (3a) it is clear that there are no differences between the methyl groups (they lie either side of a plane of symmetry), and this is confirmed by the appearance of the methyl at a single shift (0.83δ). There is only a single coupling discernible in this signal ($J = 7.1$ Hz), which is the vicinal coupling constant in free-rotating alkane chains. The chemical shift equivalence of protons on a CH_3 group results from rapid rotation around a carbon-carbon single bond. The time spent in any one rotamer is short ($\approx 10^{-6}$ s) because the energy barrier for rotation around a C-C single bond is small. The chemical shift of the methyl group is an average of the shifts of each of the three protons. The differences are therefore within the methylene groups, which contain prochiral hydrogens H^b and H^{b1} . Newman projections of the ester linkage (Scheme 23) show that the two methylene protons spend their time in different average environments.

For the non-eclipsed rotamers

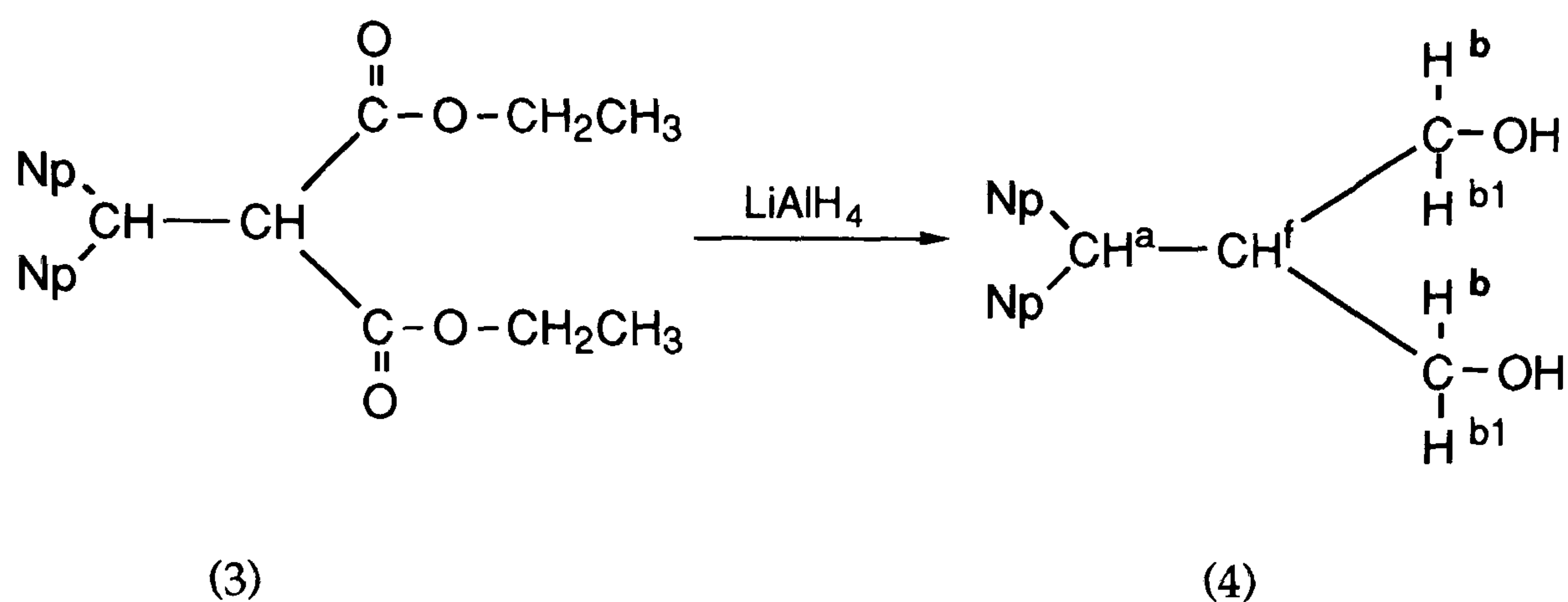


Scheme 23

The ^{13}C NMR indicates that the two methyl and methylene carbons in each moiety are equivalent, only one line appearing for each at 13.41 and 61.39 ppm, respectively. A single line for the two carbonyl groups can be seen at 168.0ppm. The C-11 has shifted higher field (58.57 ppm) because of loss of the double bond and the same happens to C-12 which changes from being a quaternary C to a tertiary and shifts from 133.25 to 40.30ppm.

The molecular ion peak in the EI-MS appears at m/z 426 (22%) and this fragments to the base peak at m/z 267 $[(Np)_2CH]^+$, due to the loss of $C_7H_{11}O_4$ $[CH(CO_2Et)_2]$.

The reduction of (3) with $LiAlH_4$ proceeded cleanly (Scheme 24) to give the diol (4) in 85% yield.



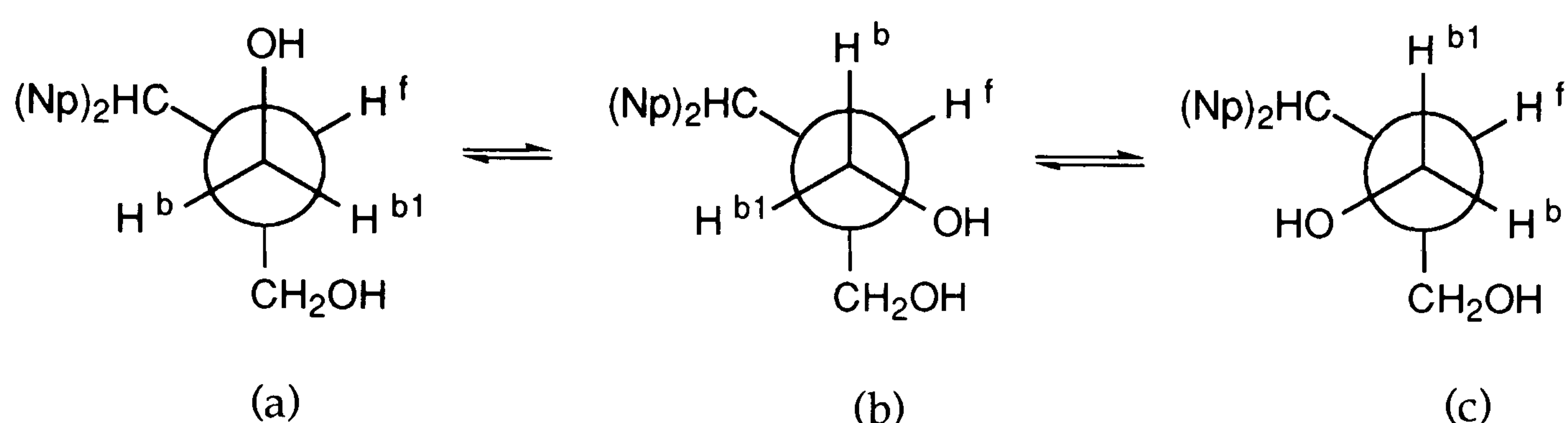
Scheme 24

Compound (4) shows a broad OH stretch at 3397 cm^{-1} in the IR spectrum and no bands in the carbonyl region which indicates that all the starting material (3) has reacted.

In the 1H NMR spectrum the signal due to the two naphthalenes has the same pattern as the signal that appears in compound (3). Proton H^a again gives a doublet as it couples to H^f but its coupling constant is 11Hz, which is similar to that in compound (3) ($J = 11.8\text{Hz}$). This doublet has shifted 0.8ppm upfield due to the loss of the two nearby ester groups. The adjacent proton H^f couples to H^a and to the two methylene protons $H^{b/b1}$ to give an overlapping doublet of triplets at 2.91δ which is not resolved using the 250MHz NMR spectrometer. This proton is highly affected by the anisotropic effect of the two ester groups in (3) so their reduction causes the signal to shift 1.64ppm to higher field. The methylene protons H^b and H^{b1} are enantiotopic. The proton H^b is split by the geminal proton H^{b1} yielding a doublet with $J = 10.3\text{Hz}$ and the same happens to the proton H^{b1} which couples to H^b ($J = 10.6\text{Hz}$). Each of the components is then split into a doublet by the proton H^f with different coupling constants $J = 3.7$ and 5.8Hz displaying a second order

spectrum ABC i.e. two doublet of doublets close together as the chemical shifts are quite similar.

The terminal CH₂OH groups can rotate through three non-eclipsed stable conformations (a, b & c). The Newman projections show that the six possible near-neighbour relationships are all different (Scheme 25). The unequal vicinal couplings suggest that there is not completely free rotation about the C-C bonds of the molecule and that conformation "a" or "c" predominates.



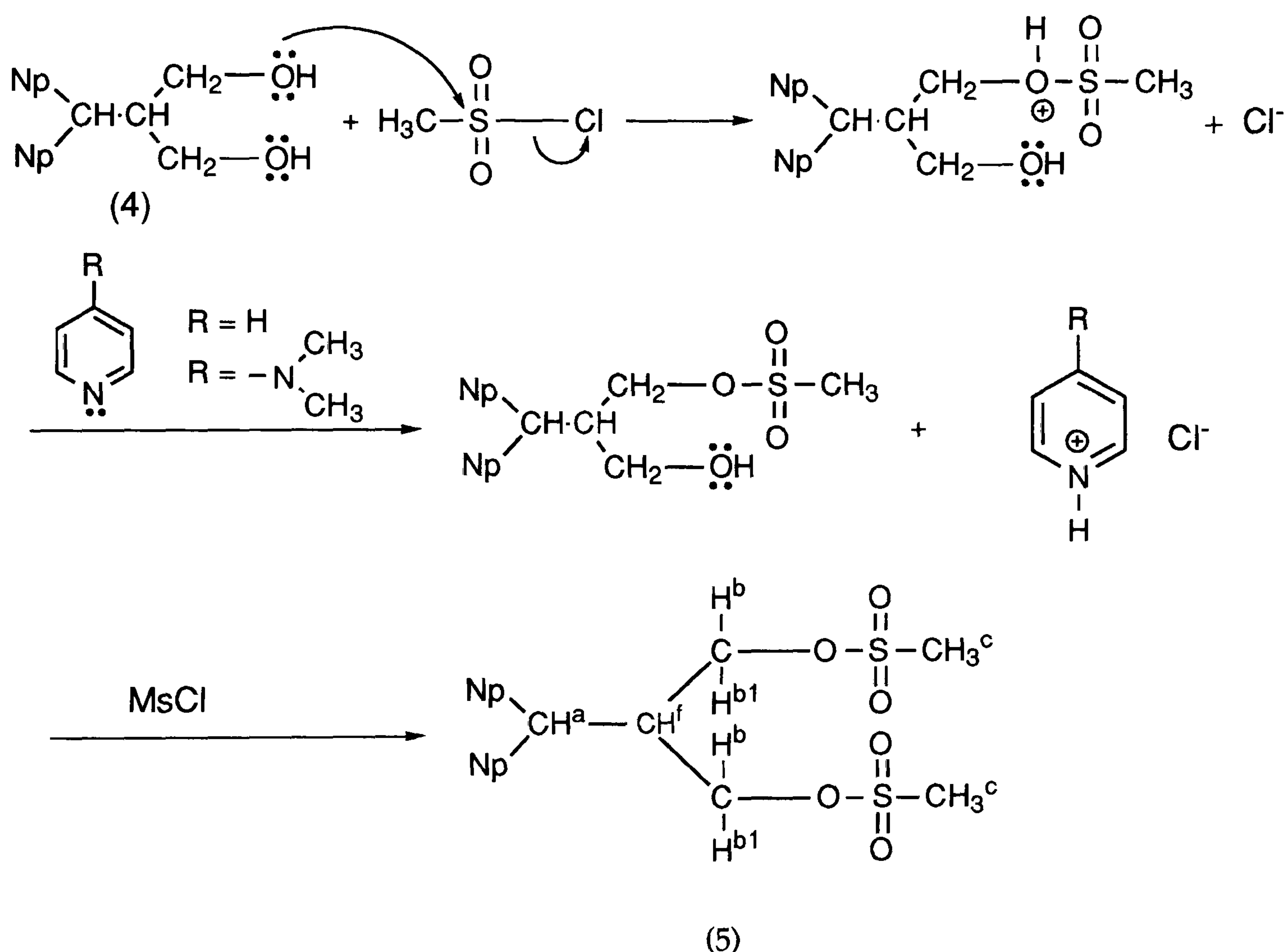
H^b : OH, H^{b1}; CH₂OH, CH(Np)₂ : δ_1 ; OH, H^{b1}; CH(Np)₂, H^f : δ_2 ; OH, H^{b1}; CH₂OH, H^f : δ_3
H^{b1} : H^b, OH; H^f, CH₂OH : δ_4 ; H^b, OH; CH₂OH, CH(Np)₂ : δ_5 ; OH, H^b; CH(Np)₂, H^f : δ_6

Scheme 25

The EI-MS gives the correct molecular ion at m/z 342 (30.3%). This ion undergoes fragmentation to give the base peak at 267 [(Np)₂CH]⁺ corresponding to the loss of C₃H₇O₂ [CH(CH₂OH)₂]. Loss of water can also be seen at 324 (1.7%) together with the loss of one and two CH₂OH chains, m/z 311 (1.5%) and 280 (3.3%).

Alcohols do not undergo S_N2 reactions because hydroxide ion is too basic (pK_a=15.7) i.e. it is not a very good leaving group. The substitution of the hydroxyl groups for better leaving groups was therefore carried out using methanesulphonyl chloride in the presence of pyridine (Scheme 26). In a small-scale run, the optimum reaction time for the synthesis of the bis-mesylester (5) was found to be 1 hour.

The product (5) showed a single spot on TLC, and the melting point agreed with the literature [75] (145°C). However, when the reaction was left longer, three spots were seen on the TLC plate. The same reaction was also examined using dimethylaminopyridine (DMAP) as a catalyst, which is a base superior to pyridine^[153]. On a small scale, the reaction in pyridine containing DMAP gave one product in 82% yield. However, when the reaction was scaled up, three spots were again observed on the TLC plate. It was not possible to follow the reaction by TLC, because pyridine has a very strong chromophore and leads to severe streaking.



Scheme 26

The production of a mixture of three products rather than the expected one on mesylation of the diol (4) represented the first serious deviation from the reported literature [75]. It was necessary to purify small quantities of the three products in order to determine their structures and establish the best way forward. Samples of the three reaction products were obtained by column chromatography using

hexane/EtOAc (50:50) as mobile phase. The most polar product, which was also present in greatest proportion in the mixture, proved to be the required bis-mesylate (5).

The IR spectrum of the pure product (5) shows two strong bands at 1184 and 1354 cm^{-1} , due to symmetric and asymmetric $-\text{O}-\text{SO}_2-$ stretches respectively.

This molecule, like (3) and (4), contains a prochiral center and a plane of symmetry. The methylene hydrogens are therefore chemically non-equivalent and appear as two doublet of doublets, but this time the difference between the two chemical shifts is big enough to see two signals (4.15 δ and 4.45 δ). Each proton in the methylene group is split by the other ($J = 10\text{Hz}$) and unequally by the neighbouring proton ($J = 6.3$ and 3.3Hz) (Fig. 58). The bulky mesyl ester groups affect the dihedral angle between H^a and H^f making it bigger. The coupling constant is increased ($J = 11.5\text{Hz}$) to the same size as when the carboxylic ester groups were present. The inductive effect of the mesyl groups is stronger than that of the OH groups, thus the multiplet of the proton H^f and the signal of the methylene protons (2dd) shift to lower field by about 0.5ppm. The singlet for the 6 protons due to the two methyl groups appears at 2.71 δ .

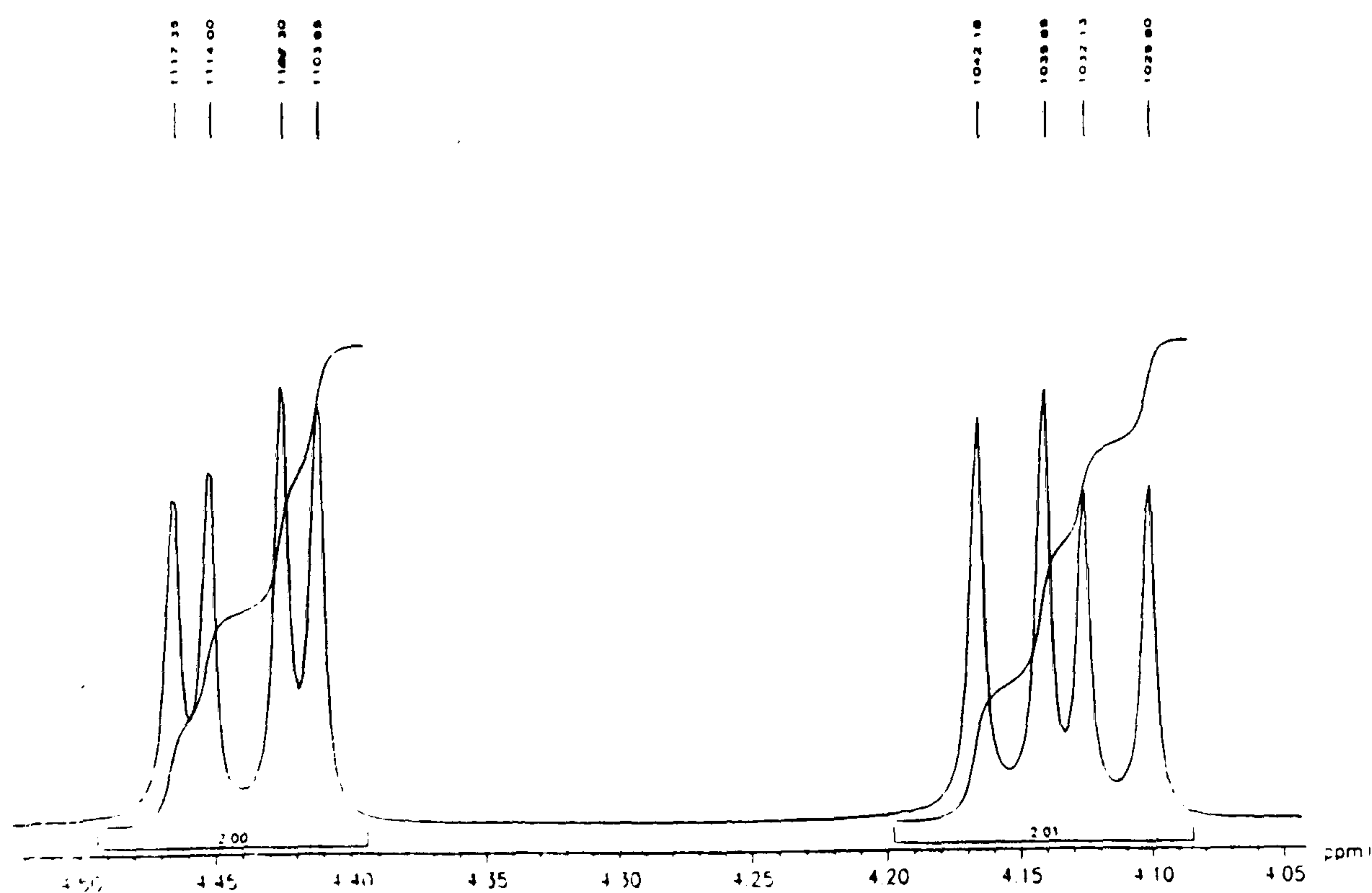
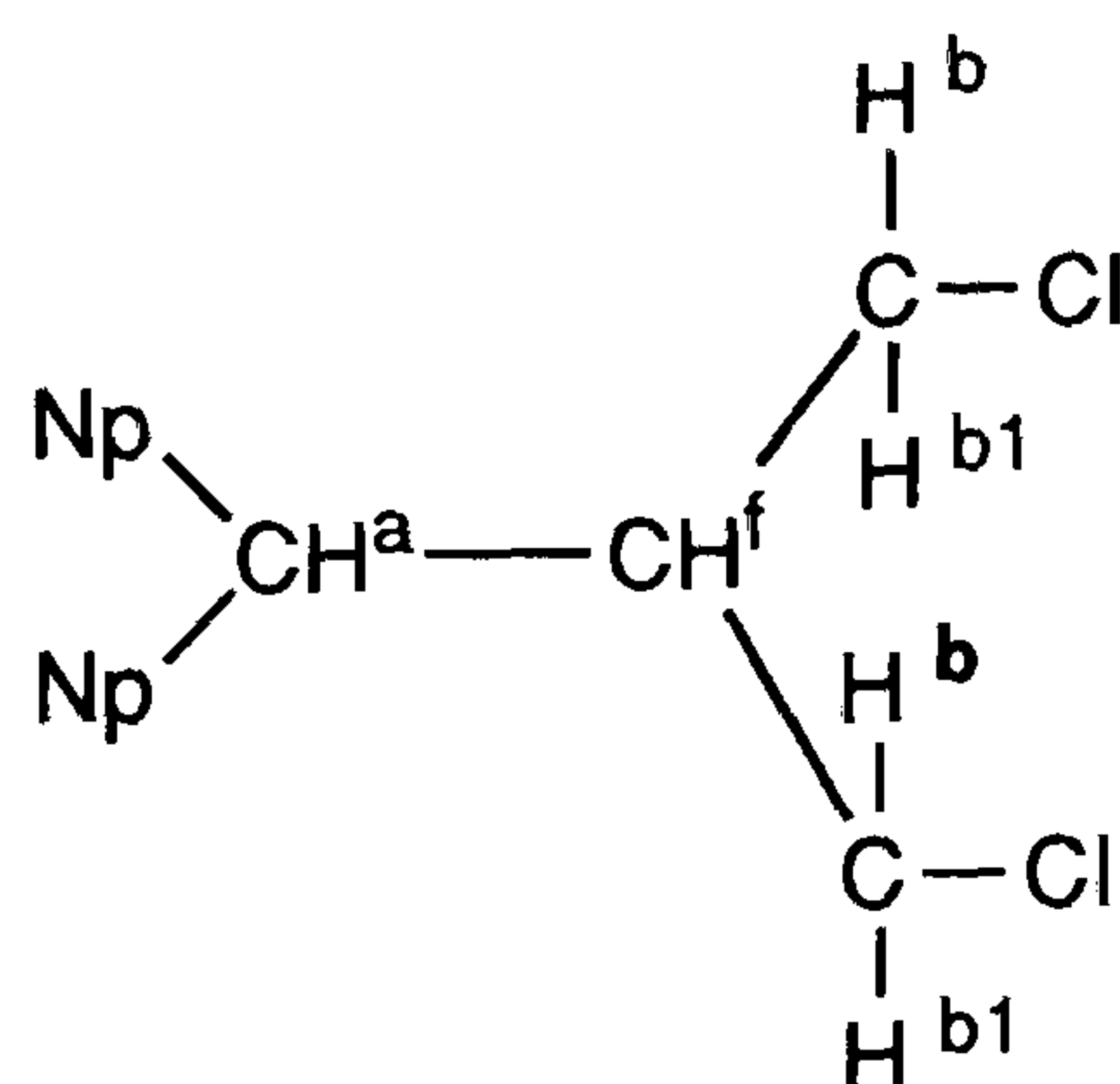


Fig. 58

IR, ^1H NMR spectra, microanalysis and mass spectrometry indicated that the least polar fraction formed in the mesylation reaction was the dichloro-substituted compound (71). A strong band at 779cm^{-1} for the C-Cl stretch appears in the IR spectrum and the ^1H NMR spectrum shows no signal for a methyl group demonstrating absence of the mesyl groups. The same pattern as for the diol (4) is displayed in the ^1H NMR for the methylene protons (ABC), giving two doublet of doublets with geminal coupling constant of $J = 11\text{Hz}$ and two different vicinal constants 6.5 and 3.2Hz. The proton H^{a} couples to H^{f} ($J = 11\text{Hz}$). The chlorines are smaller than mesylate groups and the coupling constant decreases. The multiplet due to proton H^{f} has shifted to higher field showing that the inductive effect of the chlorine groups is not as strong as that of the mesyl groups.

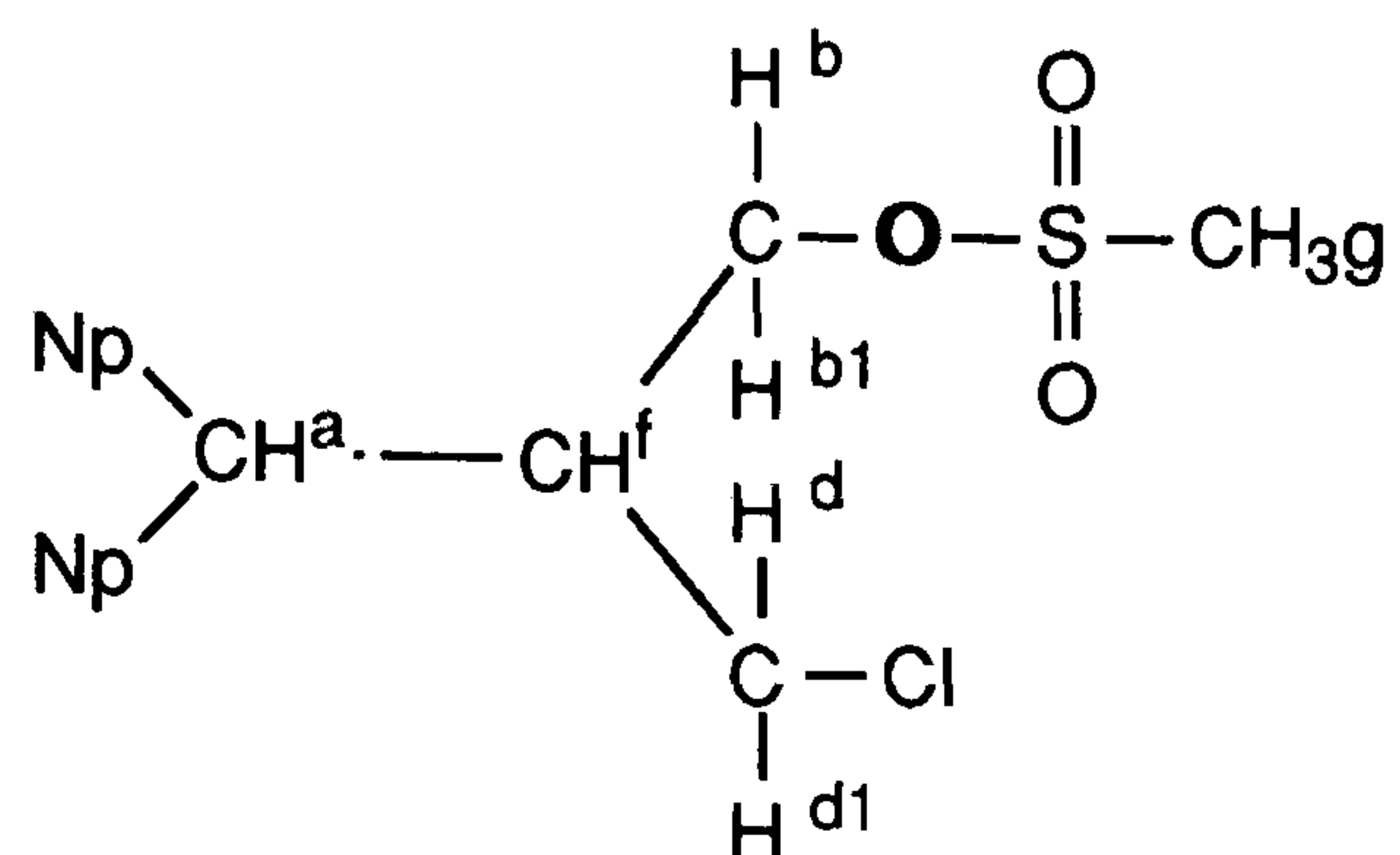
The mass spectrum of (71) shows the presence of two chlorines.



(71)

The fraction of intermediate polarity collected from column chromatography of the mesylation mixture was also analysed. The IR and ^1H NMR spectra of this fraction suggested that it was the monomesyl ester, mono-halide (72), the singlet corresponding to CH_3 now integrating for 3H. There are two bands at 1176 and 1348cm^{-1} due to S=O st symmetric and asymmetric and a strong band at 779cm^{-1} due to C-Cl st.

In the ^1H NMR spectrum a small difference in the pattern of the diastereotopic naphthalene groups can be observed. The *peri* proton of each naphthalene is affected differently by the two different groups next to the methylene groups. Two signals can be discerned, a doublet at 8.46δ and a split doublet at 8.33δ .



Since compound (72) has a chiral carbon, the methylene protons are also diastereotopic. The two methylene groups are different, as their substituents are different, and therefore appear at different chemical shifts. The protons $\text{H}^{\text{b/b1}}$ next to the mesyl group appear at lower field than the protons $\text{H}^{\text{d/d1}}$ next to the chlorine. The methylene protons $\text{H}^{\text{b/b1}}$ display the same pattern as in the mesyl derivative (5). The signal of the other methylene protons $\text{H}^{\text{d/d1}}$ is not well resolved. Only a split doublet can be seen, but its chemical shift agrees with compound (71).

Mass spectrometry confirmed that monosubstitution of mesyl by Cl had occurred, giving a peak at M^++2 due to ^{37}Cl .

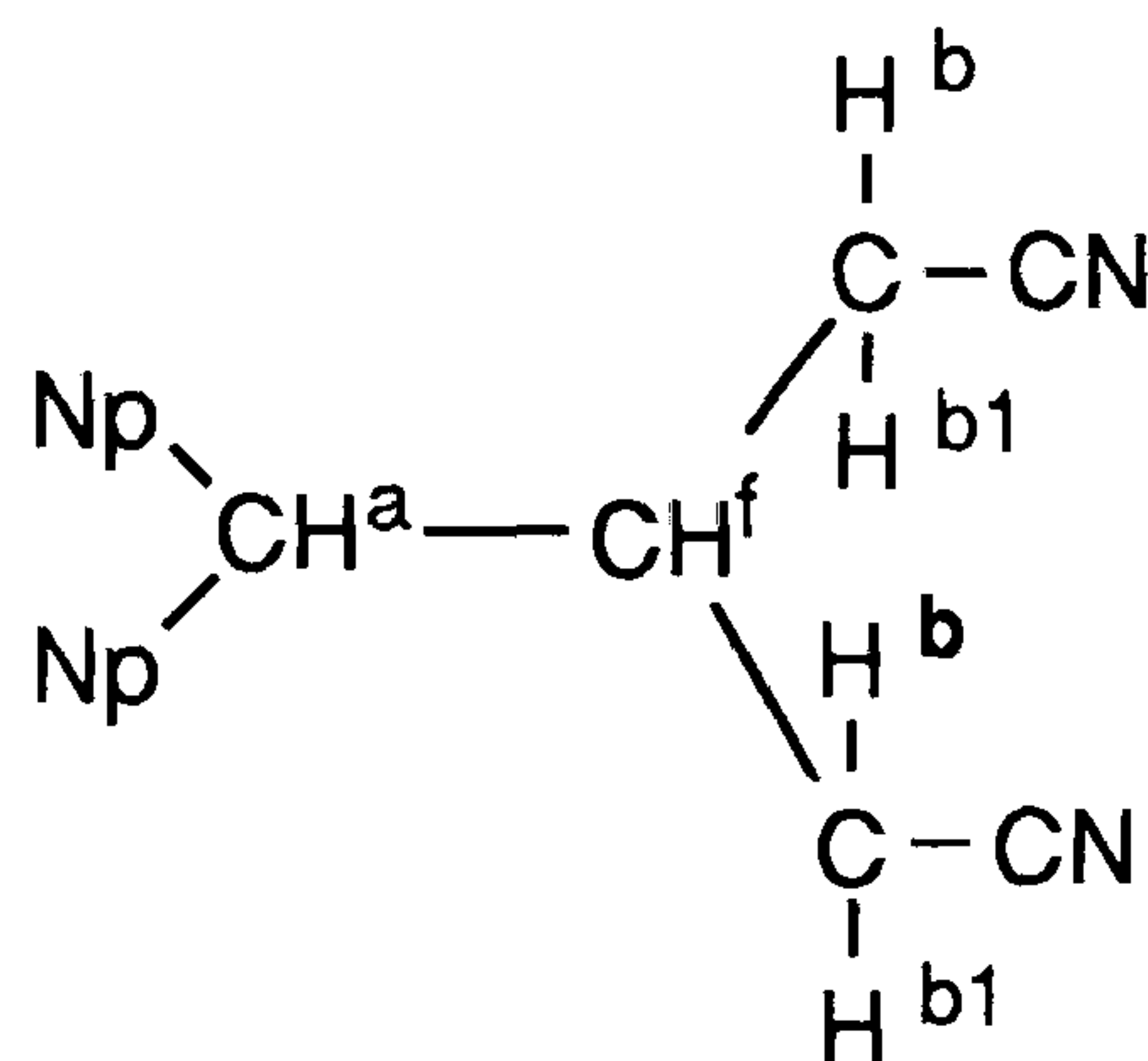
It was evident that the mesylation reaction was proceeding to completion, but that further reaction involving displacement of the mesylate groups by halide ion was occurring under the conditions used. Initially, it was felt to be desirable to use only the pure bis-mesylate ester (5) for the next stage of the synthesis and efforts were made to control the reaction in order to optimise the yield. However, attempts to follow the reaction by TLC were frustrated by the presence of pyridine, and scale-up of reactions always led to variations in product distribution.

Whilst the three reaction products from mesylation could be separated by column chromatography, this was not considered desirable for routine large-scale synthesis. Consequently, it was decided to examine another alternative, which was to use the crude mixture of compounds (5), (71), (72) for the synthesis of the dinitrile.

On small scale, it was demonstrated that reaction of KCN/KI with the bis-mesylate (5) afforded the dinitrile (6) and that this product was formed equally cleanly when using the crude mixture of (5), (71), (72).

Whilst a primary chloride is a poorer leaving group than a primary mesylate, it is evidently sufficiently reactive for the cyanide substitution to occur under the present conditions (KCN/KI, aq DMF) and it is likely that in both cases there is a common intermediate, the primary alkyl iodide.

A low intensity band for the C≡N stretch at 2244 cm⁻¹ was observed in the IR of the product (6).



(6)

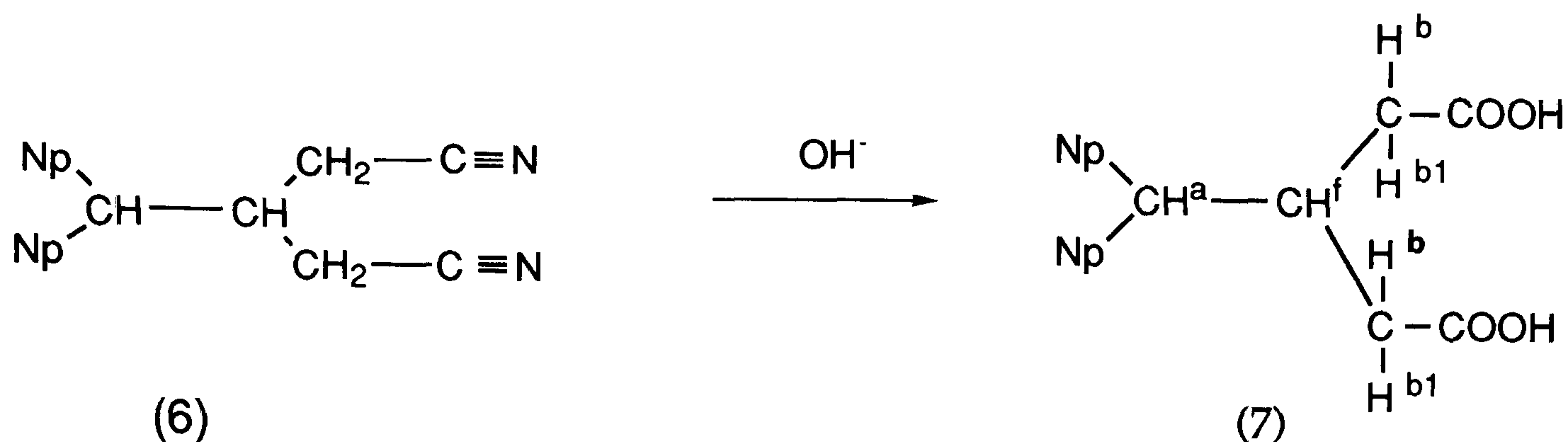
Newman projections show that the two protons of each methylene group spend their time in different environments, so the protons are enantiotopic. The ¹H NMR spectrum of (6) is similar to that of the diol (4). A second order system (ABX₃) is also displayed by the methylene protons but shifted upfield by 1ppm, as less electronegative groups were introduced. The geminal coupling constants are much bigger J = 17.2 Hz. According to the Karplus equation, the value of this J corresponds to a geminal coupling with strain. The multiplet due to H^f has moved

downfield compared to the signal in the diol (4). An analytical sample was prepared for identification but the crude product was used in the next stage.

To summarise up to this point, the first five stages of the hexahelicene synthesis had proceeded extremely well. Despite the difficulties of unexpectedly obtaining a mixture on mesylation of the diol (4) and the need to characterise the monochloride and dichloride side products and demonstrate their ability in the subsequent step, the overall yield of the dinitrile (6) obtained from 1-naphthaldehyde was 61%, compared with the literature yield of 55%

Moreover, detailed spectroscopic examination was used to confirm the structure of each intermediate and to provide reference data which would be invaluable in the assignment of the more complex structures to follow.

At the next stage in the synthesis, a serious problem was again encountered. Contrary to the literature report [75], hydrolysis of the dinitrile (6) proved to be extremely difficult. The reaction was incomplete after one hour refluxing with 10% KOH in ethylene glycol (Scheme 27). A mixture was obtained which showed a substantial retention of nitrogen on microanalysis and whose IR indicated the presence of nitrile and amide as well as carboxylic acid groups. Many small scale reactions were then carried out in order to investigate suitable conditions for obtaining complete hydrolysis. Variations in reaction time, concentration of dinitrile and KOH and extent of dryness of the solvent were made, but satisfactory results were not obtained. Further experiments were then conducted to examine the influence of solvent character and reaction temperature and eventually the best results appeared to be achieved using 20% KOH in refluxing diethylene glycol for 2hr. On a 2g scale, after work-up involving acidification, a white solid was obtained which showed only one spot on TLC.



Scheme 27

The disappearance of the band at 2244cm^{-1} ($\text{C}\equiv\text{N}$, st) in the IR and the appearance of a strong band at 1713 cm^{-1} ($\text{C}=\text{O}$ st, nonconjugated acid) indicated that the hydrolysis was completed. No trace of nitrogen was found on the microanalysis of the product.

In the ^1H NMR of (7), the proton of the methine group H^a couples to H^f to give a doublet with a coupling constant $J = 11.3\text{Hz}$. The multiplet due to the other methine group H^f appears at 3.45δ , which is a bit downfield compared to the dinitrile (6) (3.27δ).

The two protons in each of the methylene groups in the diacid are prochiral. They give two doublet of doublets at 2.37δ and 2.59δ with geminal coupling constants of 12Hz and unequal vicinal coupling constants of 4 and 8Hz , respectively. The two doublet of doublets are baseline separated and the positions have shifted to higher field compared to those in the dinitrile (2.73δ).

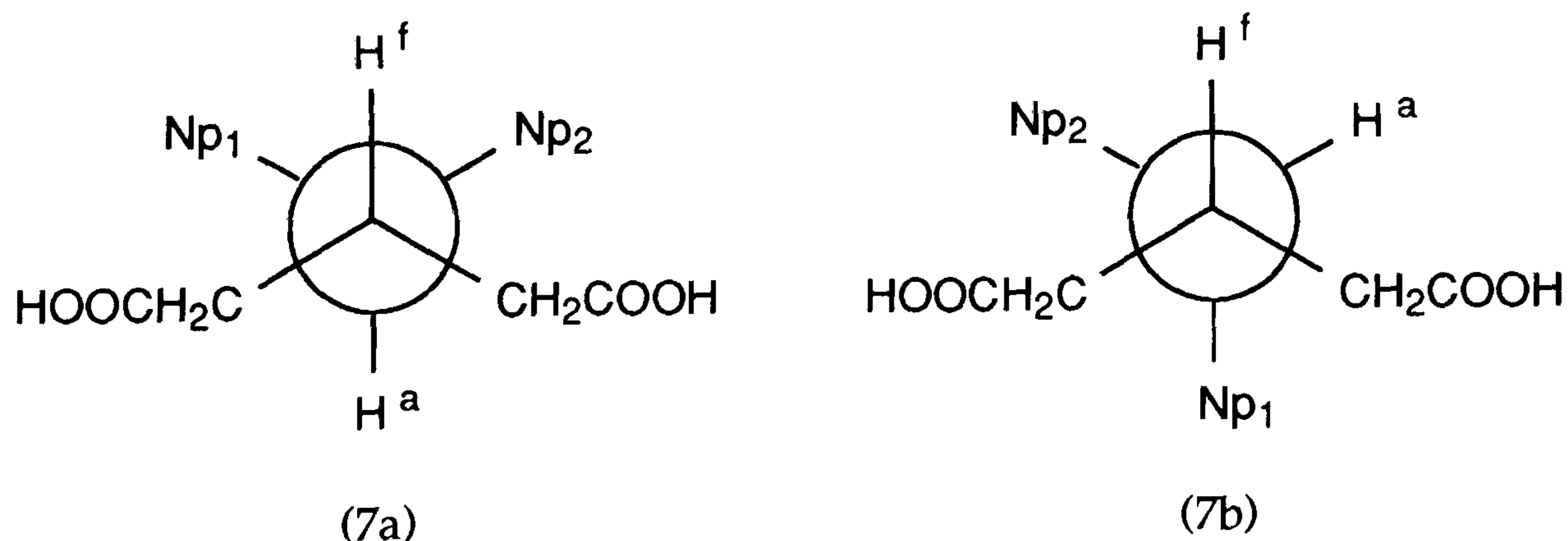
A broad peak appears at 10.3δ due to the hydroxyl group of the acid. The mass spectrometry agrees also with the expected compound (7). A molecular ion peak appears at m/z 398 (3%) and a peak due to the loss of water (m/z 380, 11%). The base peak is formed by the loss of $[\text{CH}(\text{CH}_2\text{-COOH})_2]$.

Unfortunately, problems were encountered when the hydrolysis of the dinitrile was carried out on a larger scale. It was found that the product was contaminated with amide and with the potassium salt of the required di-acid, making work-up difficult and causing substantial loss of material whilst purification

procedures were worked out. By the end of this process, the amount of pure di-acid in hand was very limited (< 5g) and, whilst sufficient for pilot-scale work on the succeeding stages, it was necessary to repeat the whole synthetic sequence to prepare larger quantities. During the repetition of the first six steps of the helicene synthesis, similar problems were again encountered in the mesylation reaction and the dinitrile hydrolysis. Considerable further effort was expended in trying to find clean conditions for the hydrolysis but yields of diacid remained poor. The consequent scarcity of di-acid meant that all further steps in the synthesis had to be carried out much more cautiously and on a smaller scale than had originally been planned and the original target of preparing tens of grams of hexahelicen-7-ylacetic acid had to be abandoned. Nevertheless, it was still hoped to obtain quantities of the resolved helicene derivative sufficient to be able to prepare at least one analytical and one preparative HPLC column for investigation of chiral separations.

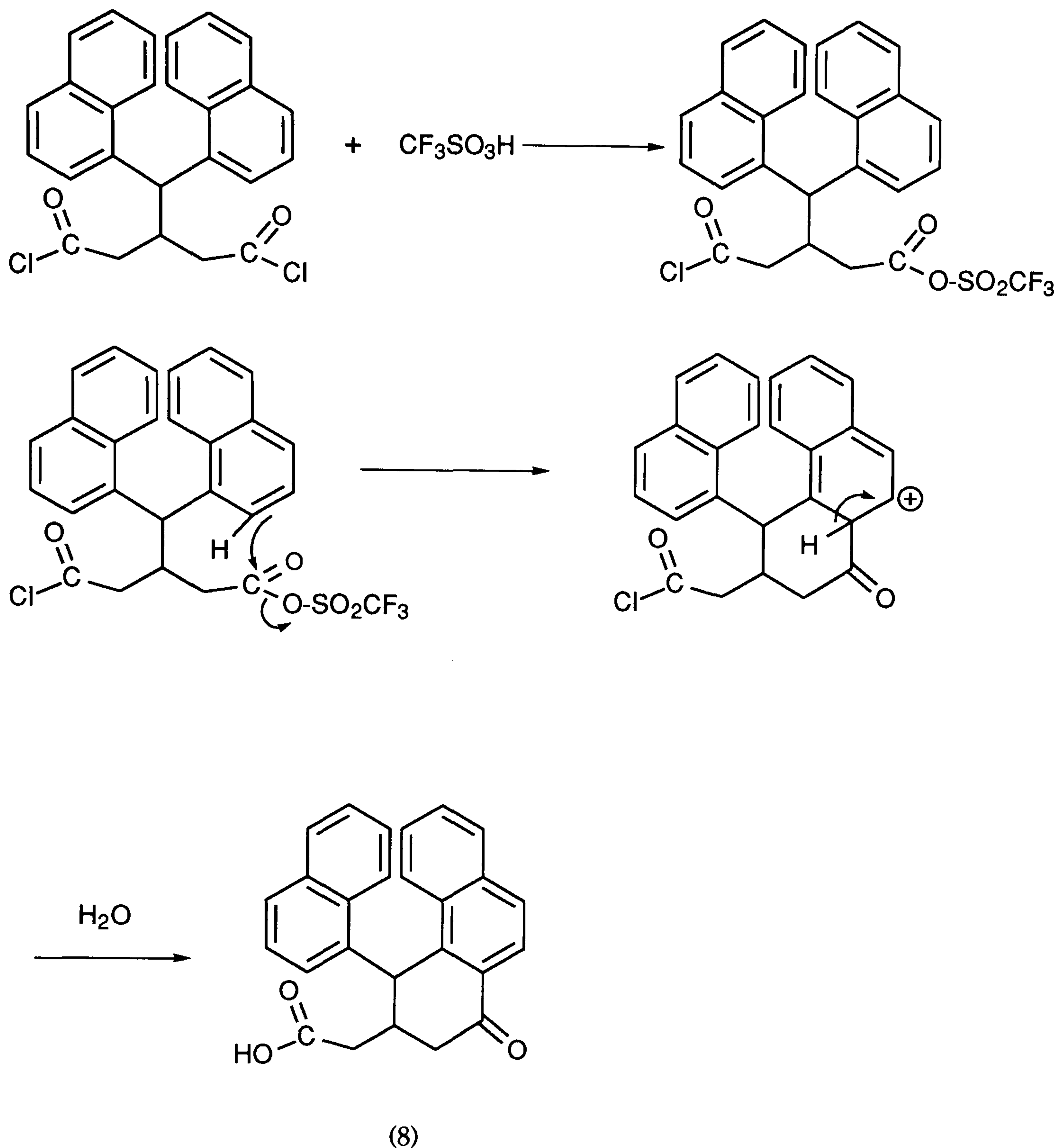
The reaction involved in the next stage was the cyclisation of one acid group of the diacid onto one of the naphthyl groups to form the corresponding cyclic ketone. According to Newman and Lednicer, concurrent double cyclisation is not possible because of steric impediments [75].

The stereochemistry of the cyclisation product is determined by the conformation undergoing ring closure. Newman projections show that conformation (7a) is less strained than conformation (7b). Consequently, the ring fusion in the product will be assumed to be *trans*.



This type of cyclisation usually proceeds in high yields [154]. However, when it was applied to the diacid (7), it proved difficult because of the increasing steric strain introduced into the molecule. The cyclisation has been effected by using strong acids such as HF [155]. In earlier work in this laboratory, the cyclisation was carried out according to Newman's method [75], using anhydrous hydrogen fluoride but no yield was reported. Moreover, the use of HF is difficult and dangerous. We therefore introduced a modification at this stage, in which the diacid was cyclised via a mixed trifluoromethanesulphonic-carboxylic acid anhydride [156].

An excess of thionyl chloride was used to ensure that the acid chloride was formed in high yield. Trifluoromethanesulphonic acid was then added to generate the mixed triflic anhydride. Trifluoromethanesulphonic acid is one of the strongest acids known because of the inductive effect of the fluorines and the trifluoromethane sulphonic-carboxylic anhydride obtained is the most powerful acylating agent known to date. It can acylate aromatic compounds without addition of a catalyst[157]. Electrophilic substitution occurs with elimination of triflic acid, which is a very good leaving group (Scheme 28).

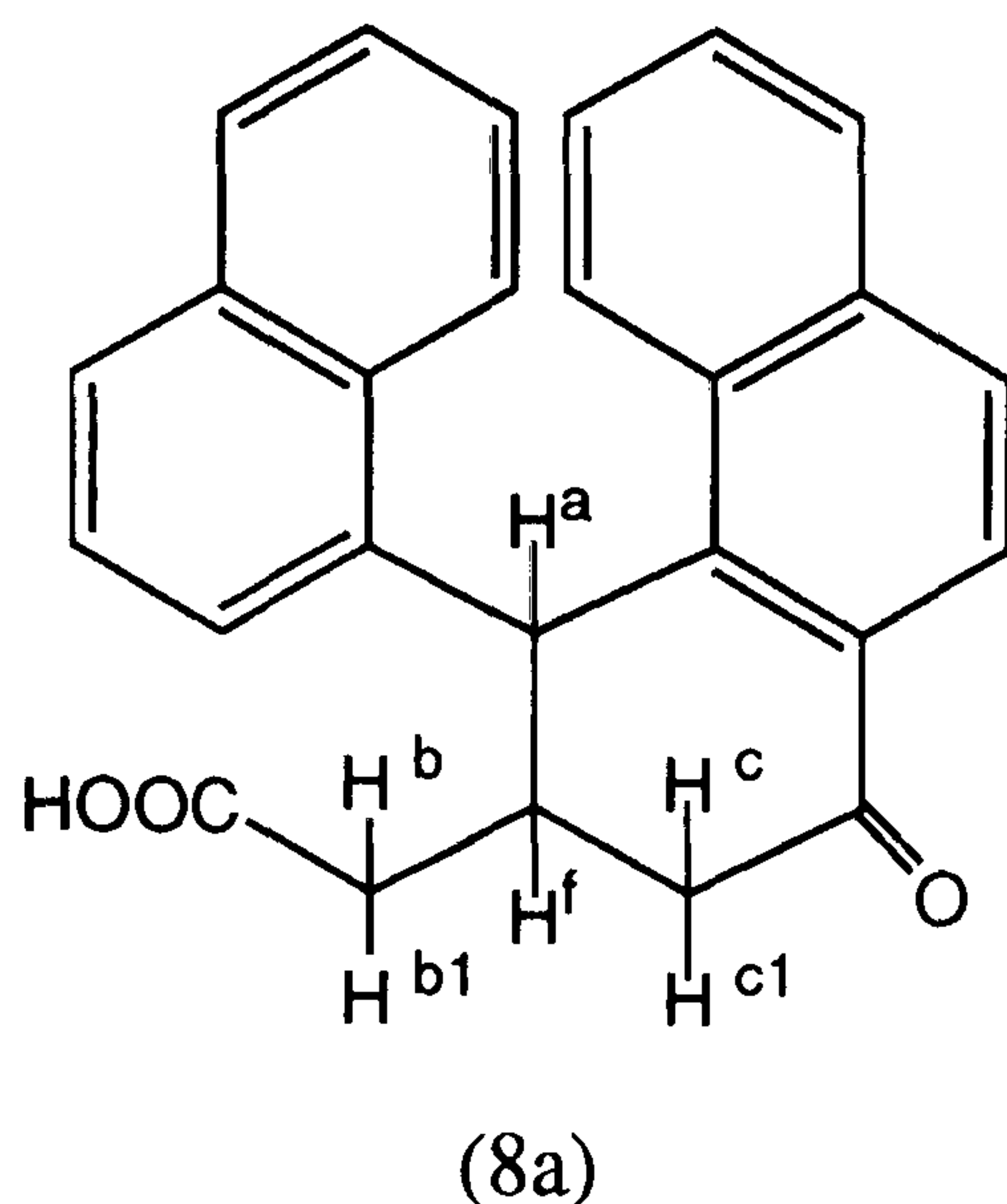


Scheme 28

Following cyclisation, the rate of the hydrolysis of the remaining acid chloride to yield the carboxylic acid (8) was slow because of the large substituent group [158]. The effect of the size of this group on the reaction rate is via its effect on solubility in water, not steric hindrance.

Two products were isolated from the reaction mixture by preparative TLC and their structures determined by spectroscopy. The more polar product formed in 55% yield, was shown to be the required ketoacid (8).

The infrared spectrum of the product (8) was consistent with the required structure (8a), displaying two strong bands at 1675 and 1730 cm^{-1} corresponding to the ketone and acid C=O stretch, respectively.

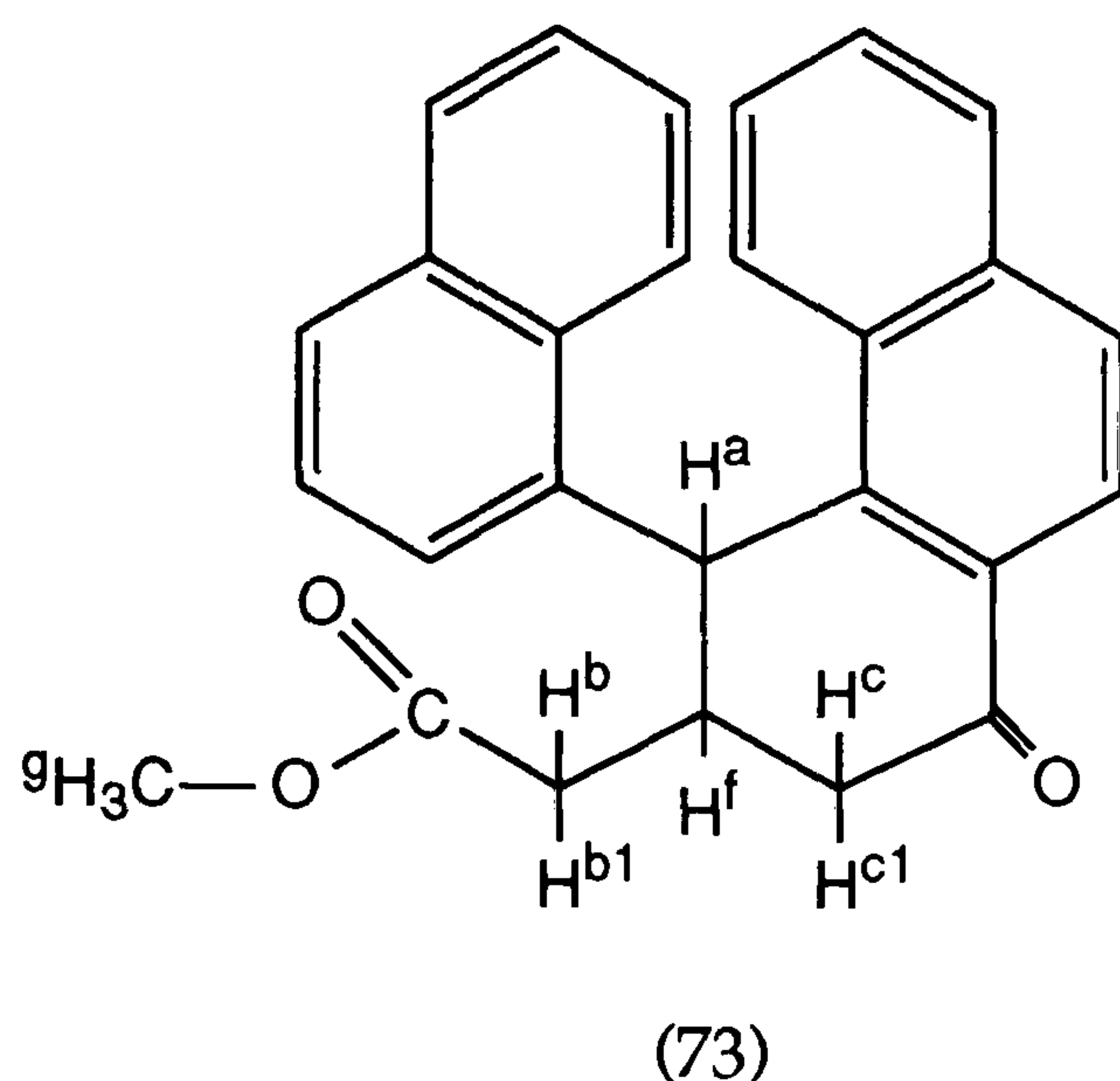


In the ^1H NMR, the proton H^a couples with proton H^f but its coupling constant is so small that it is not observable i.e. the dihedral angle must be 90° . It appears as a broad singlet at 5.97 δ . There are two chiral centers and the two methylene protons are diastereotopic. The protons of the methylene H^{b/b1} next to the acid group give two sets of dd, the first set at 2.65 δ with a geminal coupling constant of 17Hz and vicinal one of 4.4Hz. Only a doublet is observed for the second set at 2.44 δ with the same geminal coupling constant (17Hz). The two protons of the methylene H^{c/c1} next to the ketone group also give 2dd at 2.58 and 2.34 δ with a geminal constant of 15.5Hz and two different vicinal constants of 8.5 and 6.8Hz. It is possible to see the signal due to the proton of the acid at 10.6 δ .

The mass spectrum of compound (8) shows a peak at m/z 380 (M^+). The loss of the $-\text{CH}_2\text{-COOH}$ chain affords a peak at m/z 320 (100%).

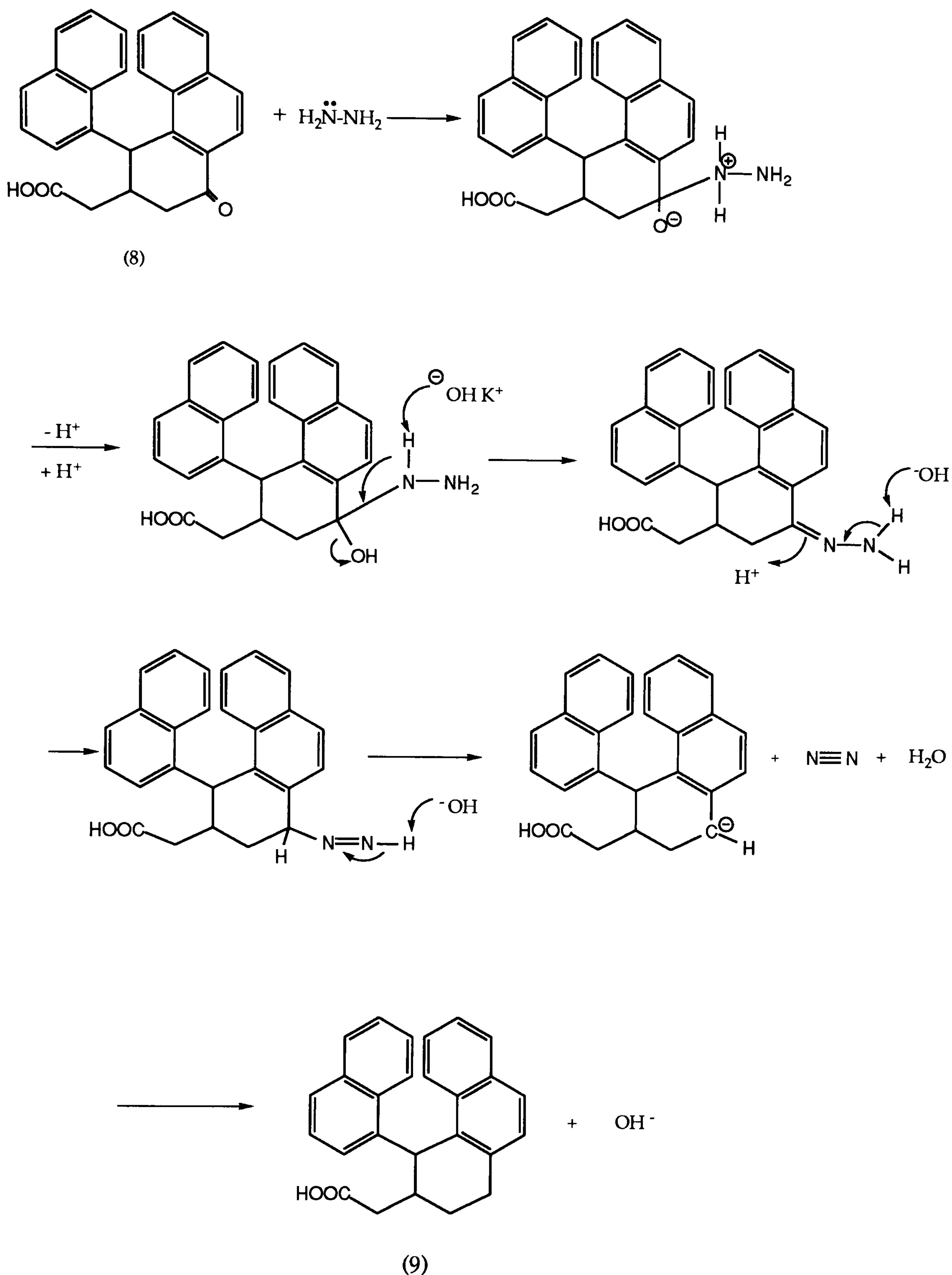
The less polar product from the cyclisation reaction was isolated in 38% yield following preparative TLC. All the spectroscopic data suggested that the compound obtained was the methyl ester derivative (73). This derivative might have been formed as a consequence of the low rate of hydrolysis of the ketoacid chloride during work-up, leaving the acid chloride to react with the MeOH present in the

mobile phase at the time of purification through preparative TLC. Two strong bands appear in the carbonyl region of the IR spectrum (1733 and 1680 cm^{-1}) which indicate the presence of the ester and conjugated ketone groups.



No major differences between the by-product (73) and the main product (8) can be seen in their ^1H NMR spectra, apart from the signals for the acid substituent. Compound (73) contains two chiral centres, therefore the two methylene groups give two sets of dd. No singlet for the proton acid is observed but a 3H singlet at 3.73 δ indicates that a methyl group is present.

The reaction involved in the next stage was the Huang-Minlon [159] modification of the Wolff-Kishner reduction. The carbonyl compound was first converted to the hydrazone, which was then heated with base in a high-boiling solvent, diethylene glycol, to convert it into the hydrocarbon with elimination of nitrogen. The reaction is fairly specific for aldehydes and ketones and can be carried out with many other functional groups present (Scheme 29).



Scheme 29

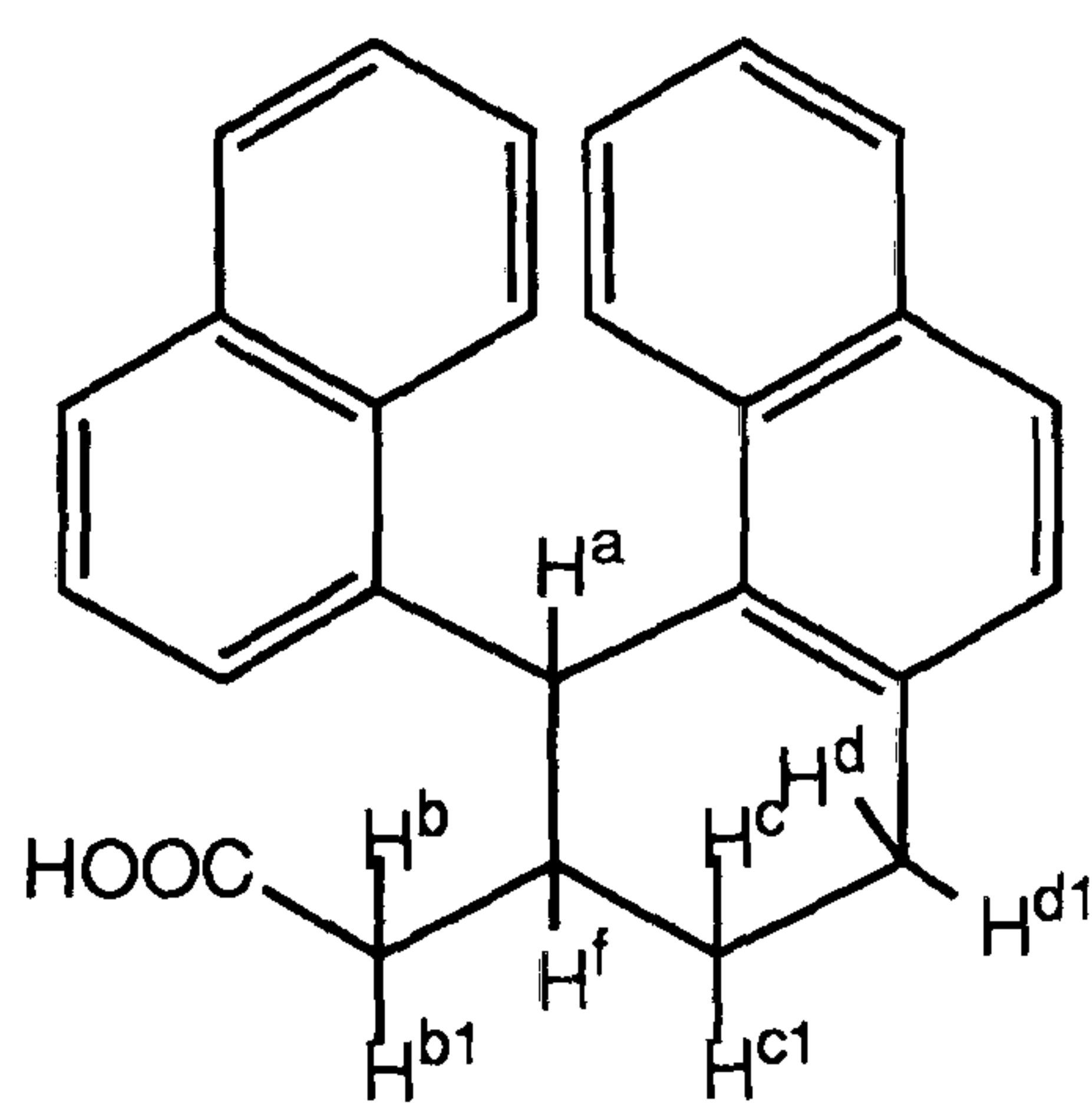
It was found that the long periods of heating often recommended [159] are not necessary. After the water produced was distilled out, the temperature began to rise rapidly. According to literature [159] if the apparatus were connected to an azotometer, it could be seen that the gas evolution was nearly theoretical and the

reaction could be complete in 15-30 minutes, and consequently that further heating after that point could be useless or harmful.

As the side product (73) of the cyclisation stage was identified as the ketone methyl ester, no further purification was performed and the mixture of (8) and (73) was used for the Huang-Minlon reduction, hydrolysis taking place during the work-up of the reaction.

The IR spectrum of the product (9) shows that the two bands at 1730cm^{-1} and 1675cm^{-1} due to the acid and the conjugated ketone in (8) are replaced by only one at 1701cm^{-1} for the acid, providing evidence that the reduction is accomplished.

The ^1H NMR indicates the presence of a new methylene group ($\text{H}^{\text{d}/\text{d}1}$). The disappearance of the ketone group makes the signal due to the methylene ($\text{H}^{\text{c}/\text{c}1}$) shift upfield by 1ppm. The hydroxyl proton of the acid appears at 10δ as a singlet.



(9)

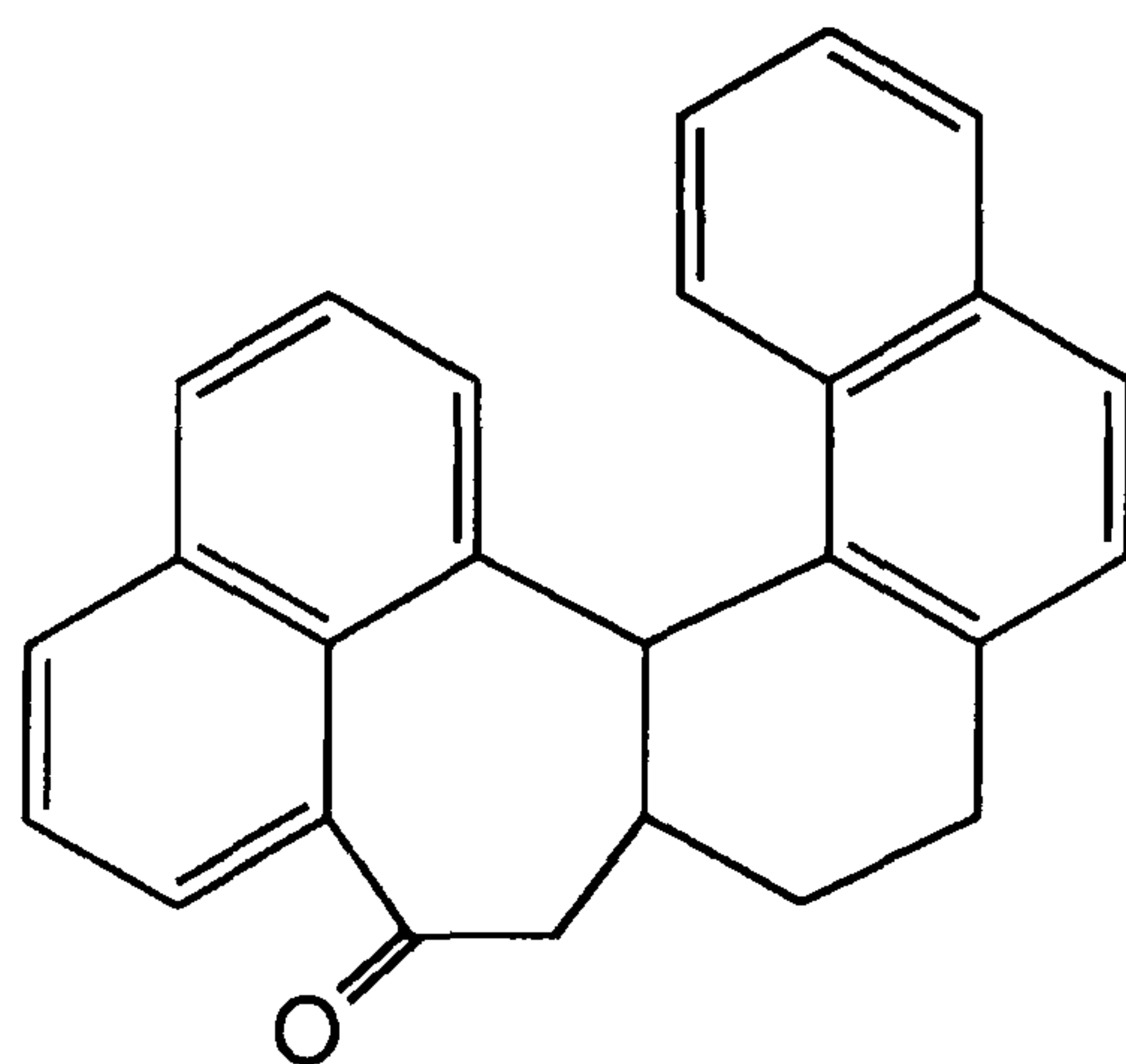
Mass spectrometry shows the required molecular ion peak m/z 366 and the base peak at m/z 306 due to the loss of the acid chain $[\text{CH}_2\text{-COOH}]$.

The cyclisation of the acid (9) was carried out via the acid chloride prepared using phosphorous pentachloride. According to literature [160], treating the acid to be cyclised with one equivalent of phosphorous pentachloride followed stannic chloride is one of the simplest and most satisfactory procedures for intramolecular Friedel-Crafts acylations. Although thionyl chloride has been widely used in the preparation of acid chlorides for cyclisation with stannic chloride, it is perhaps

less generally advantageous than phosphorous pentachloride, because it is usually necessary to remove the thionyl chloride completely before cyclisation [161]. Even a trace of the reagent, which is often almost impossible to remove without spoiling the acid chloride, may cause a noticeable decrease in the yield of the ketone. However, it is not necessary to remove the phosphorous oxychloride from the reaction mixture. On the contrary, it is even advantageous to allow it to remain in the reaction mixture [162].

The ring closure of the acid chloride of (9) to (10) proved difficult. It was finally accomplished in 55% yield by heating at 90°C for one hour with stannic chloride in *o*-dichlorobenzene. The great resistance toward formation of the hydrohexahelicene ring system can be seen as this type of cyclisation is usually very rapid for substituted γ -phenylbutyryl chlorides using stannic chloride [163] (2-12 minutes).

The ring closure of [1,2,3,4-tetrahydro-4-(1'-naphthyl)-3-phenanthryl]-acetic acid (9) results in the formation of 7,8,8a,9,10,16c-hexahydro-7-oxohexahelicene (10) from acylation at the adjacent 2-position of one of the naphthalene groups. The alternative direction of cyclisation is onto the *peri* position, involving the formation of a seven-membered ring (74). The *peri* ring closure is often predominant [164]. However, in the present case, no sign of this cyclisation was noted in the ¹HNMR spectrum.



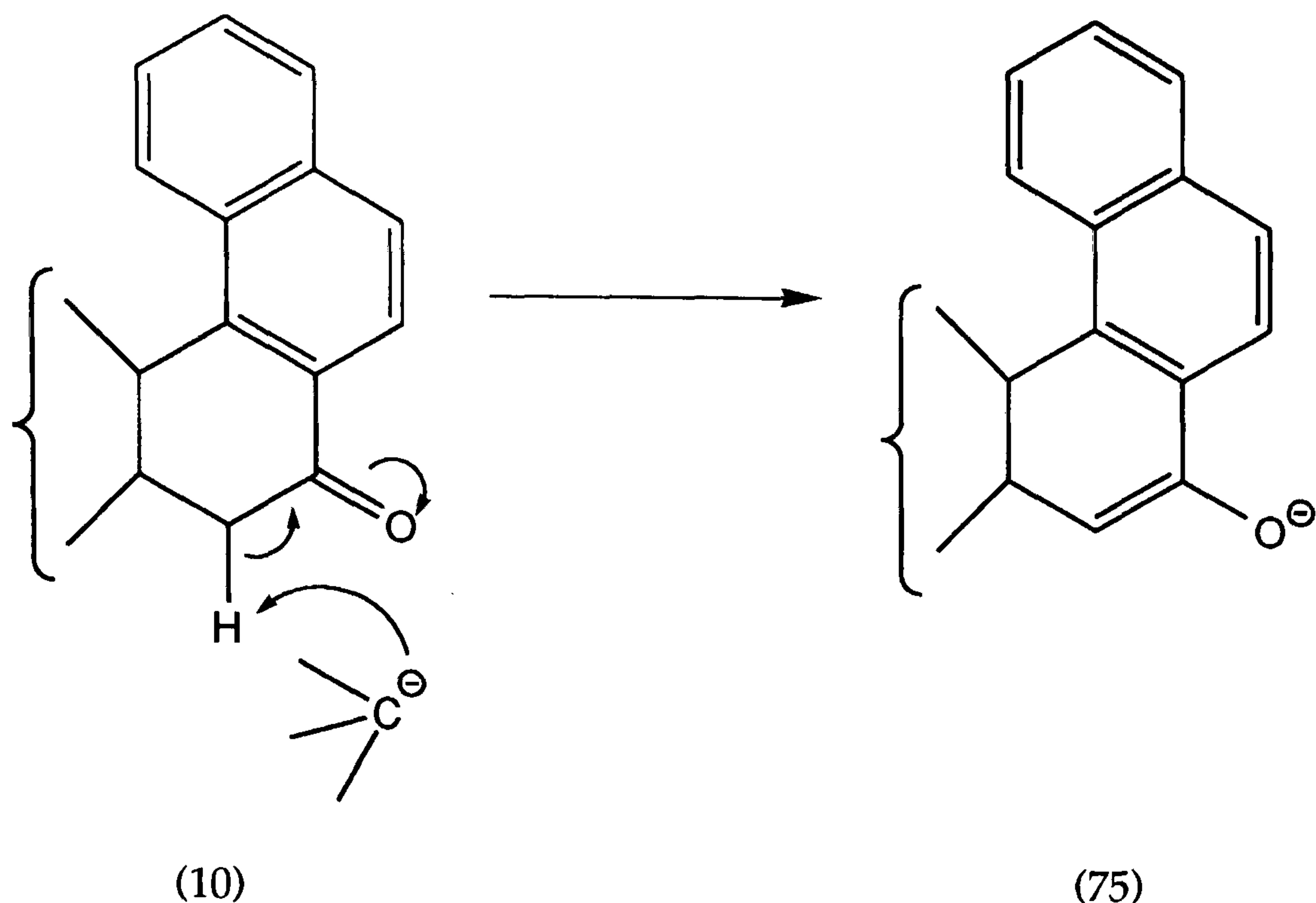
(74)

In the IR spectrum of compound (10) it is possible to see that the band at 1701 cm^{-1} due to the C=O of an acid has shifted to 1673 cm^{-1} , corresponding to the carbonyl group of a conjugated ketone.

Few differences are expected in the NMR data of ketone (10) compared to the previous compound (8). Once the second cyclisation is performed, the coupling constant H^a / H^f becomes more appreciable.

At this point, our synthesis of hexahelicen-7-ylacetic acid diverges from the pathway to hexahelicene itself which was reported by Newman and Lednicer [75]. In the published work, the synthesis of the parent hydrocarbon was completed via a second Wolff-Kishner reduction followed by dehydrogenation over a Rh/Al₂O₃ catalyst. However, our own strategy called for the attachment of an acetic side chain to C-7 by means of a nucleophilic attack on the electrophilic carbonyl group.

Numerous methods were investigated for the condensation of carbanion reagents with the hexahydroketone (10), including attack of the Reformatsky reagent derived from bromoacetic ester, Grignard reactions of alkylmagnesium halides, attack of alkyl- and acyl-lithium species, and Wittig and Peterson olefinations. In all cases, it was found that the ketone (10) was recovered in good yields and no appreciable quantities of any products of carbanion condensation could be isolated. It was concluded that, in common with many other examples for alkyl aryl ketones, the carbonyl group in compound (10) prefers to undergo enolisation to the aryl-conjugated enolate (75) rather than direct electrophilic attack. Once formed, the resonance-stabilised enolate anion (75) is protected from further nucleophilic attack and re-protonates to regenerate the ketone (10) on work up.



Scheme 30

After consulting the literature and further, extensive experimentation [165], eventually a method was found [166] which, it was hoped, would overcome this problem. This involved the generation of the anion from methyl acetate using the hindered, non-nucleophilic base, lithium bis (trimethyl silyl)amide in THF at -78°C (Scheme 31), followed by addition of the ketone.



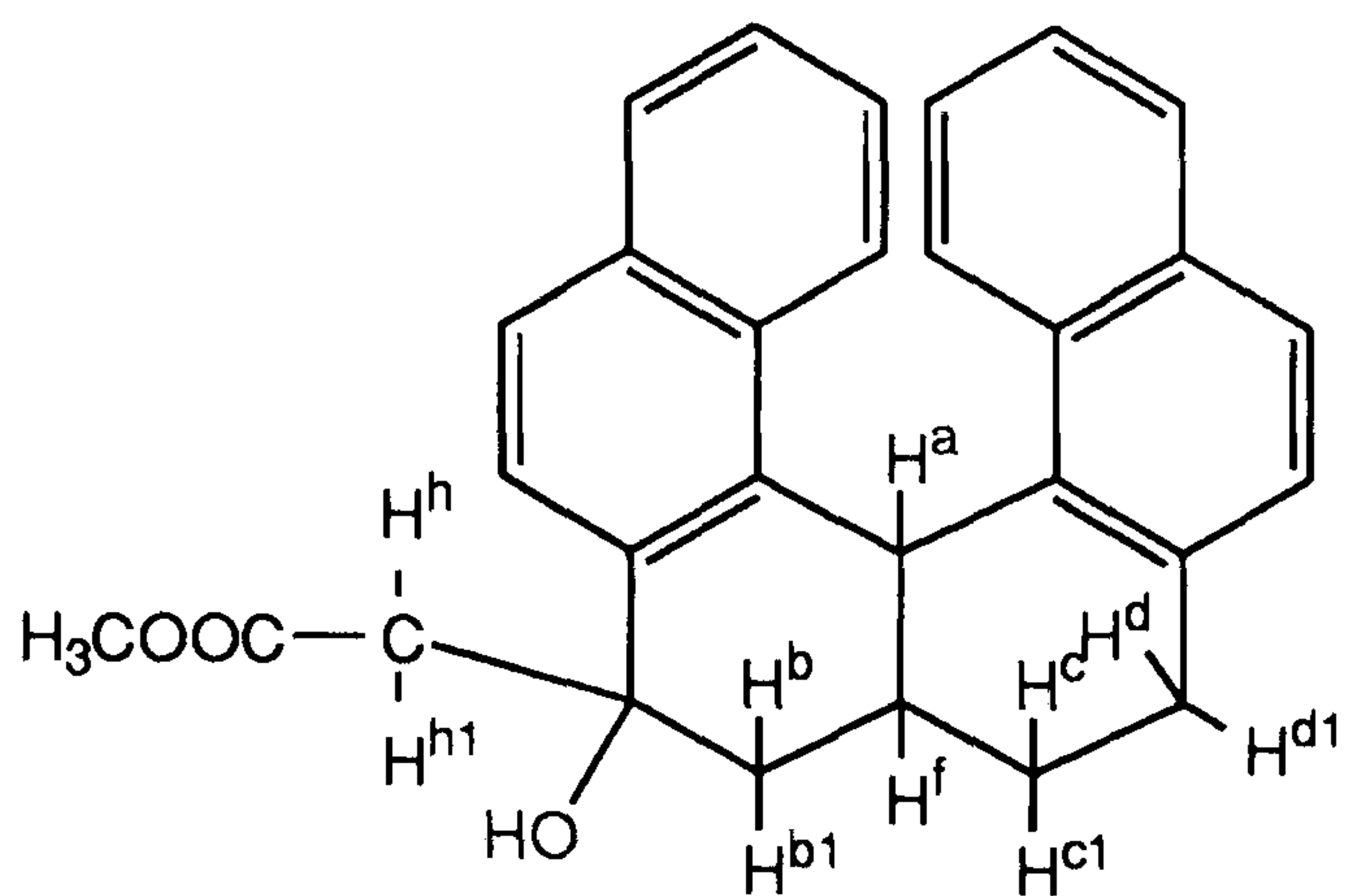
Scheme 31

The use of the lithio salt of methyl acetate presents an attractive alternative to the Reformatsky procedure. The lithio salt reaction is performed in less time than the conventional Reformatsky reaction and it does not require the preparation of the halogen derivative of the ester.

In a trial reaction, α -tetralone was used as a model and gave a good yield of the β -hydroxy methyl ester.

When the same reaction conditions were applied to the hexahydroketone (10) a crude product was obtained which showed two spots on TLC. The mixture was separated on a silica column eluted with 75:25 hexane/EtOAc. Two fractions were

collected in a 35 : 65 ratio, the first being the recovered ketone (10) and the second being consistent with the required compound (76).



(76)

The ^1H NMR of the second fraction shows two singlets at 3.78 and 4.68 δ due to the methyl group and the hydroxyl group respectively. The methylene group H^h/h^1 on the side chain shows prochirality giving two doublets (3.20 δ) with a large geminal coupling constant ($J = 15.6\text{Hz}$).

Mass spectrometry gave the required mass for hydroxyester (76). Important peaks appeared at M^+-17 due to loss of the hydroxyl group and M^+-73 due to the loss of the side chain $-\text{CH}_2-\text{COOCH}_3$.

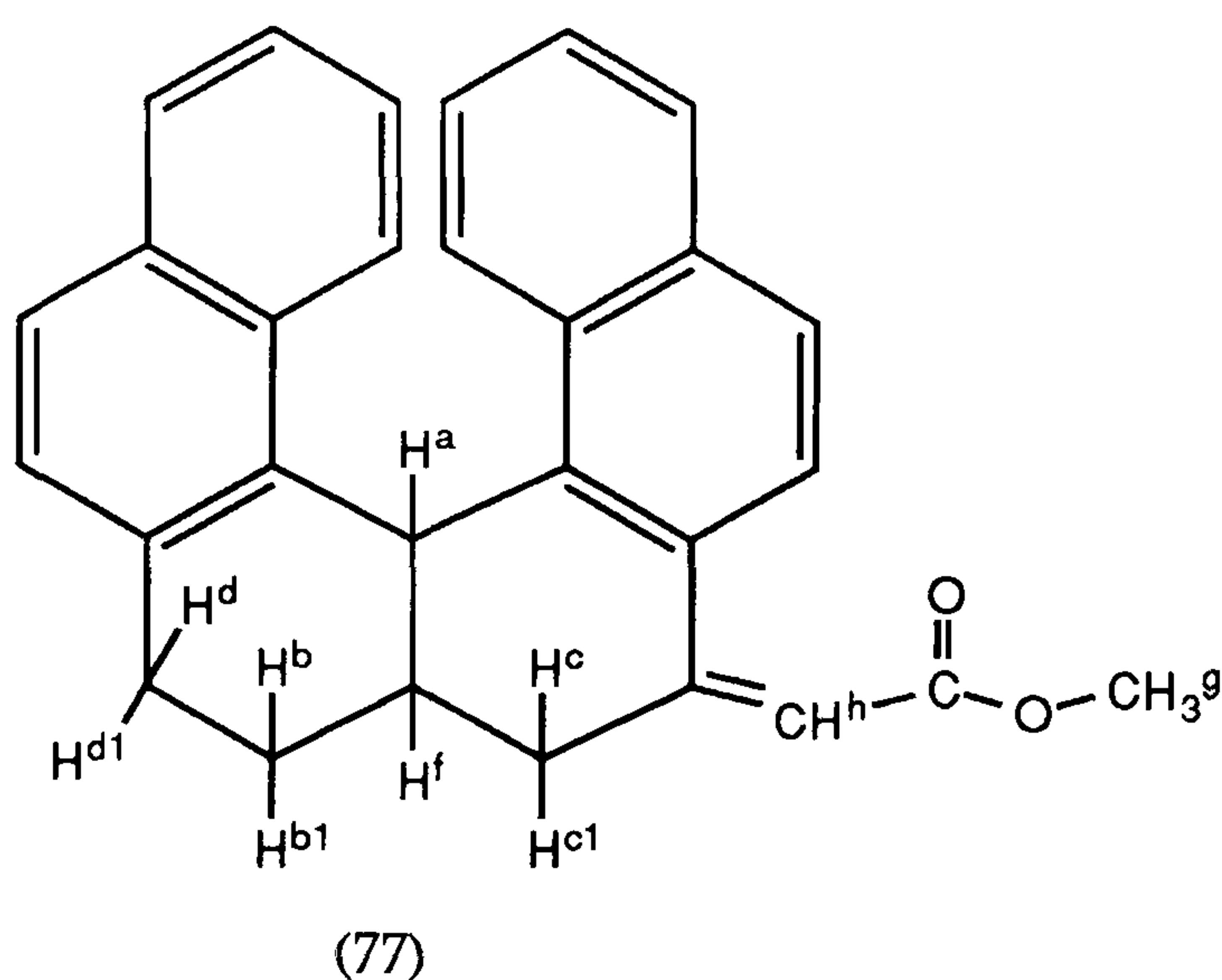
The reaction was repeated three times until most of the starting material was converted.

Generally, hydrolysis of the lithium intermediates gives the corresponding alcohol as a product, but sometimes elimination follows directly and the product is an olefin. In one of the reaction runs carried out, a mixture of conjugated and non-conjugated isomeric ene esters was obtained. IR spectrum gives two bands of equal intensity at 1732 and 1713 cm^{-1} , one for each ester.

The β -hydroxyester was then dehydrated^[167] by dissolving it in dichloromethane and drying the solution onto iron (III) chloride-silica. FeCl_3 adsorbed on chromatographic silica gel was found to be effective for rapid, high yielding and selective dehydration of alcohols^[168]. Solid adsorbents can be used as supports for selective reagents which are inefficient or inactive in solution^[169].

The dehydrations are generally very fast, taking place on contact of the substrate with the adsorbed reagent. In this case, the progress of the reaction was monitored by thin layer chromatography and took two days to complete. It was difficult to determine in which direction the dehydration occurred. The formation of three different isomers is possible: one with an endo double bond and two geometric isomers with an exo methylene group.

The IR spectrum shows two bands at 1738 and 1713 cm^{-1} due to the C=O stretch of the conjugated and non-conjugated isomers. In the ^1H NMR of the mixture a downfield singlet at 3.83 δ for the methine H^h confirmed the presence of the conjugated ester (77).



The loss of water from (76) was also confirmed by MS, but no effort was made to determine the isomeric composition of the product since further dehydrogenation was to be carried out.

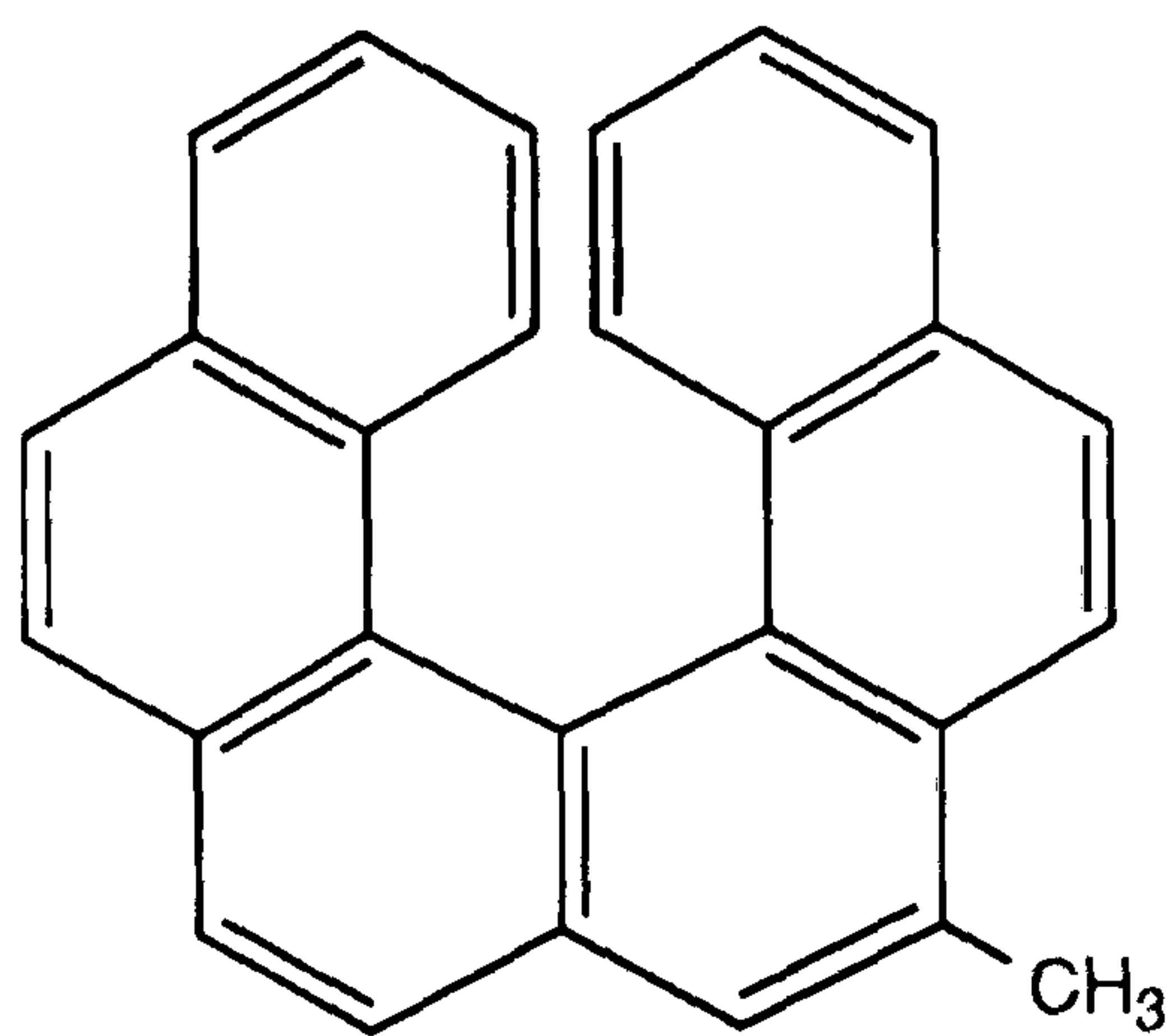
This dehydrogenation was accomplished in molten sulfur at 230°C. The mechanism of dehydrogenation by means of sulfur is not well established. The simplest explanation for the mechanism was proposed by Silverwood and Orchin for selenium dehydrogenation [170]. The abstraction of hydrogen atoms from allylic or benzylic position is believed to go through radical mechanisms. The dehydrogenation reaction occurs at temperatures at which rupture of sulfur-sulfur bonds to form sulfenyl radical is known to take place [171].



The sulfenyl radical initially abstracts hydrogen from a benzylic position to afford a hydroarene radical. The resulting radical intermediate may combine with a second sulfur radical to form, after thermal decomposition, a hydrocarbon sulfenyl intermediate, which can abstract hydrogen from the starting hydrocarbon (77) and form a double bond with an elimination of H₂S. The reaction carries on until the dehydrogenation is completed.

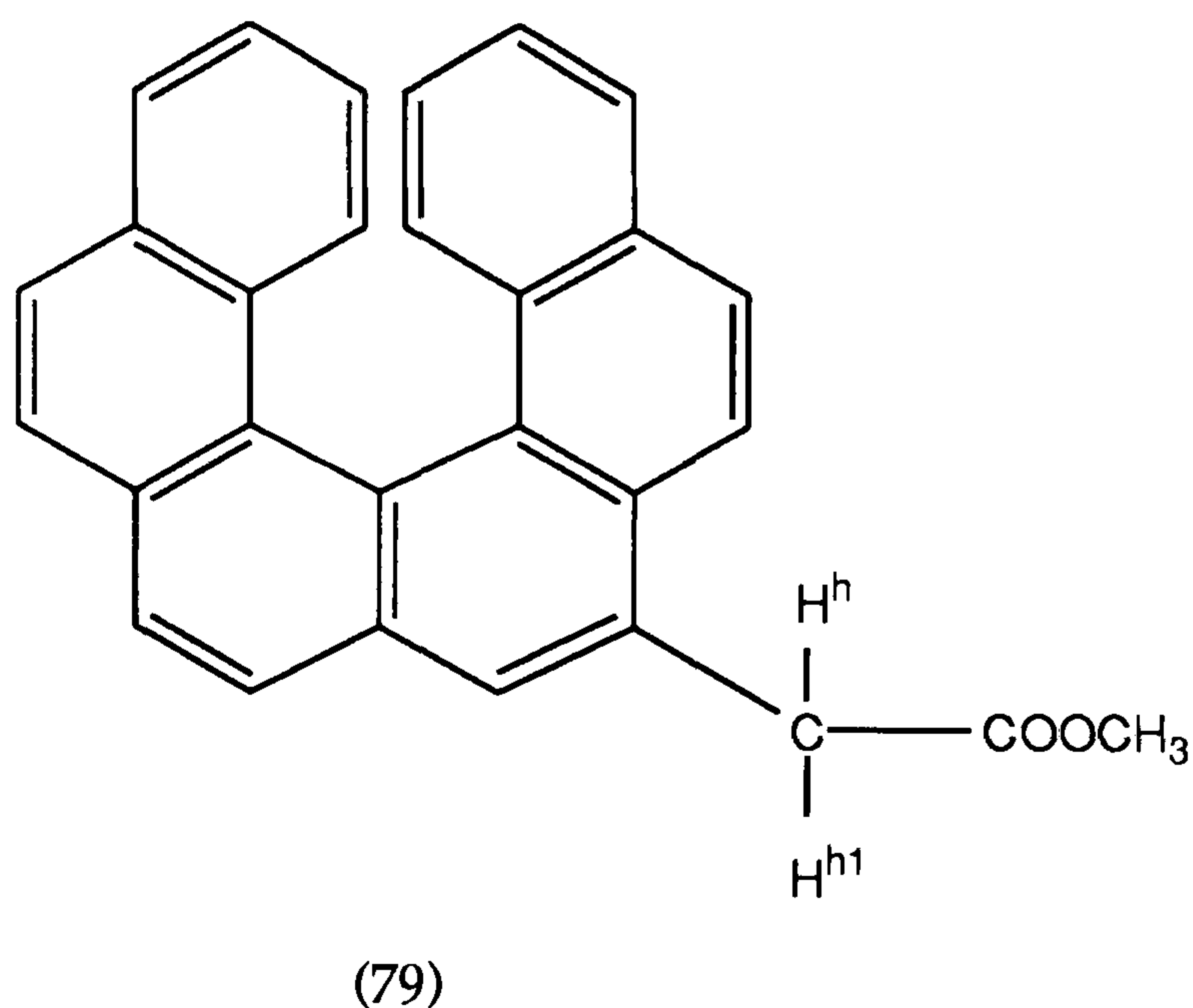
Carboxyl groups can be reduced to methyl groups in the course of their dehydrogenation [172]. This usually happens when selenium is used, as it needs a higher temperature than sulfur. However, in this case the side chain of compound (77) was reduced to a methyl group in a 8% yield even though the temperature was not so high (230°C).

Following dehydrogenation in molten sulfur, flash column chromatography was performed to separate the crude mixture using 75:25 hexane/EtOAc as an eluent. Two fractions were collected. The ¹H NMR spectrum of the less polar fraction shows a singlet at 2.85δ integrating for 3H which corresponds to a methyl group next to an aromatic group. A series of signals between 8.0-7ppm is consistent with the 15 aromatic protons of the monosubstituted hexahelicene (78).



(78)

The more polar fraction (65% yield) is consistent to the methyl ester (79). The 15 aromatic protons appear at the expected region (8.00-6.62 δ). A singlet integrating for 3 protons appears downfield (3.74 δ), corresponding to a methyl group next to an oxygen atom. The protons of the methylene group of the side chain couple to each other with a geminal constant of 15.5Hz.



Overall, a few hundred milligrams of the racemic hexahelicen-7-ylacetic acid methyl ester was obtained at the end of the sequence of reactions. This was a disappointingly small yield which reflects the unexpected difficulties encountered in the hydrolysis of nitrile (6) and the most of the subsequent reaction steps. Nevertheless, this arduous synthetic endeavour yielded sufficient material to proceed with the resolution and final linkage to a suitable support to enable HPLC investigations of the chiral phase to be conducted.

5.2- Resolution of Hexahelicen-7-ylacetic acid Derivatives.

The next synthetic step was the hydrolysis of the methyl ester to the acid. Since pure enantiomers of hexahelicen-7-ylacetic acid were required in order to prepare the chiral column, a chiral preparative separation was sought. Chiral acids are often difficult to separate due to strong hydrogen bond interactions between their polar acidic group and other polar groups on the chiral moiety or the support. This would lead to long retention times, tailing peak shapes and poor resolutions. Therefore, it was anticipated that it would be easier to separate the enantiomers at the methyl ester stage.

Matlin and Stacey^[141] had reported that it was possible to separate the hexahelicen-7-ylacetic acid methyl ester enantiomers on a stationary phase of covalently bonded (+)-TAPA on APS. Only a modest separation was achieved ($R_s=1.19$) on this analytical column, (25 x 0.45cm, 5 μ APS-(+)-TAPA; 86:10:4 v/v/v n-hexane/dichloromethane/acetonitrile; 1.0ml/min) which would limit its preparative capability. If a preparative separation were to be attempted using these conditions, only the first eluting isomer would be able to be isolated optically pure in any significant quantity. As both isomers were required for the preparation of (+) and (-) chiral phases, it was decided to examine alternative columns.

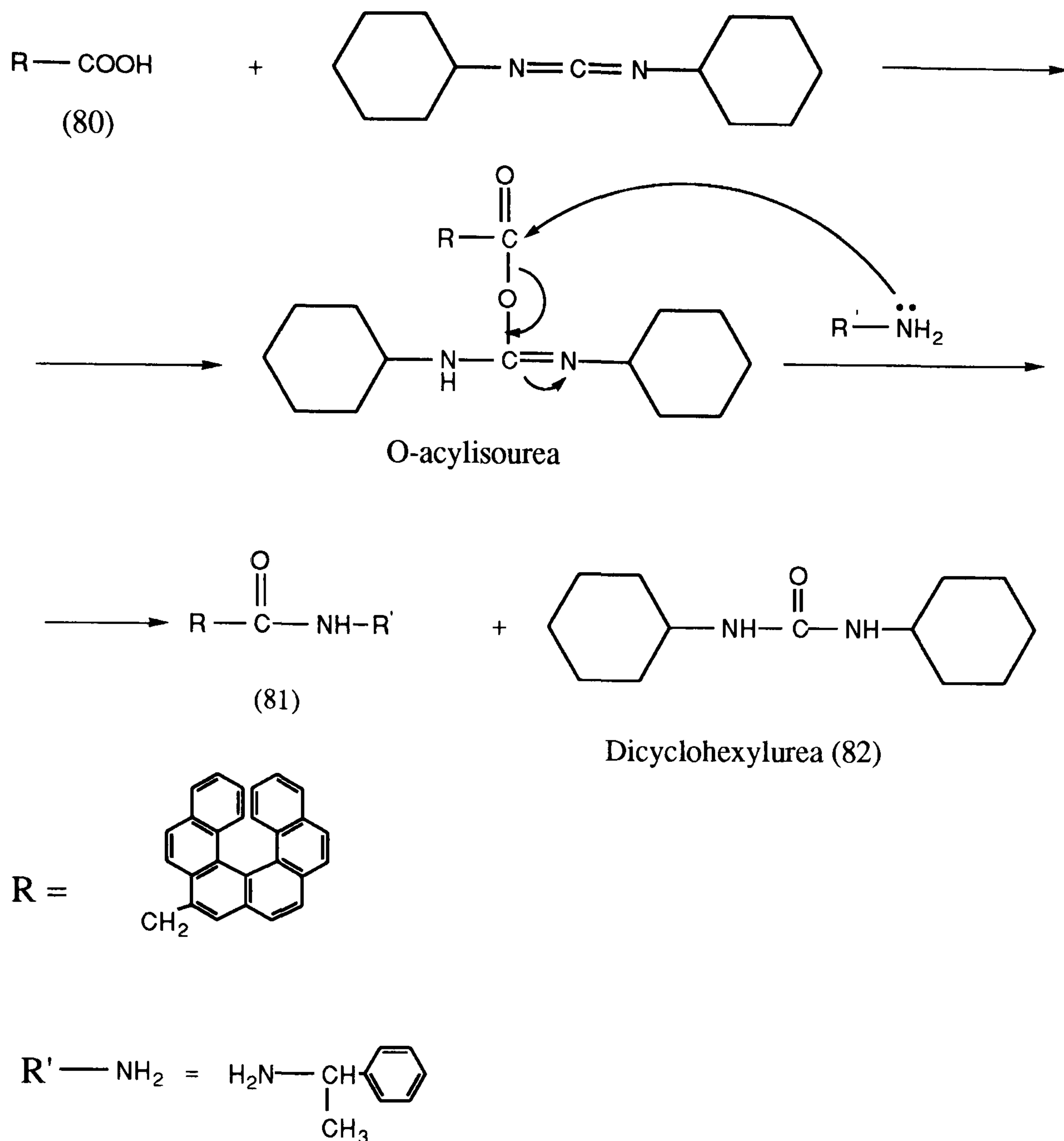
As reported in the literature (see p 70), helicenes have been resolved on several chiral HPLC phases. Hexahelicene is an highly electron rich aromatic compound and thus chiral resolution through π - π charge transfer can be achieved on electron-deficient Pirkle-type chiral phases (see Section 1.4.2). One of the most popular of these phases is that based on 3,5-dinitrobenzoylphenylglycine, either ionically or covalently bonded to APS silica. Therefore, the separation of the helicenyl methyl ester on a phase of this type (covalently bonded to 5 μ m APS; 25 x 0.46cm) was investigated. Unfortunately, resolution of the hexahelicen-7-ylacetic acid methyl ester was very poor ($R_s = 0.87$; 60:40 v/v n-hexane/IPA, 1 ml/min).

In an attempt to improve chiral resolution on the (-)-3,5-dinitrobenzoyl phenylglycine stationary phase, a new derivative of the hexahelicen-7-ylacetic acid was prepared. Optically pure R-(+)- α -methylbenzylamine was chosen, which would form a pair of diastereoisomeric amides with the racemic hexahelicen-7-ylacetic acid. It was anticipated that the amide group would further enhance the discrimination between the hexahelicene isomers by the introduction of: (i) an extra hydrogen bonding group (viz. amide NH) which can interact with the carbonyl of the amide on the stationary phase and (ii) a chirally oriented phenyl group which can interact either by π - π charge transfer with the electron deficient aromatic group on the stationary phase or by causing steric repulsion.

5.3- Preparation of (+/-)-Hexahelicen-7-yl 1'-methyl-1'-phenyl methyl amide.

The racemic hexahelicen-7-ylacetic acid methyl ester (79) was hydrolysed into the acid (80) by stirring a 0.5% (w/v) solution in methanol containing excess sodium hydroxide. The racemic acid obtained was analysed by spectroscopic methods and was consistent with the required structure. The IR spectrum gave a band at 1700 cm^{-1} due to a non-conjugated carboxylic acid. In the ^1H NMR, the methylene protons of the side chain, which are magnetically non-equivalent, gave two doublets with a geminal coupling constant of 16Hz. No singlet due to the methyl group of the ester could be seen, indicating that the hydrolysis had been completed.

The hexahelicen-7-ylacetic acid was coupled to the R-(+)- α -methylbenzyl amine using dicyclohexylcarbodiimide (DCC). The DCC forms a reactive intermediate, the O-acylisourea, which is attacked by the chiral amine to form the amide (81) and a dicyclohexylurea by-product (82) which can be easily removed. The mechanism for the formation of the amide is shown in Scheme 32^[173]:



Scheme 32

At this stage, it was decided that the amide would be characterised after separating the diastereoisomers, so that any impurities present could be removed during the resolution.

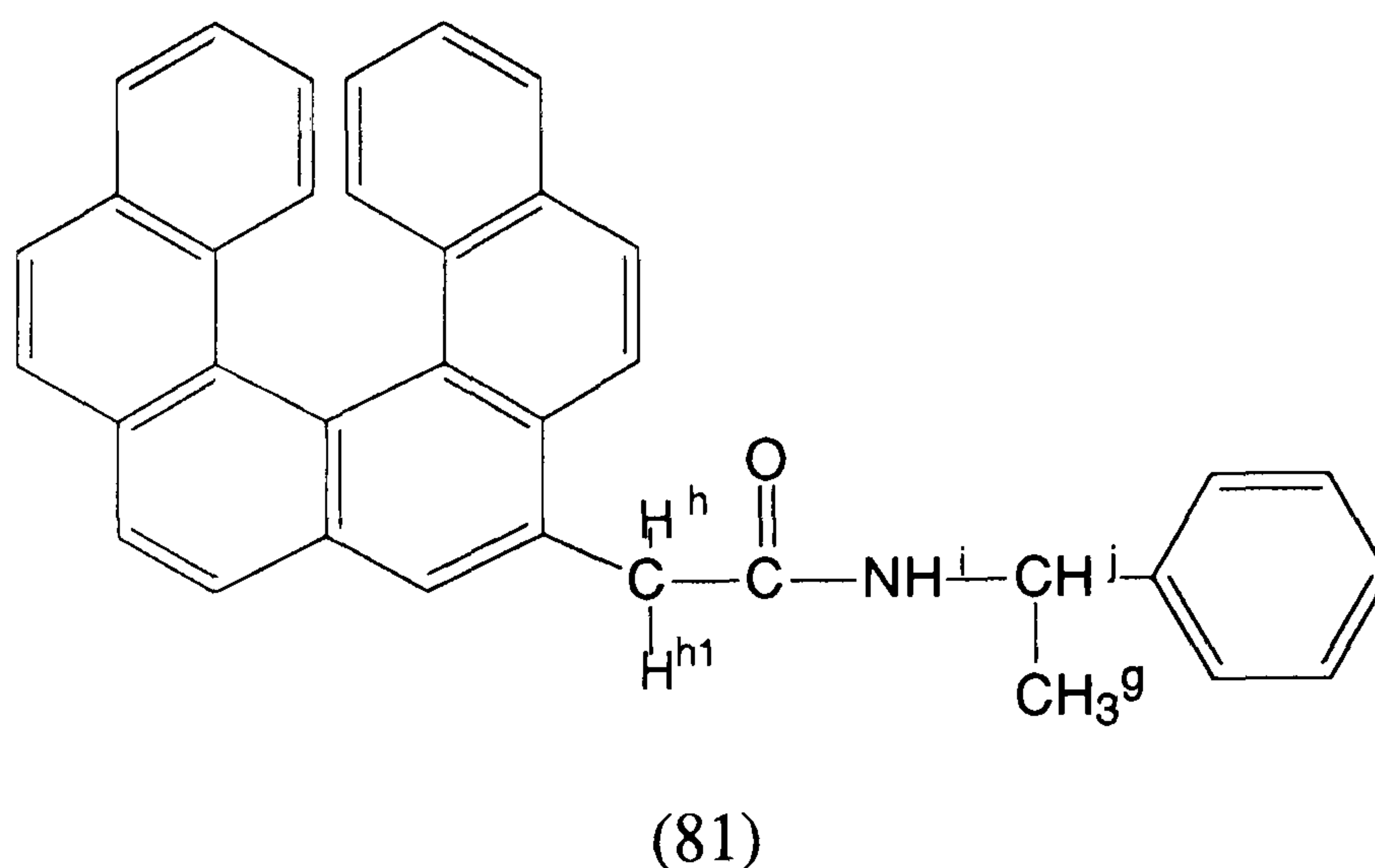
5.4- Chromatographic Resolution of the Diastereoisomers of (+/-)-Hexahelicen-7-yl 1'-methyl-1'-phenylmethanamide on a 3,5-Dinitrobenzoylphenylglycine APS Semipreparative Column.

Using 60:40 v/v n-hexane/IPA as a mobile phase on an analytical size column (25 x 0.45cm) a resolution of 1.42 was achieved (2.0 ml/min). These chromatographic conditions were transferred to the semipreparative column,

(25 x 0.7cm) of 3,5-dinitrobenzoylphenylglycine covalently bonded to APS. The optimum column loading was 3.2 mg (150 μ l). Using peak shaving and recycling of the impure fractions, a few milligrams of each diastereoisomers were isolated in highly pure form.

The first peak to elute was the (+) diastereoisomer with an optical rotation of $[\alpha]_D = +6156^\circ$. The second peak had an optical rotation of $[\alpha]_D = -6234^\circ$.

Both diastereoisomers were analysed by IR, giving a strong amide band at 1627 cm^{-1} . The ^1H NMR of both diastereoisomers (81) gave the correct signals for the 15 protons of monosubstituted hexahelicene and the 5 protons of the phenyl group. Although the protons of the methylene group $\text{H}^{\text{h/h1}}$ are not equivalent, in the (+) diastereoisomer they coincide and only a broad singlet is observed (4.2 δ). However, in the (-) diastereoisomer they appear as two doublets (4.24 δ) $J = 16.6\text{Hz}$. The amide proton (H^{i}) couples to the methine proton to give a doublet at 5.6 δ . The methine group H^{j} gives a quartet (5.2 δ) $J = 7.3\text{Hz}$. An upfield doublet integrating for 3 protons showed the presence of the methyl group.



Base hydrolysis of the two separated diastereomeric hexahelicene amides produced a few milligrams of each of the pure acid enantiomers. Only enough material was available for characterisation purposes. The spectroscopic results were consistent with previous results (Section 5.2).

It was disappointing to find that the extra time and effort required to prepare the amide derivative did not result in a better separation on the 3,5-dinitrobenzoylphenylglycine phase. The modest resolution ($R_s = 0.8$) obtained on the semipreparative column allowed only small amounts (3.2 mg) of diastereomeric amide to be processed per injection, thus hundreds of injections would be required to prepare enough isomerically pure material for the stationary phase. In view of the problems discussed above, it was decided to halt work on this method in favour of exploring further chiral columns. Fortunately, at this time, colleagues in this laboratory were becoming interested in the coated polysaccharide carbamate phases .

5.5- Chromatographic Resolution of the Enantiomers of (+/-)-Hexahelicen-7-ylacetic acid methyl ester on a Polysaccharide Column

Coated carbohydrate carbamate columns, developed by Okamoto *et al.* [174], are some of the most useful and versatile chiral columns reported in recent years. The cellulose and amylose tris(3,5-dimethylphenyl carbamate)-coated columns (commercially available as Chiralcel OD and Chiralpak AD respectively; Daicel) have been reported [175] to be able to resolve greater than 80% of all racemates tested. The structures are shown in Fig. 59.

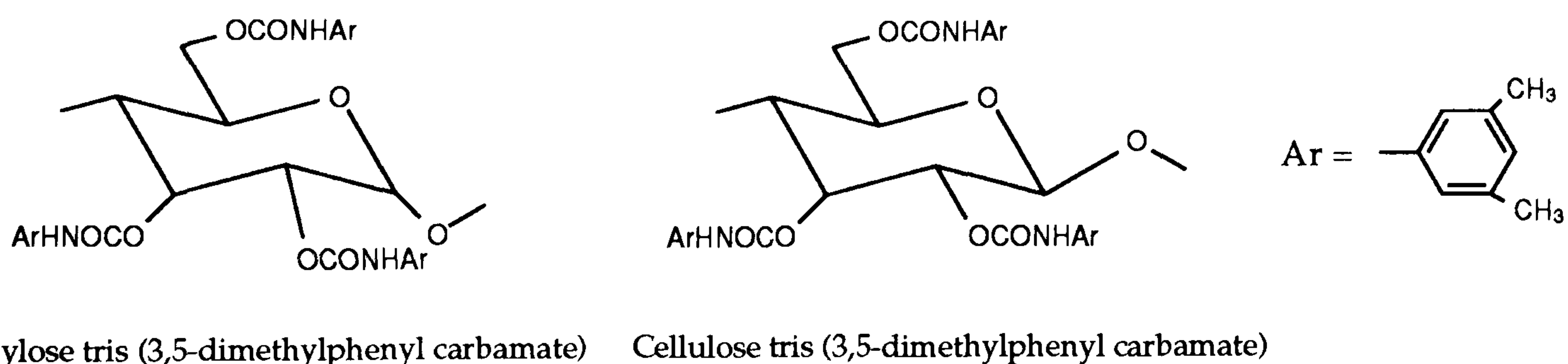


Fig. 59 Structures of amylose and cellulose tris (3,5-dimethylphenyl carbamate)

Our research group has been investigating the preparation, efficiency and mode of separation of these types of columns [176]. It was anticipated that these carbamate columns might give superior resolutions of the hexahelicen-7-ylacetic acid

methyl ester compared to those obtained on the Pirkle type column. In addition to hydrogen bonding and π - π interactions offered by the carbamate group, polysaccharide stationary phases also have a 3-D helical structure. This gives rise to chiral cavities into which one of the helical enantiomers of the hexahelicen-7-ylacetic acid methyl esters may fit more tightly than the other.

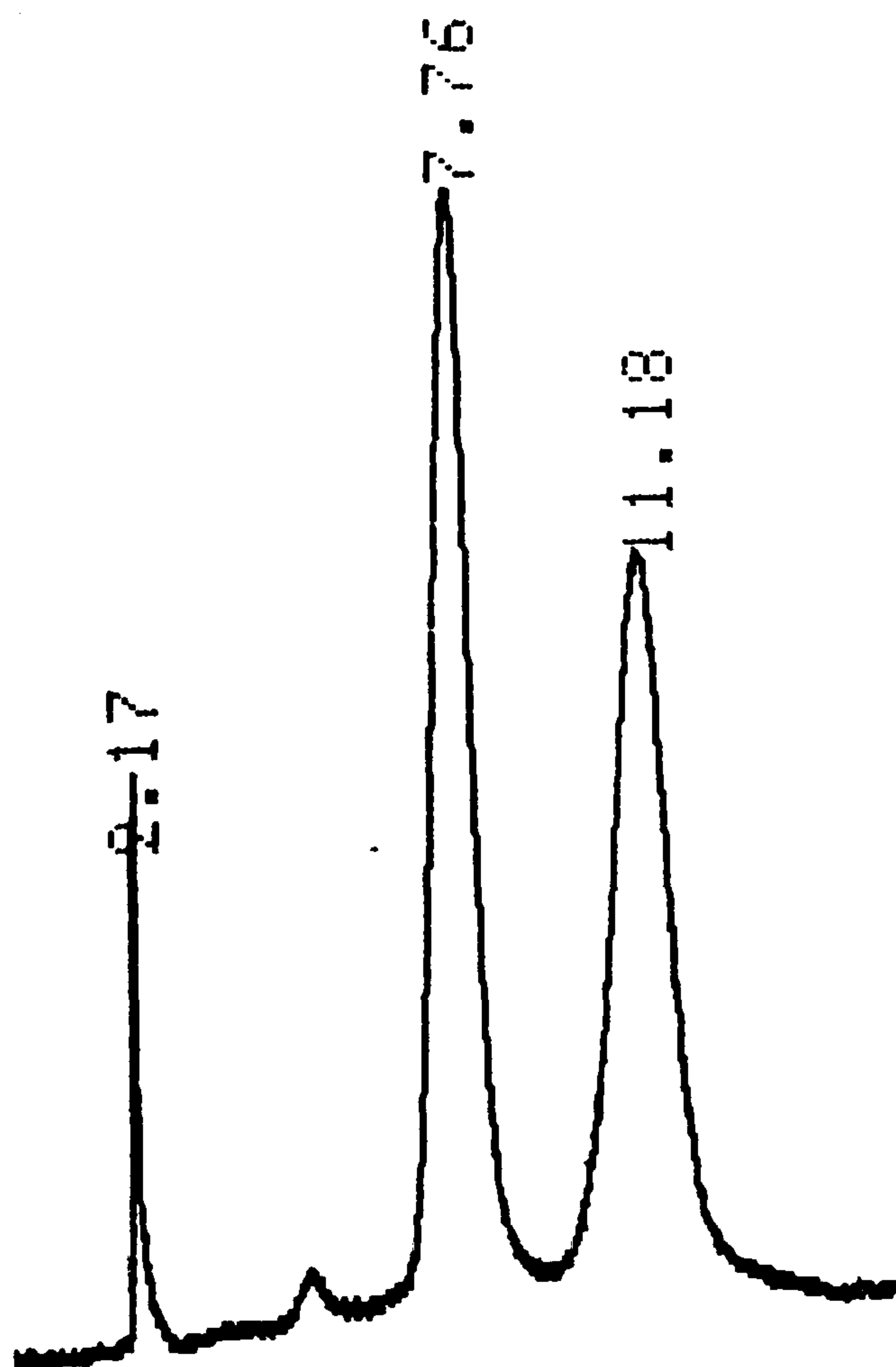
Four analytical carbohydrate carbamate-coated chiral columns, prepared by a colleague in this laboratory [177], were evaluated for the separation of hexahelicen-7-ylacetic acid methyl ester (79). The chromatographic results are shown in Table 3.

Column	Mobile phase	Flow rate ml/min	k' ₁	k' ₂	α	R _s
20% ADMPC coated on Hypersil APS (5 μ m, 120 Å) 15 x 0.46 cm	Hexane/IPA 98:2 v/v	0.5	3.36	4.43	1.32	1.88
20% CDMPC coated on Hypersil APS (5 μ m, 120 Å) 15 x 0.46cm	Hexane/IPA 95:5 v/v	0.5	2.96	3.56	1.20	1.3
	Hexane/EtOH 98:2 v/v	1.0	2.58	4.15	1.61	2.19
16.6% APC coated on Hypersil APS (5 μ m, 120 Å,) 15 x 0.46 cm	Hexane/EtOH 98:2 v/v	0.5	2.5	2.81	1.13	PR
17.5% CPC coated on Hypersil APS (5 μ m, 120 Å) 15 x 0.46 cm	Hexane/IPA 90:10 v/v	0.5	2.86		1.00	

ADMPC and CDMPC = amylose and cellulose tris(3,5-dimethylphenyl carbamate) respectively; APC and CPC = amylose and cellulose tris(phenyl carbamate) respectively.
PR = partial resolution

Table 3: The resolution of hexahelicen-7-ylacetic acid methyl ester on four carbohydrate carbamate-coated chiral columns

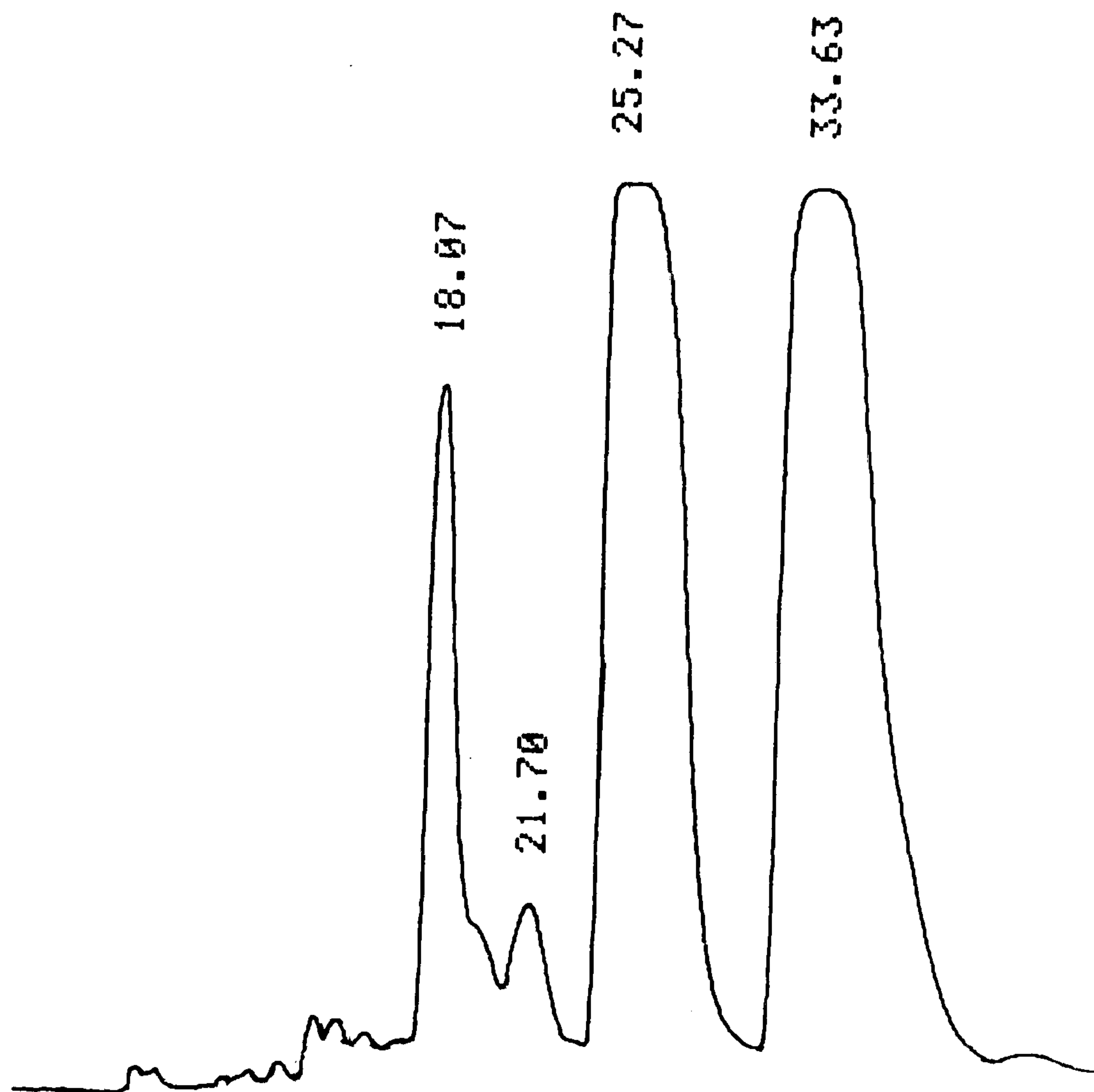
The CDMPC-coated column (mobile phase: hexane/ethanol 98:2 v/v) was found to give the best resolution of the hexahelicen-7-yl acetic acid methyl ester (79) (Fig. 60). Therefore, this column type and mobile phase conditions were chosen for the preparative separation.



Column: 20% CDMPC on 5 μ Hypersil APS
Column Size: 15 x 0.46 cm
Mobile Phase: 98:2 Hexane/EtOH
Flow Rate: 1.0 ml/min
Detection: UV-254 nm (0.2 AUFS)

Fig. 60 Analytical HPLC resolution of hexahelicen-7-ylacetic acid methyl ester.

The preparative column, (25 x 2.2 cm; packed in our laboratory) consisted of 16% w/w cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC) coated on 5 μ m Hypersil APS (120 \AA). Initially, in order to gauge the sample loading capacity of the column, a 10 mg injection of the hexahelicen-7-yl acetic acid methyl ester dissolved in absolute ethanol was made. See Fig. 61.



Column: 16% CDMPC on 5 μ Hypersil APS
 Column Size: 25 x 2.2 cm
 Mobile Phase: 98:2 Hexane/EtOH
 Flow Rate: 12 ml/min
 Detection: UV-280 nm (0.2 AUFS)

Fig. 61 Resolution of enantiomers of hexahelicen-7-ylacetic acid methyl ester on a preparative CDMPC column.

The enantiomers of the methyl ester eluted at 25.3 and 33.6 minutes. At this sample load, baseline separation was achieved and the sample loads were therefore increased gradually to find the maximum sample load that would give quantitative recovery. At a 30 mg sample load, the enantiomers were just starting to overlap.

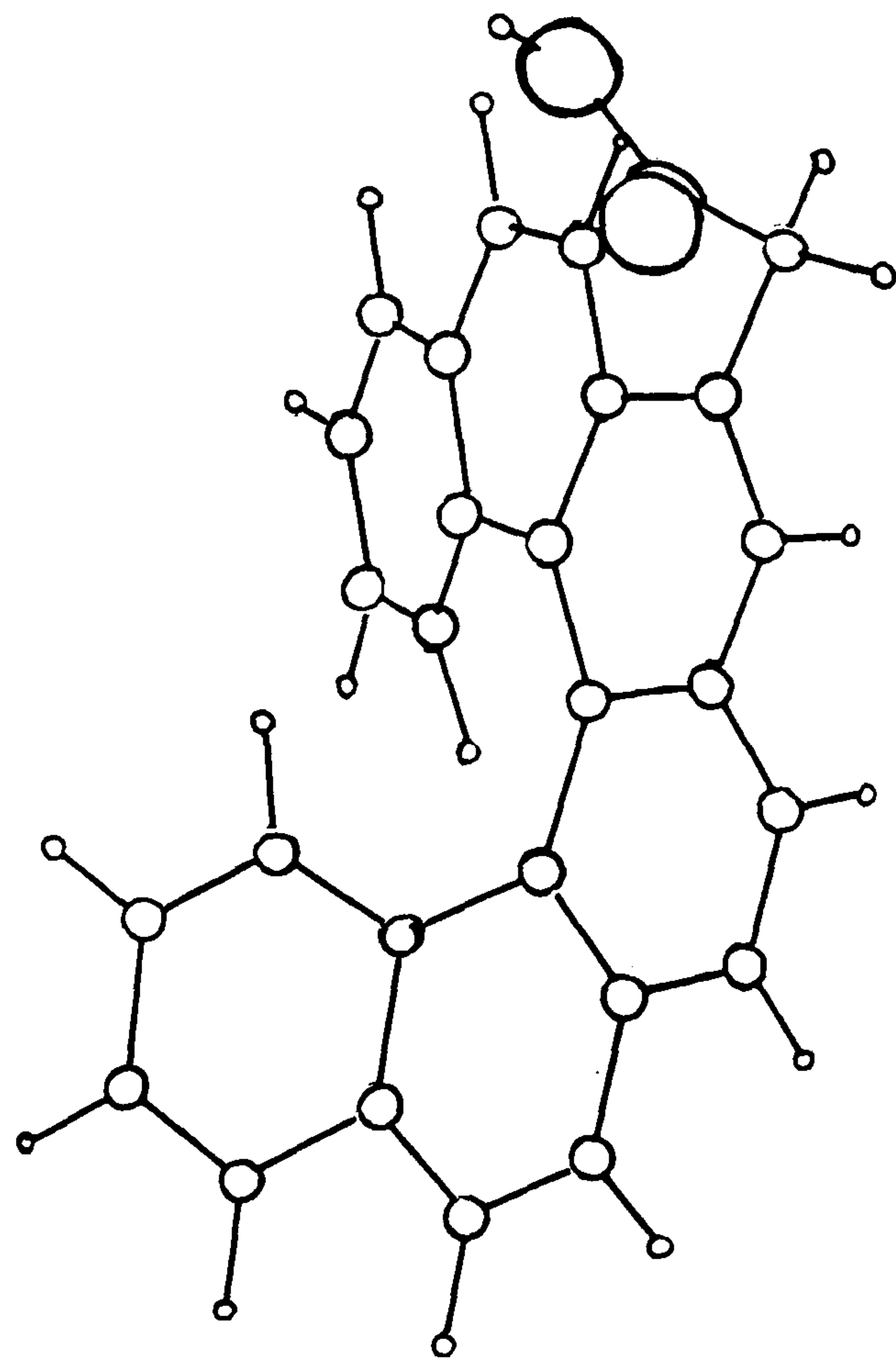
Due to the modest solubility of the product in ethanol and because very little recycling was required, a 30 mg injection was chosen as the optimum. At this sample load about 95% of the first enantiomer and 80% of the second enantiomer could be isolated in one run. The overlap fractions collected could then be evaporated and rechromatographed to obtain almost quantitative recovery of the enantiomers of hexahelicen-7-yl acetic acid methyl ester.

300 mg of the racemic hexahelicen-7-yl acetic acid methyl ester was chromatographed in this way, yielding approximately 105 mg of the first enantiomer and 85 mg of the second enantiomer. The recovery of the enantiomers was low due to the presence of impurities in the sample (18.1 min and 21.6 min) (see Fig. 61).

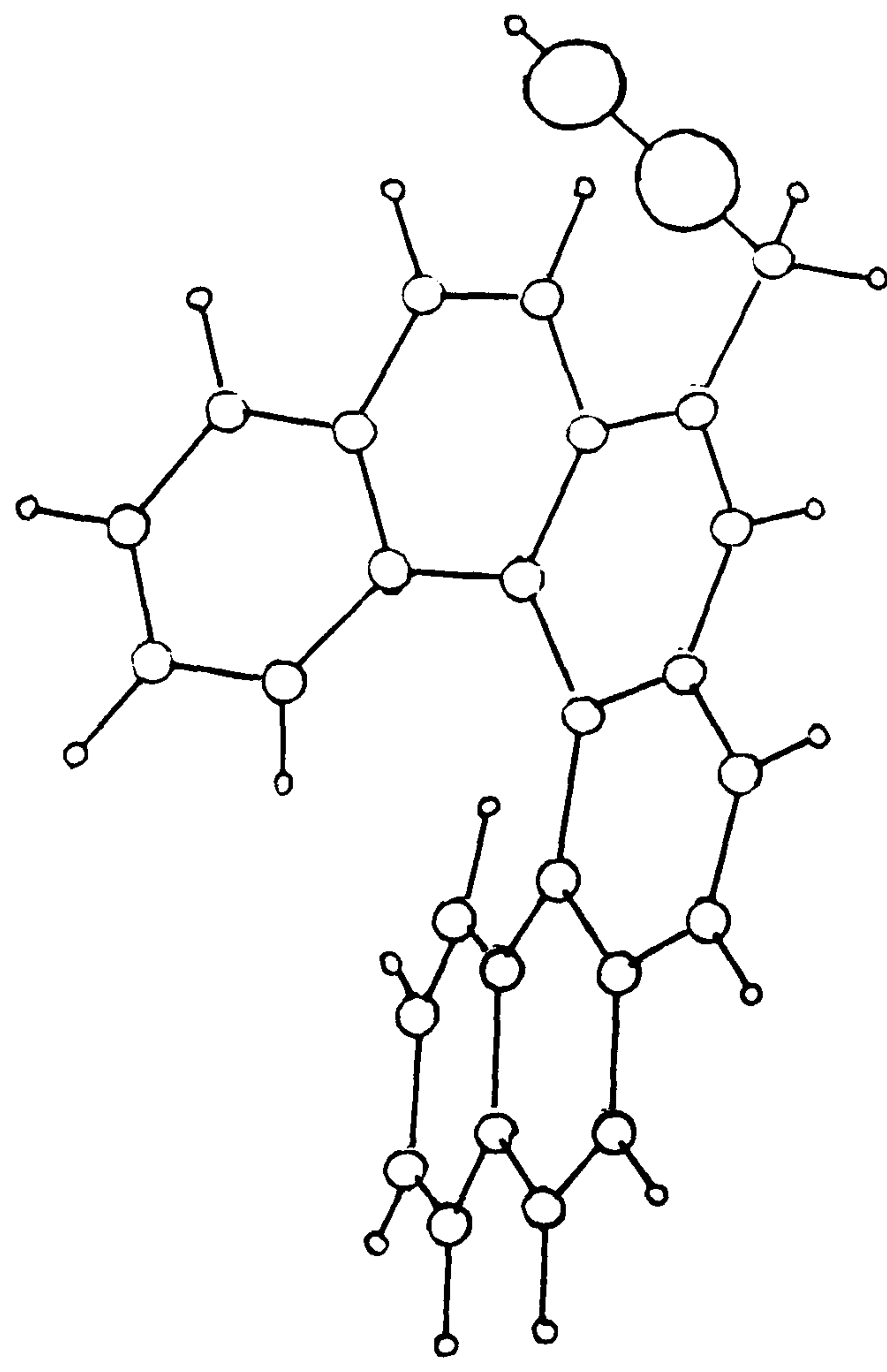
Both enantiomers were found to be >99% pure by HPLC. The optical rotations of the two enantiomers were measured. The first isomer to elute was the (-) enantiomer with $[\alpha]_D = -2557^\circ$ and the second peak was the (+) enantiomer with $[\alpha]_D = +2630^\circ$.

5.6- Hydrolysis of the Hexahelicen-7-ylacetic acid methyl ester Enantiomers

The two enantiomers were hydrolysed separately into their respective acids (Fig.62). The samples were dissolved in 0.5% (w/v) solution in methanol with excess of sodium hydroxide and left stirring overnight. A pale yellow solid was obtained in 98% yield. Each enantiomer was analysed by spectroscopic methods and the data obtained were consistent with those for the racemic acid analysed previously (see Section 5.2).



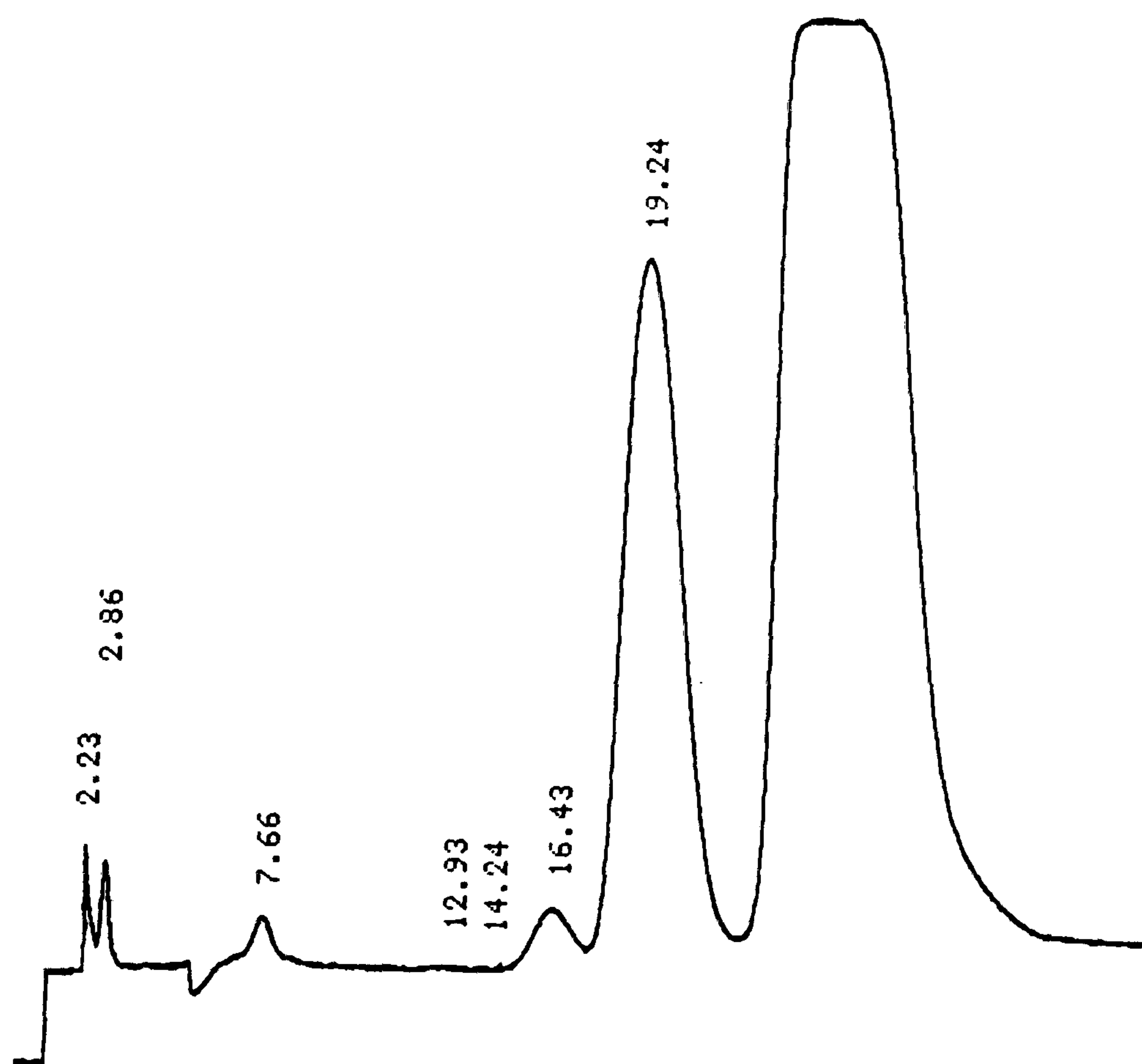
M(-)-Hexahelicen-7-ylacetic acid



P(+)-Hexahelicen-7-ylacetic acid

Fig. 62 Molecular modelling of (+/-)-hexahelicen-7-ylacetic acid enantiomers

The enantiomers of hexahelicene-7-ylacetic acid could be partially resolved on an analytical CDMPC coated column using a mobile phase of trifluoroacetic acid/ethanol/hexane 0.5:2:98 v/v/v. Therefore, it was possible to check whether the methyl esters had racemised. As anticipated, neither of the hexahelicene-7-ylacetic acid enantiomers had racemised. However, although the chromatogram for the (+)-hexahelicene-7-yl acetic acid showed only one peak, there was an impurity (~10%) at 19.24 minutes in the (-)-hexahelicene-7-yl acetic acid chromatogram (see Fig. 63). This impurity must have been co-eluting with the (-)-hexahelicene-7-ylacetic acid methyl ester enantiomer during the preparative separation.



Column: 16% CDMPC on 5 μ Hypersil APS
Column Size: 25 x 2.2 cm
Mobile Phase: 98:2 Hexane/EtOH + 0.5% TFA
Flow Rate: 12 ml/min
Detection: UV-280 nm (0.2 AUFS)

Fig. 63 HPLC preparative separation of the impurity from (-)-hexahelicene-7-ylacetic acid

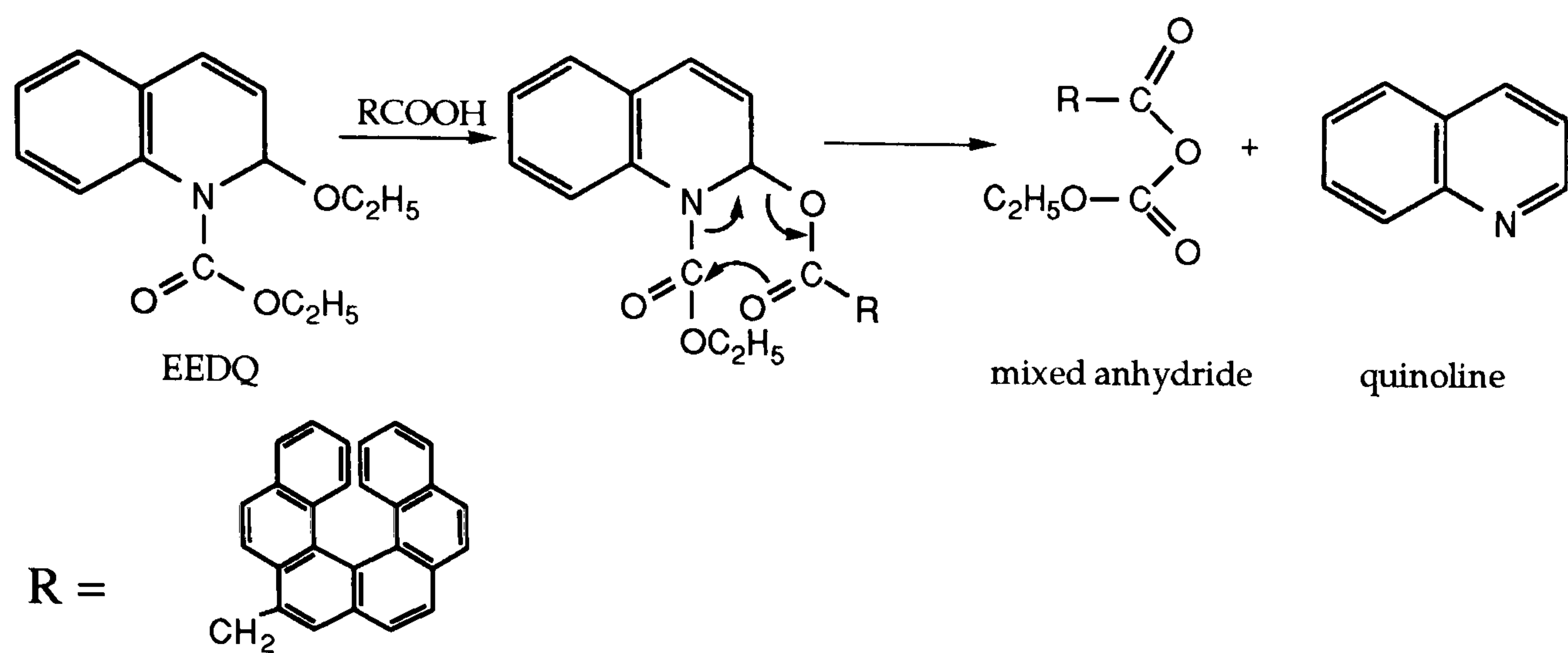
Fortunately, the resolution between the impurity and the (-)-hexahelicen-7-yl acetic acid was very good. Therefore, a separation of the impurity and the enantiomer was attempted on the preparative CDMPC chiral column used previously. Three 35 mg injections were made and the impurity and (-)-hexahelicen-7-ylacetic acid fractions collected separately. Both fractions were evaporated, washed several times with dichloromethane to remove the trifluoroacetic acid and then freeze dried. The final weight of the (-)-hexahelicen-7-ylacetic acid was 90 mg.

5.7- Bonding the Hexahelicen-7-ylacetic acid to Hypersil APS.

In view of the relatively small amount of hexahelicen-7-ylacetic acid isomers obtained and the requirement to optimise bonding procedures (See Section 4.2), it was decided to apply a different reagent to the preparation of the chiral bonded phase for each enantiomer.

The conditions used by Stacey [141] were repeated for the coupling of the (+)-hexahelicen-7-ylacetic acid isomer to APS. (+)-Hexahelicen-7-ylacetic acid and 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-4-toluenesulphonate (CDI) in acetonitrile were stirred overnight at room temperature with aminopropylated silica. The mechanism is similar to that illustrated for DCC (Scheme 32). Microanalysis showed that 40.9% of the available amino groups had reacted with the acid. This coverage was higher than that obtained by Stacey (33%).

An alternative coupling procedure was investigated for the (-)-hexahelicen-7-ylacetic acid isomer. (-)-Hexahelicen-7-ylacetic acid and N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) [178] in THF were stirred overnight at 30°C with aminopropylated silica reacting via a mixed anhydride intermediate (Scheme 33). Microanalysis showed that 11.9% of the available amino groups had reacted with the acid. From this limited comparison, it appears that CDI is a much more effective coupling agent than EEDQ for this application.



Scheme 33

5.7.1- Calculation of the Surface Coverage

The amount of the stationary phase bonded to the support is an important parameter. It is used to evaluate the extend of bonding or percentage of coverage of the surface achieved after reaction. Reporting only the total per cent carbon content (elemental analysis) is not a satisfactory procedure for characterising the bonded phase as it gives incomplete information. The surface coverage can be reported in terms of the number of aminopropyl groups per square nanometer of aminopropyl silica. The number (n) of organic groups bonded per nm² of silica backbone can be calculated using the following equation [179] (a):

$$n = \frac{\{(C_{obs}^{\%} \times 10^{-2}) \times 602300\}}{\left\{SA \times C_p^{wt} \times \left[1 - (C_{obs}^{\%} \times 10^{-2}) \times \left(\frac{Mw}{C_p^{wt}}\right)\right]\right\}}$$

where:

$C_{obs}^{\%}$ = Elemental analysis data for carbon.

C_p^{wt} = Mass of carbon in 1 mole of organic monolayer chains.

Mw= Molecular weight of organic monolayer chain.

SA = Surface area of silica backbone.

In this calculation, the surface area of aminopropyl silica SA is taken as $180 \times 10^{18} \text{ nm}^2 \text{ g}^{-1}$, the molecular mass of the organic monolayer in the case of aminopropyl silica corresponding to $\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$ is 58, the elemental analysis data for carbon is 1.9% and the mass of carbon in 1 mole of organic monolayer chain is 36. This results in $n = 1.82$ groups per nm^2 .

After establishing the number of aminopropylsilane groups (n) present due to modification (i.e. aminopropylation) of the silica surface, the extent of the reaction of the amino function of the spacer chain ($\text{Si}(\text{CH}_2)_3\text{NH}_2$) with a substrate can be monitored by elemental analysis. This determination can be expressed as the percentage of initial aminopropyl groups reacted (x):

$$x = \frac{\left[C_{obs}^{\%} \frac{Mw_1 + 602300}{SA \times n} \right] - 100C_1}{(C_2 - C_1) - \left(\frac{Mw_2 - Mw_1}{100} \right) C_{obs}^{\%}}$$

Where:

Mw_1 = Molecular weight of bonded chiral selector before reaction

C_1 = mass of carbon in organic monolayer before reaction

Mw_2 = Molecular weight of bonded chiral selector after reaction

C_2 = Mass of carbon in organic monolayer after reaction

n = Groups of organic monolayer per nm^2 of silica backbone

$C_{obs}^{\%}$ = Elemental analysis data for carbon

x = Percentage groups reacted

SA = Surface area of silica backbone.

The number of aminopropyl groups reacted with (+)-hexahelicen-7-ylacetic acid per nm^2 of silica : $x = 40.9\%$ and for (-)-hexahelicen-7-ylacetic acid, $x = 11.9\%$.

5.8- Separations of Chiral Test Solutes on the (+)- and (-)-Hexahelicene Bonded Phases.

5.8.1- Design of the Chiral Test Analytes

A series of nitroaryl chiral analytes were synthesised to investigate the relationship between the structure of the chiral solutes and the enantioselectivity and retention behaviour of the (+)- and (-)-hexahelicene stationary phase. Stacey [141] had investigated the resolution of the 2,4-dinitrophenyl ether and 3,5-dinitrobenzoyl ester of (+/-)-phenethyl alcohol and found that both types of derivative could be resolved on the (+)-hexahelicene-7-ylacetic acid phase.

In the present work, the more symmetrically substituted 3,5-dinitrobenzoyl (3,5-DNB) ester was selected as the model. 3,5-Dinitrobenzoyl chloride was reacted with a series of arylalkanols to prepare solutes (N, Scheme 34) displaying the following features:

- 1- Increasing length of the alkyl chain at the chiral centre from methyl to propyl (y parameter).
- 2- Spacer between the aromatic group and the chiral centre (x parameter).
- 3- Variation of the aromatic group at the chiral centre.

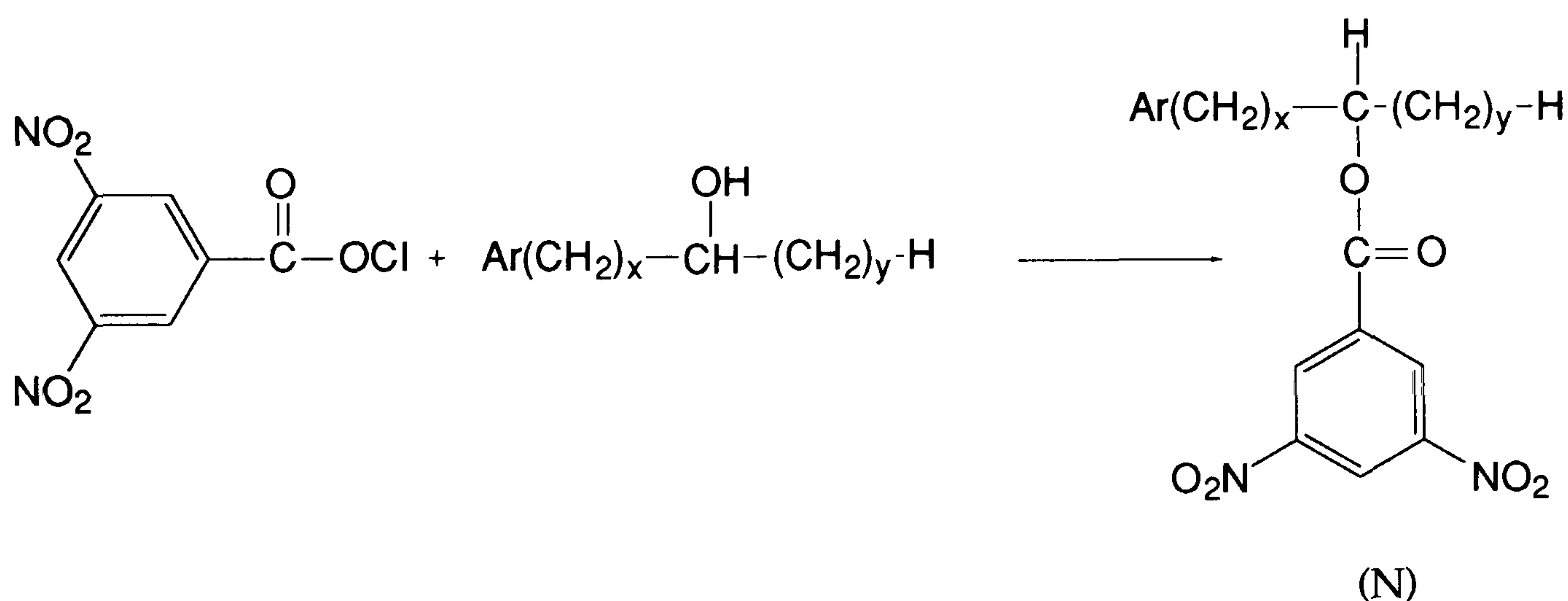


Fig. 64 Design of the chiral test solutes

3,5-DNB-derivatives	Ar	x spacer	y alkyl group
1-phenylethanol	phenyl	0	1
1-phenylpropan-1-ol	phenyl	0	2
1-phenylbutan-1-ol	phenyl	0	3
1-phenylbutan-2-ol	phenyl	1	1
4-phenylbutan-2-ol	phenyl	2	1
1-naphthylethanol	naphthyl	0	1

Table 4 Series of 3,5-dinitrobenzoyl derivatives prepared

The 3,5-dinitrobenzoyl derivatives of optically pure (S) and (R)-1-phenylethanol and 1-phenylbutan-1-ol were prepared so that the order of elution could be determined.

5.8.2- Development of the Chromatographic Conditions for the Resolution of the Tests Analytes.

Stacey used isopropanol/n-hexane (10:90 v/v) at a flow rate of 2 ml/min to elute the derivatives she prepared. However, since the column length and diameter in our columns were reduced (10 x 0.32 cm versus 25 x 0.45 cm) the mobile phase and flow rate were optimised. Ethanol was found to give sharper peak shapes and thus better resolutions than isopropanol and the flow rate was reduced to 0.4 ml/min. The mobile phase composition was adjusted to give similar capacity factors ($k'_1 \approx 4$) for each 3,5-dinitrobenzoyl derivative. The HPLC results are shown in Table 5.

3,5-dinitrobenzoyl derivative	Mobile phase Hexane/EtOH	k'_1	k'_2	α	R_s
1-phenylethanol	92:8	3.73	4.03	1.08	0.80
1-phenylpropan-1-ol	98:2	4.0	4.52	1.13	1.02
1-phenylbutan-1-ol	98.5:1.5	4.06	4.75	1.17	1.22
1-phenylbutan-2-ol	99.5:0.5	4.07	4.27	1.05	0.65
4-phenylbutan-2-ol	99.5:0.5	4.5	-	1.0	-
1-naphthylethanol	85:15	3.93	4.17	1.06	0.71

Table 5 HPLC results on the (+)-hexahelicene bonded phase

3,5-dinitrobenzoyl derivative	Mobile phase Hexane/EtOH	k'_1	k'_2	α	R_s
1-phenylethanol	99.75:0.25	3.8	4.2	1.08	0.73
1-phenylpropan-1-ol	99.75:0.25	3.06	3.53	1.15	0.88
1-phenylbutan-1-ol	99.75:0.25	2.73	3.13	1.15	0.97

Table 6: HPLC results on the (-)-hexahelicene bonded phase.

5.8.3- Discussion of Results:

The chiral resolution and retention behaviour obtained for the 3,5-DNB derivatives of the arylalkanol chiral solutes on the (-)- and (+)-hexahelicene chiral columns can be correlated with the structural differences of this series of structurally related chiral compounds. This correlation should improve the understanding of which structural factors in these chiral solutes affect the enantiodiscrimination power of these hexahelicene phases. The effect of the following structural differences of the chiral solutes under study are discussed with respect to the chiral resolution observed on the (-)- and (+)-hexahelicene chiral phases.

1. Effect of the length of the alkyl chain at the chiral centre on chiral resolution

The results in Tables 7 and 8 demonstrate that increasing the length of the alkyl chain at the chiral centre improves significantly the chiral discrimination (increased R_s values). The increased enantiodiscrimination of the 3,5-DNB arylalkanol chiral solutes with increasing alkyl chain length may result from the increased steric hindrance between one enantiomer and the hexahelicene chiral phases. While hindrance due to a methyl group on one enantiomer and not the other is not large enough to obtain high chiral discrimination on this hexahelicene chiral phase, the bulky butyl group results in high chiral resolution. Almost baseline resolution was achieved for the 3,5-DNB of 1-phenylbutan-1-ol.

3,5-DNB-derivatives	x spacer	y alkyl group	α	R_s	Elution order
1-phenylethanol	0	1	1.08	0.80	3
1-phenylpropan-1-ol	0	2	1.13	1.02	2
1-phenylbutan-1-ol	0	3	1.17	1.22	1

Table 7: HPLC results on (+)-hexahelicene bonded phases

3,5-DNB-derivatives	x spacer	y alkyl group	α	R_s	Elution order
1-phenylethanol	0	1	1.08	0.73	3
1-phenylpropan-1-ol	0	2	1.15	0.88	2
1-phenylbutan-1-ol	0	3	1.15	0.93	1

Table 8: HPLC results on (-)-hexahelicene bonded phases

2- Effect on chiral resolution of the distance from the aromatic group to the chiral centre

Increasing the distance between the aromatic group and the chiral centre resulted in reduced chiral discrimination. Table 9 list two sets of examples. Both sets have the same structural difference. While the same alkyl chain at the chiral centre is maintained, the phenyl group is separated from the chiral centre by none and one and two methylene groups, respectively.

In the first example, both chiral solutes contain a methyl alkyl group at the chiral centre. For the 3,5-DNB of 1-phenylethanol, the phenyl group is adjacent to the chiral centre and a resolution of 0.80 is obtained. For the 3,5-DNB of 4-phenylbutan-2-ol, the phenyl group is separated by an ethylene group from the chiral centre and no resolution is obtained.

This trend is also present to some degree in the second example, where both solutes contain an ethyl group at the chiral centre. The chiral discrimination is significantly decreased between the 3,5-DNB of 1-phenylpropan-1-ol where the phenyl group is adjacent to the chiral centre and the 3,5-DNB of 1-phenylbutan-2-ol, where the phenyl group is separated from the chiral centre by a methylene group. In contrast to the first example, some resolution is still obtained for the poorest resolved chiral analyte. This may be the result of two additive effects. The enantiodiscrimination of the chiral analyte is enhanced by the steric hindrance of a large ethyl alkyl group and resolution is not decreased significantly by having the phenyl group only separated from the chiral centre by one methylene group.

3,5-DNB-derivatives	x spacer	y alkyl group	α	R _s	Elution order
1-phenylethanol	0	1	1.08	0.80	2
4-phenylbutan-2-ol	2	1	1.0	-	1
1-phenylpropan-1-ol	0	2	1.13	1.02	2
1-phenylbutan-2-ol	1	2	1.05	0.65	1

Table 9: HPLC results on (+)-hexahelicene bonded phases

The decreased chiral resolution observed on the 3,5-DNB of the arylalkanols with the increasing distance of the phenyl group from the chiral centre may imply that for chiral resolution to be effective, the phenyl group has to interact with the hexahelicene chiral stationary phase. This interaction may result through π - π stacking. Additional to this interaction, for chiral resolution of these solutes to take place, there must be the main interaction, a π - π charge transfer between the electron rich (π -base) hexahelicene chiral selector in the stationary phase and the electron poor (π -acid) DNB group in the chiral solute. As in the case of 3,5-DNB of 4-phenylbutan-1-ol, for those chiral solutes with the phenyl group separated by two or more methylene groups from the chiral centre, it is not possible for both the DNB group and the phenyl group to interact simultaneously with the hexahelicene chiral selector and therefore no resolution is obtained.

Looking at the three dimensional structure of the 3,5-DNB of these arylalkanols, the two aromatic groups in these solutes can be rotated like propellers so that they can be arranged in a helix-like structure. This helical structure may allow one solute to have better interaction with the helical structure of the chiral hexahelicene selector and be retained longer while the other enantiomer will not and therefore will elute earlier.

3- Effect of the aromatic group at the chiral centre on chiral resolution

By comparing the chiral resolution of the 3,5-DNB of 1-phenylethanol with that of 1-naphthylethanol, it can be concluded that changing the aromatic group adjacent to the chiral centre from phenyl to naphthyl does not significantly affect chiral resolution (Table 10). Although in the previous section, it was implied that for chiral resolution to take place, there was a need to have some simultaneous interactions of both the DNB group and the aromatic group at the chiral centre, this interaction appears not to be significantly affected by the size and aromatic character of this aromatic group.

3,5-DNB-derivatives	x spacer	y alkyl group	α	Rs	Elution order
1-phenylethanol	0	1	1.08	0.80	1
1-naphthylethanol	0	1	1.06	0.71	2

Table 10: HPLC results on (+)-hexahelicene bonded phases

5.8.4- Retention Mechanism of the Hexahelicene Chiral Stationary Phases

Using a predominantly non polar mobile phase composition (hexane modified with EtOH or IPA), the increase in aliphatic character of the analyte due to the increase in alkyl chain length (see Table 5, 6, 7 and 8) or due to the increase in the length of alkyl spacer between the aromatic group and the chiral centre (see Table 5 and 9) has lead to a consistent and significant reduction in retention times. This retention behaviour is consistent with a normal phase mechanism where the interactions take place between the polar groups in the analyte and the polar groups in the stationary phases. This must mean that despite the hexahelicene chiral stationary phase having a significant hydrophobic character, the predominant interactions are polar. In the case of these hexahelicene phases, the retention must be due to a contribution of the unreacted silanol and aminopropyl groups and the amido (dipole, H-bonding) and aryl (π , charge transfer) groups. The retention data shown in Table 5 and 10 points to the operation of a π -electron based mechanism. The change from phenyl to naphthyl group adjacent to the chiral centre in chiral solutes increased the retention time of the solutes significantly. This data can be explained by a π - π stacking retention mechanism between the aromatic group in the chiral analyte and the hexahelicene chiral stationary phase.

5.8.5- Comparison of the Resolution of the Chiral Test Solutes on (+) -and (-)-Hexahelicene Phases

In order to do a comparative study of the chiral resolving power of the columns prepared with the (+)- and (-)-hexahelicene, three solutes which had been separated well on the (+)-hexahelicene bonded phase were tested. The three solutes were: 1-phenylethanol, 1-phenylpropan-1-ol and 1-phenylbutan-1-ol. Whereas, with the (+)-hexahelicene phase, three mobile phases with increase polarity had to be used to elute the chiral analytes with increasing lipophilic character, the three analytes could be eluted on the (-)-hexahelicene column using the same mobile phase. This was due to the significantly lower coverage of hexahelicene on the (-) phase. The analytes eluted much faster and with reduced resolution on the (-) column compared to the (+)-hexahelicene bonded phase. Fig. 64 shows the resolution of the 1-phenylbutan-1-ol derivative on the (+)- and (-)-hexahelicene column using the conditions described in Table 5 and 6.

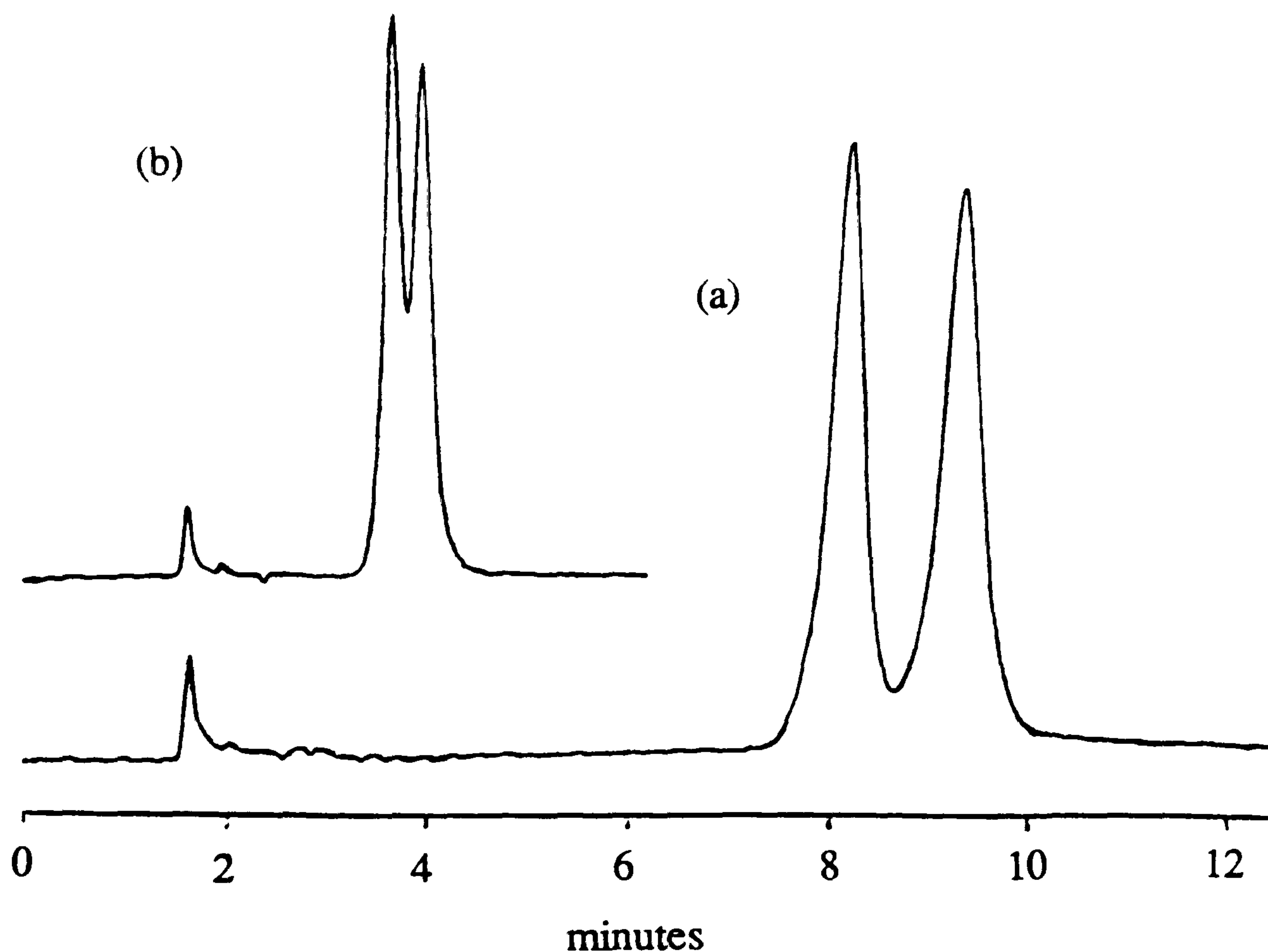


Fig. 64 Resolution of the 3,5-DNB of 1-phenylbutan-1-ol on (a) (+) and (b) (-)-hexahelicene bonded phases using ethanol/n-hexane (1.5:98.5 v/v) eluting at 0.4ml/min.

The lower coverage of the (-) versus the (+)-hexahelicene bonded phase (20 and 41% w/w, respectively) results in reduced resolution of the (-) phase.

5.8.6- Order of Elution

The elution order was tested using the 3,5-dinitrobenzoyl derivatives of optically pure (S) and (R) 1-phenylethanol and 1-phenylbutan-1-ol. The order of elution was (S) before (R) on the (+)-hexahelicene bonded phase and the opposite on the (-)-hexahelicene bonded phase. Fig. 65.

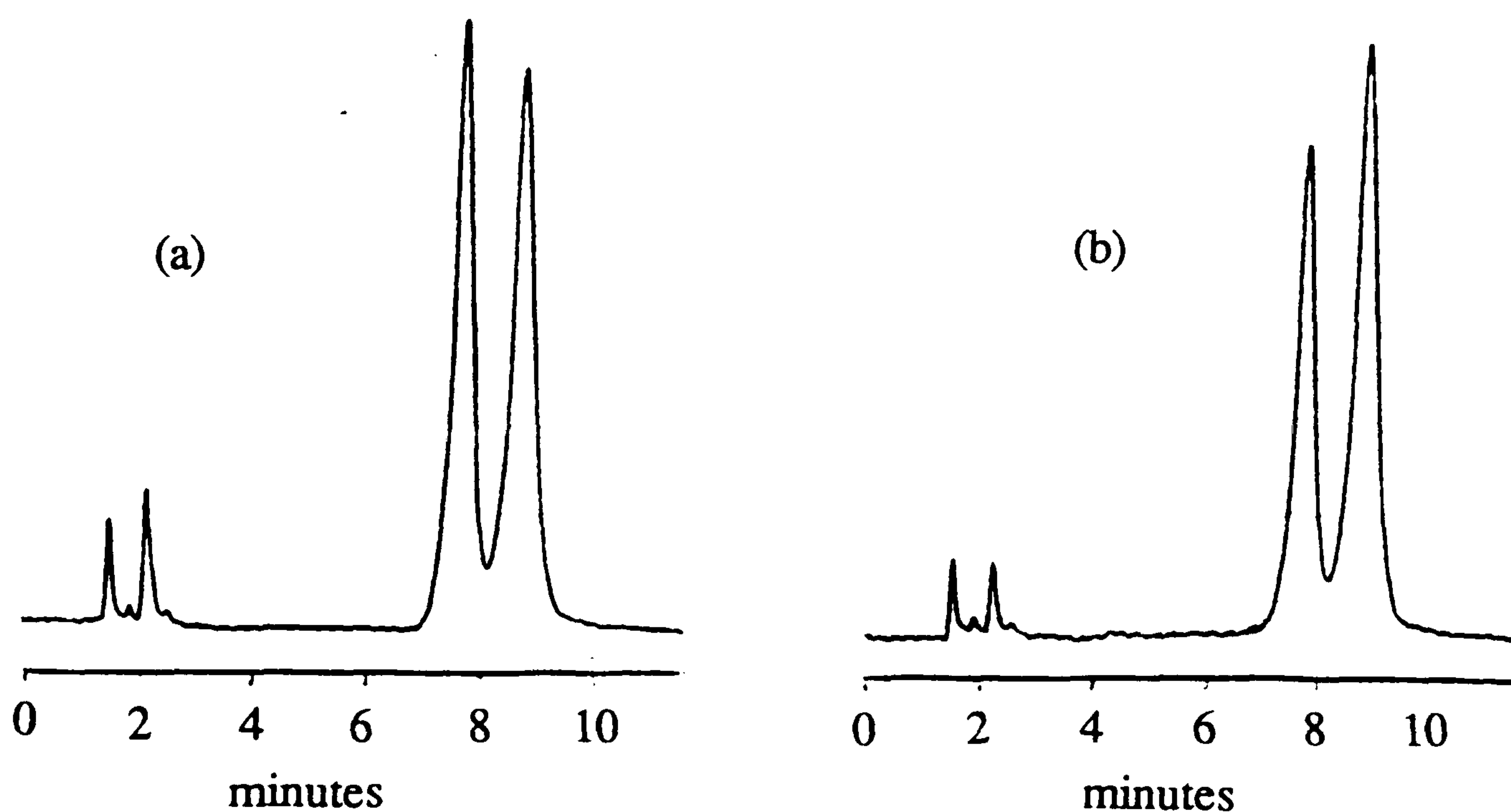


Fig. 65 Resolution of the 3,5-dinitrobenzoyl derivative of 1-phenylbutan-1-ol (a) as a racemate and (b) racemate spiked with (R) enantiomer using ethanol/n-hexane (1.5:98.5 v/v)

5.8.7- Discussion

Hexahelicene chiral bonded phases have been demonstrated to be effective in the chiral resolution of nitroaryl analytes. Chiral resolution of these nitroaryl analytes has been found to increase with an increase in the length of the alkyl chain at the chiral centre. This effect is believed to be the result of steric hindrance between one analyte enantiomer and the chiral hexahelicene phase. It does not take place with the other

analyte enantiomer. The chiral resolution of these nitroaryl solutes appears to rely heavily on the π - π charge transfer between the electron rich hexahelicene chiral selector of the stationary phase and the electron deficient nitroaryl moiety of the analyte. Additionally, for chiral resolution to take place, the degree of proximity of another aromatic group to the chiral centre appears to be important. Best resolution is obtained with nitroaryl analytes containing an additional aromatic group adjacent to the chiral centre. Resolution was completely lost for analytes with the aromatic group separated from the chiral centre by two methylene groups. This may imply that for chiral resolution to take place, the phenyl group must be able to interact with the hexahelicene, probably by π - π stacking. From the chromatographic data it appears that change in the size of the aromatic group adjacent to the chiral centre does not significantly affect chiral resolution. The (-)- and (+)-hexahelicene phases have been demonstrated to switch the order of elution of chiral analytes which is consistent with expectations.

The achiral retention mechanism of these nitroaryl solutes on the hexahelicene phases decreases significantly with increased aliphatic character of the analytes. The hexahelicene phases are used with a predominantly non polar mobile phase composition (hexane modified with EtOH or IPA). This could imply that the retention mechanism may act predominantly through a normal phase mechanism. Despite the high lipophilic content of this phase due to the polyaromatic hexahelicene chiral selector, the overall retention mechanism of solutes appears to result from the polar interactions between groups present in the analytes and various groups present in the stationary phase. This explanation is also consistent with the decreased retention of chiral on the (-)-hexahelicene phase with only 20% surface coverage versus the higher surface coverage of 40% of the (+)-hexahelicene phase. Reduced coverage exposes more unreacted polar aminopropyl silica and silanol groups, increasing the overall polarity of the phase. An increased size of aromatic group of the analyte mainly in proximity to the chiral centre increases retention time. This may be the result of increased π - π stacking interaction with the hexahelicene moiety. It could be therefore

concluded that the achiral retention mechanism of the hexahelicenes chiral phases is predominantly by normal phase mechanism with a π - π stacking contribution.

Chapter Six

EXPERIMENTAL

All chemicals, including solvents, were either used as purchased (Aldrich), or purified according to literature methods [180]

Melting points (°C) were recorded on a Gallenkamp apparatus and are uncorrected.

Infra-red spectroscopy was performed on samples contained in NaCl windows, either as nujol mulls (solids) or as thin-films or CHCl₃ solutions (liquids), in a Perkin-Elmer 983 or 580-B Infra-red Spectrometer. Frequencies are quoted in wavenumbers (cm⁻¹).

UV spectra were recorded on a Philips PU 8720 UV/Vis scanning spectrophotometer and are quoted as λ_{\max} / nm (ϵ / dm³ mol⁻¹ cm⁻¹).

¹H and ¹³C NMR spectroscopic studies were performed in a Bruker WH 250 or 400 MHz spectrometer on CDCl₃ or CD₃COCD₃ solutions. Chemical shifts are quoted in ppm, downfield from an internal reference (TMS), coupling constants, (J) are in Hz, and multiplicities are abbreviated as follows: s, singlet; b. s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

Thin Layer Chromatography (TLC) was routinely used to follow the course of reactions; the measurements were carried out on 0.2mm thick Merck Kieselgel GF254 plates and monitored by means of ultraviolet irradiation at 254nm.

Column chromatography was carried out using Kieselgel 60 (230-400 mesh).

Elemental analysis was obtained using a Carlo Erba model 1106 machine.

EI and FAB mass spectra were recorded on a Kratos MS 80 spectrometer.

The term *in vacuo* refers to the removal of organic solvent under reduced pressure on a Büchi rotary evaporator.

6.1- Synthesis of (+/-)-Hexahelicen-7-ylacetic acid methyl ester

6.1.1- Diethyl 2-(1'-naphthylmethylene)malonate (2) [75]

A solution of 1-naphthaldehyde (100 g, 0.64 mol) and diethyl malonate (120g, 0.75 mol) in dry benzene (100ml) was brought to a reflux in a Dean and Stark apparatus. Following slow addition of a solution of benzoic acid (7g, 0.06 mol) and piperidine (7g, 0.08 mol) in dry benzene (15ml), water (12ml, 100% theoretical) was collected in the trap. The reaction mixture was allowed to cool and was then extracted with 1:1 ether/benzene (100ml). The organic layer was separated and washed with 10% hydrochloric acid (3 x 50ml), followed by 10% aqueous sodium bicarbonate (2 x 50ml) and finally, saturated sodium chloride (2 x 50ml). It was then dried with anhydrous sodium sulphate and the solvent was removed *in vacuo*. The orange-brown oil remaining was vacuum distilled at 5 mm Hg pressure and the fraction collected between 148 and 210°C was then redistilled, also at 5 mm Hg pressure. A viscous light yellow oil was collected between 205 and 207°C (152g, 85% yield) which on standing formed a sticky solid. This was recrystallised from EtOH to give the title compound (2) as a pale yellow solid (80% yield). TLC (50:50 hexane/EtOAc), R_f : 0.6; m.p. 36-37°C, (lit.[75] b.p. 203-205°C, 2mm Hg); found, C 72.45, H 6.12, (C₁₈ H₁₈ O₄ requires C 72.47, H 6.08); IR (nujol mull, ν_{max}), 1713 (C=O, st, ester), 1633 (C=C, olefinic), 1633, 1580, 1500, and 1450 (C=C, skeletal aromatic), 1212 (C-O, st), 800 and 775 cm⁻¹ (1-naphthalene substitution, C-H bending out of plane); δ_H (400MHz, CDCl₃), 8.47, 1H (s, H^a), 8.00, 1H (d, J = 8Hz, H^{b'}), 7.85, 2H (m, H^{c'}, H^{d'}), 7.58-7.49, 3H (m, H^{e'}, H^{f'}, H^{g'}), 7.41, 1H (t, J = 7.9Hz, H^{h'}), 4.36, 2H (q, J = 7.1Hz, H^b), 4.15, 2H (q, J = 7.1Hz, H^c), 1.37, 3H (t, J = 7.1Hz, H^d), 1.05, 3H (t, J = 7.1Hz, H^e); EI-MS, m/z 298 (M⁺, 51%) and 152 (M⁺- 2COOCH₂CH₃, 100%); ¹³C NMR, (CDCl₃) 166.01, C13 (C=O, ester), 163.84, C14 (C=O, ester), 141.11, C11 (H^aC=), 133.25, C12 (=C<), 131.24, C1, 130.73, C9, 130.34, C5 or 4, 129.18, C10, 128.55, C4 or C5, 126.81, C6, 126.30, C7&2, 125.07, C3, 123.96, C8 (naphthalene), 61.65, C15 (CH₂^b), 61.35, C16

(CH₂^c), 14.10 C17 (CH₃^d), 13.65, C18 (CH₃^e). UV in EtOH 220nm (38,000); 244nm (12,000); 323nm (9,000).

6.1.2- *1-Naphthylmagnesium bromide* [75]

Dried magnesium turnings (10g, 0.41 mol, special for Grignard reactions), were placed in a dry 2-litre 3-necked round bottom flask fitted with a condenser, mechanical stirrer and dropping funnel. Dry THF was added via the dropping funnel until the magnesium turnings were just covered, then a crystal of iodine was added and the mixture allowed to stand for several minutes while the iodine etched the surface of the magnesium. A solution of 1-bromonaphthalene (79.6g, 0.385 mol) in dry THF (250ml) was placed in the dropping funnel. Part of the solution was added dropwise to the flask without stirring, after which the flask was warmed with a heat-gun to encourage the reaction to start. Once the reaction was established, the stirrer was started and the rest of the 1-bromonaphthalene solution was slowly added. When the reaction mixture cooled down, an off-white precipitate formed. The mixture was used immediately in the next reaction.

6.1.3- *Diethyl 2-(1,1'-dinaphthylmethyl)malonate (3)* [75]

A solution of diethyl 2-(1'-naphthylmethylene)malonate (100g, 0.34 mol) in dry benzene (150ml) was added dropwise to the 1-naphthylmagnesium bromide. The mixture was heated to a gentle reflux for 1h and then left to cool down with continuous stirring. Any excess Grignard reagent was decomposed by adding water (50ml) followed by 50% hydrochloric acid (100ml). An off-white precipitate formed to which a large amount of water was added. The supernatant liquid (orange) was decanted into a separating funnel, diluted with more water and the aqueous layer was neutralised by 5% sodium bicarbonate solution. The organic layer was separated and the aqueous layer was extracted again with a small amount of benzene. The combined organic extracts were dried over anhydrous magnesium sulphate and evaporated to dryness to leave an off-white solid (122.5g, 86%). This was then recrystallised from

ethanol to give (3) as a clean white solid. TLC (50:50 hexane/EtOAc), R_f : 0.65; m.p. 115-116°C, (lit.[75] 109-113°C); found, C 78.82, H 6.17, (C₂₈H₂₆O₄ requires C 78.85, H 6.14); IR (nujol mull, ν_{\max}), 1756 (C=O, st), 1726, 1697, 1598, 1447 (C=C, skeletal aromatic), 1251 (C-O, st), 804 and 788 cm⁻¹ (1-naphthalene substitution, oop); δ_H (400MHz, CDCl₃), 8.43, 2H (d, J = 8.4Hz 2H^{b'}), 7.80, 2H (d, 2H^{d'}), 7.72, 2H (d, J = 8.2Hz, 2H^{c'}) 7.54, 2H (d, J = 7.2Hz, 2H^{e'}), 7.50-7.38, 6H (m, 2H^{f'}, 2H^{g'}, 2H^{h'}), 6.55, 1H, (d, J = 11.8Hz, H^a), 4.57, 1H, (d, J = 11.8Hz, H^f), 3.80, 4H, (m, J = 7.1 & 3.6Hz, H^{b/b1}), 0.83, 6H, (t, J = 7.1Hz, H^c); EI-MS, m/z 426 (M⁺, 22%), 267 (M⁺-159, 100%); ¹³C NMR, (CDCl₃) 168.03 C13&14 (C=O), 137.55, C1&1', 133.96, C9&9', 131.46, C5&5' or C4&4', 128.76, C10&10', 127.63, C4&4' or C5&5', 126.30, C6, 125.55, C7&7' or C2&2', 125.15, C2&2' or C7&7', 124.82, C3&3', 123.51, C8&8', (2 naphthalenes), 61.39, C15&16 (CH₂^{b/b1}), 58.57, C11 or C12 (>CH^{a-}), 40.30, C12 or C11 (-CH^f<), 13.41, C17&18 (CH₃^g); UV in MeOH 219nm (65,000), 284nm (16,000).

6.1.4- 2- (1,1'-Dinaphthylmethyl)-1,3-propanediol (4) [75]

A solution of diethyl 2-(1,1'-dinaphthylmethyl)malonate (60g, 0.14 mol) in dry benzene (300ml) was added slowly and with vigorous stirring to a suspension of lithium aluminium hydride (15g, 0.4mol) in refluxing dry ether (900ml). The mixture was left stirring overnight, following which the excess of reagent was destroyed with water (200ml) and the Lewis-complex broken up by the addition of 50% sulphuric acid (200ml). The aluminium and lithium salts precipitated and the yellow organic layer was decanted and filtered through a bed of anhydrous magnesium sulphate. The ether solution was evaporated to dryness to yield a white solid which was recrystallised from ethanol. Yield of compound (4) 41g (85%). TLC (50:50 hexane/EtOAc), R_f : 0.30; m.p. 71°C, (lit.[75] 75°C); found, C 84.10, H 6.55, (C₂₄H₂₂O₂ requires C 84.18, H 6.47); IR (nujol mull, ν_{\max}), 3397(O-H, st); 1206cm⁻¹ (C-O, st); δ_H (250MHz, CDCl₃) 8.38, 2H (d, J_o = 8.3Hz, J_m = 1.8Hz, 2H^{b'}), 7.84, 2H (dd, J_o = 7.7Hz, J_m = 1.3Hz, 2H^{d'}), 7.68, 4H (m, 2H^{c'}, 2H^{e'}),

7.45, 6H (m, 2H^{f'}, 2H^{g'}, 2H^{h'}), 5.75, 1H (d, J = 11Hz, H^a), 3.80, 4H (2dd, J_g = 10.3&10.6Hz, J_v = 3.7 & 5.8Hz, H^{b/b1}), 2.91, 1H, (m, H^f), 2.2, 2H (s, OH); EI-MS m/z 342 (M⁺, 30.3%), 267 (M⁺-75, 100%). UV in MeOH 218nm (52,000), 285nm (6,000), 325nm (400).

6.1.5- 2-(1, 1'-Dinaphthylmethyl)-1,3-propanediol di-mesyl ester (5) [75]

2-(1,1'-Dinaphthylmethyl)-1,3-propanediol (3,75g, 0.01mol) was dissolved in dry pyridine (40ml) and cooled in an ice bath. Methanesulphonyl chloride (4g, 0.035mol) was added dropwise with stirring, and stirring was continued for a further 1h 15min. Pyridine hydrochloride separated from the yellow solution and was filtered out. The filtrate was poured into 1L of distilled water and stirred. The mass of crystals which formed on standing was collected by filtration, washed with copious quantities of water, and dried *in vacuo* to give a white solid. TLC (50:50 hexane/EtOAc), showed one spot (R_f : 0.44). The solid was recrystallised from CH₂Cl₂/MeOH giving colourless needles of compound (5). Yield 4.5g (82%). m.p. 145°C, (lit.[75]145-150°C); found, C 62.41, H 5.27, (C₂₆H₂₆O₆S₂ requires C 62.63, H 5.26); IR (nujol mull, ν_{max}), 1354 & 1184 cm⁻¹ (-SO₂O-, st, asymmetric and symmetric); δ_H (250MHz, CDCl₃) 8.38, 2H (d, J = 8.4Hz 2H^{b'}), 7.85, 2H (dd, J_o=8Hz, J_m = 1.5Hz, 2H^{d'}), 7.77, 2H (d, J = 8.2Hz, 2H^{c'}), 7.70-7.45, 8H (m, 2H^{e'}, 2H^{f'}, 2H^{g'}, 2H^{h'}), 5.77, 1H (d, J = 11.5Hz, H^a), 4.45, 2H (dd, J_g = 10Hz, J_v = 3.3Hz, 2H^b or 2H^{b1}), 4.15, 2H (dd, J_g = 10Hz J_v = 6.3Hz, 2H^{b1} or 2H^b), 3.38, 1H (m, H^f), 2.71, 6H (s, H^g); EI-MS, m/z 498 (M⁺, 4.79%), 267 (M⁺-231, 100%); ¹³C NMR, (CDCl₃) 136.72. C1&1', 134.04, C9&9', 131.63, C4&4' or C5&5', 129.22, C10&10', 127.98, C5&5' or C4&4', 126.73, C6&6', 125.82, C7&7', 125.48, C2&2', 125.06, C3&3', 122.93, C8&8', (2 naphthalenes), 67.51, C13&14 (2CH₂^{b/b1}), 43.54, C11 (>CH^{a-}), 37.11, C12 (-CH^f<), 13.41, C15&16 (CH₃^c); UV in MeOH 219nm (56,000), 285nm (9,000), 325nm (500).

The reaction was repeated on a larger scale. Compound (4) (64g, 0.13mol) was dissolved in pyridine (650ml) and MeSO₂Cl (65ml) was added. The procedure was used exactly as before. TLC (50:50 hexane/EtOAc) as eluent, showed 3 spots. The crude mesyl ester (compound (5)) was purified by chromatography using hexane/ethyl acetate (50:50v/v), and a column (40cm x 7cm) packed with silica gel 60 (40-63 μ m, 230-400 mesh). The product was separated into 3 fractions which were collected and evaporated to dryness. The residues were recrystallised from aqueous ethanol and analysed.

Fraction 1 (71) : 35% yield. TLC (50:50 hexane/EtOAc), R_f : 0.88; m.p. 145-147°C; found, C 76.74, H 5.52, (C₂₄H₂₀Cl₂ requires C 76.19, H 5.29); IR (nujol mull, ν_{\max}), 779cm⁻¹ (C-Cl, st); δ_{H} (250MHz, Cl₃CD) 8.42, 2H (d, J=8.4Hz, 2H^{b'}), 8.00, 2H (dd, J=7.6Hz J=1.1Hz, 2H^{d'}), 7.88, 2H (dd, J=8Hz J=1.2Hz, J=1.7Hz, 2H^{c'}), 7.77, 2H (d, J=8.1Hz, 2H^{e'}), 7.67-7.44, 6H (m, 2H^{f'}, 2H^{g'}, 2H^{h'}), 6.02, 1H (d, J = 11Hz, H^a), 3.88, 2H (dd, J_g = 11Hz, J_v = 6.5Hz, 2H^b), 3.77, 2H (dd, J_g=11Hz, J_v=3.2Hz, 2H^{b1}), 3.29, 1H (m, H^f); EI-MS, m/z 382 (M⁺⁺ 4, 2.3%), 380 (M⁺⁺ 2, 11.1%, ³⁷Cl), 378 (M⁺, 16%) ³⁵Cl), 267 (M⁺ -111, 100%); UV in MeOH 218 (95,000), 276nm (21,000).

Fraction 2 (72) : 23% yield. TLC (50:50 hexane/EtOAc), R_f : 0.72; m.p. 105-110°C; found, C 69.02, H, 5.27, (C₂₅H₂₃O₃SCl requires C 68.4, H 5.25); IR (nujol mull, ν_{\max}), 1358 & 1176cm⁻¹(SO₂O-, st, asymmetric and symmetric); δ_{H} (250MHz, CDCl₃) 8.46, 1H (d, J=8.5Hz, H^{b'}), 8.33, 1H (m, H^{b''}), 7.88-7.41, 12H (m), 5.87, 1H (d, J = 11.2Hz, H^a), 4.40, 1H (dd, J_g = 10Hz, J_v = 3.4Hz, H^b or H^{b1}), 4.25, 1H (dd, J_g = 10Hz, J_v = 6Hz, H^{b1} or H^b), 3.77, 2H (d, split J = 3.7, H^{d/d1}), 3.34, 1H (m, H^f), 2.66, 3H (s, H^g); EI-MS, m/z 440 (M⁺⁺2, ³⁷Cl, 4.1%), 438 (M⁺, ³⁵Cl, 10.5%); UV in MeOH 222nm (67,000), 285nm (18,000).

Fraction 3 (5) : 42% yield

This corresponded to the dimesyl ester (5) and gave analytical data as previously detailed.

6.1.6- 3 -(1,1'-Dinaphthylmethyl) glutaronitrile (6) [75]

A solution of the dimesylate derivative (5) (60.5g, 0.12mol) in dimethylformamide (460ml) was added to a stirred solution of potassium cyanide (45.4g, 0.7mol) and potassium iodide (1.51g, 8×10^{-3} mol) in water (300ml) which had been heated to 80-90°C and held at that temperature for 3.5h. The mixture was maintained at 90°C for a further hour and then allowed to cool to room temperature. The solution was poured into water (5L) and a pale brown solid precipitated. This was collected by filtration and dried to yield 37g (85%) of crude dinitrile which was then recrystallised from EtOH. TLC (50:50 hexane/EtOAc), R_f : 0.7; m.p. 150-154°C, (lit.^[70] 156-159°C); found, C 85.31, H 5.67, N 7.82 (C₂₆H₂₀N₂ requires C 86.66, H 5.55, N 7.77), IR (nujol mull, ν_{max}), 2244 cm⁻¹ (C-N, st); δ_H (250MHz, CDCl₃) 8.38, 2H (d, J=8.5Hz, 2H^{b'}), 7.88, 2H (dd, $J_o=8$ Hz, $J_m=1.4$ Hz, 2H^{d'}), 7.80, 2H (d, J=8Hz, 2H^{c'}), 7.62-7.45, 8H (m, 2H^{e'}, 2H^{f'}, 2H^{g'}, 2H^{h'}), 5.77, 1H (d, J = 11.3Hz, H^a), 3.27, 1H (m, H^f), 2.73, 4H (2dd, $J_g = 17$ Hz, $J_v = 6.5$ Hz & 4.5Hz, 2H^{b/b1}); EI-MS, m/z 360 (M⁺, 55.9%), 267 (M⁺-93, 100%); UV in MeOH 218, (100,000), 284nm (19,000).

6.1.7- 3 -(1,1'-Dinaphthylmethyl) glutaric acid (7) [75]

The crude nitrile (10g, 0.028mol) was refluxed for 1h with 10% potassium hydroxide in ethyleneglycol (100ml) [75]. The reaction mixture was poured into dilute hydrochloric acid with vigorous stirring. A solid precipitated and was extracted into dichloromethane (50ml). The bulked organic extracts were dried over anhydrous magnesium sulphate and evaporated to dryness to give a dark brown solid. Microanalysis showed a significant level of nitrogen which suggested that hydrolysis

was incomplete. Found C 77.33, H 6.20, N 2.61 (C₂₆H₂₂O₄ requires C 78.37, H 5.56).

The reaction was repeated using more drastic conditions. The nitrile (2g, 0.005 mol) was refluxed for 2h with 20% potassium hydroxide in diethyleneglycol (30ml). The reaction mixture was poured into dilute hydrochloric acid with vigorous stirring. A solid precipitated and was filtered off, washed several times with water, and dried under vacuum. A pale brown solid was obtained which was recrystallised from EtOH/H₂O to afford a white solid (75% yield). TLC 75:25 (CH₂Cl₂/MeOH), gave one major spot (R_f: 0.56) with no trace of starting material. M.p. 220°C (lit.^[75] 217-221°C); found, C 77.91, H 5.59, (C₂₆H₂₂O₄ requires C 78.37, H 5.56); IR (nujol mull, ν_{\max}), 1713cm⁻¹ (C=O, st, non-conjugated acid); δ_{H} (250MHz, DMSO-d₆) 10.3, 2H (s, COOH), 8.42, 2H (d, J=8.5Hz, 2H^{b'}), 7.82, 2H (dd, J =8Hz, J =1.3Hz, 2H^{d'}), 7.68, 4H (2d, J = 8Hz, 2H^{c'}, 2H^{e'}), 7.58-7.43, 6H (m, 2H^{f'}, 2H^{g'}, 2H^{h'}) 6.15, 1H (d, J = 12Hz, H^a), 3.45, 1H (m, H^f), 2.59, 2H (dd, J_g = 12Hz, J_v = 8Hz, 2H^b or 2H^{b1}), 2.37, 2H (dd, J_g = 12Hz, J_v = 4Hz, 2H^{b1} or 2H^b); EI-MS m/z 398 (M⁺, 3%), 380 (M⁺- H₂O, 11%), 267 (M⁺- 131, 100%). UV in MeOH 219 (98,000), 285nm (15,000).

6.1.8- [1,2,3,4-tetrahydro -4- (1'-naphthyl)-1-oxo-3-phenanthrenyl]-acetic acid (8) [154]

The diacid (7) (1g, 0.0025mol) and thionyl chloride (1ml, 0.0086mol) were heated to reflux in dry benzene (approx. 30ml) for 2h, after which the solvent was removed by evaporation. Further dry benzene was added, sufficient to dissolve the solid, and the solution was evaporated to dryness; this was repeated three times. Sufficient dry dichloromethane was added to dissolve the solid, and the solution was again evaporated to dryness. Finally, the solid was dissolved in CH₂Cl₂ (approx. 30ml) and cooled to -78°C in an acetone/Carbice bath. Via a septum seal, trifluoromethanesulphonic acid (0.25ml, 0.0028mol) was added dropwise from a syringe with stirring. When addition was complete, the mixture was allowed to warm

to room temperature and left stirring over night. The solution was poured into iced water, and the aqueous layer was separated and re-extracted once with dichloromethane. The combined organic extracts were washed successively with 5% NaHCO₃, water, and brine. The solution was then dried over sodium sulphate before evaporating to dryness to give a dark brown solid. Crude yield 81%.

TLC of the sample, using 80:20 v/v CH₂Cl₂/MeOH as eluent, gave four spots at: R_f : 0.97, 0.82, 0.73, 0.65. To test for the presence of a ketone group in the products, the TLC plate was sprayed with a 2,4-dinitrophenylhydrazine solution (prepared by dissolving the 2,4-dinitrophenylhydrazine in methanolic H₂SO₄). A red orange colour was seen in the spots at R_f : 0.65 and 0.97 .

When the reaction was scaled up, TLC of the product showed two spots only at R_f : 0.65 and 0.9 (80:20 v/v CH₂Cl₂/MeOH). These two components were separated by preparative TLC and each band collected and analysed. The more polar (R_f : 0.65) was the ketoacid (8) and the less polar (R_f : 0.9) was the ketone-methyl ester (73).

[1,2,3,4-tetrahydro-4-(1'-naphthyl)-1-oxo-3-phenanthrenyl] acetic acid (8):

55% yield. R_f : 0.65; m.p.: 265-268°C, (Lit.^[75] 263°-267°C); found, C 81.89, H 5.13, (C₂₆H₂₀O₃ requires C 82.1, H 5.26); IR (nujol mull, ν_{max}), 1730 (C=O, st, non-conjugated acid), 1675 cm⁻¹ (C=O, st, conjugated ketone); δ_H (400MHz, DMSO-d₆) 10.6, 1H (s, COOH), 8.96, 1H (d, J = 8.3Hz, H^{a'}), 8.12, 1H (d, J = 8.7Hz, H^{b'}), 8.02-7.95, 3H (m, H^{c'}, H^{d'}, H^{e'}), 7.75, 2H (m, H^{g'}, H^{h'}) , 7.60, 3H (m, H^{i'}, H^{j'}, H^{k'}), 7.34, 1H (m H^{l'}), 7.18 1H (t, J = 7.5Hz, H^{m'}), 6.54, 1H (d, J = 7.1Hz, H^{n'}), 5.97, 1H (s, H^a), 2.97, 1H (m, H^f), 2.65, 1H (dd, J_g = 17Hz, J_v = 4.4, H^b), 2.58, 1H (dd, J_g = 15.5Hz, J_v = 8.5Hz, H^c), 2.44, 1H (d, J_g = 16.5Hz, H^{b1}), 2.34, 1H (dd, J_g = 15.5Hz, J_v = 6.8Hz, H^{c1}); EI-MS, m/z 380 (M⁺, 3.1%), 320 (M⁺-60, 27.9%), 267 (M⁺-113, 100%); UV in MeOH 224nm (84,000), 253nm (49,000), 348nm (2,000).

[1,2,3,4-tetrahydro-4-(1'-naphthyl)-1-oxo-3-phenanthrenyl] acetic acid methyl ester (73) :

38% yield. R_f : 0.9; IR (nujol mull, ν_{\max}), 1733 (C=O, st, ester), 1680 cm^{-1} (C=O, st, conjugated ketone); δ_{H} (250MHz, CDCl_3), 8.78, 1H (d, $J = 8.5\text{Hz}$, $\text{H}^{\text{a}'}$), 8.30, 1H (d, $J = 8.7\text{Hz}$, $\text{H}^{\text{b}'}$), 8.01-7.50, 8H (m, $\text{H}^{\text{c}'}$, $\text{H}^{\text{d}'}$, $\text{H}^{\text{e}'}$, $\text{H}^{\text{g}'}$, $\text{H}^{\text{h}'}$, $\text{H}^{\text{i}'}$, $\text{H}^{\text{j}'}$, $\text{H}^{\text{k}'}$), 7.31, 1H (2d, $J_{\text{O}} = 7\text{Hz}$, $J_{\text{m}} = 1.3\text{Hz}$, $\text{H}^{\text{l}'}$), 7.15, 1H (t, $J = 7.4\text{Hz}$, $\text{H}^{\text{m}'}$), 6.68, 1H (d, $J = 7\text{Hz}$, $\text{H}^{\text{n}'}$), 5.92 1H (s, H^{a}), 3.73, 3H (s, H^{g}), 3.26, 1H (m, H^{f}), 2.95, 1H (dd, $J_{\text{g}} = 17.3\text{Hz}$, $J_{\text{v}} = 4.9\text{Hz}$, H^{b}), 2.76, 1H (dd, $J_{\text{g}} = 16.3\text{Hz}$, $J_{\text{v}} = 6.4\text{Hz}$, H^{c}), 2.6, 1H (dd, $J_{\text{g}} = 16.3\text{Hz}$, $J_{\text{v}} = 8.4\text{Hz}$, $\text{H}^{\text{c}1}$), 2.50, 1H (dd, $J_{\text{g}} = 17.3\text{Hz}$, $J_{\text{v}} = 1.6\text{Hz}$, $\text{H}^{\text{b}1}$); EI-MS, m/z 394 (M^+ , 41.2%), 320 ($\text{M}^+ - 74$, 100%), 265 ($\text{M}^+ - 129$, 49.1%). UV in MeOH 227nm (98,000), 284nm (18,000).

6.1.9- [1,2,3,4-Tetrahydro-4-(1'-naphthyl)-3-phenanthrenyl] acetic acid (9) [159]

The crude ketoacid (8) (0.74g, 0.002mol), hydrazine hydrate (2ml), potassium hydroxide (0.4g) and diethyleneglycol (bp: 245°C) (10ml), were heated in a 50ml two-necked round-bottomed flask fitted with a condenser, a Dean and Stark trap and a nitrogen bubbler (on the condenser) to indicate nitrogen evolution. The temperature gradually increased to 200°C and the water distilled off. The solution was then heated at constant reflux for four hours. Following this, the reaction mixture was cooled and poured into water and the mixture extracted with ether. The alkaline aqueous layer was acidified with hydrochloric acid and extracted further with ether several times. The ether layers were bulked, dried over sodium sulphate and evaporated to dryness to yield 0.46g (64.5%) of a white solid. TLC 80:20 hexane/EtOAc showed 2 spots, one on the baseline and the other with R_f : 0.70.

The crude product (0.4g) was purified by column chromatography using 80:20 hexane/EtOAc as eluent. The less polar (9) was collected, analysed and recrystallised from EtOH/ H_2O (67%). TLC R_f :0.70; m.p. 242-243°C

(lit.^[75] 237-238°C); found C 84.66, H 6.02, (C₂₆H₂₂O₂ requires C 85.24, H 6.01); IR (nujol mull, ν_{\max}), 1701cm⁻¹ (C=O, st, acid); δ_{H} (250MHz, CDCl₃) 8.70 1H (d, J = 8.4Hz, H^{a'}), 7.96, 1H (dd, J_o = 8.1Hz, J_m = 1Hz, H^{b'}), 7.84-7.63, 4H (m, H^{c'}, H^{d'}, H^{e'}, H^{g'}), 7.59-7.52, 2H (m, H^{h'}, H^{i'}), 7.41-7.32, 2H (m, H^{j'}, H^{k'}), 7.22-7.14, 2H (m, H^{l'}, H^{m'}), 6.745-6.71, 1H (dd, J_o = 7.2Hz, J_m = 0.8Hz, H^{n'}), 5.52, 1H (s, H^a), 3.18-3.11, 2H (m, H^f, H^b), 2.90, 1H (m, H^{b1}), 2.70, 2H (m, H^{d/d1}), 2.05, 1H (m, H^c or H^{c1}), 1.75, 1H (m, H^{c1} or H^c); EI-MS, m/z 366 (M⁺, 81.4%), 306 (M⁺- 60, 100%); UV in MeOH 227nm (91,000), 284nm (16,000).

The baseline fraction was discarded.

6.1.10- 7, 8, 8a, 9, 10, 16c-Hexahydro-7-oxohexahelicene (10)

The acid (9) (29.0g, 0.08 moles) and phosphorus pentachloride (17.3g, 0.083 moles) were heated to reflux in dry benzene (580ml) for 1h. The benzene was removed by evaporation and dry o-dichlorobenzene (465ml) was added and the mixture cooled to 0°C in an ice bath. Stannic chloride (18.5ml, 41.3g, 0.16 moles) was added and the mixture was left to stir for a few minutes and then heated at 60°C for approximately 50 min. The reaction was quenched by addition of 50cm³ of 1M HCl. The solution was then extracted with 1:1 ether/benzene and the organic layer was separated and washed with 1M NaOH (three times), with H₂O (twice) and the solvent was removed *in vacuo*. The crude product showed two spots in TLC (75:25 hexane/EtOAc), R_f: 0.5 and 0.34. Column chromatography was used to separate the two components, by using 75:25 hexane/EtOAc as mobile phase. The second fraction (R_f:0.34) proved to be the required ketone compound (10). This was recrystallised from EtOH/H₂O to give yellow flakes (80%). m.p. 224°C (lit.^[75]; 219-221°C); found C 89.08, H 5.61 (C₂₆H₂₀O requires C 89.65, H 5.74); IR (nujol mull, ν_{\max}) 1673cm⁻¹ (C=O, st, conjugated ketone); δ_{H} (400MHz, CDCl₃), 7.92, 1H (d, J = 8.4Hz, H^{a'}), 7.70-7.65, 3H (m, H^{b'}, H^{c'}, H^{d'}), 7.58, 1H (d, J = 8.2Hz, H^{e'}), 7.29, 1H, (d, J = 8.4Hz, H^{g'}), 7.16-7.05, 3H (m, H^{h'}, H^{i'}, H^{j'}), 6.87-6.83, 1H (m, H^{k'}), 6.64, 1H (d, J = 8.9Hz, H^{l'}), 6.52-6.48, 1H (m, H^{m'}), 4.88, 1H (d, J = 9.4Hz, H^a), 3.10-2.83, 3H

(m, H^f, H^{b/b1}), 2.68-2.59, 2H (m, H^{d/d1}), 2.03, 1H (dd, J_g=12.4Hz, J_v=3.5Hz, H^c), 1.52, 1H (dd, J_g=12.4Hz, J_v=3.2Hz, H^{c1}); FAB-MS, m/z 348 (M⁺, 3.1%), 307 (M⁺-41, 18%). UV in MeOH) 229nm (60,000), 258nm (28,000), 356nm (2,000).

6.1.11- 3,4,4a,5,6,12c-Hexahydro-3-hydroxy-phenanthro[3,4-c]phenanthren-3-ylacetic acid methyl ester (76) [166]

A dry 250ml flask equipped with septum inlet and magnetic stirrer was flushed with nitrogen. A solution of lithium bis-(trimethylsilyl)amide in tetrahydrofuran (Aldrich) (77ml of a 1M solution, 0.41 moles) was injected and the flask immersed in a Cardice/acetone bath. Dry methyl acetate (6ml) was added dropwise to the silylamide over a period of two minutes and the solution was stirred for an additional 15 minutes to allow complete the formation of the lithiomethyl acetate. The ketone (10) (13.2g, 0.038 moles) was dissolved in dry THF (100ml) and added to the mixture. After two hours stirring at -78°C, 8ml of 20% HCl was injected to hydrolyse the lithium salts. The reaction mixture was diluted with water and extracted several times with pentane. The bulked pentane layers were then washed with water, dried over Na₂SO₄ and the solvent was evaporated to give a light brown solid. This showed two spots in TLC (75:25, hexane/EtOAc), one corresponding to the starting material (R_f: 0.34) and the other to the β-hydroxy ester (R_f:0.2). These were separated by column chromatography using silica gel 60 (40-63mm, 230-400 mesh eluting at 75:25 hexane/EtOAc as mobile phase. 35% of the starting material was recovered.

Compound (76) 55% yield. m.p. 144-148°C; found C 82.3, H 6.00, (C₂₉H₂₆O₃ requires C 82.46, H 6.16); IR (nujol mull, ν_{max}), 1732 (C=O, st, non-conjugated ester), 1713 (C=O, st, conjugated ester), 1376 cm⁻¹ (C-O, st); δ_H (250MHz, CDCl₃) 7.97-6.55, 12H (m, aromatics), 4.68, 1H (s, OH), 4.59, 1H (d, J = 8.8Hz, H^a), 3.78, 3H (s, CH₃^g), 3.20, 2H (dd, J = 15.6Hz, H^{h/h1}), 2.85, 2H (2t, J = 3.2Hz, H^{b/b1}), 2.57-2.25, 3H (H^f, H^{d/d1}), 2.14, 1H (dd, H^c, J_g = 6.2Hz, J_v = 3Hz), 1.50,

^1H (2dd, $J_g = 12\text{Hz}$, $J_v = 3.6\text{Hz}$, H^{c1}); MS-FAB, m/z 422 (M^+ , 6.4%), 405 ($\text{M}^+ - 17$, 30.5%), 349 ($\text{M}^+ - 73$, 3.9%).

6.1.12- 4a,5,6,12c-Tetrahydrophenanthro[3,4-c]phenanthren-3-ylacetic acid methyl ester (77) [167]

Anhydrous $\text{FeCl}_3\text{-SiO}_2$ reagent was prepared as follows [167]:

In a 250ml flask, chromatographic grade silica gel (50g) (70-230 mesh) and ground anhydrous ferric chloride (4g) (8% of the weight of SiO_2) were vigorously stirred, without solvent, at room temperature for 24 hours in order to achieve homogeneous adsorption. A pale yellowish-green powder was obtained and used directly for the dehydration reaction.

The hydroxyester (76) (3.5g, 8×10^{-3} moles) was weighed into a 250ml round-bottomed flask and dissolved in a small amount of CH_2Cl_2 . $\text{FeCl}_3/\text{SiO}_2$ (10.5g) was added and the CH_2Cl_2 was removed from the slurry on a rotatory evaporator. The sample was placed in a desiccator over P_2O_5 for two days. It was then suspended in CH_2Cl_2 and filtered through a bed of silica. The solution was dried over MgSO_4 and evaporated to dryness. The ^1H NMR showed a mixture of isomers.

IR (nujol mull, ν_{max}), 1738 (C=O, st, non-conjugated ester), 1713 (C=O, st, conjugated ester), 1615 (C=C, st, olefinic), 1251cm^{-1} (C-O, st); δ_{H} (250MHz, CDCl_3), 7.98-6.59, 12H (m, aromatics), 4.48, 1H (d, $J = 10.6\text{Hz}$, H^{a}), 3.83, 1H (s, $=\text{CH}^{\text{h}}$), 3.76, 3H (s, CH_3^{g}). FAB-MS m/z 404 (M^+ , 6.7%), 343 ($\text{M}^+ - 59$, 2.0%), 329 ($\text{M}^+ - 73$, 1.7%).

6.1.13- Hexahelicen-7-ylacetic acid methyl ester (79)

Compound (77) (1.5g, 0.0037 moles) and sulfur (0.2g, 0.0037 moles) were placed together in a pear-shaped flask and mixed thoroughly. The flask was fitted with a bubbler, lowered into a silicone oil bath, heated to 230°C , and left for 5 hours. The mixture was allowed to cool to room temperature and then dissolved in CH_2Cl_2 . This

solution was filtered through a bed of silica. TLC (75:25 hexane/EtOAc) as eluent showed 2 spots (R_f : 0.47 and 0.32).

7-methyl hexahelicene (78) : 15% yield. TLC (75:25 hexane/EtOAc), R_f : 0.47; ^1H NMR (250MHz, CDCl_3) 8.05-, 7.80, 8H (m), 7.61, 2H (2d, $J = 8.3\text{Hz}$), 7.23, 2H (m), 6.77, 2H (m), 2.85, 3H (s, CH_3 g).

Hexahelicen-7-ylacetic acid methyl ester (79): 65% yield. TLC (75:25 hexane/EtOAc), R_f : 0.32; IR (nujol mull, ν_{max}), 1734cm^{-1} (C=O, st, non-conjugated ester); δ_{H} (400MHz, CDCl_3) 8.09, 1H (d, $J = 8.9\text{Hz}$), 7.97-7.90, 6H (m), 7.80, 2H (dd, $J_{\text{O}} = 7.4\text{Hz}$, $J_{\text{m}} = 1.1\text{Hz}$), 7.58-7.49, 2H (2d, $J = 8.3\text{Hz}$), 7.22-7.17, 2H (m), 6.67-6.62, 2H (m), 4.35-4.21, 2H, (2d, $J = 15.5\text{Hz}$, $\text{CH}_2^{\text{h/h'}}$), 3.74, 3H (s, CH_3 g). FAB-MS, m/z 400 (M^+ , 31.6%), 341 ($\text{M}^+ - 59$, 24.6%);

6.2- Synthesis and Resolution of (+/-)-Hexahelicen-7-yl 1'-methyl-1'-phenylmethanamide

6.2.1- Hydrolysis of the Racemic Hexahelicen-7-ylacetic acid methyl ester (80)

The racemic ester (79) (0.2g, 5×10^{-4} moles) was warmed in MeOH (40ml) in a 100ml round-bottomed flask fitted with a magnetic stirrer. Saturated methanolic NaOH (5ml) was added to the methanol solution and the mixture was stirred and heated in an oil bath at 50-60°C for 24h. The reaction was quenched by adding 1M (aq) HCl (50 ml) and the product (80) was extracted four times into CH_2Cl_2 . The organic layer was dried over anhydrous sodium sulphate and evaporated to dryness to give (80), 80% yield; found C 86.9, H 4.7, ($\text{C}_{28}\text{H}_{18}\text{O}_2$ requires C 87.04, H 4.63); IR (nujol mull, ν_{max}), 1700cm^{-1} (C=O, st, acid); δ_{H} (250MHz, CDCl_3) 7.98, 1H (d, $J = 8.9\text{Hz}$), 7.87-7.79, 6H (m), 7.69, 2H (d, $J = 7.3\text{Hz}$), 7.48-7.37, 2H (2d, $J = 8.5\text{Hz}$), 7.10, 2H (m), 6.55, 2H (m), 4.27-4.10, 2H, (2d, $J = 15.9\text{Hz}$, $\text{CH}_2^{\text{h/h'}}$); EI-MS m/z 386 (M^+ , 66.9%), 341 ($\text{M}^+ - 45$, 44.6%), 327 ($\text{M}^+ - 59$, 29.4%).

6.2.2- *Synthesis of (+/-)-Hexahelicen-7-yl 1'-methyl-1'-phenylmethanamide (81)* [173]

The acid (80) (0.1g, 2.6×10^{-4} moles) and R-(+) α -methylbenzylamine (27×10^{-3} g, 26×10^{-5} moles) were dissolved in 100ml of CH_2Cl_2 . The solution was cooled to 0°C and dicyclohexylcarbodiimide (53×10^{-3} g, 26×10^{-5} moles) was added. The mixture was stirred for 30 minutes, after which the ice bath was removed and the reaction allowed to warm to room temperature. After stirring for a further 2-4 hours, the N, N'-dicyclohexylurea which had precipitated was filtered out and washed with a small volume of CH_2Cl_2 . The CH_2Cl_2 filtrate was washed with 10% citric acid solution, 10% NaHCO_3 (aq) solution and then with brine until the washings were neutral. The CH_2Cl_2 solution was dried over anhydrous magnesium sulphate, filtered and evaporated to dryness to give a bright yellow coloured compound (yield 98%). m.p. 112°C ; IR (nujol mull, ν_{max}), 1627 cm^{-1} (C=O, st, amide).

6.2.3- *Resolution of (+/-)-Hexahelicen-7-yl 1'-methyl-1'-phenylmethanamide on 3,5-Dinitrobenzoylphenylglycine*

A sample of (+/-)-hexahelicen-7-yl 1'-methyl-1'-phenylmethanamide was resolved on an analytical 3,5-dinitrobenzoylphenylglycine column (25 x 0.45cm) bought from Capitol HPLC covalently bonded to aminopropyl silica using 60:40 v/v hexane/IPA as mobile phase, 2.0ml/min flow rate and 254nm detection, giving $\alpha = 1.40$; $R_s = 1.42$; $k'_1 = 7.43$; $k'_2 = 10.43$.

(+/-)-Hexahelicen-7-yl 1'-methyl-1'-phenylmethanamide (60×10^{-3} g, 125×10^{-3} mols) was then chromatographed on a semipreparative 3,5-dinitrobenzoylphenylglycine / APS Hypersil column (25 x 0.7cm) using the conditions above, 60:40 v/v hexane/IPA as mobile phase and increasing the flow rate to 5.0 ml/min. The sample load was 3.2mg (150 μ l). 18 and 15mg of the first and second diastereoisomer were obtained.

The first peak was analysed and corresponded to (+)-hexahelicen-7-yl 1'-methyl-1'-phenylmethanamide. Optical rotation $[\alpha]_D = +6156^\circ$. δ_H (400MHz, $CDCl_3$) 8.07-7.89, 7H (m), 7.83-7.79, 2H (dd, $J_o = 7\text{Hz}$, $J_m = 1.2\text{Hz}$), 7.56, 1H (d, $J = 8.7\text{Hz}$), 7.45, 1H (d, $J = 8.1\text{Hz}$), 7.24-7.18, 2H (m), 7.01-6.77, 5H (phenyl protons), 6.70-6.63, 2H (m), 5.63, 1H (d, -NH-, $J = 8.3\text{Hz}$), 5.18, 1H, (q, $J = 7.3\text{Hz}$, -CH-), 4.24 2H (s, $-CH_2^{h/h1}$), 1.28, 3H (d, CH_3^g); EI-MS, m/z 489 (M^+ , 76.4%), 341 ($M^+ - 148$, 100%). The second diastereoisomer was also analysed and corresponded to (-)-hexahelicen-7-yl 1'-methyl-1'-phenylmethanamide δ_H (400MHz, $CDCl_3$) 8.04-6.63, 20H (aromatics, hexahelicene and phenyl protons), 5.62, 1H (d, $J = 9.2\text{Hz}$), 5.18, 1H (q, $J = 7.6\text{Hz}$), 4.31-4.18, 2H (2d, $J = 16.6\text{Hz}$), 1.25, 3H (d, $J = 6.6\text{Hz}$).

6.2.4- Hydrolysis of (+) and (-)-Hexahelicen-7-yl 1'-methyl-1'-phenylmethanamide (80)

The (+) and (-) enantiomers of hexahelicen-7-yl 1'-methyl-1'-phenylmethanamide (15 and 10mg, respectively) were separately hydrolysed as described in section 6.2.1. The spectroscopic results for the (+) and (-)-hexahelicen-7-ylacetic acid were consistent with the results obtained previously (6.2.1).

6.3- Direct Resolution of (+/-) Hexahelicen-7-ylacetic acid methyl ester

6.3.1- Analytical Resolution of (+/-)-Hexahelicen-7-ylacetic acid methyl ester using Carbohydrate Carbamate Columns.

Analytical HPLC was carried out with a Waters 6000A pump, Rheodyne 7125 injector (20 μ l loop) and Cecil 2112 detector (8ml flowcell) at 254nm connected to a Linseis recorder. Four different analytical columns were tested:

1)- Amylose tris (3,5-dimethylphenyl carbamate) (ADMPC) [177] (20%) coated on Hypersil APS (5 μ m, 120 \AA). HPLC conditions: 98:2 v/v hexane/IPA as mobile phase, flow rate = 0.5ml/min. Results: $k'_1 = 3.36$; $k'_2 = 4.43$; $\alpha = 1.32$; $R_s = 1.88$.

2)- Cellulose tris (3,5-dimethylphenyl carbamate) (CDMPC) [177] (20%) coated on Hypersil APS (5 μ m, 120 \AA). HPLC conditions:

- a)- 95:5 v/v hexane/IPA as mobile phase, flow rate = 0.5ml/min. Results: $k'_1 = 2.96$; $k'_2 = 3.56$; $\alpha = 1.20$; $R_s = 1.3$.
- b)- 98:2 v/v hexane/IPA as mobile phase, flow rate = 1.0ml/min. Results: $k'_1 = 2.58$; $k'_2 = 4.15$; $\alpha = 1.61$; $R_s = 2.19$.
- 3)- Amylose tris (phenyl carbamate) (APC) (16.6%) coated on Hypersil APS (5 μ m, 120Å). HPLC conditions: 98:2 v/v hexane/IPA as mobile phase, flow rate = 0.5ml/min. Results: $k'_1 = 2.5$; $k'_2 = 2.81$; $\alpha = 1.13$; PR = partial resolution.
- 4)- Cellulose tris (phenyl carbamate) (CPC) (17.5%) coated on Hypersil APS (5 μ m, 120Å). HPLC conditions: 90:10 v/v hexane/IPA as mobile phase, flow rate = 0.5ml/min. Results: $k'_1 = 2.86$; $\alpha = 1$.

6.3.2.- Preparative Resolution of (+/-)-Hexahelicen-7-ylacetic acid methyl ester

Racemic hexahelicen-7-ylacetic acid methyl ester (3×10^{-3} g, 75×10^{-2} mols) was dissolved in absolute alcohol (40ml) and resolved on a preparative column (25 x 2.2cm) of 16% w/w cellulose tris (3,5-dimethylcarbamate) (CDMPC) coated on 5 μ m Hypersil APS (120Å) using the analytical conditions.

A Q1 Metripump (Metering Pumps Ltd, London), was used. The Rheodyne injector was fitted with a 5ml loop and the detector with a preparative (1mm pathlength) flowcell. The two enantiomers eluted at 25.3 and 33.6 minutes , respectively. They were collected and evaporated to dryness. Yield: 95% of the first enantiomer and 80% of the second enantiomer.

6.3.3- Hydrolysis of Hexahelicen-7-ylacetic acid methyl ester Enantiomers

The (-)-hexahelicen-7-ylacetic acid (0.1g, 0.26moles) was dissolved in a solution of methanol (0.5% w/v) with excess of sodium hydroxide in a 50ml round-bottomed flask fitted with a magnetic stirrer. The mixture was left to stir overnight. The reaction was quenched by adding 1M HCl (25ml) and the product was extracted four times into CH₂Cl₂. The organic layer was dried over anhydrous sodium sulphate and evaporated to dryness to give a pale yellow solid in 98% yield. The spectroscopic data were the same as those obtained for the racemic ester. (See Section 6.2.1). The same procedure was used for (+)-hexahelicen-7-ylacetic acid (0.08g, 0.2moles).

6.4- Bonding of (+)-Hexahelicen-7-ylacetic acid (-)-Hexahelicen-7-ylacetic acid to APS

A solution of (+)-hexahelicen-7-ylacetic acid (0.08g, 0.21mmoles) was prepared in far-UV grade acetonitrile (15ml) and added dropwise to a solution of 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-4-toluene sulphonate [181] (0.085g, 2×10^{-4} moles) (CDI Aldrich Chemicals) in the same solvent (10ml) followed by aminopropyl silica (0.73g). The slurry was left stirring overnight at room temperature. The solid was filtered off, washed several times with acetonitrile/dichloromethane 1:1, then dried *in vacuo*.

Microanalysis: aminopropyl silica: found, C 1.90, H 0.48, N 0.58, required C 1.8, H 0.8, N 0.6; (+)-Hexahelicen-7-ylacetic acid : found, C 8.72, H 0.97, N 0.76 consequently 41% of amino groups reacted with the acid.

N-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (0.063g, 0.25×10^{-3} moles) was dissolved in HPLC grade THF (10ml) and added dropwise with stirring to a solution of (-)-hexahelicen-7-ylacetic acid (0.090g, 0.23×10^{-3} moles) in the same solvent (10ml). APS silica (0.83g) was then added and left stirring overnight at 30°C. The solid was filtered off, washed sequentially with THF (100ml), EtOH (50ml), CH₂Cl₂ (50ml), ether (25ml) and dried *in vacuo*.

Microanalysis: aminopropyl silica: found, C 4.03, H 0.55, N 0.44, required C 1.8, H 0.8, N 0.6; (-)-Hexahelicen-7-ylacetic acid : consequently, about 12% of the amino groups reacted with the acid.

6.5- Packing of (+) and (-)-Hexahelicen-7-ylacetic acid Bonded Stationary Phases

The two columns were packed in stainless steel column (15 x 0.2cm) using high pressure slurry packing method [182].

The chiral stationary phases, (+)-hexahelicen-7-ylacetic and (-)-hexahelicen-7-ylacetic acid bonded to APS (0.75g) were slurried in hexane/IPA 50:50 v/v and sonicated to achieve total dispersion. The top end of the column to be packed was attached to a slurry reservoir using a coupling connector tube. A nitrogen gas driven constant-pressure Haskel 780-3 HPLC packing pump, which could operate up to 10000 psi, was used. All the liquid lines were primed with the packing solvent (hexane/IPA, 80:20 v/v) prior to packing. The pressure was held at the packing pressure (8000 psi) by closing a liquid shut off valve ready to start packing. The slurry was poured into the top of the reservoir (connected to the column) until full. A top column end fitting was then fitted to the top of the reservoir and the reservoir was attached to the liquid line of the packer. The liquid valve on the pump was opened to start the packing. After 10-15min packing, the pressure was slowly reduced and the column was carefully detached from the reservoir, so as not to disturb the packing material at the top of the column. A column end fitting was attached to the column. This column was allowed to stabilise overnight before use.

6.6- Synthesis of the Chiral Analytes for Testing

A solution of each alcohol (1m eq) in pyridine (10ml) was added to a solution of 3,5-dinitrobenzoyl chloride (1.5m eq) in dry benzene (10ml). The mixture was refluxed for half an hour and allowed to cool. Ether was added and the ethereal layer washed with 2M hydrochloric acid to remove the pyridine, then with 2M sodium

hydroxide to remove the 3,5-dinitrobenzoic acid and finally with water. The ethereal-benzene solution was dried over sodium sulphate and evaporated to dryness. Pale yellow solids were obtained (95% yield).

Chapter Seven

REFERENCES

- [1] Hubbard, J. W., Ganes, D., Lim, H. K., Midha, K. K., *Clin. Biochem.*, (1986), **19**, 107.
- [2] Köhler, F., Meise, W., Okenfels, H., *Experimentia*, (1971), **27**, 1149.
- [3] a) Chiral Separations by HPLC: "*Applications to pharmaceutical compounds*," Krstulovic, A.M., (Ed.), Ellis Horwood Ltd, Chichester, (1989) pp 1-548.
b) *Chiral Liquid Chromatography*, Lough, W. J., (Ed.) Blackie, Glasgow, (1989), pp 1-276.
- [4] Danvankov, V. A., Kurganov, A. A., Rogozhin, S.V., *Usp. Khim.*, (1974), **43**, 764.
- [5] Pirkle, W. H., House, D.W. *J. Org. Chem.*, (1979), **44**, 1957.
- [6] Helfferich, F. G., *Nature*, (1961), **189**, 1001.
- [7] Davankov, K. A., Zolotarev, Y. A., *J. Chromatogr.*, (1979), **155**, 1226.
- [8] Davankov, K. A., Navratil, J. D., Walton, H. F., "*Ligand exchange chromatography*", CRC Press, (1988).
- [9] Hare, P. E., Gil-Av, E., *Science*, (1979), **204**, 1226.
- [10] Pirkle, W. H., House, D.W., Finn, J. M., *J. Chromatogr.*, (1980), **192**, 143.
- [11] Pirkle, W. H., Hyun, M. H., *J. Chromatogr.*, (1985), **322**, 287.
- [12] Oi, N., Kitahara, T., Doi, T., Yamamoto, S. *Bunseki Kagaku* (Jap. Anal.), (1983), **32**, 345.
- [13] Pirkle, W. H., Pochapsky, T. C., Mahler, G. S., Corey, D. E., Rene, D. S., Alessi J., *Org. Chem.*, (1986), **51**, 4991.
- [14] Lowe, C. R., Dean, P. G. D., "*Affinity Chromatography*", Wiley, London, (1974).
- [15] Allenmark, S., Bomgren, B., Boren, H., *J. Chromatogr.*, (1983), **264**, 63.
- [16] Stewart, K. K., Doherty, R. F., *Proc. Nat. Acad. Sci.*, (1973), **70**, 2850.
- [17] Mc Menamy, R. H., Oncley, J. L., *J. Biol. Chem.*, (1958), **233**, 1436.

- [18] Allenmark, S., Bomgren, B., Boren, H., *J. Chromatogr.*, (1983), **365**, 63.
- [19] Allenmark, S., *J. Liq. Chromatogr.*, (1986), **9**, 425.
- [20] Allenmark, S., Bromgren, B., *J. Chromatogr.*, (1982), **252**, 297.
- [21] Steinhardt, J., Reynolds, J. A., "*Multiple Equilibria in Proteins*", Academia Press, New York, 1969.
- [22] Miwa, T., Miyakawa, T., Kayano, M., Miyake, Y., *J. Chromatogr.*, (1987), **408**, 316.
- [23] Crowley, S. C., Walters, R. R., *J. Chromatogr.*, (1983), **226**, 157.
- [24] Muller, W. E. in "*Drug Stereochemistry*" Wainer, I. W., Drayer, D. E., eds. Marcel Dekker, (1988), pp 227-244.
- [25] Wainer, I. W., Stiffin, R. M., *J. Chromatogr.*, (1988), **424**, 15.
- [26] Lagercrantz, C., Larsson, T., Denfors, I., *Comp. Biochem. Physiol. [C]*, (1981), **690**, 375.
- [27] Hermansson, J., *J. Chromatogr.*, (1983), **269**, 71.
- [28] Arai, K., Ohara, Y., Takakuwa, Y., Izumi, T., US Pat.4417070, (1983) (to Nissan Chem. Ind. Ltd).
- [29] Schill, G., Wainer, I. W., Barkan, S.A. *J. Chromatogr.*, (1986), **365**, 73.
- [30] Lindgren, G., (1985), LKB Produkter AB, Bromma, Sweden.
- [31] Hermansson, J., *J. Chromatogr.*, (1984), **298**, 67.
- [32] Miwa, T., Ichikawa, M., Hattori, T., Miyakawa, T., Kayano, M., Miyake, Y., *Chem. Pharm. Bull.*, (Tokyo), (1987), **35**, 682.
- [33] Allenmark, S., Bomgren, B., *J. Chromatogr.*, (1984), **316**, 617.
- [34] Sebille, B., Thuand, N., *J. Liq. Chromatogr.*, (1980), **3**, 299.
- [35] Pettersson, C., Arvidsson, T., Karlsson, A. L., Marle, I., *J. Pharm. Biomed. Anal.*, (1986), **4**, 221.
- [36] Hermansson, J., *J. Chromatogr.*, (1984), **316**, 537.
- [37] a) Bender, M. L., Komiyama, M., "*Cyclodextrin*," Spinger-Verlag, Berlin, (1978).

- b) Ward, T. J., Armstrong, D. W., "Cyclodextrin-bonded phases in chromatographic chiral separation", Crane, L. J., Zief, M. (Eds.), Marcel Dekker, New York, (1987).
- [38] Hinze, W. L., "Separation and Purification Methods," Van Oss, C.J. (Ed.), Vol.10. Marcel Dekker, New York, (1981), p 159.
- [39] Street, Jr. K. W., *J. Chromatogr.*, (1987), **10**, 655.
- [40] Chang, C. A., Wu, Q., Eastman, M. P., *J. Chromatogr.*, (1986), **371**, 269.
- [41] Ward, T. J., Armstrong, D. W., *J. Liq. Chromatogr.*, (1986), **9**, 407.
- [42] Seeman, J. I., Secor, H. V., Armstrong, D. W., Timmons, K., Ward, T., *Anal. Chem.*, (1990), **62**, 332.
- [43] Han, S. M., Han, Y. I., Armstrong, D.W., *J., Chromatogr.*, (1988), **441**, 376.
- [44] Chang, C. A., Wu, Q., *Anal. Chim. Acta*, (1986), **189**, 293.
- [45] Armstrong, D.W., Li, W., *Chromatography*, (1987), **2**, 407.
- [46] Maguire, J. H., *J. Chromatogr.*, (1987), **387**, 453.
- [47] Chang, C. A., Wu, Q., *J. Liq. Chromatogr .*, (1987), **10**, 1359.
- [48] Armstrong, D.W., Yang, X., Han, S. M., Menges, R. A., *Anal. Chem...*, (1987), **59**, 2594.
- [49] Armstrong, D. W., Han, S. M., Han, Y. I., *Anal. Biochem.*, (1987), **167**, 261.
- [50] Armstrong, D. W., Stalcup, A. M., Hilton, M. L., Ducan, J. D., Faulkner, J.R., Chang, S. C., *Anal. Chem.*, (1990), **62**, 1600.
- [51] Beesley, T. E, *Amer Lab.*, (1985), May, 78.
- [52] Pirkle, W. H., Pochapsky, T. C., *J. Am. Chem. Soc.*, (1986), **108**, 5627.
- [53] Armstrong, D. W., Ward, T., Armstrong, R. D., Beesley, T., *Science*, (1986), **237**, 1132.
- [54] Nobuhara, Y., Hirano, S., Nakanishi, (1983), *J. Chromatogr.*, (1983), **258**, 276.
- [55] Hesse, G., Hagel, R. *Chromatographia*, (1973), **6**, 277.
- [56] Lindner, K.R. & Mannschreck, A., *J. Chromatogr.*, (1980), **193**, 308.

- [57] Mintas, M., Mannschreck, A., Scheider, M.P., *J. Chem. Soc. Chem. Commun.*, (1979), 602.
- [58] Ichida, A., Shibata, T., Okamoto, I., Yuki, Y., Namikoshi, H., Toga, Y. *Chromatographia.*, (1984), **19**, 280.
- [59] Francotte, E., Romain, M., Lohmann, D., Mueller, R., *J. Chromatogr.*, (1985), **347**, 25.
- [60] *Instruction sheet*. Daicel Chemical Industries. California 90017, USA.
- [61] a) Okamoto, Y., Sakamoto, H., Hatada, K., Irie, M., *J. Chromatogr.*, (1986), 983.
b) Okamoto, Y., Kawashima, M., Hatada, K., *J. Chromatogr.*, (1986), **363**, 173.
- [62] Okamoto, Y., Kawashima, M., Hatada, K., *J. Amer. Chem. Soc.*, (1984), **106**, 5357.
- [63] Okamoto, Y., Suzuki, K., Ohta, K., Hatada, K., Yuki, H. *J. Amer. Chem. Soc.*, (1979), **101**, 4763.
- [64] Yuki, H., Okamoto, Y., Okamoto, I., *J. Amer. Chem. Soc.*, (1980), **102**, 6356.
- [65] Okamoto, Y., Honda, S., Hatada, K., Okamoto, I., Toga, Y., Kodayashi, S., *Bull. Chem. Soc. Japan.*, (1984), **57**, 1681.
- [66] Okamoto, Y., Honda, S., Hatada, K., Yuki, H. *J. Chromatogr.*, (1985), **350**, 127.
- [67] Okamoto, Y., Mohri, H., Nakamura, M., Hatada, K., *Nippon Kagakukaishi*, (1987), 435.
- [68] Kinkel, J. N., Fraenkel, W., Blaschke, G., *Kontacte*, (1987), **1**, 3.
- [69] Pedersen, C. J., *J. Amer. Chem. Soc.*, (1967), **89**, 2495 & 7017.
- [70] Pedersen, C.J., Frensdorff, H.K., *Angew. Chem. Int. Ed.*, (1972), **11**, 16.

- [71] a) Sousa, L.R., Hoffman, D.H., Kaplan, L., Cram, D.J., *J. Amer. Chem. Soc.*, (1974), **96**, 7100.
b) Lingerfelter, D. S., Helgeson, R. C., Cram, D. J., *J. Org. Chem.*, (1981), **46**, 393.
- [72] Sousa, L.R., Sogah, G. D. Y., Hoffman, D.H., Cram, D.J., *J. Amer. Chem. Soc.*, (1978), **100**, 4569.
- [73] Shinbo, T., Yamaguchi, T., Nishimura, K., Sugiura, M., *J. Chromatogr.*, (1987), **405**, 145.
- [74] a) Martin, R. H., *Angew. Chem. Int. Ed. Eng.*, (1974), **13**, 649.
b) Prinsen, W. J. C., Laarhoven, W.H., *J. Chromatogr.*, (1987), **393**, 377.
- [75] Newman, M. S., Lednicer, D., *J. Amer. Chem. Soc.*, (1956), **78**, 4755.
- [76] Bogaert-Verhoogen, D., Martin, R. H., *Tetrahedron Lett.*, (1967), 3045.
- [77] Helwett, C. H., *J. Chem. Soc.*, (1938), 1286.
- [78] a) Laarhoven, W. H., *Recl. Trav. Chim. (Pays-Bas)*, (1983), **102**, 185.
b) Mallory, F. B., Mallory, C. W. in: *Org. Reactions*, Willey, J. & Sons, New York. (1984), Vol. **30**, pp.1.
- [79] a) Martin, R. H., Marchant, M. J., Baes, M., *Helv. Chem. Acta*, (1971), **54**, 358.
b) Siegrist, A. E. *et al.*, *Helv. Chem. Acta*, (1969), **52**, 1282.
- [80] Martin, R. H., Flammang-Barbieux, M., Cosyn, J.P., Gelbaker, M., *Tetrahedron Lett.*, (1968), 3507.
- [81] Wisnoski-Knittel, T., Fischer, E., *Mol. Photochem.*, (1978), **9**, 67.
- [82] Wood, C. S., Mallory, F.B., *J. Org. Chem.*, (1964), **29**, 3373.
- [83] Laarhoven, W. H., Veldhuis, R. G. M., *Tetrahedron*, (1972), **28**, 1811.
- [84] Vanest, J. M., Gorsane, M., Libert, V., Pecher, J., Martin, R. H., *Chimia*, (1975), **29**, 343.
- [85] Jutz, C., Loebering, H. G., *Angew. Chem.*, (1975), **87**, 418.
- [86] Le Bel, L. A., *Bull. Soc. Chim. Fr.*, (1874), **22**, 347.

- [87] Kagan, H., Moradpour, A., Nicoud, J. F., Balavoine, G., Tsoucaris, G., *J. Amer. Chem. Soc.*, (1971), **93**, 2353.
- [88] Bernstein, W. J., Calvin, M., Buchardt, O., *J. Amer. Chem. Soc.*, (1972), **94**, 494.
- [89] Laarhoven, W. H., Cuppen, Th. J. H. M., *J. Chem. Soc. Chem. Comm.*, (1977), 47.
- [90] Cahn, R. S., Ingold, C. K., Prelog, V., *Angew. Chem., Int. Edn.*, (1966), **5**, 385.
- [91] De Jong, M. H., Laarhoven, W. H., *Rec. Trav. Chim.*, (1973), **92**, 673.
- [92] Laarhoven, W. H., Cuppen, Th. J. H. M., *J. Chem. Soc. Perkin*, (1978), **2**, 315.
- [93] Hilbert, M., Solladie, G., *J. Org. Chem.*, (1980), **45**, 5393.
- [94] Seuron, P., Solladie, G., *J. Org. Chem.*, (1980), **45**, 715.
- [95] Vanest, J., Martin, R. H., *Recl. Trav. Chim. (Pays-Bas)*, (1979), **98**, 113.
- [96] Boyd, D. R., Neill, D. C., *J. Chem. Soc. Chem. Commun.*, (1977), 51.
- [97] Martin, R. H., Schurter, J. J., *Tetrahedron*, (1972), **28**, 1749 & *Tetrahedron Lett.* (1969), 3679.
- [98] Nicoud, J. F., Moradpour, A., Balavoine, G., Kagan, H., Martin, R. H., Deblecker, M., *J. of Chromatogr.*, (1972), **66**, 43.
- [99] Mackay, I. R., Robertson, J. L., Sime, J. G., *J. Chem. Soc. Chem. Comm.*, (1969), 1470.
- [100] De Rango, C., Tsoucaris, G., Declerq, J.P., Germain, G., Putzeys, J.P., *Cryst. Struct.*, (1973), **2**, 189.
- [101] Herraiez, M. A., Sanchez, F., *Anales Real Soc. espan., Fis-Quim.*, (1965), **61**, 13, 952.
- [102] Coulson, C. A., Senet, S., *J. Chem. Soc.*, (1955), 1819.
- [103] Newman, M. S., Lutz, W. B., Lednicer, D., *J. Amer. Chem. Soc.*, (1955), **77**, 3420.

- [104] a) Klemm, L. H., Reed, D., *J. Chromatogr.*, (1960), **3**, 364.
 b) Laarhoven, W. H., Cuppen, Th. J. H. M., Nivard, R. J. F., *Tetrahedron Lett.*, (1974), **30**, 3343.
- [105] Nakazaki, M., Yamamoto, K., Maeda, M., *J. Org. Chem.*, (1981), **46**, 1985.
- [106] Nakazaki, M., Yamamoto, K., Maeda, M., *Chem. Lett.*, (1980), 1553.
- [107] Mikes, F., Boshart, G., Gil-Av, E., *J. Chem. Soc. Chem. Comm.*, (1976), 99.
- [108] Mikes, F., Boshart, G., Gil-Av, E., *J. Chromatogr.*, (1976), **122**, 205.
- [109] a) Numan, H., Helder, R., Wijnberg, H., *Recl. Trav. Chim. (Pays-Bas)*, (1976), **95**, 211.
 b) Nakagawa, H., Ogashiwa, S., Tanaka, H., Yamada, K., Kawazura, H., *Bull. Chem. Soc. Japan*, (1981), **54**, 1903.
- [110] Souter, R. W., "*Chromatographic separations of stereoisomers*," CRC Press. Inc. (1985).
- [111] a) Mikes, F., Boshart, G., *J. Chem. Soc. Chem. Comm.*, (1978), 173.
 b) Mikes, F., Boshart, G., Gil-Av, E., *J. Chromatogr.*, (1978), **149**, 455.
- [112] Rau, H., Schuster, O., *Angew. Chem.*, (1976), **88**, 90.
- [113] Martin, R. H., Eyndels, C., Defay, E., *Tetrahedron Letters*, (1972), 2731.
- [114] Matlin, S. A., Tito-Lloret, A., Lough, W. J., Bryan, D. G., Browne, T., Mehani, S. J., *High Resolution Chromatography and Chromatographic Commun.*, (1981), **4**, 81.
- [115] Kim, Y. H., Balan, A., Tishbee, A., Gil-Av, E., *J. Chem. Soc. Chem. Comm.*, (1982), 1336.
- [116] Lochmuller, C. H., Ryall, R. R., *J. Chromatogr.*, (1978), **150**, 511.
- [117] Kim, Y. H., Tishbee, A., Gil-Av, E., *J. Amer. Chem. Soc.*, (1980), **102**, 5915.
- [118] Kim, Y. H., Tishbee, A., Gil-Av, E., *Science*, (1981), 1379.
- [119] Weil-Malherbec, H., *Biochem. J.*, (1946), **4**, 351.
- [120] Sundaralingam, M., "*Structure and Conformation of Nucleic Acids and Protein-Nucleic Acid Interactions*," Sundaralingam, M. and Rao, S. T., Eds. University Park Press, Baltimore, (1975), pp 487-524.

- [121] Martin, R. H., Marchant, M. J., *Tetrahedron Lett.*, (1972), 3707.
- [122] Martin, R. H., Marchant, M. J., *Tetrahedron*, (1974), **30**, 347.
- [123] Lindner, H. J., *Tetrahedron.*, (1975), **31**, 281.
- [124] Borkent, J. H., Laarhoven, W. H., *Tetrahedron* , (1978), **34**, 2565.
- [125] a) Haigh, C. W., Mallion, R. B., *Mol. Phys.*,(1971), **22**, 945 & 955.
 b) Van der Hark, Th. E. M., Noordik, J. H., *Cryst. Struct. Comm.*, (1973), **2**, 643.
- [126] a) Martin, R. H., Defay, N., Zimmermann, D., *Tetrahedron Lett.*, (1971), 1871.
 b) Martin, R. H., Morian, J., Defay, N., *Tetrahedron .*, (1974), **30**, 179.
- [127] a) Boekelheide, V., Schmidt, W., *Chem. Phys. Lett.*, (1972), **17**, 410.
 b) Heilbronner, E., Maier, J. P., *Helv. Chim. Acta*, (1974), **57**, 151.
- [128] Obenland, S., Schmidt, W., *J. Amer. Chem. Soc.*, (1975), 6633.
- [129] Boschi, R., Clar, E., Schmidt, W., *J. Chem. Phys.*, (1974), **60**, 4406.
- [130] Brown, J. M., Field, I. P., Sidebottom, P. J., *Tetrahedron Lett.*, (1981), **22**, 486.
- [131] Birks, J. B., Birch, D. J. S., Cordemans, E., Van der Donckt, E., *Chem. Phys. Lett.*, **43**, 3.
- [132] Kim, S. S., Weissman, S. I., *J. Amer. Chem. Soc.* , (1979), **101**, 5863.
- [133] Dougherty, R. C., *J. Amer.. Chem. Soc.*, (1968), **90**, 5788.
- [134] Martin R. H., Jaspers, J., Defay, N., *Helv. Chim. Acta*, (1975), **58**, 776.
- [135] Martin R. H., Jaspers, J., Defay, N., *Tetrahedron Lett.*, (1975), 1093.
- [136] a) "*Friedel Crafts and Related Reactions*," Balaban and Menitzesen, Olah Ed. Interscience Publishers Inc. New York (1963), Vol **II**, pp 979.
 b) Borkent, J. H., Rouwette, P. H. F. M., Laarhoven, W. H., *Tetrahedron.*, (1978), **34**, 2569.
- [137] Streitwieser, A., "*Molecular Orbital Theory for Organic Chemists*", John Wiley, New York, (1961), pp 175.

- [138] Op den Brouw, P. M., Laarhoven, W. H., *Recl. trav. Chim. (Pays-Bas)*, (1978), **97**, 265.
- [139] Archer, W. J., El Din Shafiq, Y., Taylor, R., *J. Chem. Soc. Perkin 2*, (1981), 675.
- [140] Diesveld, J. W., Borkent, J. H., Laarhoven, W. H., *Recl. Trav. Chim. (Pays-Bas)*, **99**, 3921.
- [141] Matlin, S. A., Stacey, V. E., *J. Chromatography*, (1988), **450**, 157.
- [142] Hamilton, T. C., Weir, F. W., Weston, A. M., *Brit. J. Pharmacol.*, (1986), **88**, 103.
- [143] Ashwood, V. A., Buckingham, R. E., Cassidy, F., Evans, J. M., Faruk, E. A., Hamilton, T. C., Nash, D. J., Stemp, G., Wollcocks, K., *J. Med. Chem.*, (1986), 29, 2144.
- [144] Evans, J. M., Lough, W. J., Oliver, R. S., Matlin, S. A., Stacey, V. E., Manuscript in preparation.
- [145] Lough, W. J., Matlin, S. A., Stacey, V. E., "Hexahelicene Chiral Stationary Phase Part I" in preparation.
- [146] Lough, W. J., Matlin, S. A., Stacey, V. E., *UK Patent Applic. 8809821*, 26th April 1988.
- [147] a) Matlin, S. A., Tinker, J. S., Tito-Lloret, A., Lough, W. J., Chan, L., Bryan, D. G., *Pro. Anal. Div. Chem. Soc.*, (1979), **16**, 354.
b)- Matlin, S.A., Zhou, R. H., *J. High Resolution Chromatography and Chromatographic Commun.*, (1984), **7**, 629.
- [148] Han, Lapworth, *J. Chem. Soc.*, (1904), **85**, 46.
- [149] Patai, S., Israeli, Y., *J. Chem. Soc.*, (1960), 2020 & 2025.
- [150] Dyer, J. R., *Applications of Absorption Spectroscopy of Organic Compounds*, Prentice-Hall (1965).
- [151] Kohler, Reimer, *J. Amer. Chem.*, (1905), **33**, 333.

- [152] a) Royals, E. E., " *Advanced Organic Chemistry*". Prentice-Hall, (1954), p 698.
- b) Wright, G. F., " *Steric Effects in Organic Chemistry*", J. Wiley, New York, (1956), p 406.
- [153] Hofle, G., Steglich, W. Vortruggen, H, *Angew. Chem., Int. Ed. Engl.* (1978), **17**, 569.
- [154] Hulin, B., Koreeda, M., *J. Org. Chem.*, (1984), **49**, 207.
- [155] Johnson, W. S., *Org. React.*, (1949), **2**, 114.
- [156] Effenberger, F., Epple, G., *Angew. Chem., Int. Ed.*, (1972), **11**, 299.
- [157] Effenberger, F., Epple, G., *Angew. Chem., Int. Ed., Engl.* (1980), **19**, 17.
- [158] March, J., *Advanced Organic Chemistry* (3rd Ed), pp714.
- [159] Huang-Minlon, *J. Amer. Chem. Soc.*, (1949), **71**, 3301.
- [160] Newman, M. S., Anderson, H. V., Takemura, K. H., *J. Am. Chem. Soc.* , (1953), **75**, 347.
- [161] Bachmann, Chemerda, *J. Org. Chem.*, (1941), **6**, 36.
- [162] Fieser, Hershberg, Long, Newman, *J. Am. Chem. Soc.*, (1937), **59**, 475.
- [163] Newman, M.S., Zahm, H. V., *J. Am. Chem. Soc.*, (1943), **65**, 1097.
- [164] Fieser, Novell, *J. Am. Chem. Soc.*, (1940), **62**, 1855.
- [165] Stacey, V., unpublished observations.
- [166] Rathke, R.W., *J. Amer. Chem. Soc.*, (1970), **92**, 3222.
- [167] Fadel, A., Salaun, J., *Tetrahedron* , (1985), **41**, 413.
- [168] Keiman, E., Mazur, Y., *J. Amer. Chem. Soc.*, (1977), **99**, 3861.
- [169] Kakis, F. J., Fetizon, M., Douchkine, N., Golfier, M., Mourgues, P., Prange, T., *J. Org. Chem.*, (1974), **39**, 523.
- [170] Silverwood, H.A., Orchin, M., *J. Org. Chem.*, (1962), **27**, 3401.
- [171] Gardner, D. M., Fraenkel, G. K., *J. Am. Chem. Soc.*, (1956), **78**, 3279.
- [172] Windaus, Thiele, *Ann*, (1936), **521**, 160.
- [173] Khorana, H. G., *Chem. Revs.*, (1953), **53**, 145.
- [174] Okamoto, Y., Aburatani, Hatada, K., *J. Chromatogr.*, (1987), **389**, 95.

- [175] Okamoto, Y., Kaida, Y., *J. Chromatogr.*, (1994), **666**, 403.
- [176] Grieb, S. J., Matlin, S. A., Phillips, J. G., Belenguer, A. M., Richie, H. J., *Chirality*, (1994), **6**, 129.
- [177] Matlin, S. A., Tiritan, M. E., Crawford, A. J., Cass, Q. B., *Chirality*, (1994), **6**, 129.
- [178] Belleau, B., Martel, R., Lacasse, G, Menard, M. , Weinberg, N. L., Perron, Y. G., *J. Am. Chem. Soc.*, (1968), **90**, 823.
- [179] Etherington, G. W., Ph D Thesis, (1995), University of Warwick.
- [180] Perrin, D. D., Armarego, W. L. F., Perrin, D. R. "*Purification of Laboratory Chemicals*" 2nd Edition , Pergamon Press.
- [181] Lin, L.J., Foster, J. F., *Anal. Biochem.*, (1975), **63**, 485.
- [182] Kirkland, J. J., Synder, L. R., "*Introduction to Modern Liquid Chromatography*", Willey-Interscience Publication, John Willey & Sons Ltd, USA. 2ndEd, (1979).

ABSTRACT

The thesis presents a review of the literature on the HPLC resolution of enantiomers using chiral stationary phases and discusses the mechanisms of separation and the utility of these phases. The synthesis and chemical, chiroptical and spectroscopic properties of helicenes are also reviewed and the potential utility of helicene-based for the HPLC resolution of enantiomers is discussed.

The work carried out involved the preparation of a hexahelicene-based chiral stationary phase and demonstrations of its utility for the resolution of chiral analytes. This phase was chemically bonded rather than physically coated, in order to make it stable to hydrolysis and solvent stripping, and therefore to permit its employment with a wide range of mobile phases.

This thesis describes the synthesis of hexahelicen-7-ylacetic acid methyl ester, including confirmation of the structure of the key synthetic intermediates by spectroscopic analysis, and the investigation of several procedures for the resolution of hexahelicen-7-ylacetic acid methyl ester into its enantiomers, one of which enabled around 100 mg amounts of each of (+) and (-) enantiomers of hexahelicen-7-ylacetic acid to be obtained in highly chemically and optically pure form. The thesis also gives an account of several synthetic approaches to covalently bonding of the chiral selector (hexahelicen-7-ylacetic acid) to a modified silica stationary phase.

Chiral stationary phases were prepared from each of the enantiomers in sufficient amount to permit the packing of analytical columns of these phases. Chiral solutes containing nitroaryl functionalities were synthesised to investigate the chiral resolving power of these novel phases. Adequate separations were obtained with both chiral stationary phases and, as anticipated, the eluting order of chiral analytes was reversed between the (+) and (-) stationary phases. The work demonstrated the utility of these columns for the resolution of nitroaryl-containing chiral analytes.