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New methods for the enantiomeric excess determination using NMR

Hulst, Anthony Jacques Ronald Lambert

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
1994

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Hulst, A. J. R. L. (1994). *New methods for the enantiomeric excess determination using NMR*. s.n.

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NEW METHODS FOR THE ENANTIOMERIC EXCESS DETERMINATION USING NMR

This investigation was supported by DSM Research, Geleen

RIJKSUNIVERSITEIT GRONINGEN

NEW METHODS FOR THE ENANTIOMERIC EXCESS DETERMINATION USING NMR

PROEFSCHRIFT

ter verkrijging van het doctoraat in de
Wiskunde en Natuurwetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus Dr. S. K. Kuipers
in het openbaar te verdedigen op
vrijdag 18 februari 1994
des namiddags te 2.45 uur precies

door

ANTHONY JACQUES RONALD LAMBERT HULST

geboren op 20 december 1959
te Utrecht

1994
Drukkerij van Denderen B.V.
Groningen

Promotor: Prof. Dr. B.L. Feringa
Referent: Dr. Ir. N.K. de Vries

Aan allen die hebben bijgedragen aan het tot stand komen van dit proefschrift betuig ik mijn hartelijke dank.

We live as we dream...
Alone

Joseph Conrad

ter herinnering aan mijn vader

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CHAPTER 1

Introduction

1.1 *Chirality*

In 1874, Jacobus van 't Hoff and Joseph Le Bel simultaneously[1] but independently formulated a bold hypothesis about the structure of organic molecules. They realized that when four different groups are attached to the four corners of a tetrahedron, the groups can assume two and only two possible arrangements that are related as one's right and left hands[#2,3]. Similar yet distinct, the two structures are nonsuperimposable; they are mirror images. The carbon atom, bearing the four non-identical substituents, is *asymmetric* and the mirror images are called *enantiomers* (Greek *enantio*, opposite). Two enantiomeric molecules have so called opposite *configurations*. Structures that contain two or more stereogenic centers can exist in more than two stereoisomeric configurations. The number of possible *stereoisomers* (Greek *stereos*, solid), all having the same structural formula, can theoretically be equal to 2^n, where n is the number of stereogenic centers in the structure. The relationship among the stereoisomers, however, may be one of mirror images (enantiomers) or stereoisomers that are not mirror images of each other. These are called *diastereomers*[4].

In nature virtually all the essential compounds possess at least one stereocenter and hence can be *chiral*[5] (Greek *chiros*, handedness). Complex organic compounds, like sugars, having more than one asymmetric center, mostly exist in several diastereomeric forms. If only one asymmetric center is present, like in most α-amino acids, nature enjoined, however, a predominant preference for only one enantiomeric form. Biological systems, like enzymes, themselves being chiral, non-racemic compounds, interact with the enantiomers in a diastereomeric, hence different, way[6]. Because of this, it is desirable to have access to both of the substrate enantiomers in order to understand and influence the biological activity and the natural processes, or to use such compounds as pesticides or pharmaceuticals. Although more than 50% of the commercial drugs are chiral, less than half of these are marketed in an enantiomerically pure form and only about 10% of the synthetic chiral drugs are available in the enantiomerically pure form[7]. Some notorious examples of the dramatic pharmacological response differences of the two enantiomers are known: (*S*)-propranolol **1.1** (Scheme **1.1**) is an antihypertensive and antiarythmic used in the treatment of heart disease, while the *R* enantiomer acts as a contraceptive[8]. The *R* enantiomer of asparagine **1.2** (Scheme **1.1**) tastes sweet whereas the *S* enantiomer tastes bitter[9]. Thalidomide **1.3** (Scheme **1.1**), commercially known as Softenon, was originally

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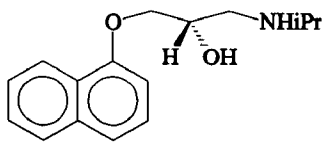
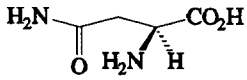
[#] This hypothesis, however, was not the first discovery in this respect. In 1815 Jean Baptiste Biot discovered optical activity, while Louis Pasteur isolated a pair of mirror-imaged nonsuperimposable crystals in 1848.

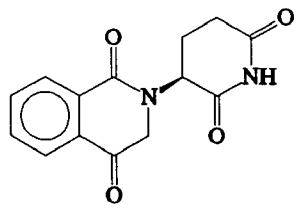
used as a racemate, whereas only the *R* enantiomer is responsible for the desired (sedative) therapeutic effect and the *S* enantiomer causes teratogenic effects[10].



1.1



1.2



1.3

Scheme 1.1

Examples like this are causing the industry to market chiral compounds as single enantiomers, addressing the requirements being imposed by the regulatory authorities in Europe and the United States[11]. This led to an dramatic increase in research efforts and development of (new) chiral synthons, catalysts and procedures leading to the synthesis of enantiomerically pure products.
In particular the growing interest and improvement in *enantioselective synthesis* led to an increased demand for accurate, reliable and convenient methods of measuring the enantiomeric composition.

1.2 *Methods for the determination of enantiomeric composition*

The enormous improvement in enantioselective synthesis[12] by means of *e.g.* kinetic resolution, stoichiometric or catalytic asymmetric transformations and biomimetic synthesis makes the availability of reliable analytical techniques for the correct determination of the enantiomeric composition increasingly important in order to cope with the needs of contemporary stereochemistry.
Regardless of the synthetic method used, the *enantiomeric excess* (*e.e.*)[#] of the obtained products always provides the quantitative criterion for the success of the asymmetric transformation. The method used to determine the enantiomeric composition should be able to render the correct composition in the two borderline cases, that is, the determination of a small enantiomeric excess and the determination of a small enantiomeric impurity.
An inherent difficulty in analyzing enantiomeric compositions is the fact that enantiomers have, apart from their chiroptical characteristics, identical physical and chemical properties

[#] For definitions see references 4, 13, 30 and 38.

(in an achiral environment). Up to the 60's, the enantiomeric purity of a chiral molecule was most frequently determined by making use of its chiroptical behaviour. Usually this involved measuring the optical rotation of the sample under accurately defined conditions. The obtained value was then compared with the known rotation for an enantiomerically pure compound, measured under the same conditions. The so determined *optical purity* is often equated with the *enantiomeric purity*, though several examples are known in which uncritical use of absolute rotations quoted in the literature led to incorrect statements concerning the optical yield of an enantioselective reaction#.

Any other method of distinguishing enantiomers must rely on the use of a chiral environment via diastereoisomer formation or diastereomeric interactions. Diastereomeric interactions can be created by means of reaction with a chiral auxiliary compound, the use of chiral solvating agents or by means of self association.

In 1967 Raban and Mislow[13] distinguished four general approaches to the determination of the optical purity of a mixture of enantiomers *R* and *S*. The determination may or may not involve a separation of *R* and *S*. Furthermore, the determination may be performed on the enantiomers themselves, or the enantiomers may be transformed into a pair of diastereomers *RR'* and *SR'* to facilitate the determination. Thus it follows that the determination may be carried out on enantiomers without separation (*class A,1*), on enantiomers with separation (*class A,2*), on diastereomers without separation (*class B,1*) and on diastereomers with separation (*class B,2*). A further distinction can be made depending on whether the determination of the enantiomeric composition can be performed without an auxiliary probe (*a*) or only in the presence of a nonracemic chiral probe (*b*). Some examples are known using an auxiliary achiral probe (*c*), which reacts twice with the enantiomers, giving diastereomers.

Any chemical operation which involves the separation of enantiomers or diastereomers or the conversion of enantiomers into diastereomers is very closely related to the methods used for resolution, and hence may involve potential sources of error. The formation of the diastereomers must occur under conditions which rigorously exclude the possibility of racemization or of kinetic resolution. Also, there must be no change in the ratio of diastereomers due to fractionation of the diastereomers by incomplete recovery, decomposition and losses arising during the isolation and sample handling. During the purification of diastereomeric products only methods must be used that avoid selective enrichment of one of the diastereomers.

1.2.1 *Class A,1,a: on enantiomers without separation and without auxiliary probe*

1) The Isotope Dilution Method: A test sample of unknown enantiomeric composition is diluted with an isotopically labelled sample of known enantiomeric composition of the same compound[14]. The isotopic content of an isolated aliquot is measured and the enantiomeric composition of the test sample is calculated from the dilution of the isotopic tracer, independent of the yield obtained in the isolation[15]. Radioactive or nonradioactive

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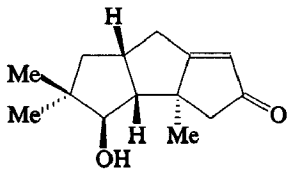
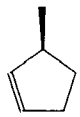
See for a short discussion Chapter 1.2.1 and 1.2.2.

isotopes may be used for this labeling, like *e.g.* ^{14}C, ^{2}H and ^{15}N. It is obvious that this methodology is rather tedious in that isotopically labeled tracers have to be synthesized with known degrees of isotopical labelling.

2) Polarimetry: The *optical purity* is determined by the comparison of the chiroptical properties of an optically active compound of given enantiomeric composition with that of the 100 % pure enantiomer[4,14,15]. Several methods are known, using *e.g.* the specific rotation of plane polarized light at a specific wavelength (*polarimetry*), or that over a range of wavelengths (*optical rotation dispersion, ORD*). Asymmetric compounds exhibiting a Cotton effect can be analyzed by the difference in their absorption of right and left handed polarized light (*circular dichroism, CD*). Although these techniques probably are the most popular methods for the determination of the optical purity *p* and the evaluation of the enantiomeric composition, several problems are connected with the use of chiroptical techniques. Firstly, the optical purity and the enantiomeric purity are not necessarily equivalent[16]. Horeau[17] demonstrated that the optical rotation does not vary linearly with the enantiomeric composition when the enantiomers undergo molecular self-association, although this phenomenon is also reported to be seen in more polar solvents at low concentrations, making oligomerisation less probable. Secondly, variations with any of the parameters like *e.g.* wavelength, temperature, concentration, and the solvent used (when not measured neat) may occur[18]. The pH of the solvent, the purity of both the sample and the solvent are other variables[#]. Thirdly, the requirement that the specific rotation of the pure enantiomer, $[\alpha]_{max}$, is known with certainty imposes a great restriction on these techniques. This, however, is a serious problem since literature is known to be infected with many examples of incorrect optical rotations for compounds which were considered to be enantiomerically pure[19]. For example, enone **1.4** (Scheme **1.2**) has been shown to have a rotation of $[\alpha]_D^{20}$= +34° (*c* 1, $CHCl_3$), whereas its enantiomer has been reported to have a rotation of $[\alpha]_D^{20}$= −115.4° (*c* 0.2, $CHCl_3$)[19,20].



1.4



1.5

Scheme 1.2

Enantiomerically pure 3-methylcyclopentene **1.5** (Scheme **1.2**), was up to 1974 believed to have $[\alpha]_D^{20}$= 78° (neat) as determined by means of rotation and empirical calculation[21]. An independent determination of the *enantiomeric excess* (*e.e.*) showed that the rotation was $[\alpha]_D^{20}$= 174.5° (neat) for the enantiopure material[22].

[#] See also Schurig, V., *Kontakte (Darmstadt)* **1985**, *1*, 54.

Finally, the optical purity relates to experimental conditions, whereas the enantiomeric purity is defined by making use of the enantiomeric ratio, without using physical measurements; direct comparison is hence more troublesome (or not allowed)[13].

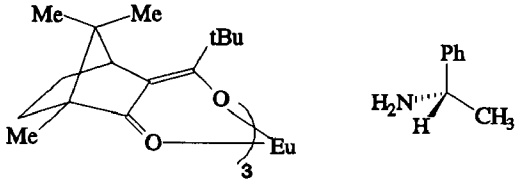
3) Calorimetric Methods: The enantiomeric composition of optically impure substances can be determined by means of differential microcalorimetry[23]. The method depends on the phase relationship of the component enantiomers, which is established by comparing the heats of fusion or melting points of the enantiomeric mixture with that of the racemic form. This methodology has a high precision though its applicability is rather limited.

4) NMR spectroscopy: Although NMR is an *inherently achiral technique*[#] and usually a chiral auxiliary is needed, sometimes and under very strict conditions the sample can serve as its own chiral probe[24,25]. The self association between *homochiral* and *heterochiral* molecules of *e.g.* amino acids[26] and thiophosphoric amides[27] will produce diastereomeric interactions for the pure enantiomers (*R,R* or *S,S*) and the racemate (*S,R* or *R,S*) making a direct *e.e.* determination possible.

5) Enzymatic techniques: Many enzymes are capable of discriminating completely between a pair of enantiomers[28], particularly in the case of amino acids. So techniques based upon the enzymatically catalyzed transformations of *e.g.* amino acids are useful for an exact determination of the enantiomeric purity. It is possible to detect as little as 0.1 % of an enantiomer with high accuracy.

1.2.2 *Class A,1,b: on enantiomers without separation in the presence of a chiral probe*

NMR spectroscopy: The use of chiral paramagnetic (lanthanide) shift reagents or chiral solvating agents and chiral solvents renders the *externally enantiotopic* nuclei into *externally diastereotopic*[##] nuclei which, in principle, can be distinguished.



1.6

Scheme 1.3

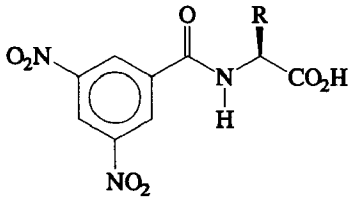
[#] The methodology using NMR techniques for the determination of enantiomeric excess will be discussed in detail in Chapter 1.3.

[##] The concept of stereotopicity, as introduced by Mislow and Raban, will be discussed in Chapter 1.3.1.

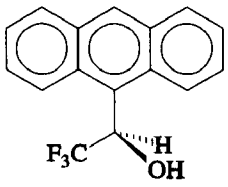
Since 1970, when Whitesides and Lewis[29] demonstrated that the application of the chiral europium complex tris-(3-*t*-butyl-hydroxymethylene-(1*R*)-camphorato)-europium (III) **1.6** (Scheme **1.3**) resolved the externally enantiotopic methyl, methine and ortho aromatic protons of α-phenylethylamine of both enantiomers, the use of chiral lanthanide shift reagents became very popular. All kind of lanthanide shift reagents are known nowadays, including several water-soluble systems[30,31,32].
Though not very widely recognized, diastereomeric salts also form dynamic systems showing external diastereotopicity provided that the spectra are recorded in nonpolar solvents[33].

1.2.3 *Class A,2,b: on enantiomers with separation in the presence of a chiral probe*

Chromatographic methods: In 1938, Karagunis and Coumoulos[34] reported the first resolution of a racemic selectand on a chiral selector using the principle of *enantioselective adsorption* in flow systems. At the same time Henderson and Rule[35] were able to separate racemic *d,l-p*-phenylene-bis-iminocamphor on an adsorption column containing solid *d*-lactose. In 1959 Karagounis and Lippold[36] were able to separate the racemic mixtures of 2-butanol and of 2-bromo-*n*-butane on optically active stationary phases making use of gas-chromatographic methods. It took, however, seven more years before the first example of a fully reproducible separation of enantiomers by means of gas-chromatography was published[37]. Over the past years these methods proved to be very sensitive and widely applicable for the determination of the *enantiomeric excess*[38]. The enormous number of chiral selector-selectand systems developed since that time, *e.g.* by Pirkle and co-workers[39], made these methods probably the most powerful in the field of enantiomer separation[40]. A breakthrough in this respect was made by Pirkle and co-workers by the introduction of *N*-(3,5-dinitrobenzoyl)amino acids **1.7** (Scheme **1.4**) as immobilized, chiral charge transfer acceptor ligands used for high pressure liquid chromatography[41]. The development of (*R*)-2,2,2-trifluoro-1-(9-anthryl)ethanol **1.8** (Scheme **1.4**), known as *Pirkle's alcohol*, used for the resolution of a large variety of substrates, preceded this methodology[42].



1.7



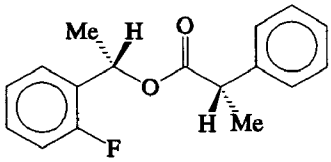
1.8

Scheme 1.4

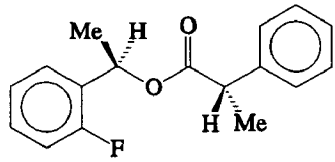
Chiral stationary phases based upon the ability to separate enantiomers via hydrogen bonding or via coordination are now readily available[43].
In general, the chromatographic methods tend to be very fast, sensitive (often very high resolutions are obtained), precise and reproducable, making them very attractive for the modern chemist.

1.2.4 *Class B,1,b: on diastereomers without separation and with a chiral probe*

NMR spectroscopy: The use of enantiomerically pure chiral derivatizing agents to convert enantiomers into a pair of diastereomers with nonequivalent external diastereotopic nuclei prior to the NMR analysis was first reported by Mislow and Raban in 1965[44]. They observed chemical shift nonequivalences in the proton NMR spectra of the diastereomeric 1-(methylphenyl)ethanoic acid esters of 1-(2-fluorophenyl)ethanol **1.9** (Scheme **1.5**), although some racemization occurred during the preparation of the diastereomers, making an exact determination of the enantiomeric composition impossible.



1.9a



1.9b

Scheme 1.5

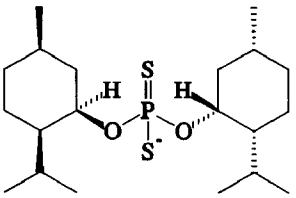
A large variety of chiral derivatizing agents has since then been developed making use of all kind of NMR active nuclei[31]. A problem, however, is the necessity that the derivatizing agent has to be enantiomerically pure since a small enantiomeric impurity of the compound will reduce the obtained values for the enantiomeric purity drastically, making exact determinations less easy to perform.

1.2.5 *Class B,2,b: on diastereomers with separation and with a chiral probe*

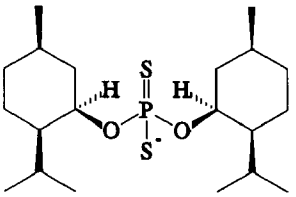
Chromatographic methods: When achiral stationary phases are used the enantiomers have to be converted into a pair of diastereomers by chemical reaction with an enantiomerically pure auxiliary prior to the chromatographic analysis[37]. Because commercial HPLC columns may be employed for the separation of diastereomers, this indirect technique has in the past enjoyed wider utility than the direct resolutions on chiral stationary phase columns, although at this moment the situation has changed.
As for NMR, all kinds of enantiomerically pure chiral auxiliaries have been developed over the last few years.

1.2.6 *Class B,1,c: on diastereomers without separation and with an achiral probe*

NMR spectroscopy: In a number of examples the auxiliary is not an enantiomerically pure compound but an achiral derivatizing agent capable of reacting or complexing with two molecules of the mixture to be analyzed[45]. This methodology relies upon differences in inherent chirality between an enantiomerically pure and (partly) racemic substance.
When *e.g.* $(PhO)_2P(S)SH$ is allowed to react with *d,l*-menthol, diastereomeric *O,O*-dialkylphosphorodithioates are formed[46]. The phosphorus atom in the diastereomeric derivatives **1.10** (Scheme **1.6**) is stereogenic but achirotopic, so two signals are observed in the proton decoupled ^{31}P NMR.



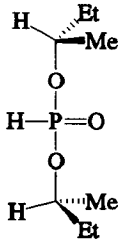
1.10 racemate (RR shown)



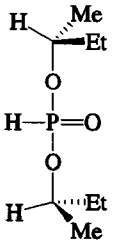
1.10 meso

Scheme 1.6

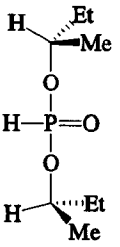
When however PCl_3 is used in the corresponding coupling reaction with 2-butanol, four stereoisomers **1.11** (Scheme **1.7**) are formed, giving three singlets in the proton decoupled ^{31}P NMR[47]. Two resonances are obtained for the meso compound, due to the pseudochiral phosphorus atom.



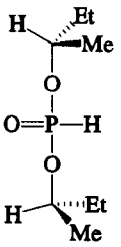
1.11 (SS)



1.11 (RR)



1.11 (SR)



1.11 (SR)

Scheme 1.7

1.2.7 *Class B,2,c: on diastereomers with separation and with an achiral probe*

Chromatographic methods: Several methods exist in which two enantiomers are converted into diastereomers with the aid of an achiral derivatizing agent. An outstanding example forms the reaction of CH_3POCl_2 with *e.g.* alcohols[48], following the same methodology as for analysis by means of NMR (see Chapter 1.2.6, *Class B,1,c*).

1.3 *NMR spectroscopy and the determination of enantiomeric composition*

NMR is incapable of distinguishing between enantiomers without the aid of an auxiliary chiral probe#. The nuclei of enantiomers are *isochronous*, hence no differentiating quantitative or qualitative analyses can be obtained. As already discussed, direct and indirect methods are available to transform enantiomers into diastereomers with *aniso-chronous* nuclei, which should in principle give different signals in the NMR spectra. Before discussing the methodologies of transformation, it seems appropriate to become familiar with the symmetry criteria that play an important role in the determination of enantiomeric composition by means of NMR.

1.3.1 *Stereochemical considerations*

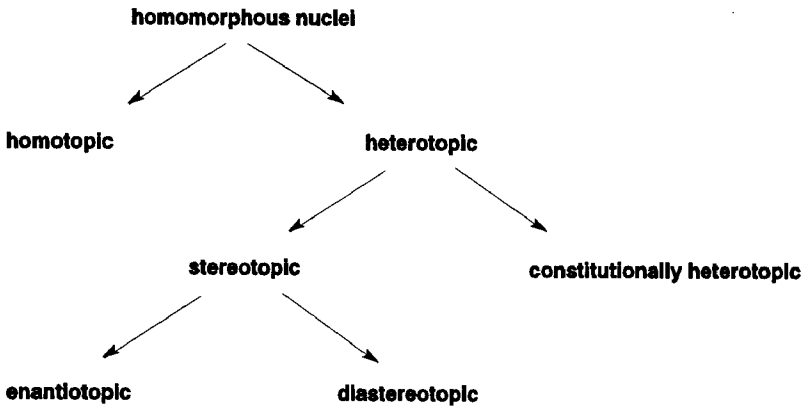
In 1967, Mislow and Raban[13] introduced the concept of *stereotopicity*, providing an excellent tool for the prediction of magnetic shielding anisochrony by means of symmetry considerations. Spatial relationships between portions of molecules are simularly termed as is common for stereoisomeric molecules##.

In terms of stereotopicity we differentiate between *homotopic* (Greek *homos*, same and *topos*, place) and *heterotopic* (Greek *hetero*, different) nuclei. The homomorphous nuclei are called homotopic when they can be interchanged by a proper axis of rotation (C_n); when not, we call them heterotopic. *Enantiotopic* are the stereotopic nuclei which can be interchanged by: reflection symmetry through a plane of symmetry (C_s), an improper axis (S_n) or through a symmetry center (C_i). When nuclei are not permutable by any symmetry operation (C_1) they are called *diastereotopic*.

These symmetry rules are easily related to NMR chemical shift equivalence, *isochrony*, arising from the symmetry equivalence of enantiotopic or homotopic nuclei and at the other hand NMR chemical shift nonequivalence, *anisochrony*, arising from the diastereo-meric nonequivalence of diastereotopic nuclei. The symmetry equivalence of two or more nuclei is a sufficient but not a necessary condition for the isochrony, whereas symmetry nonequivalence of such nuclei is a necessary though not a sufficient condition for anisochrony, since symmetry nonequivalent nuclei may be accidentally isochronous[49].

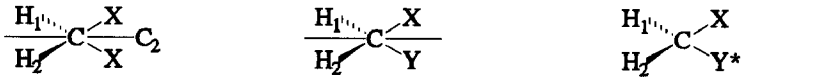
Sometimes self association renders the enantiomers into diastereomers which can be analyzed without the aid of a chiral auxiliary, see Chapter 1.2.2.

For stereochemical definitions and discussion, see reference 4.



Scheme 1.8 Topic relationships between homomorphous nuclei

In defining the spatial relationships of molecular substructures we make a distinction between *internal* and *external* comparison[50,51]. In the former, homomorphous nuclei or subsets of nuclei in the same molecule are compared (intramolecularly), while in the latter the comparison takes place between homomorphous nuclei or subsets of nuclei, which are part of different molecules (intermolecularly). This means that substructures that are related by external comparison are not necessarily related by internal comparison, though substructures that are related by internal comparison are always relatable by external comparison. It is important to note that externally enantiotopic nuclei belonging to a pair of enantiomers are altered into externally diastereotopic nuclei in the presence of non-racemic chiral additives#.



1 **2** **3**

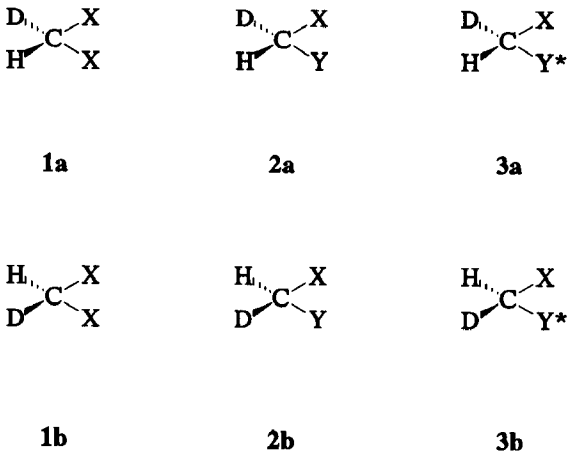
H_1/H_2: homotopic **H_1/H_2: enantiotopic** **H_1/H_2: diastereotopic**

*Scheme 1.9 Internal topicity (*Y is a chiral ligand)*

Certain theories describe a molecule as a set of atoms which are either bonded or non-bonded to the atom of interest. The bonded atoms contribute to the local diamagnetic and paramagnetic components of the screening constant. The non-bonded atoms contribute only by long-range effects. Using this description it is obvious that constitutional heterotopicity and diastereotopicity are fundamentally different.

Internal homotopic protons, *e.g.* like in molecule **1** (Scheme **1.9**), are related by a C_2 rotational axis, while the protons in molecule **2** are internally enantiotopic because they are related by a plane of symmetry C_s. The plane of symmetry is destroyed in molecule **3**, and the protons are internally diastereotopic.

In Scheme **1.10** three sets of molecules illustrate the different possibilities of external topicity. In the *homomers* $\mathbf{1^a/1^b}$ the homomorphous nuclei are equivalent, hence externally homotopic. The nuclei of the enantiomers $\mathbf{2^a/2^b}$ are externally enantiotopic because they are related by a plane of symmetry (this plane of symmetry lies in the plane of the paper). The plane of symmetry, however, is destroyed by the introduction of a chiral ligand Y* and the resulting diastereomers are $\mathbf{3^a}$ and $\mathbf{3^b}$ are externally diastereotopic.



1a **2a** **3a**

1b **2b** **3b**

Scheme 1.10 *External topicity (*Y is a chiral ligand)*

A convenient heuristic for the distinction between two diastereotopic atoms involves the substitution of such an isotopic atom by a test group to form diastereomers[13]. The heuristic converts internal into external diastereotopism. Furthermore, it seems appropriate to define *chirotopic* as any atom or any point segment of the molecule residing in a chiral environment, *achirotopic* as any one that does not[13,52].

The identity of an asymmetric carbon as a stereocenter depends on models of bonding since both stereoisomers must have the same connectivity. It must be emphasized that the character of such an atom as a chirotopic center is separate and distinct from its character as a stereocenter, so *stereogenicity and chirotopicity are conceptually distinct.*[#]

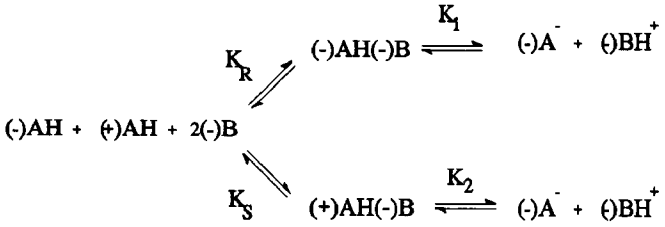
[#] For example, the halogen atoms in CHBrClF are chirotopic but nonstereogenic and the CHCl groups of 1,2-dichloroethene are achirotopic but stereogenic[47].

1.3.2 *Methods for the enantiomeric excess determination*

For the determination of the enantiomeric excess by means of NMR techniques mostly nonracemic chiral auxiliaries are needed to transform the isochronous enantiotopic nuclei into anisochronous diastereotopic nuclei. As long as there is a large enough chemical shift nonequivalence $\Delta\delta$, to give baseline resolution of the appropriate signals, integration gives a direct measure of the diastereomeric composition of the sample under investigation. The diastereomeric composition can subsequently be related directly to the enantiomeric composition.

There are three types of chiral auxiliary that are used. *Chiral solvating agents* and *chiral lanthanide shift reagents* form diastereomeric complexes *in situ* that allow a direct determination of the diastereomeric composition. The use of *chiral* (or *achiral*, see Chapter 1.2.6, *Class B.1.C*) *derivatizing agents* requires the separate formation of diastereomers prior to the NMR analysis.

1) Chiral solvating agents: The chiral solvating agents form diastereomeric solvatation complexes which solute enantiomers in competition with the bulk solvent in a rapid equilibrium[53,32]. Detailed studies have shown that factors like temperature, concentration and the optical purity of the chiral solvating agent do not change the magnetic nonequivalence but only the magnitude#. The relative value of the diastereomeric complexation constants K_R and K_S, the equilibrium constant between the undissociated salt and its components in the diastereomeric chiral acid versus chiral amine systems, are not equal ($K_R \neq K_S$)(Scheme **1.11**).



Scheme 1.11

Since the dissociation equilibria are relatively fast on the NMR time scale, NMR nonequivalences are observed only in the enantiomerically enriched part of the salts. The observed resonances derived from each enantiomer δ_R(obs) and δ_S(obs) represent population weighted averages of the chemical shift contributions for the chiral and achiral solvates δ_R, δ_S and δ_{ach} respectively. Given that Φ_R and Φ_S are the fractional populations of achiral solvate, so that $K_R = (1-\Phi_R)/\Phi_R$, then;

$$\delta_R(\text{obs}) = \Phi_R\delta_{ach} + (1-\Phi_R)\delta_R$$

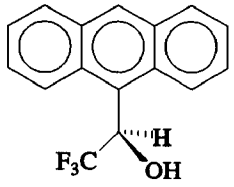
For a detailed study see references 25 and 33.

$$\delta_S(obs) = \Phi_S\delta_{ach} + (1-\Phi_S)\delta_s$$

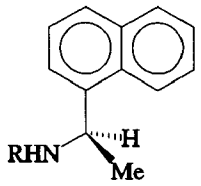
hence

$$\Delta\delta = \Phi_R(\delta_{ach} + K_R\delta_R) - \Phi_S(\delta_{ach} + K_S\delta_S)$$

Since the enantiomeric composition of components in the system affects the ratio of lifetimes of bonded and nonbonded components, the changes are reflected by different $\Delta\delta$ values proportionally with changes of enantiomeric purity *S*, being only zero for a racemic base[49].

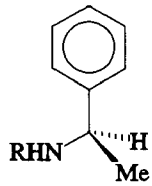


1.13



R = H or Me

1.14



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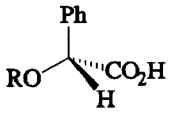
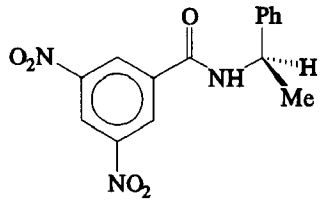


R= H, Me, Ac

1.16



1.17

Scheme 1.12 Common Chiral Solvating Agents

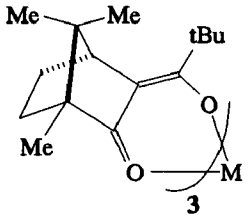
The advantages of this method are clear; it is quick and relatively simple to perform, without the problems associated with kinetic resolution. A disadvantage is the low solubility of these salt–systems in less polar solvents that are usually required for this type of measurements[#]. In Scheme **1.12** several examples of chiral solvating agents are collected, including acids, amines and alcohols[32].

[#] Only a limited range of solvents may be used. Nonpolar solvents tend to maximize the observed anisochrony between the diastereomeric complexes, though they lower the solubility of the complexes drastically. The more polar solvents preferentially solvate the solute and the $\Delta\delta$ falls to zero.

2) Chiral lanthanide shift reagents: The addition of a lanthanide shift reagent to an organic compound may result in diastereomeric shift differences[30,31,32]. The lanthanide reagent forms a weak addition complex that is in fast exchange with the non-bonded organic substrate on the NMR time scale. The induced shifts are caused by a difference in the magnetic susceptibility tensors for the seven-coordinate complex. The McConnell-Robertson equation[54] defines the relationship between the induced shift $\Delta\delta$, the distance from the metal center and the angle between the principal magnetic symmetry axis and the nucleus in question on the basis of pseudo contact shifts#.

$$\Delta\delta = \textit{pseudo contact shift difference} = k^*(1-3\cos^2\theta)r^{-3}$$

Apart from practical uses, lanthanide shift reagents have a great potential for yielding information on the constitution, configuration and conformation of molecules in solution.



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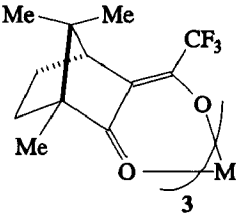
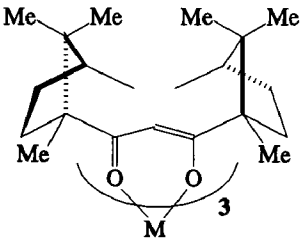


1.19

Lanthanide= Eu, Pr, Yb



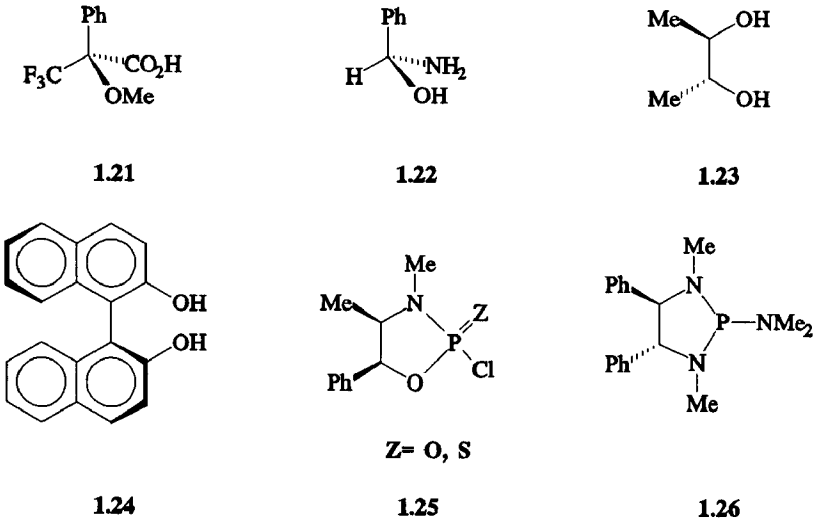
1.20

Scheme 1.13 Common Chiral Lanthanide Shift Reagents

Scheme **1.13** contains several typical lanthanide shift reagent systems. As with chiral solvating agents the chiral lanthanide shift reagents need not be enantiomerically pure, though they should not be racemic when used for the determination of the enantiomeric composition[30,31,32].

Lanthanide shift reagents are in general less useful at high field. Under the fast exchange conditions line broadening is proportionally to B_0^2, giving a larger line broadening with an increasing field strength.

3) Chiral Derivatizing Agents: The determination of the enantiomeric composition by means of chiral derivatizing agents is probably the most widely used NMR technique[55]. The discrete diastereomeric complexes that are formed give an observed chemical shift nonequivalence $\Delta\delta$ that is typically five times higher then for related chiral solvating agents. The formation of the diastereomers must occur under strictly defined conditions, excluding racemization or kinetic resolution. When purification is necessary only methods that exclude potential selective enrichment of one of the diastereomers may be used.
In spite of all these restrictions, many chiral derivatizing agents are known and commonly used[32,51] (Scheme **1.14**).



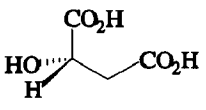
Scheme 1.14 Common Chiral Derivatizing Agents for ^{1}H, ^{19}F and ^{31}P NMR analysis

The chiral derivatizing agents often contain more than one NMR active nucleus, *e.g.* ^{31}P, ^{19}F, ^{29}Si or ^{77}Se nuclei[56]. This enhances the applicability of the chiral derivatizing agent used.

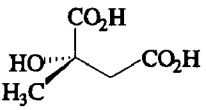
1.4 *Enantiomeric excess determination of amino acids by means of NMR*

Although amino acids are usually classified into two groups, the *hydrophobic* and *hydrophilic* amino acids, according to the nature of their side chain, they have the most important features in common[57]. Amino acids have an acidic and a basic group attached to the α-carbon. The α-carboxyl group has a pK_a that typically is close to 2, while the amino group has a pK_a that is between 9 and 10. At physiological pH (ph 7) free amino acids exist largely as *zwitterions*, having both a quaternary ammonium and a carboxylate ionic group. The nitrogen as well as the oxygen can act as electron donating group for the

formation of a dative bond, thus creating a capacity to serve as a chelating agent. These features lead to the major restriction in the enantiomeric excess determination of amino acids by means of NMR. Water is normally the solvent of choice, due to the very low solubility of amino acids in other (organic) solvents.
Given these restrictions the enantiomeric excess determination usually follows two main routes; the direct enantiomeric excess determination in polar solvents or the derivatization and subsequent enantiomeric excess determination.
The Direct Enantiomeric Excess Determination: The direct enantiomeric excess determination in polar solvents like water is closely related to the development of water-soluble shift reagents[58]. Reuben[#,59] used a 'self resolution' approach to resolve the enantiomeric nuclei of the α-hydroxycarboxylates **1.27** and **1.28**, using several lanthanide ions (Scheme **1.15**).



1.27



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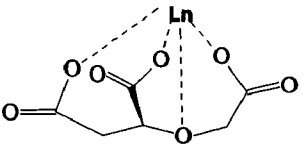
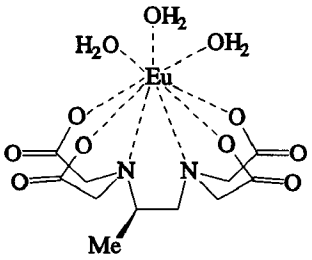


1.29

Scheme 1.15

Upon addition of lanthanide (III) chlorides, chiral chelate complexes are formed, in which a hindered rotation of the carboxylate groups affords the water-soluble shift reagent. The first genuinely water-soluble shift reagent, however, was reported by van Bekkum and co-workers[60] who used lanthanide derivatives of (*S*)-[(carboxymethyl)oxy]succinic acid **1.29** (Scheme **1.15**). The enantiomeric nuclei of hydroxy carboxylic acids and amino acids could be resolved using this reagent, giving resolutions up to 7 Hz. Although the system is stable over a large pH range (pH 3-10), it is shown that its tendency to self-association limits the use as shift reagent.
The Eu^{3+} complex of (*R*)-propylenediaminetetraacetate **1.30** as reported by Kabuto and Sasaki[61] appears to be very suitable as a chiral shift reagent for the enantiomeric excess determination of α-hydroxy acids and amino acids (Scheme **1.16**).
This reagent is also applicable to substrates having polar substituents on their side chains, for which so far no simple method for the determination of the absolute configuration had been reported. The ΔΔδ values show a strong pH dependence, giving optimal peak separations *e.g.* for lactic acid at pH 4 and for several amino acids at pH 12.

[#] Self association is a typical property of lanthanide carboxylate complexes, limiting their use to a restricted pH range, see Chapter 2.1 for a discussion.



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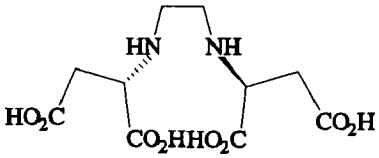
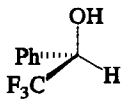


1.31

Scheme 1.16

The most potent chiral shift reagent for aqueous solutions, however, probably is the Eu^{3+} complex of (*S,S*)-ethylenediamine-*N,N'*-disuccinic acid **1.31** (Scheme **1.16**), which was prepared by the condensation of two molecules *L*-aspartic acid with dibromoethane[62]. This system affords excellent peak separation for the enantiomeric nuclei of several amino acids (see Chapter 2 for a detailed discussion).

Enantiomeric Excess Determination via Derivatization: The derivatization of amino acids usually proceeds via two possible routes: (a) the derivatization towards a in a nonpolar medium soluble derivative and subsequent enantiomeric excess determination by one of the 'classical' methods (like a chiral shift reagent or derivatizing agent), or: (b) reactions with a chiral or achiral reagent forming diastereomers which can be analyzed directly.



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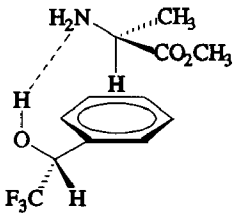
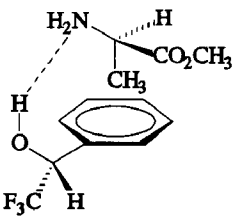


1.32a

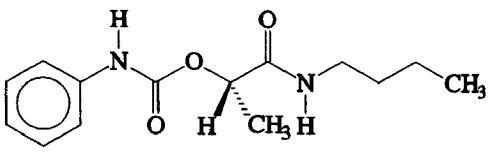


1.32b

Scheme 1.17

After esterification of the α-amino acids with MeOH/HCl, the enantiomeric excess of the methyl esters can easily be determined by means of α-methoxy-α-(trifluoromethyl)-phenylethanol **1.32** (Scheme **1.17**)[63].

Presumably, a complex between the amino acid ester and the chiral solvating agent is formed, only these conformations that can bring the enantiotopic nuclei above or below the phenyl ring system show spectral nonequivalence.
When Eu^{3+} chiral shift reagents are used it is often more appropriate to enhance the chelating capacity of the amino acid derivative, *e.g.* by transforming them into amides instead of esters[30]. An example of this type of methodology is the enantiomeric excess determination of the *N*-acetyl derivatives of amino acids, which can be easily formed after reaction with acetic anhydride[64].
The more potent chelating capacities of amides was also demonstrated by Pini[65], who uses the *N*-(*n*-butylamide) of (*S*)-2-(phenylcarbamoyloxy)propionic acid **1.33** as a chiral solvating agent for *N*-(3,5-dinitrobenzoyl)amino acid methyl esters **1.34** (Scheme **1.18**). Especially the amide protons show an significant $\Delta\delta$, for the diastereomeric associates, up to 0.54 ppm.



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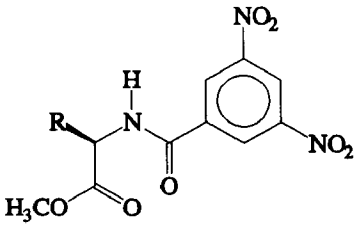
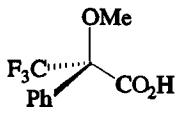


1.34

Scheme 1.18

These types of approach, however, are rather tedious in that first derivatives have to be prepared and subsequently a successful procedure to determine the enantiomeric excess has to be found, making the 'direct' derivatization into diastereomers more attractive.



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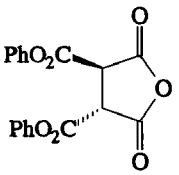

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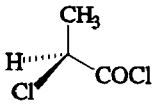


1.37

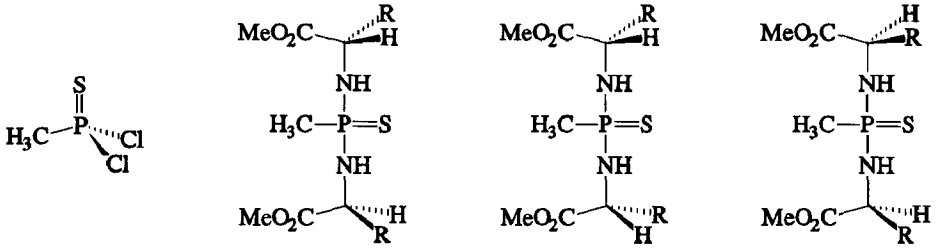
Scheme 1.19

Mosher's reagent[66], α-methoxy-α-(trifluoromethyl)phenylacetic acid **1.35**, is probably the most potent chiral derivatizing agent known and can be used for the derivatization of unprotected amino acids as well[67] (Scheme **1.19**). The enantiomeric excess can be determined by means of ^{1}H or ^{19}F NMR techniques.

The enantiomeric excess of amino acid esters can also be determined after derivatization with (*S*)-di-benzoyltartaric anhydride **1.36** (Scheme **1.19**). Simple ^{1}H NMR analysis of the tartaric imides affords the enantiomeric excess[68].

Not only the unprotected α-amino acids, but also sterically hindered α-alkylated amino acids can be analyzed by means of (*S*)-2-chloropropionyl chloride **1.37** (Scheme **1.19**)[69]. This reagent is easily obtained from (*S*)-alanine. As with most other methods this method is also applicable for chiral amines, alcohols and thiols, affording excellent peak separation in the ^{1}H NMR[69].

A very elegant method uses the principle of Horeau[45], in which two amino acid esters are reacted with the achiral methylphosphothiodichloride **1.38** (Scheme **1.20**)[70]. The resulting derivatives are easily analyzed using ^{31}P NMR techniques#. The advantages of the use of ^{31}P NMR are clear, there are only three singlets observed (see Chapter 1.2.6) while the ^{1}H NMR gives a much more crowded spectrum making analysis troublesome.



1.38 **1.39** **1.40** **1.41**

Scheme 1.20

A disadvantage these methods have in common is the fact that they are not universally applicable, *e.g.* with α-hydroxy-amino acids severe side product formation is observed. Also, often a derivatization to the esters has to be performed prior to the derivatization with the chiral reagent itself.

1.5 *Objective of this thesis and survey of its contents*

Although several methods are known to determine routinely the enantiomeric excess of amino acids by means of NMR, the rather laborious routes for the enantiomeric excess determination are far from ideal. A further restriction is the fact that most methods are not able to analyze α-alkylated amino acids, a class of compounds that currently attracts a lot of interest. The aim of the research, described in this thesis, is to develop new and simple routes for determining the enantiomeric excess of α-amino acids and α-alkylated amino

Several reagents of this type, bearing a sulfur or oxygen substituent, have been developed.

acids, their derivatives and products by means of NMR techniques, and to evaluate the factors that govern the diastereomeric chemical shift differences $\Delta\delta$. Different approaches have been used to reach the proposed objective. As became evident during the course of our research, thorough mechanistic study was required in order to understand important *side* reactions and phenomena that were found.

In Chapter 2, strategies for the design of new, aqueous chiral shift reagents based upon the use of enantiomerically pure γ-menthyloxyfuranone is described. Based upon Horeau's principle, Co^{III}-porphyrine systems represent achiral water-soluble shift reagents. The synthesis and the characteristics as chiral shift reagents are discussed.

Chapter 3 deals with the structural investigation of trivalent phosphorus containing chiral derivatizing agents and their use as enantiomeric excess determining agents for amines, alcohols, thiols and esters of amino acids. These reagents and the diastereomeric adducts formed, being moisture and oxygen stable, turned out to be an interesting framework for a study towards the relationship between the conformation of the diastereomers and their chemical shift differences $\Delta\delta$. These studies were performed using 2D NMR techniques (COSY, NOESY and ^{31}P COSY), combined with modelling studies (still under investigation).

The use of pentavalent phosphorus containing chiral derivatizing agents is the basis of the research described in Chapter 4. Pentavalent phosphites and the corresponding phosphoric acid chlorides are used for the enantiomeric excess determination of amines, alcohols and amino acids.

The use of modified cyclic phosphites as alternative for the derivatization agents as used in Chapter 4, is described in Chapter 5.

Eight phosphoric acid esters and amides were studied using a combination of 2D NMR, ^{31}P NMR, X-ray and modeling techniques, resulting in the development of a model predicting the chemical shift dependencies as a function of the conformation, as is described in Chapter 6.

In Chapter 7 a detailed mechanistic study is described of the reaction of cyclic phosphoric acid chlorides or phosphites and *e.g.* amines. Intermediates are characterized and a model is postulated for the formation of the pyrophosphate side product.

1.6 *References*

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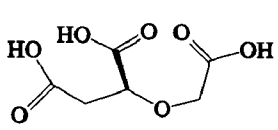
CHAPTER 2

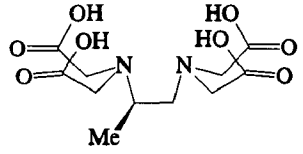
Towards the design, synthesis and use of new aqueous shift reagents for the enantiomeric excess determination of amino acids

2.1 *The concepts of known chiral aqueous shift reagents*

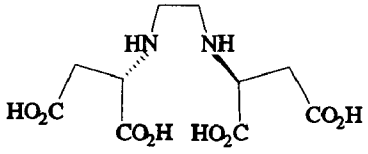
Although many chiral lanthanide shift reagents are known for use in *organic* media, studies performed on water-soluble chiral shift reagents are very limited. Since Reuben[1], in 1980, introduced his self-resolution approach to resolve the enantiomeric nuclei of α-hydroxy carboxylic acids by means of paramagnetic lanthanide ions, only three other systems have been developed for the determination of the enantiomeric excess of free amino acids in water. Van Bekkum[2] and co-workers used a lanthanide derivative of (*S*)-[(carboxy-methyl)oxy]succinic acid **2.1** to resolve the enantiomeric nuclei of α-amino acids and α-hydroxy carboxylic acids. Very promising is the system presented by Kabuto and Sasaki[3] who demonstrated the utility of the Eu^{3+} complex of (*R*)-propylene-1,2-diaminetetraacetate **2.2** as a powerful chiral shift reagent for aqueous solutions. The most simple and promising chiral water-soluble shift reagent probably is the system prepared by Kido[4] and co-workers. The ligand system, (*S,S*)-ethylenediamine-*N,N'*-disuccinic acid **2.3**, is easily prepared by the condensation of two molecules of *L*-aspartic acid with dibromoethane[5] followed by complexation with Eu^{3+}.
These systems, though basically different, have some features and characteristics in common that are typical for aqueous lanthanide shift reagents.



2.1



2.2



2.3

Scheme 2.1 Three known ligand systems for the preparation of water-soluble shift reagents

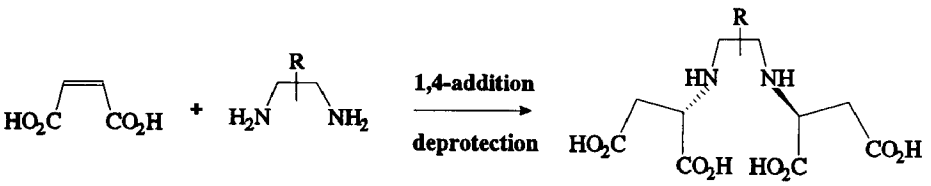
All are potentially tetra- or hexadentate ligands, containing either one ether and three carboxylate binding sites (as in **2.1**) or two nitrogen and four carboxylate binding sites (as in **2.2** and **2.3**), affording a strong coordinating ligand towards the lanthanide ion.
The lanthanide induced shift can be interpreted in terms of structural properties of the metal–ligand system. One component is the *contact interaction* which involves electron delocalization through chemical bonds[6]. The second, and more important, component is the *dipolar interaction*, which is a "through space" interaction depending upon the orientation of the nucleus with respect to the metal[7]. In this way, the induced shift is mainly the result of a rapid equilibrium between the hydrated lanthanide–ligand system and the complex in which one water molecule is replaced by a chelating amino acid[8]. The hydrated state is found to be dependent on the pH of the solution[9], and as the amino acids reside at the sites where the water molecules usually are located, the coordinating behavior of the amino acids also shows a large pH dependence, making the observed chemical shift nonequivalences pH dependent[#].
Furthermore, the Eu complex of **2.1** *e.g.* appears to be strongly associated above pH 6, with the oligomerization reaching a maximum value at pH 8[10]. Although the system dissociates between pH 8 and 11, the monomeric state is probably never reached again. This behavior limits the use of this lanthanide shift reagent to a rather restricted pH domain (pH 1–6).
Binding of an amino acid to the lanthanide–ligand system can give rise to substantial steric interactions when both ligand and amino acid bind at the inner coordination sphere of the lanthanide ion, as was shown for system **2.2**[11]. The stereochemical information obtained would reflect the nature of the perturbed system, making comparisons between "diastereomeric" complexes troublesome, because they would contain information about systems which structure is not known with certainty.
Above pH 8 the lanthanide complex of **2.3**, however, has been shown to be a monomeric complex, containing a hexadentate chiral ligand and three bonded water molecules, not exhibiting any oligomerization tendencies[12]. The three water molecules are easily replaced, allowing an amino acid to coordinate while, moreover, the existence of ternary complexes with water that is not replaced by the coordinating amino acid, has been demonstrated.
Together, all these features of the Eu complex of **2.3** form exactly *what would be required for a well–behaved chiral shift reagent*[4].

2.2 *Chiral aqueous shift reagents based on menthyloxyfuranone*

Because of the feasibility of Eu^{3+} complexes of (*S,S*)–ethylenediamine–*N,N'*–disuccinic acid **2.3** as chiral aqueous shift reagents, it would be of great interest when (slightly) modified systems, having the same basic characteristics, were available for structure–chemical shift correlation studies. These basic characteristics are primarily determined by

[#] The difference in the formation constants for the diastereomeric amino acid complexes is probably responsible for the observed diastereomeric nonequivalence.

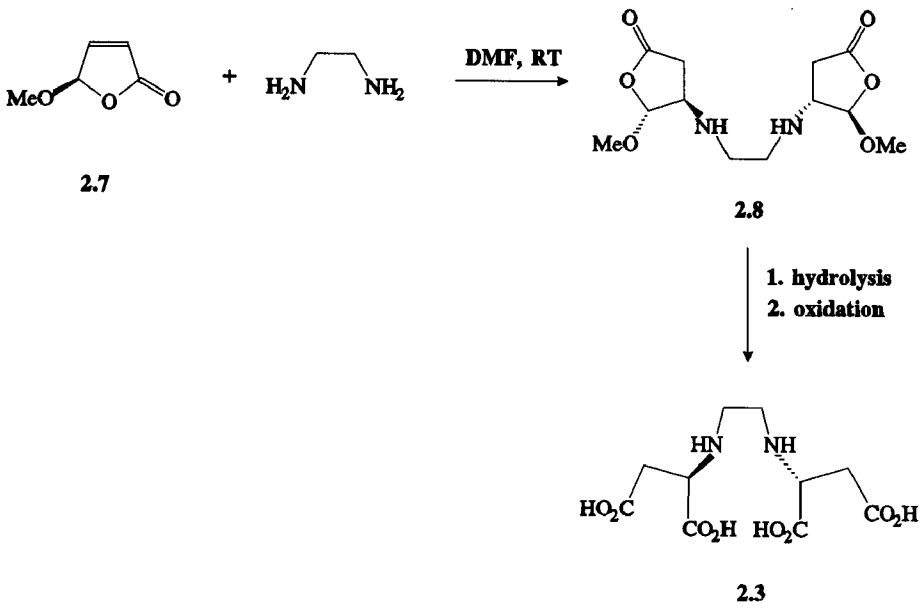
the four carboxylic acid and two nitrogen groups, affording an ideal chelating area for the lanthanide ion. Although the original synthesis as described by Major, and modified by Neal and Rose[5], from *L*-aspartic acid and dibromoethane guarantees the ready availability of the ligand, the synthetic methodology allows no easy structural differentiations in *e.g.* the ethylene bridge or both of the di-acid moieties. It would therefore be of importance when alternative synthetic routes were developed, allowing more structural flexibility.
An useful alternative would be the stereoselective, double 1,4-addition of a suitable diamine to a masked, α,ß-unsaturated di-acid **2.4**, as depicted in Scheme **2.2**. This would allow more structural freedom in the ethylene part, for instance the introduction of substituents and chirality in the bridging moiety is easily feasible. Several diamines that would be suitable for such an approach are readily available, like ethylenediamine, piperazine and 1,2-diamino-1,2-diphenylethane (stilbenediamine).



2.4 **2.5** **2.6**

Scheme 2.2 A possible route to bridged tetra acids as ligand systems

Since we were familiar with the 1,4-additions of amines and thiols to 5-alkoxy-2(5H)-furanones **2.7** (Scheme **2.3**) as described by de Lange and Feringa[13], the choice of the α,ß-unsaturated system is clear: only trans adducts are obtained in the 1,4-addition of various amines as reported[13], whereas hydrolysis and subsequent oxidation or direct hydrolytic oxidation would render the 1,4 substituted furanone systems **2.8** into the desired tetra-carboxylic acids **2.3**. In fact, the lactone system in **2.7** serves as a *masked* chiral aspartic acid moiety.
The most frequently used chiral 5-alkoxy-2(5H)-furanone system probably is 5-(menthyloxy)-2(5H)-furanone **2.9**, which is readily available in optically pure form using both 5-hydroxyfuranone and *l*- or *d*-menthol[14]. With this system, addition of amines is reported to proceed diastereoselectively[13], while subsequent reduction affords amino diols without racemization at the 2-position[13].
Although the proposed procedure seems to be rather straightforward, it harbours some potential problems. Firstly, the direct oxidation of a lactone system, such as **2.8**, into a di-acid under mild acidic conditions seems not to be a known procedure for the system used. Secondly, the presence of an amine moiety can easily lead to the oxidation of the amine functionality under these conditions. Thirdly, racemization at the stereogenic centers, which actually are the positions α to the carbonyl formed in the oxidation process, is very likely to take place.



2.7 **2.8** **2.3**

Scheme 2.3 A possible route to chiral tetra acids

2.2.1 *The synthesis of (R,R)-1,2-ethylenediamino-N,N'-disuccinate, (R,R)-1,2-diamino-bis-ethylene-N,N,N',N'-disuccinate and (S,S)-(1,2-(1S,2S)-diamino-diphenylethylene)-N,N'-disuccinate from 2-[5H]-menthyloxyfuranone*

When *l*-menthyloxyfuranone **2.9** was treated with ethylenediamine or piperazine, a nearly quantitative 1,4-addition took place at room temperature (as indicated by ^{1}H NMR) using CH_2Cl_2 or DMF as solvent, affording the ethylene and piperazine bridged bis-menthyloxyfuranone products **2.10** and **2.11**, respectively (Scheme **2.4**).

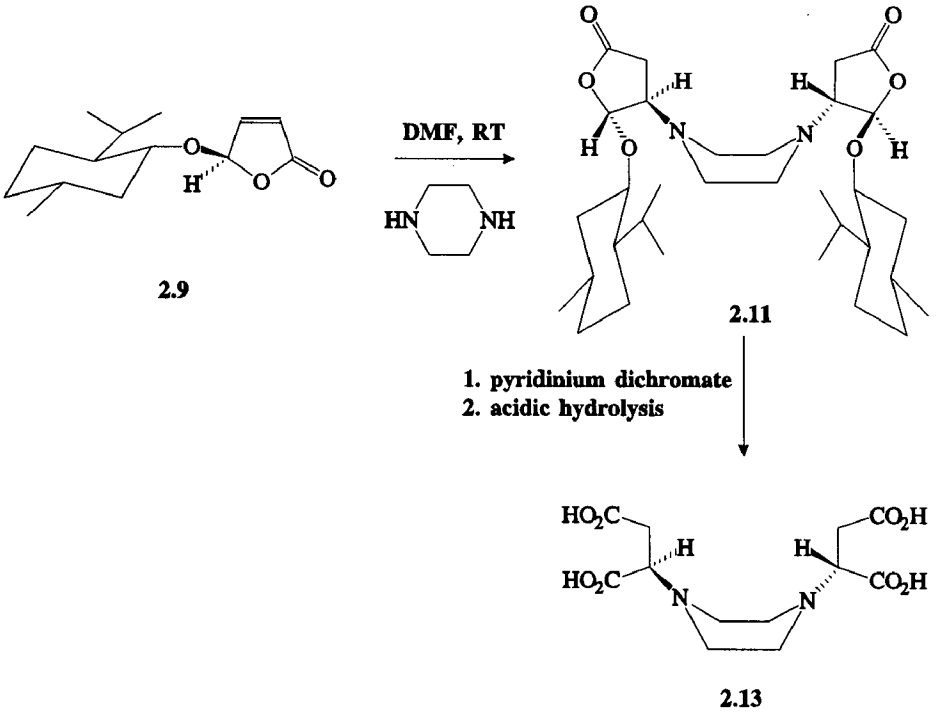
The reaction of stilbenediamine with *d*- or *l*-menthyloxyfuranone **2.9** proceeded more slowly, after 7 days at RT about 25% conversion was reached in DMF. The reaction is best performed using no solvent at all, giving product **2.12** in moderate yield (34%).

(*S,S*)-Stilbenediamine reacted in the 1,4-addition reactions only with *d*-menthyloxyfuranone **2.9**, probably because of severe steric hinderance by the two phenyl groups (both the furanone and the bisamine are chiral)[#].

In the reactions mentioned, a substantial amount of the cis-trans and even cis-cis addition products was formed, indicating that the amine 1,4-additions were not stereospecific, as previously reported[13,15].

Surprisingly, no traces of mono-substituted products were found. This unexpected result urged us to take a closer look at the stereospecificity of the 1,4-additions.

[#] The same observations were made by Drs Mariel Zwaagstra, unpublished results.

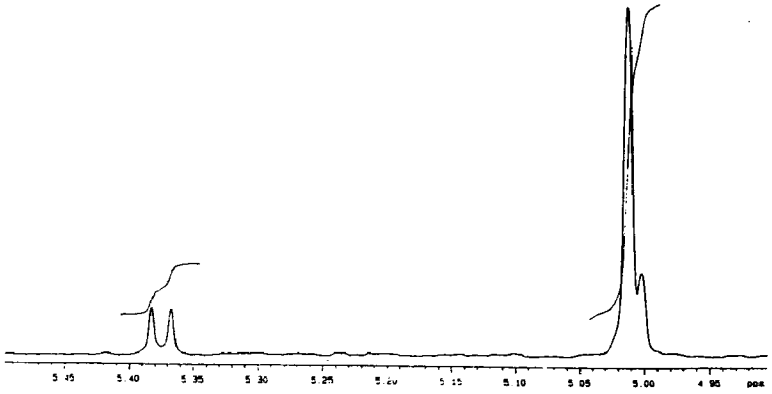


Scheme 2.4 The three step route to chiral tetra-acid 2.13

When *l*-menthyloxyfuranone **2.9** was allowed to react with benzylamine or α-phenyl-ethylamine ratios of between 90–10 and 95–5 (trans vs cis products) were found for both diastereomers according to the ^{1}H NMR#. For the products of ethylenediamine and piperazine typical ratios of 85–15 were found (trans–trans vs trans–cis), while no traces of cis–cis adducts were observed. The addition of (*S,S*)-stilbenediamine gave the most complex situation, with ratios of 65–29–6 for the trans–trans, trans–cis and cis–cis products, respectively. In Scheme **2.5** part of the spectrum of the crude reaction mixture of product **2.12** is given, clearly showing the three diastereomeric products.

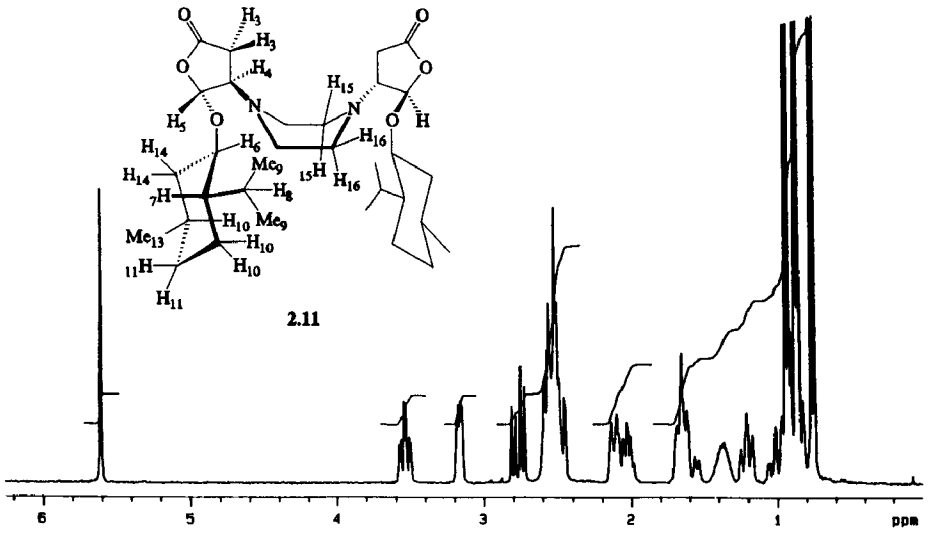
Contrary to the mono-amine 1,4-adducts, the bis-amine adducts are much less easily purified, except for adduct **2.11**, which appeared to be insoluble in DMF and crystallized spontaneously under the reaction conditions. Crystallization from ethanol (for **2.11**), or chromatography followed by crystallization from ethanol (for **2.10** and **2.12**) afforded the epimerically pure materials in 83, 71 and 34% yield, respectively.

The acetal proton usually gives two (or more) signals for the diastereomers. In case of the reaction mixture of adduct **2.12** deconvolution techniques were used to ensure proper quantification of the signals.

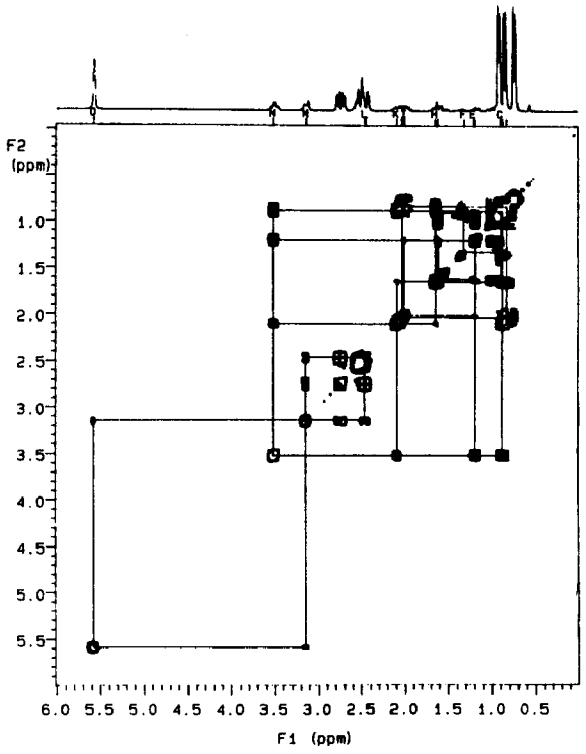


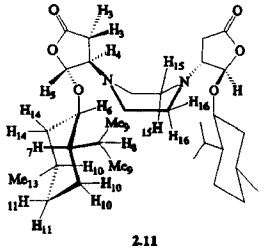
Scheme 2.5 Part of the ^1H NMR spectrum of crude 2.12 recorded in $CDCl_3$

Based on the very small coupling constants (^3J < 1.5 Hz) of the acetal proton of adducts **2.10**, **2.11** and **2.12** (indicating a trans arrangement of proton 5 and proton 4[16]), we conclude that the addition of the amines has taken place primarily from the less hindered side of the molecule. Unfortunately, it appeared not possible to obtain crystals suitable for X-Ray analysis. Hence, the trans configuration (and other structural information) of the main products were established by means of extensive 2D NMR studies (COSY, NOESY and HetCor).

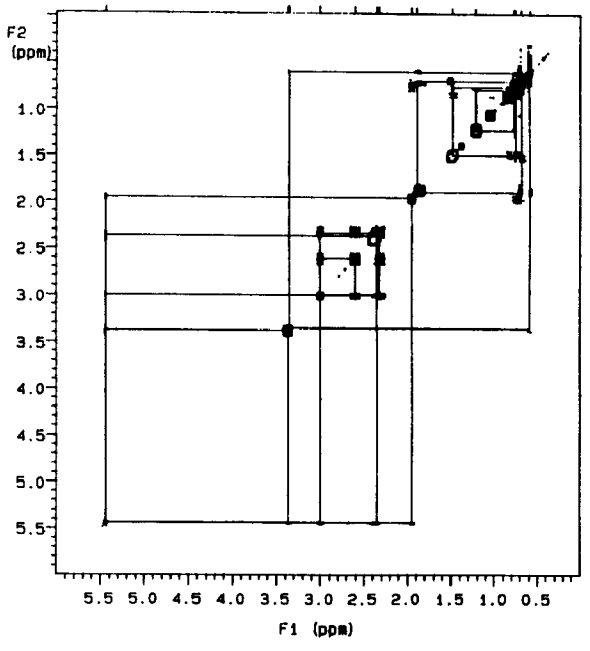


Scheme 2.6 The ^1H NMR spectrum of 2.11 recorded in $CDCl_3$



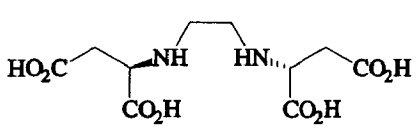


2.11

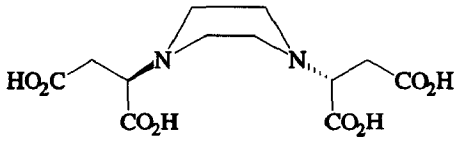


Scheme 2.7 The COSY and NOESY spectra of 2.11 recorded in $CDCl_3$

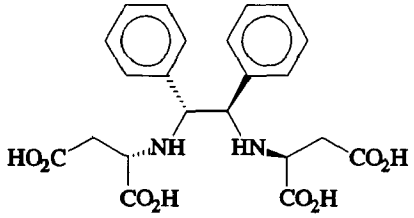
The ^{1}H NMR of **2.11** (Scheme **2.6**) clearly shows a doublet at δ 5.53 ppm (^{3}J= 1.46 Hz) for acetal proton 5, while the absorption at δ 3.47 ppm belongs to proton 6. Proton 4 at δ 3.10 ppm couples with three other protons: the acetal proton at δ 5.53 ppm (^{3}J= 1.46 Hz), the 3-cis proton at δ 2.81 ppm (^{3}J= 8.05 Hz) and the 3-trans proton at δ 2.41 ppm (^{3}J= 3.66 Hz). The 3-cis and 3-trans protons couple with each other ($^2J_{AB}$= 17.95 Hz). The protons of the ethylene bridges are located around δ 2.60 ppm, but the obtained resolution did not allow further assessment. The other absorptions belong to the menthyl part of the molecule, and are assigned in the experimental section.
The ^{1}H COSY spectrum (Scheme **2.7**) clearly shows the correlation between acetal proton 5 and proton 4. The protons 4, 3-cis and 3-trans are also correlated, as can be easily seen. Using the same methodology the menthyl group signals are also designated, showing little or no differentiation from the menthyl residue in menthyloxy-furanone itself (as in **2.10** and **2.11**, not in **2.12**, which shows large differences due to the anisotropic effect of the two aromatic systems).
From the ^{1}H NOESY experiment (Scheme **2.7**), the most important interactions of acetal proton 5 were designated; interactions with protons 6, 4, 14-equatorial and one of the ethylene bridge protons. At this stage it is not completely clear whether the interaction of proton 5 and 4 is due to a direct interaction within one menthyloxyfuranone moiety of the molecule or between the two parts of the molecule. For the further assignment of the NMR data of the ligands **2.10**, **2.11** and **2.12** we refer to the experimental section, where the numbering is used as shown in Scheme **2.6** for adduct **2.11**.



2.3



2.13



2.14

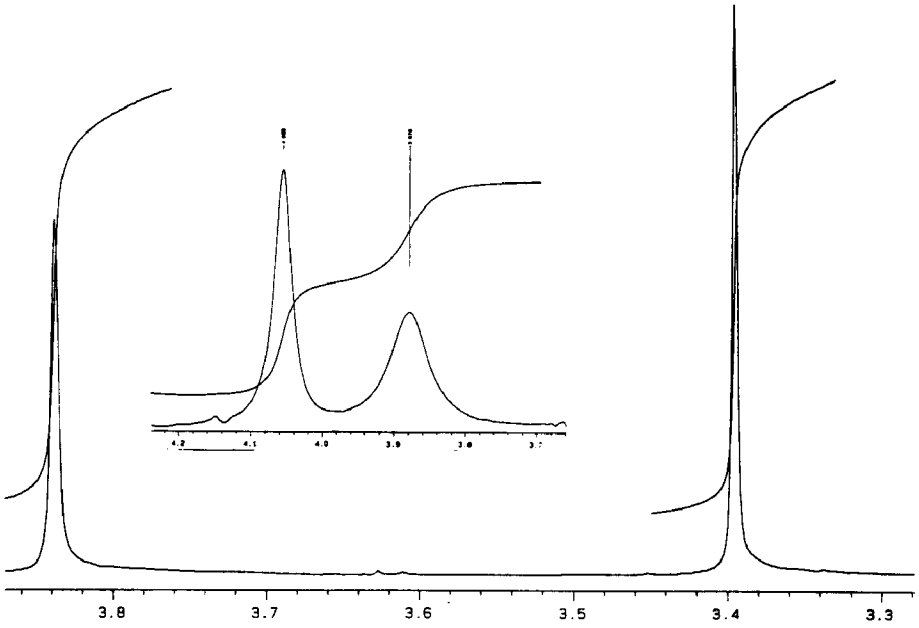
***Scheme 2.8** The three new ligand systems 2.3, 2.13 and 2.14*

Oxidation of the adducts **2.10**, **2.11** and **2.12** with pyridinium-dichromate in DMF or acetone and basic workup afforded the tetra-acids **2.3**, **2.13** and **2.14** respectively, in

moderate yield after crystallization from dilute acid[4,5]. The analytical data of (*S,S*)-**2.3** appeared to be in excellent agreement with the data of Kido and co-workers[4].
Attempts to prepare (*R,R*)-**2.11** (and subsequently (*R,R*)-**2.13**) by means of alkylation of (*R,R*)-**2.10** with dibromoethane were not successful, indicating that the methodology described is a very convenient one.

2.2.2 *N,N'- bridged disuccinate europium complexes as chiral shift reagents*

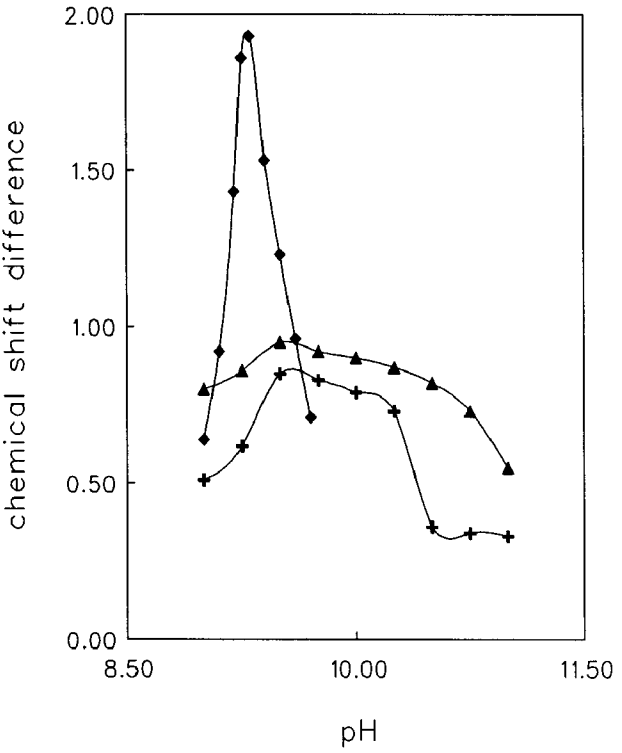
It seemed appropriate to determine the applicability of the europium derivatives of **2.13** and **2.14** as chiral shift reagents relative to (*S,S*)-**2.3**, which was already extensively documented in the literature[4]. Therefore, measurements were also performed using **2.3**, that was synthesized according to the procedure as described above.
For NMR studies, separate solutions were prepared of the amino acids and the Eu-complexes of **2.3**, **2.13** and **2.14**, and combined in the desired ratios. Each amino acid was dissolved in D_2O with an equivalent amount of NaOD. An appropriate amount of NaCl was added to keep the final NaCl concentration at 2 M after the combination with the Eu-**2.3**, **2.13** or **2.14** solutions. These were prepared by dissolving the desired ligand in D_2O with an appropriate amount of NaOD and adding the solution so obtained to a D_2O solution of $EuCl_3.6H_2O$, according to the method as described by Kido and co-workers[4].



Scheme 2.9 *^{1}H NMR spectra of D_2O solutions (0.02 M) Eu-2.3 (inset) and Eu-2.13 with d,l-phenylglycine*

The ^{1}H NMR spectra of 0.1 M D_2O solutions of *d,l*-phenylglycine in the presence of 0.02 M Eu-ligand **2.3** and **2.13** (inset) are given in Scheme **2.9**, clearly showing the larger

upfield shift of the α-proton of phenylglycine for the *L* isomer relative to the *D* isomer when Eu-(*S,S*)-**2.3** or Eu-(*S,S*)-**2.13** complexes are used.
The magnitude of the lanthanide-induced shift was found to increase with the europium-complex : phenylglycine ratio, although the line broadening increased also, making a correct determination of the resonance position impossible at higher concentrations.
The pH dependence of the induced chemical shift by the Eu-complexes was investigated using *d,l*-phenylglycine as the substrate molecule, at $[S_0]$= 0.1 and [L]= 0.02 M typically. Using Eu-(*R,R*)-**2.3** the resonances of the α-proton of *d,l*-phenylglycine were shifted upfield, giving the largest shift difference for *l*-phenylglycine and a somewhat smaller shift difference for *d*-phenylglycine. Both induced chemical shifts Δδ showed a large dependence upon the pH of the solution (Scheme **2.10**) in the pH range 9-11 with a bell shaped profile having a maximum induced chemical shift Δδ at pH 9.5-10.



Scheme 2.10 *pH dependence versus chemical shift difference Δδ of the europium complexes of 2.3 (+), 2.13 (▲) and 2.14 (♦) with D-phenylglycine*

The sharp decrease in Δδ on both the acidic and the basic side probably corresponds to oligomerization and hydroxo complex formation, respectively, of the Eu–(*R,R*)–**2.3** complex[12]. The Eu–(*R,R*)–**2.13** complex showed the same type of behavior, also giving a maximum induced chemical shift Δδ at pH 9–10, although the bell–shaped dependence is much less outspoken.

For the Eu–(*S,S*)–**2.14** system the situation is more complex, primarily due to the limited solubility of this complex as a function of the pH. The Δδ maximum is found to be located at pH 9.0–9.5, with a sharp decrease at both sides of the pH curve, though this decrease is probably due to limitations in solubility, and not to the factors noted before. This behavior makes it impossible to compare ligand **2.14** with the ligand systems **2.3** and **2.13**, that do not show such a large solubility restriction. Since all solutions were turbid in the pH range below 9 and above 11, experiments could only be conducted in the pH range 9–11 (measurements at pH 10), except for the experiments using **2.14**, where all measurements were performed at pH 9.25.

The ^{1}H NMR data permitted the determination of the formation constants for the ternary complexes, assuming that only 1:1 Eu–ligand : phenylglycine complexes are formed[17,18], with equilibrium constant *K*;

$$S_0 = (L_0 + [LS]^2/S_0)(\Delta_b/\Delta\delta) - (L_0 + 1/K) \qquad \text{eq } \mathbf{1}$$

In eq **1**, Δ_b is the bonded shift of the formed complex and L_0 and S_0 represent the total concentration of lanthanide shift reagent and substrate. The observed chemical shifts of the nucleus in the presence and absence of shift reagent are given by δ_{obs} and δ_0, respectively. At (very) low lanthanide to substrate ratios, $[LS]^2/S_0$ must be negligible in comparison with L_0 and eq **1** simplifies to eq **2** and **3**;

$$S_0 = (L_0\Delta_b/\Delta\delta) - (L_0 + 1/K) \qquad \text{eq } \mathbf{2}$$

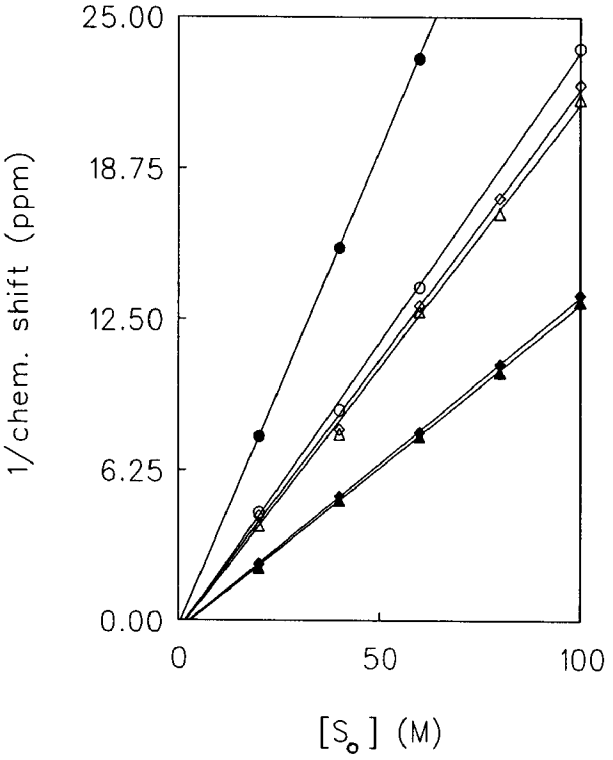
$$1/\Delta\delta = \{(S_0 + 1/K)/\Delta_b\}(1/L_0) + 1/\Delta_b \qquad \text{eq } \mathbf{3}$$

A plot of the reciprocal of the induced shift Δδ against the substrate concentration S_0 (at a constant lanthanide concentration) affords both the bonded shift (slope/L_0) and the association constant [−1/(intercept + L_0)]. In Scheme **2.11** the plots are given for the three europium ligand systems used, at $[L_0] = 0.03$ M, with both *d*– and *l*–phenylglycine.

For the Eu–(*R,R*)–**2.3** system it was found that the data were in good agreement with the data as determined by Kido and co–workers[4]. The association constant for the *L* isomer (K_L= 5.9) was found to be greater than that for the *D* isomer (K_D= 3.5), while the bonded shift for the *L* isomer (Δ_{bL}= 7.2) was also greater than that for the *D* isomer (Δ_{bD}= 4.4). When Eu–(*R,R*)–**2.13** was used as shift reagent, these data were not altered in sign, but only in magnitude; K_L= 6.2 and K_D= 3.7 whereas Δ_{bL}= 7.4 and Δ_{bD}= 4.5. Due to solubility limitations, complex Eu–(*S,S*)–**2.14** could not be investigated with the same degree of

accuracy as the two other complexes: the data were determined to be K_L= 3.2, K_D= 5.4, Δ_{bL}= 7.9 and Δ_{bD}= 13.1, respectively.

These data clearly indicate that for the systems Eu-(*R,R*)-**2.3** and Eu-(*R,R*)-**2.13** the ternary complexes with the *L* isomer of phenylglycine are geometrically more favorable, resulting in more stable complexes. For the Eu-(*S,S*)-**2.14** system the situation is not clear, the formation constants are smaller compared to the other two systems, but the observed shift differences are larger. This phenomenon is probably related to the anisotropy effect of the aromatic ring systems, inducing an additional shift-inducing factor with regard to the observed shift differences in comparison with the two other systems.



Scheme 2.11 *for L-phenylglycine; Eu-2.3 (△), 2.13 (◇), 2.14 (○); for D-phenylglycine; Eu-2.3 (▲), 2.13 (◆), 2.14 (●)*

Some of the results on the diastereomeric shift differences of various racemic amino acids using the europium-complexes **2.3**, **2.13** and **2.14** are summarized in Table **2.1**. Typically, [S_0]= 0.1 M and [L]= 0.02-0.03 M at pH 10 or 9.25, were used for the measurements (see forgoing discussion for details). Phenylglycine, used as the test substrate, is spectroscopically the most simple chiral amino acid, and the signals of the α-proton are readily

resolved using one of the three ligand systems, with Eu–(*S,S*)–**2.14** giving the largest ΔΔδ values. Also using alanine as the substrate, resolved signals could be obtained with all the three europium complexes giving signal separation for the ß protons; the α–proton signals appeared as quartets, which could not be easily assigned. Again, Eu–(*S,S*)–**2.14** gave the largest shift differences ΔΔδ.

substrate	Eu–2.3 (ppm)	Eu–2.13 (ppm)	Eu–2.14 (ppm)
phenylglycine	0.41 (α)	0.51 (α)	1.31 (α)
alanine	0.08 (ß)	0.14 (ß)	0.31 (ß)
serine	0.19 (α)	0.24 (α)	0.43 (α)
threonine	0.27 (α)	0.37 (α)	0.54 (α)
phenylalanine	0.36 (ß)	0.33 (ß)	1.56 (ß)
tyrosine	0.38 (ß)	0.34 (ß)	1.53 (ß)
tryptophan	0.21 (ß)	0.17 (ß)	0.89 (ß)
proline	0.08 (α)	0.16 (ß)	0.23 (ß)
lysine	0.15 (α)	0.23 (α)	0.48 (α)
histidine	0.25 (ß)	0.31 (ß)	0.74 (ß)
valine	0.18 (α)	0.26 (α)	0.52 (α)
α–Me–PG	0.14 (ß)	0.27 (ß)	0.89 (ß)
α–Me–Phe	0.19 (ß)	0.29 (ß)	0.64 (ß)
α–allyl–PG	0.12 (ß)	0.17 (ß)	0.93 (ß)
α–Me–Val	0.23 (ß)	0.25 (ß)	0.69 (ß)
α–allyl–Ala	0.20 (ß)	0.22 (ß)	0.99 (ß)

Table 2.1 *The ΔΔδ values of several amino acids compared for the systems Eu–2.3, Eu–2.13 and Eu–2.14 (nucleus with greatest Δδ), [S_0]= 0.01 M*

Phenylalanine also yielded the best results when Eu–(*S,S*)–**2.14** was used, giving the largest shifts for the ß–protons of the *D* isomer, although, due to line broadening (and probably π–stacking), no assignment could be made for the phenyl protons as in the two other systems. When α–alkylated amino acids were used, the line broadening was extensive, so that the assignment could not always be made, regardless of the europium ligand system used. Also when primary amino acid–amides were used, these being precursors for several amino acids, no assignment could be made. In these cases the europium ion was probably transferred to the primary amide entity, giving rise to severe

line broadening. Furthermore, it appeared not to be possible to identify definite trends in the observed ΔΔδ values in relation to the structural elements of the amino acid.
In Table **2.2**, several ratios of racemic and partially enriched amino acids, as determined using the three Eu–ligand complexes, are compared with the data obtained by the α-chloropropionyl chloride method[19] and the sec-butylphosphite method[20] and are in excellent agreement. Although the systems **2.13** and **2.14** are now readily available, the results of their use as chiral shift reagents are not impressive in comparison with the known Eu-(*S,S*)-**2.3** complex.

substrate	measured ratio	α-chloropropionyl chloride[19]	phosphite[20]
Eu-2.3			
phenylglycine	74:26	74.7:25.3	74.4:25.6
alanine	63:57	64.1:35.9	64.2:35.8
α-Me-PG	49:51	49.5:50.5	49.7:50.3
Eu-2.13			
phenylglycine	74.2:25.8		
alanine	64:36		
α-Me-PG	49:51		
Eu-2.14			
phenylglycine	74.1:25.9		
alanine	63:57		
α-Me-PG	49:51		

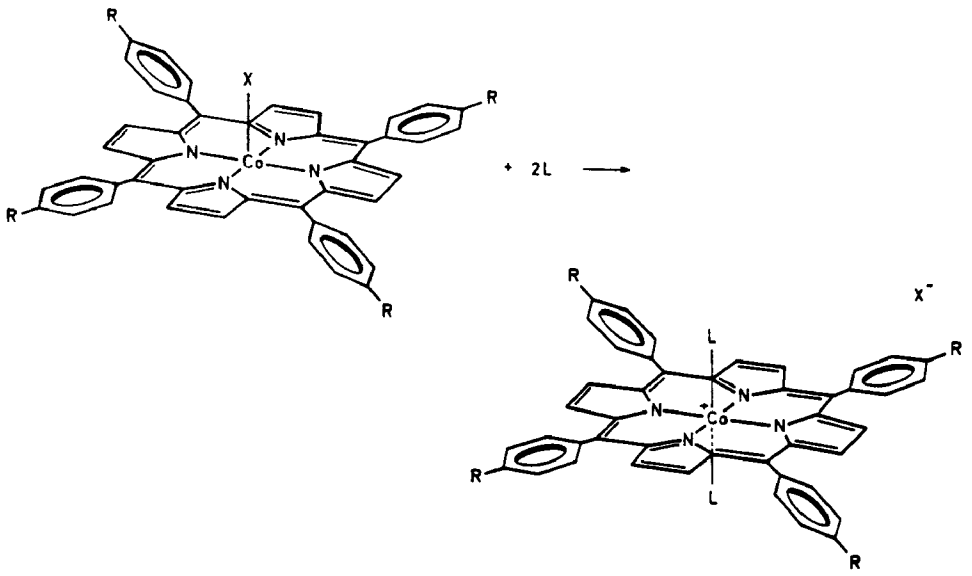
Table 2.2 *The observed diastereomeric ratios as determined by means of the Eu-complexes compared with the data obtained by other methods[19,20]*

The observed ΔΔδ values for the Eu-(*R,R*)-**2.13** are comparable with the known system Eu-(*S,S*)-**2.3**, although the pH dependence of the observed Δδ values is not that large, making this system more easy in use. The rather large pH dependency of Eu-(*R,R*)-**2.14** makes this system less easy to handle, although the observed chemical shift differences ΔΔδ are far superior compared to the other two systems.
Using this methodology, however, both enantiomers of the ligand systems **2.3**, **2.13** and **2.14** are readily available from *d*- or *l*-menthyloxybutenolide **2.9**. Furthermore, the followed synthetic route offers the flexibility to introduce substituents and, if desired, additional chirality into the bridging ethylene moiety.

2.3 *Cobalt^{III}-porphyrin systems as shift reagent*

The extensive studies of metal porphyrin complexes have provided not only a potential new class of metal-centered probes and mimics for the study of biological systems, such as enzymatic systems (like vitamin B_{12}), heme proteins and cytochrome P-450[21], but also gave rise to their development as shift reagent for water (!) and anionic and cationic substrates[22].

Especially Co^{III}-porphyrin systems proved to be useful as shift reagent, because of their complexing power (*e.g.* towards amines) as well as the fact that the cobalt porphyrin systems, unlike the naturally occurring porphyrins, do *not* possess planar chirality. These systems can form complexes possessing two nitrogeneous ligands in the axial positions (Scheme **2.12**). This feature makes it possible, owing to the slow ligand exchange on the NMR time scale in the six-coordinate complex, to observe in the ^1H NMR spectrum both diastereomeric complexes that will be formed when using a racemic amine (*i.e. RR* or *SS* and *RS* or *SR*)[23], according to the principle introduced by Horeau[24] (for a discussion, see Chapter 1).



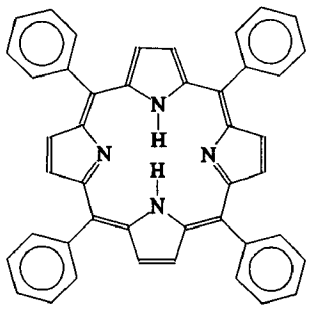
Scheme 2.12 Co^{III}-porphyrin system as shift reagent for amine (L) containing substrates

The complex of *R* amines with Co^{III}-porphyrin, is optically active and exhibits planar chirality through the porphyrin ring plane, whereas the meso *RS* adduct is optically inactive due to the pseudo-chirality or pseudo-asymmetry about the porphyrin ring plane[25].

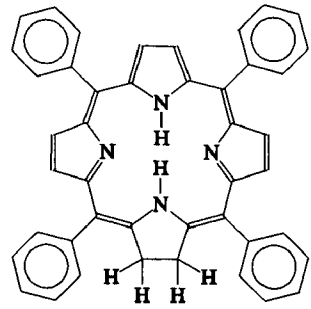
These features should make it possible to use Co^{III}-porphyrin systems as shift reagents for amine containing chiral compounds[23]. Abraham and co-workers[23] were able to determine the enantiomeric composition of α-phenylethylamine and levamisole using this simple methodology, using chloroform as solvent. Although the observed shifts are usually very large (in the order of several ppm's), the ΔΔδ values between the diastereomers, being around 0.005 ppm typically, are only of significance when high field strengths are applied. In view of the feasibility of this method, we wondered whether it would be possible to use the same methodology to determine the enantiomeric composition of amino acids in polar solvents like water or water-alcohol mixtures using *water-soluble* Co^{III}-porphyrin systems#.

2.3.1 *The synthesis of water-soluble porphyrins*

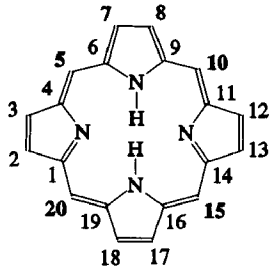
About 50 years ago, Rothemund[26] was the first to synthesize tetraphenylporphyrin $H_2(TPP)$[27##] **2.15** by reaction of benzaldehyde and pyrrole in pyridine, using a sealed vessel in the absence of oxygen at 140-220 °C for 24 hours or longer.



2.15



2.19



Scheme 2.13 Structures of tetraphenylporphyrin 2.15 and chlorin 2.19. The numbering of the porphyrin skeleton is as given

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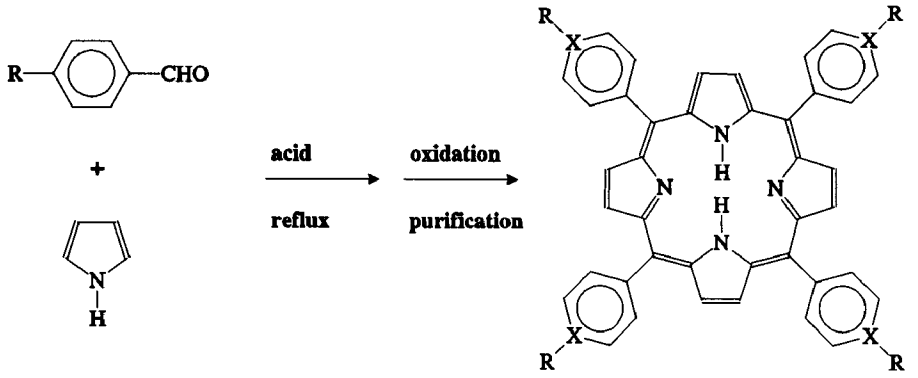
Amine complexes of Co^{III}-porphyrin systems are unaffected when dissolved in polar solvents such as methanol or DMSO[18].

Several systems of nomenclature exist, see ref 27 for the IUPAC convention.

The yields were very low, and several new synthetic routes have been developed; the Adler[28], Badger[29] and Lindsey[30] variations being the most important improvements. Nonetheless, several problems are connected with the synthesis of porphyrin systems. The yields are usually low (between 5 and 20% for the free base products), while contamination with the corresponding tetraphenylchlorin (2,3-dihydroporphyrin) can give rise to problems. Also, considerable amounts of polymeric products are usually obtained, making the purification time consuming and sometimes impossible.



2.15 X= C; R= H, Me, COOH, OMe

2.16 X= C; R= SO_3H **2.17** X= N

Scheme 2.14 Synthesis of tetraphenylporphyrin systems 2.15 and 2.16

Being aware of these difficulties, we decided to use the Adler protocol for all the syntheses and subsequently perform a reaction with DDQ[31] to oxidize the formed chlorin. The insertion of Co^{II} followed by the oxidation to Co^{III} using known techniques[32] would convert the porphyrin systems to the desired Co^{III}-porphyrin shift reagents.

Reaction of benzaldehyde and pyrrole in propionic acid afforded tetraphenyl-porphyrin **2.15** (R= H) in good yield (18%)[32]. By the same strategy, using *4*-pyridinecarboxaldehyde and *4*-carboxybenzaldehyde, the porphyrin systems **2.17** and **2.15** (R= CO_2H) were readily synthesized[33]. The meso-tetra-(*p*-sulfonatophenyl)porphyrin **2.16** was synthesized from **2.15** (R= H) by means of a sulfonation reaction using fuming sulfuric acid followed by a rather laborious purification to remove mono-, di- and trisulfonated products[34,35].

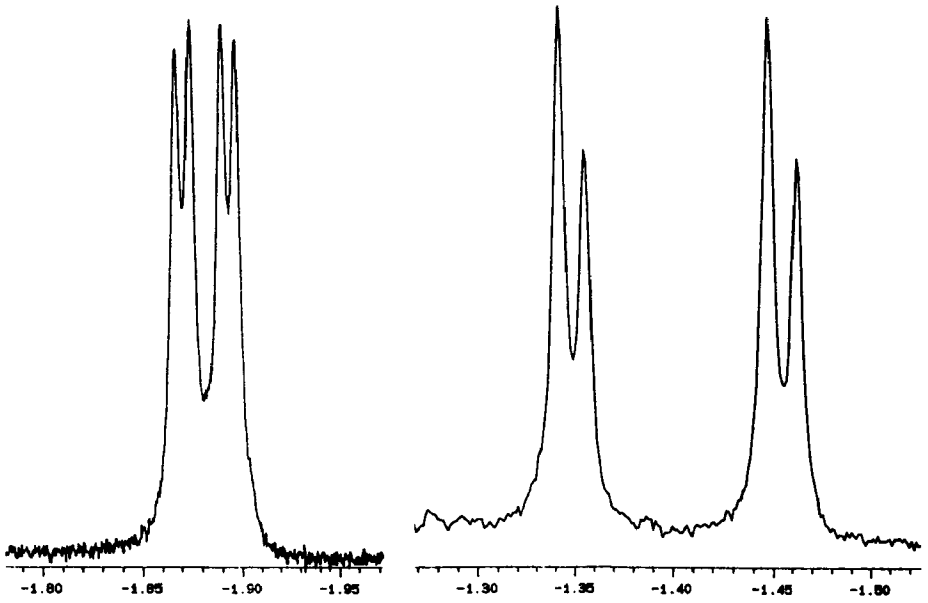
The insertion of Co^{II} by reaction with Co^{II}-acetate in refluxing acetic acid was monitored by UV/VIS spectrometry and proceeded nearly quantitatively. Subsequent oxidation with concentrated HBr, while oxygen was passed through the solution, afforded the desired Co^{III}-porphyrin systems in reasonable yield (50%), which were purified by means of chromatography[32]. The purification of these systems is very important. Only Co^{III}, Co^{I}, Co^{0} and Co^{-I} compounds are leading to high resolution NMR spectra while traces of Co^{II} give

rise to a severe line broadening in the ^1H NMR spectra. Therefore, removal of all Co^{II} components is essential. The Co^{III} containing porphyrin systems were characterized by means of ^1H NMR, ^{59}Co NMR and UV/VIS spectroscopy.

2.3.2 *Co^{III}-porphyrins as shift reagents for amines, amino alcohols and amino acids*

Upon the admixture of Co^{III}-**2.15** (R= H, 0.1 mmole) and *d,l*-α-phenylethylamine (0.2 mmole) in $CDCl_3$ (2 mL) several proton resonances in the ^1H NMR spectrum separated, due to the formation of diastereomeric association complexes, as is clearly shown in Scheme **2.15**.

The separation of the *RS* and *RR* adducts is not very large, as expected, and in fact the only signal separation of analytical importance is the methyl resonance signal, giving two doublets at δ −1.88 ppm with a ΔΔδ of 0.007 ppm, in accordance with the results of Abraham and co-workers[23].



Scheme 2.15 ^1H NMR spectra of Co^{III}-2.15 (R= H, 0.1 mmol, $CDCl_3$) and Co^{III}-2.16 (0.05 mmol, D_2O/Na_2CO_3) and amine containing ligands

The enantiomeric excess of several amino alcohols and esters of amino acids was determined using this system; the results are collected in Table **2.3**. Typical concentrations used for this type of experiments are in the order of [L]= 0.05 to 0.1 mmole with a substrate concentration of [S]= 0.1 to 0.2 mmole.

The measurements appear to be in good agreement with those obtained by Abraham and co-workers, although they applied a somewhat higher field strength (400 MHz instead of 300 MHz[23]). As was stated by these authors, the substrate protons that are closest to the

complexation site and which are expected to be most sensitive to the nature of the apical ligands, do not show any separation at the field strength used, including the NH and δ–CH protons.

Because of solubility reasons only the *water–soluble* Co^{III}–**2.16** system was used for enantiomeric excess analysis of amino acids. The two other systems are not sufficiently soluble to permit reasonably fast measurements, due to the long pulsing times required.

For nonracemic alanine, using Co^{III}–**2.16** two doublets were obtained for the methyl group, situated at δ –1.39 ppm, although the exact position is somewhat pH dependent, giving shift deviations of about Δδ 0.5 ppm over the pH range 8–12. All the further measurements were performed at constant pH 10.0 (using Na_2CO_3 as a base).

The other determinations nearly always show a diastereomeric peak separation, although the shift differences are in most situations relatively small.

In Table **2.3** the ΔΔδ of several amino acids, α–alkylated amino acids and amino alcohols are collected. Sufficient resolution is obtained to allow analysis, especially when α–alkylated amino acids are used. Using Co^{III}–**2.16**, solubility restrictions appear to be strongly substrate dependent, and hence shifts are strongly variable.

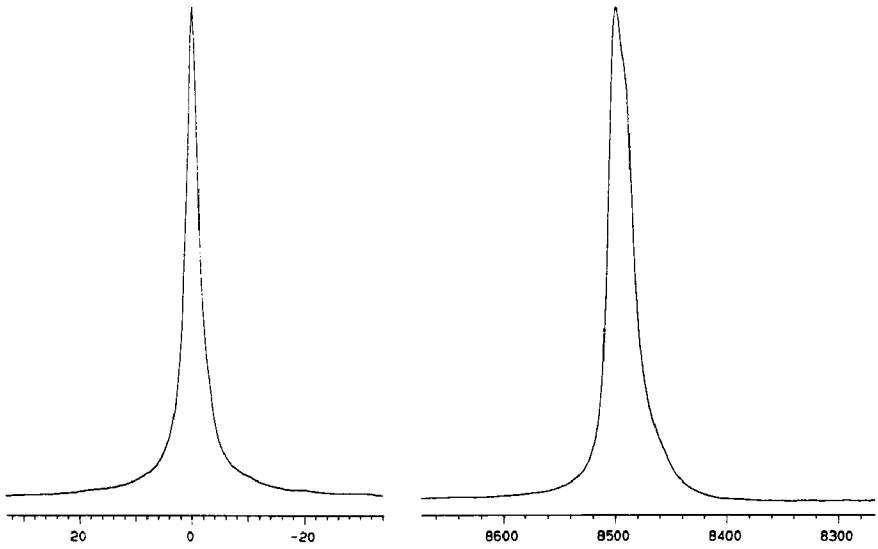
substrate	Co^{III}–2.15 R=H	Co^{III}–2.16
alanine		0.06 (ß)
phenylalanine		0.08 (ß)
valine		0.06 (γ)
serine		0.04 (ß)
phenylglycine		0.02 (α)
tyrosine		0.09 (ß)
α–Me–PG		0.11 (ß)
α–Me–Phe		0.09 (ß)
α–allyl–PG		0.12 (ß)
α–ethylphenylamine	0.05 (ß)	
valinol	0.07 (γ)	
ephedrine	0.11 (ß)	

Table 2.3 *The ΔΔδ values of racemic amine derivatives using Co^{III}–2.15 (R= H, $CDCl_3$) and Co^{III}–2.16 (D_2O/Na_2CO_3)(nucleus of interest)*

For alanine, phenylglycine, α-methyl-phenylalanine and valinol the diastereomeric ratios were compared with those obtained with the α-chloropropionyl chloride[19] and sec-butylphosphite methods[20], and appeared to be in good agreement (within 2%).
It is clear, however, that only those substrates giving simple resonances (at least for some protons) in the ^{1}H NMR spectrum, as is shown for the Co^{III}**-2.15** (R= H) system, show enough resolution to allow analysis of the diastereomeric ratios. This restriction is quite common when shift reagents are used, because the diastereomeric shift differences are usually observed in the signals belonging to the substrate. This, however, urged us to investigate ^{59}Co NMR as an alternative for ^{1}H NMR.

2.3.3 *^{59}Co NMR; an useful alternative for ^{1}H NMR ?*

A Co^{III}-porphyrin system bearing two apical ligands, like an amine or amino acid, normally possesses only three different NMR active nuclei: the ^{1}H, ^{13}C and ^{59}Co nuclei.
The ^{1}H resonances are often split into multiplets, making this option sometimes useless as stated above. The ^{13}C nucleus is not an attractive one, long acquisition times are needed to obtain spectra of sufficient quality, while also long relaxation times are required to ensure complete relaxation. Also, for porphyrin systems some of the ^{13}C nuclei give rise to broad signals in the NMR spectrum.
The only nucleus that should in fact give a single or double resonance signal when substituted by *e.g.* a racemic amine is the ^{59}Co nucleus.



Scheme 2.16 *^{59}Co NMR spectra of $K_3Co(CN)_6$ ($CDCl_3$) and Co^{III}-**2.16** complexed with racemic alanine (D_2O/Na_2CO_3)*

Many Co^{III}–porphyrin systems have been studied using ^{59}Co NMR techniques[36,37], and it has been shown that the observed chemical shift δ and linewidth $\omega_{1/2}$ data can be used to probe the interaction between the central metal and substituents on the porphyrin and axial amine ligands. The ^{59}Co nucleus appears to be very sensitive to temperature changes, impurities (like traces alcohol in nonpolar solvent systems), solvents used and the complexing axial ligands on the Co^{III} nucleus[37], this last point being of great importance for the present discussion.
In Scheme **2.16** the ^{59}Co NMR spectra are given of $K_3Co(CN)_6$ (the reference material) and of Co^{III}–**2.16** complexed by racemic alanine. As can be seen, no diastereomeric separation was found using this porphyrin system. Also when other axial ligands were tested, no diastereomeric differentiation was observed. It is, however, not completely understood whether the signals cannot be seen separately because the $\Delta\Delta\delta$ values are too small in comparison with the large line broadening or that the shifts are too big to capture them in one window, being primarily a technical problem.
We might conclude that ^{59}Co NMR is not an useful alternative for the ^1H NMR techniques when enantiomeric excess determinations are to be performed, mainly due to the fact that broad window NMR equipment is not a standard facility in most laboratoria.

2.4 *Conclusions*

Although water–soluble Eu^{III} shift reagents, as described by Kido and co–workers[4] are probably the most promising at the moment, the restricted *synthetic* freedom to alter the systems must be seen as a disadvantage.
As shown in Chapter 2.2, we succeeded in developing a new synthetic route that easily garantees the availability of new chiral tetra–acids **2.3**, **2.13** and **2.14** from menthyloxy–furanone **2.9**. The Eu^{III} complexes of the tetra–acids **2.3**, **2.13** and **2.14** appear to be suitable as chiral shift reagents for amino acids in water systems, although they appear to be only slightly better than the known system **2.3** (as for **2.13**). Using system **2.14**, the use is limited to a rather restricted pH domain, although the observed chemical shift differences $\Delta\Delta\delta$ are far superior compared to known systems. The methodology used, the synthesis from menthyloxyfuranone **2.9**, tolerates structural variations, contrary to the methodology presented by Kido and co–workers[4]. Although the results as described are not as promising as we had hoped, further research using other (chiral) bisamines to synthesize chiral tetra–acids probably will be successful.
In Chapter 2.3 the synthesis and use of Co^{III}–porphyrin systems **2.15**, **2.16** and **2.17** is described. Using both systems we were able to determine the enantiomeric composition of a number of amine containing substrates, like amines, amino alcohols and amino acids in $CDCl_3$ and D_2O. To the best of our knowledge, Co^{III}–porphyrine systems have never been used before in the enantiomeric excess determination of amino acids in water containing solvent systems. The use of ^1H NMR techniques, however, limits the utility of this approach.
We therefore tried to make use of ^{59}Co NMR techniques, which appeared not to be successful in fulfilling the goals we were aiming at.

2.5 *Experimental Section*

General remarks

Melting points (uncorrected) were determined on a Reichert Melting Point apparatus equipped with a microscope. Infrared spectra were recorded on a Perkin Elmer 257 Grating Spectrophotometer or on a Mattson Instruments 4020 GALAXY Series FT-IR, equipped with a Hewlett-Packard 7550 Graphics Plotter. Rotations were measured on a Perkin Elmer 241 MC polarimeter at room temperature at the Na D-line, under the conditions as defined. The UV spectra were recorded on a Perkin Elmer λ 5 UV/VIS spectrophotometer. The pH determinations were done on a Radiometer Copenhagen PHM 82 standard pH meter, using a Corning calomel combination electrode. Buffer solutions (pH 4.0, pH 7.0 and pH 10.0) were used to calibrate the pH meter before each series of measurements.

Mass spectra were recorded on a AEI-MS-902 mass spectrometer by EI (acc. voltage 8 kV, voltage 70 eV) by Mr. A. Kiewiet. Elemental analyses were performed in the Microanalytical Department of this laboratory by Mr. H. Draayer, J. Ebels, J.E. Vos and J. Hommes.

All NMR spectra were taken thermostatted at 30° C (± 0.1° C), unless stated otherwise, using the Varian temperature control unit. ^{1}H and ^{13}C NMR (APT) spectra were recorded on a Varian VXR-300 spectrometer at 300 and 75.43 MHz respectively. Proton spectra were generated using 32768 data points (zero filling was used) with a line broadening used of 0.01 typically. Chemical shifts are denoted in δ-units (ppm) relative to the solvent and converted to the TMS scale. The splitting patterns are designated as follows: s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), m (multiplet) and br (broad). Chemical shifts (in ppm) are positive in lowfield direction.

When references are made towards the text designation follows (multiplicity, signal form, *number in the text*, number of protons).

^{1}H decoupled ^{31}P NMR spectra were recorded on a Varian VXR-300 spectrometer operating at 121.42 MHz. The chemical shifts are denoted either relative to $(NPCl_2)_3$ at δ 19.91 ppm (for the solvents $CDCl_3$ and C_6D_6) or to phosphoric acid at δ 0 ppm (for the solvents D_2O, DMSO-d_6 and CD_3OD).

^{59}Co NMR spectra were recorded on a Varian VXR-300 spectrometer at 71.17 MHz. Maximum sw was used (sw= 100000) with a typical d1= 0 s and a pulse width of 15 μsec which corresponds to a pulse angle of 90°. Shifts were denoted relative to $K_3Co(CN)_6$ at δ= 0 ppm and recalculated from the tuning shift necessary to cover the entire spectral range (≈ 30000 ppm).

The experiments with lanthanide shift reagents were performed on a Varian Gemini-200 system, operating at 200 MHz for ^{1}H NMR, using a d1= 5 s for optimal recovery. This system was also thermostatted at 30° C (± 0.1° C) using the Varian temperature control unit.

Before measuring the 2D spectra the exact 90° pulse width was determined. T_1 relaxation times were calculated with the inversion recovery method, using a standard Varian microprogram. All the COSY and NOESY spectra were recorded at 30° C (± 0.1° C) on the Varian VXR-300 spectrometer. The shift correlated 2D NMR (COSY) spectra were recorded using standard Varian microprograms. The data matrix used was 2048x2048 and the transformations were carried out with sine-bell window functions in both dimensions

and zero filling in the F_1 dimension. After a careful analysis of the non-symmetrized spectrum symmetrization was performed on the final data matrix.
NOESY (incoherence transfer) spectra were recorded using a data matrix of 2048x2048 which was processed using shifted sinusoidal multiplication in each dimension and was not further symmetrized.
Both spectrometers were operating in the Fourier transform mode using the ^2H resonance of the solvent as field-frequency lock.
DMF was distilled from CaH_2 and stored over 3 Å mol sieves. Ether, CH_2Cl_2, hexane and pentane were distilled from P_2O_5 and stored over 3 Å mol sieves. $CHCl_3$ was dried by means of column chromatography (Al_2O_3 80, basic, act I-III) and stored over 3 Å mol sieves after a few drops of ethanol were added. Toluene and benzene were distilled from Na (benzophenone) under a N_2 atmosphere and stored over Na wire. THF was distilled just prior to use from $LiAlH_4$ under a N_2 atmosphere. Methanol and ethanol were distilled from Mg turnings and stored over Na wire. Et_3N was stored on KOH pellets. HMPT was distilled under a N_2 atmosphere and used directly in synthesis.
Deuterated solvents for NMR were dried by converting them through a small column (Al_2O_3) into oven dried (180° C) NMR tubes, except for D_2O that was used as such. NaOD was prepared just prior to use by reacting Na with D_2O. The concentration was determined by titration. All other commercially available chemicals were used without further purification.

***5-Hydroxy-2(5H)-furanone*[38]**
A solution of freshly distilled furfural (50.0 g, 0.50 mol) in methanol (300 mL) was photooxygenated using a few milligrams of methylene blue as sensitizer. A stream of oxygen was introduced through a glass filter (P2) into the reaction vessel[#]. The reaction was followed by ^1H NMR, taking samples from the solution at regular intervals. When all furfural had reacted, the mixture was taken to dryness and 5-hydroxy-2(5H)-furanone was obtained as an oil, which solidified upon standing. This material was crystallized from butyl acetate, affording a white crystalline material. Yield 39.5 g (0.38 mol, 76%).
Mp 57.1-59.6 °C (lit[38] 58.0-60.0 °C); ^1H NMR ($CDCl_3$): δ 5.71 (s, br, 1H), 6.18 (s, 1H), 6.22 (d, $^3J_{AB}$= 6.0 Hz, 1H), 7.31 (d, $^3J_{AB}$= 6.0 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 99.09 (CH), 124.29 (CH), 152.68 (CH), 172.22 (C).

***(5R)-5-(l-Menthyloxy)-2(5H)-furanone (R)-(2.9)*[14]**
A solution of 5-hydroxy-2(5H)-furanone (25.0 g, 0.25 mol) and *l*-menthol (50.0 g, 0.32 mol) was heated at 100 °C for 24 h. Unreacted *l*-menthol was removed by distillation. Subsequently, the residue was distilled (bp 120-124 °C, 0.01 mm Hg), affording a yellowish oil consisting of two diastereomers (ratio 60:40, based upon the ^1H NMR of the acetal proton at δ 5.91 and 6.04 ppm), which solidified on standing at room temperature. After two crystallizations from petroleum-ether (40-60) diastereomerically pure (R)-**2.9** was obtained, as was determined by ^1H NMR. The mother liquors were combined and gave a second crop of diastereomerically pure (R)-**2.9**. Combined total yield 19.8 g (0.14 mol, 56%). Mp 70.4-70.8 °C (lit[14] 70.5-70.7 °C); $[\alpha]_D^{20}$= -136.3° (c 1.0, abs. ethanol); ^1H NMR ($CDCl_3$): δ 0.80 (d, ^3J= 7.33 Hz, *H9*, 3H), 0.86 (m, *H11*^{ax}, 1H), 0.87 (d, ^3J= 7.33

[#] Kaptan 500 H was used as UV and blue filter and a Hanau Q 700 lamp served as a light source.

Hz, *H9*, 3H), 0.91 (m, *H14^{ax}*, 1H), 0.94 (d, ^{3}J= 6.59 Hz, *H13*, 3H), 0.99 (m, *H10^{ax}*, 1H), 1.07 (m, *H7*, 1H), 1.28 (m, *H12*, 1H), 1.63 (m, *H10^{eq}*, 1H), 1.67 (m, *H11^{eq}*, 1H), 2.07 (m, *H8*, 1H), 2.12 (m, *H14^{eq}*, 1H), 3.63 (dt, ^{3}J= 10.62 Hz, ^{3}J= 4.39 Hz, *H6*, 1H), 6.07 (d, ^{3}J= 1.10 Hz, *H5*, 1H), 6.18 (d, $^3J_{AB}$= 4.39 Hz, *H3*, 1H), 7.14 (dd, $^3J_{AB}$= 4.39 Hz, ^{3}J= 1.10 Hz, *H4*, 1H); ^{13}C NMR ($CDCl_3$): δ 15.52 (CH_3), 20.56 (CH_3), 21.93 (CH_3), 22.86 (CH_2), 25.05 (CH), 31.16 (CH), 33.92 (CH_2), 40.03 (CH_2), 47.45 (CH), 78.76 (CH), 100.24 (CH), 124.35 (CH), 150.80 (CH), 170.54 (C); HRMS calcd 238.155, found 238.156.

(5S)-5-(d-Menthyloxy)-2(5H)-furanone (S)-(2.9)

This compound was synthesized from 5-hydroxy-2(5H)-furanone and *d*-menthol in the same way as described for (5R)-5-(*l*-menthyloxy)-2(5H)-furanone (R)-**2.9** and was kindly provided by Mr Ebe Schudde. Mp 74.1-74.3 °C; $[\alpha]_D^{20}$= +139.7° (c 1.0, $CHCl_3$).

***Hydrobenzamide*[39,40]**

A mixture of freshly distilled benzaldehyde (100.0 g, 0.94 mol) and liquid ammonia was stirred for 3 h while occasionally ammonia was bubbled through. The mixture was allowed to stand at room temperature until all ammonia had evaporated. The crude product was recrystallized twice from cyclohexane, yield 80.4 g (0.27 mol, 86%) of a white powder.
Mp 100-101 °C (lit[39,40] 101-102 °C); ^{1}H NMR ($CDCl_3$): δ 6.05 (s, 1H), 7.35-7.50 (m, 9H), 7.61 (d, ^{3}J= 6.60 Hz, 2H), 7.92-7.94 (m, 4H), 8.65 (s, 2H); ^{13}C NMR ($CDCl_3$):
δ 94.41 (CH), 127.03 (CH), 127.61 (CH), 128.34 (CH), 128.49 (CH), 128.74 (CH), 130.81 (CH), 135.75 (C), 141.52 (C), 160.45 (CH); HRMS calcd 298.147, found 298.147.

***cis-2,4,5-Triphenyl-3-imidazolidine, amarine*[41]**

Hydrobenzamide (75.0 g, 0.25 mol) was refluxed in 20 mL benzene for 5 h. The temperature was not allowed to exceed 130 °C, benzene was added occasionally to maintain this temperature. At the end of the heating period benzene was added (50 mL) and the mixture was cooled to room temperature with vigorous stirring. The pale yellow product was slurried and filtered twice with hexane and dried in vacuum at 50 °C.
Yield 52.0 g (0.17 mol, 70%) of a white powder. Mp 127-130 °C (lit[41] 128-131 °C); ^{1}H NMR ($CDCl_3$): δ 4.20 (s, br, 1H), 5.42 (s, br, 1H), 6.96-7.04 (m, 9H), 7.41-7.58 (m, 4H), 7.98-8.01 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 69.92 (br, CH), 126.57 (CH), 127.09 (CH), 127.33 (CH), 127.42 (CH), 128.46 (CH), 129.93 (C), 130.84 (CH), 138.83 (C), 164.29 (C); HRMS calcd 298.147, found 298.147.

***trans-2,4,5-Triphenyl-2-imidazolidine, isoamarine*[40,42]**

A mixture of 11.0 g (0.27 mol) KOH, H_2O (6.5 mL), 35 mL of diethylene glycol and 50.0 g (0.17 mol) amarine was heated in an open beaker until the temperature reached 155 °C. This temperature was maintained for 1 h with vigorous stirring, during which time the sodium salt of isoamarine precipitated and the solution became a thick slurry. After cooling, the slurry was treated with 50 mL glacial acetic acid with some heating. When most of the product had dissolved 150 mL of 96% ethanol was added and the solution was refluxed for 15 min. After cooling, the solution was neutralized with excess concentrated aqueous ammonia and the volume was concentrated to about 250 mL. The isoamarine precipitated slowly as tiny white crystals. This was filtered, washed twice with hexane, recrystallized from ethanol, and subsequently dried in vacuum at 50 °C. Yield 32.0 g (0.11 mol, 65%) of small white needles. Mp 199-201 °C (lit[40,42] 202 °C); ^{1}H NMR ($CDCl_3$): δ 4.93 (s, br, 1H), 5.64 (s, br, 1H), 7.11-7.55 (m, 13H), 7.96 (d, ^{3}J= 7.33

Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 80.05 (br, CH), 126.64 (CH), 127.38 (CH), 128.53 (CH), 128.59 (CH), 128.71 (CH), 130.14 (C), 130.99 (CH), 145.53 (C), 163.07 (C); HRMS calcd 298.147, found 298.147.

Racemic N-benzoyl-N'-acetyl-1,2-diamino-1,2-diphenylethane[42]

A solution of 1.50 g (18 mmole) sodium-acetate (free of water), 30.0 g isoamarine (0.10 mol) and 65 mL of acetic-anhydride was heated at 150 °C during 3 h. The solution was cooled to 65 °C, H_2O (100 mL) and 10 mL of concentrated HCl was added subsequently under stirring. This mixture was warmed at 100 °C during 3 h affording a slurry which was filtered hot and was washed with hot water. The white material was recrystallized twice from methanol. Yield 35.1 g (0.10 mol, 98%) of small white needles. Mp 251 °C (lit[42] 251 °C); ^1H NMR (CD_3OD): δ 1.85 (s, 3H), 5.48 (dd, ^3J= 8.05 Hz, ^3J= 8.43 Hz, 1H), 5.56 (dd, ^3J= 8.05 Hz, ^3J= 8.44 Hz, 1H), 7.03-7.53 (m, 13H), 7.81 (t, ^3J= 8.79 Hz, 2H), 8.51 (d, ^3J= 8.44 Hz, 1H), 8.77 (d, ^3J= 8.43 Hz, 1H); ^{13}C NMR (CD_3OD): δ 22.71 (CH_3), 56.93 (CH), 57.84 (CH), 126.79 (CH), 127.22 (CH), 127.28 (CH), 127.37 (CH), 127.86 (CH), 128.26 (CH), 131.19 (CH), 134.67 (C), 140.71 (C), 140.73 (C), 166.19 (C), 168.94 (C); Analysis calcd for $C_{23}N_2O_2H_{22}$, C: 77.07, N: 7.82, H: 6.19. Found, C: 76.76, N: 7.85, H: 6.13. HRMS calcd 340.157 ($-H_2O$), found 340.156.

Racemic 1,2-diamino-1,2-diphenylethane, stilbenediamine[42]

A mixture of racemic N-benzoyl-N'-acetylstilbenediamine (30.0 g, 0.08 mol), 45 mL of glacial acetic acid and 90 mL of 48% HBr was refluxed for 48 h. The volume was then concentrated to 10 mL and cooled to -20 °C. The precipitate was filtered, washed with cold ether and dissolved in 75 mL of water. The solution was cooled to 10 °C and neutralized with 40% aqueous sodium hydroxide after which the amine precipitated. The mixture was extracted with three portions of ether (75 mL) and the combined ether layers washed with water. The ether layer was dried over Na_2SO_4 and concentrated to a volume of 40 mL and petroleum-ether (40-60) was added. On cooling, racemic stilbenediamine separated as white needles which were dried in vacuum at 50 °C. Yield 2.54 g (12 mmole, 15%). Mp 81-82 °C (lit[42] 83 °C); ^1H NMR ($CDCl_3$): δ 2.53 (s, br, 4H), 4.02 (s, 2H), 7.01-7.25 (m, 10H); ^{13}C NMR ($CDCl_3$): δ 61.83 (CH), 126.81 (CH), 126.89 (CH), 128.09 (CH), 143.38 (C). HRMS calcd 106.066 (molecule breakdown), found 106.066.

2-Spirocyclohexane-4,5-diphenylisoimidazole[43]

A solution of 100.0 g (0.47 mol) benzil, 46.7 g (0.47 mol) cyclohexanone and 400 g (5.20 mol) ammonium acetate in acetic acid (1 L) was refluxed during 1 h. The color changed from yellow to green during this period. The mixture was diluted with 1.5 L of water with vigorous stirring. The precipitated brown material was filtered and washed with water, diluted in hot methanol-water (90-10) and left at room temperature for 12 h. The yellowish crystals were filtered off and dried in vacuum at 80 °C. Yield 131.4 g (0.46 mol, 97%). Mp 105-107 °C (lit[43] 106-108 °C); ^1H NMR ($CDCl_3$): δ 1.61-1.69 (m, 2H), 1.70-1.75 (m, 2H), 1.84-2.01 (m, 6H), 7.22-7.50 (m, 10H); ^{13}C NMR ($CDCl_3$): δ 24.13 (CH_2), 26.00 (CH_2), 34.32 (CH_2), 104.11 (C), 128.07 (CH), 128.95 (CH), 130.01 (CH), 133.03 (C), 163.92 (C); HRMS calcd 288.163, found 288.162.

2-Spirocyclohexane-4,5-trans-diphenylimidazolidine[44]

A solution of 100.0 g (0.35 mol) 2-spirocyclohexane-4,5-diphenylisoimidazole and 1 L of THF-NH_3 (liq) (4:5) was cooled to -40 °C with vigorous stirring. Lithium was added

in small portions over a period of 2 h upon which the solution colored intense blue. After the addition of 4 equivalents of lithium the solution was stirred for 1 h at 20 °C. NH_4Cl was added until the formation of gas stopped and the mixture was poored on ice. This mixture was extracted with three portions of THF (250 mL), the THF layers were combined and washed with brine and subsequently dried over Na_2SO_4. After the solvent was evaporated the brown material was recrystallized from ethyl acetate–hexane affording white crystals. Yield 95.0 g (0.32 mol, 93%). Mp 92–93 °C (lit[44] 93 °C); ^{1}H NMR ($CDCl_3$): δ 1.41–1.58 (m, 2H), 1.61–1.82 (m, 6H), 2.02–2.09 (s, br, 2H), 4.20 (s, 2H), 7.03–7.18 (m, 10H); ^{13}C NMR ($CDCl_3$): δ 23.99 (CH_2), 25.61 (CH_2), 39.85 (CH_2), 69.76 (CH), 76.49 (C), 127.17 (CH), 127.32 (CH), 128.38 (CH), 140.88 (C); HRMS calcd 292.194, found 292.193.

Racemic 1,2-diamino-1,2-diphenylethane, stilbenediamine[42,44]
A solution of 75.0 g (0.26 mol) 2-spirocyclohexane-4,5-transdiphenylimidazolidine in 300 mL of CH_2Cl_2 was stirred with 300 mL of a 3 N HCl solution for 1 h, during which time a white solid precipitated. The mixture was subsequently refluxed for 12 h yielding a clear, brown solution. The mixture was cooled to room temperature and made basic (pH 10, 5 N NaOH solution). The mixture was extracted with three portions CH_2Cl_2 (300 mL), the combined CH_2Cl_2 layers were washed with water and dried over Na_2SO_4. After the solvent was evaporated the residue was recrystallized twice from petroleum-ether (40–60), affording white crystals. Yield 53.5 g (0.25 mol, 97%). Mp 80–81 °C (lit[42,44] 83 °C).
Further spectral data as quoted above.

(-)(S,S)-Diamino-1,2-diphenylethane, (-)-stilbenediamine[43,44]
Racemic stilbenediamine (25.8 g, 0.12 mol) was dissolved in 135 mL of 96% ethanol. To this stirred solution a warm solution of 18.6 g (0.12 mol) *l*-tartaric acid in 96 % ethanol was added. This mixture was stirred for 1 h, affording large lumps of white material which were filtered off and subsequently dissolved in 140 mL of water at 60 °C. To the stirred solution was added 140 mL of 96% ethanol after which the solution was allowed to reach room temperature, affording white crystals which were filtered off and were washed with absolute ethanol. The sequence was repeated and the so obtained crystals were dried under vacuum at 40 °C. $[\alpha]_D^{20}$= −12.06° (c 1.0, water)(lit[41,42] −11.0°).
The salt (25.0 g, 69.06 mmol) was dissolved in 350 mL of water at 0 °C and powdered KOH (70.0 g, 1.75 mol) was added over a 30 min period. The mixture was stirred for 1 h and extracted with ether, dried over Na_2SO_4 and evaporated to dryness. The residue was recrystallized twice from petroleum-ether (40–60) affording small white needles which were dried in vacuum at 40° C. Yield 6.00 g of white needles (28.30 mmol, 82%). Mp 81–82 °C (lit 83 °C); $[\alpha]_D^{20}$= −86.57° (c 1.0, ether)(lit[41,43] −87°, c 1.0, ether); ^{1}H NMR ($CDCl_3$): δ 2.53 (s, br, 4H), 4.02 (s, 2H), 7.01–7.25 (m, 10H); ^{13}C NMR ($CDCl_3$): δ 61.83 (CH), 126.81 (CH), 126.89 (CH), 128.09 (CH), 143.38 (C). Analysis calcd for $C_{14}N_2H_{16}$, C: 79.20, N: 13.20, H: 7.60. Found, C: 79.01, N: 13.04, H: 7.35; HRMS calcd 10.066 (molecule breakdown), found 106.066.

(4R,4'R)-1,2-Ethylenediamino-N,N'-((5R)-(5-(l-menthyloxy)-butyrolactone)), (R,R)-(2.10)
A solution of 1.00 g (4.20 mmole) *l*-menthyloxyfuranone **2.9** and 0.13 g (2.10 mmole) ethylenediamine in 10 mL of DMF was stirred for 12 h at room temperature. The solution was taken to dryness, taken up in CH_2Cl_2 (25 mL) and washed 5 times with water (5 mL)

and concentrated after which the residue was purified by column chromatography (silicagel, ethyl acetate–ethanol 95:5) followed by crystallization from ethanol (twice), affording white needles, which were dried in vacuum at 40 °C for 2 h. Yield 0.80 g (1.49 mmole, 71%). Mp 114–115 °C; $[\alpha]_D^{20}$= −151.30° (c 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.80 (d, ^{3}J= 6.84 Hz, *H9*, 6H), 0.89 (m, *H11^{ax}*, 2H), 0.90 (d, ^{3}J= 6.84 Hz, *H9*, 6H), 0.91 (m, *H14^{ax}*, 2H), 0.97 (d, ^{3}J= 6.35 Hz, *H13*, 6H), 1.07 (m, *H10^{ax}*, 2H), 1.25 (m, *H7*, 2H), 1.43 (m, *H12*, 2H), 1.49 (s, br, *NH*, 2H), 1.69 (m, *H10^{eq}*, 2H), 1.70 (m, *H11^{eq}*, 2H), 2.05 (m, *H8*, 2H), 2.12 (m, *H14^{eq}*, 2H), 2.29 (dd, $^2J_{AB}$= 17.58 Hz, ^{3}J= 2.93 Hz, *H3^{trans}*, 2H), 2.76 (s, *H15*, 4H), 2.87 (dd, $^2J_{AB}$= 17.58 Hz, ^{3}J= 6.84 Hz, *H3^{cis}*, 2H), 3.34 (dd, ^{3}J= 6.84 Hz, ^{3}J= 2.93 Hz, *H4*, 2H), 3.35 (dt, ^{3}J= 10.74 Hz, ^{3}J= 3.91 Hz, *H6*, 2H), 5.44 (s, *H5*, 2H); ^{13}C NMR ($CDCl_3$): δ 15.59 (CH_3), 20.74 (CH_3), 22.12 (CH_3), 23.12 (CH_2), 25.52 (CH), 31.29 (CH), 34.24 (CH_2), 34.92 (CH_2), 39.70 (CH_2), 46.80 (CH_2), 47.72 (CH), 59.99 (CH), 76.85 (CH), 103.71 (CH), 174.91 (C); HRMS calcd 534.367 (– 4H), found 534.366; Analysis calcd for $C_{30}H_{54}N_2O_6$, C: 66.88, N: 5.20, H: 10.10. Found, C: 66.51, N: 5.22, H: 10.25.

(4S,4'S)–1,2–Ethylenediamino–N,N'–((5S)–(5–(d–menthyloxy)–butyrolactone)), (S,S)–(2.10)

Prepared from *d*–menthyloxyfuranone **2.9** in the same way as described for (*R,R*)–**2.10**. Mp 116–118 °C; $[\alpha]_D^{20}$= +152.61° (c 0.1, $CHCl_3$).

(4R,4'R)–1,2–Diaminobisethylene–N,N,N',N'–((5R)–(5–(l–menthyloxy)–butyrolactone)), (R,R)–(2.11)

A solution of 1.00 g (4.20 mmole) *l*–menthyloxyfuranone **2.9** and 0.18 g (2.10 mmole) piperazine in DMF (10 mL) was stirred for 1 h at room temperature, after which time crystals formed spontaneously. The crystals were filtered off and washed with small amounts of water and shortly dried in vacuum at 40 °C. Recrystallization (twice) from ethanol afforded small white needles. Yield 0.97 g (1.74 mmole, 83%). Mp 152–153 °C; $[\alpha]_D^{20}$= −173.5° (c 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.76 (d, ^{3}J= 6.95 Hz, *H9*, 6H), 0.83 (m, *H11^{ax}*, 2H), 0.87 (d, ^{3}J= 6.95 Hz, *H9*, 6H), 0.91 (m, *H14^{ax}*, 2H), 0.94 (d, ^{3}J= 6.22 Hz, *H13*, 6H), 1.01 (m, *H10^{ax}*, 2H), 1.21 (m, *H7*, 2H), 1.38 (m, *H12*, 2H), 1.63 (m, *H10^{eq}*, 2H), 1.66 (m, *H11^{eq}*, 2H), 2.02 (m, *H8*, 2H), 2.10 (m, *H14^{eq}*, 2H), 2.46 (dd, $^2J_{AB}$= 17.95 Hz, ^{3}J= 4.03 Hz, *H3^{trans}*, 2H), 2.50 (m, *H15*, 4H), 2.58 (m, *H16*, 4H), 2.77 (dd, $^2J_{AB}$= 17.95 Hz, ^{3}J= 8.06 Hz, *H3^{cis}*, 2H), 3.16 (ddd, ^{3}J= 8.06 Hz, ^{3}J= 4.03 Hz, ^{3}J= 1.46 Hz, *H4*, 2H), 3.53 (dt, ^{3}J= 10.61 Hz, ^{3}J= 4.39 Hz, *H6*, 2H), 5.53 (d, ^{3}J= 1.46 Hz, *H5*, 2H); ^{13}C NMR ($CDCl_3$): δ 15.57 (CH_3), 20.76 (CH_3), 22.17 (CH_3), 23.03 (CH_2), 25.42 (CH), 31.28 (CH), 31.30 (CH_2), 34.20 (CH_2), 39.52 (CH_2), 47.66 (CH), 49.33 (CH_2), 65.87 (CH), 77.02 (CH), 101.26 (CH), 174.60 (C); HRMS calcd 560.382 (– 4H), found 560.382; Analysis calcd for $C_{32}H_{56}N_2O_6$, C: 68.05, N: 4.96, H: 9.99. Found, C: 67.94, N: 4.88, H: 9.97.

(4S,4'S)–1,2–Diaminobisethylene–N,N,N',N'–((5S)–(5–(d–menthyloxy)–butyrolactone)), (S,S)–(2.11)

Prepared from *d*–menthyloxyfuranone **2.9** in the same way as described for (*R,R*)–**2.11**. Mp 153–154 °C; $[\alpha]_D^{20}$= +175.00° (c 0.1, $CHCl_3$).

(4S,4'S)-(S,S-1,2-Diaminodiphenylethylene)-N,N'-((5S)-(5-(d-menthyloxy)-butyrolactone)), (S,S)-(2.12)

A mixture of 1.00 g (4.20 mmole) *d*-menthyloxyfuranone **2.9** and 0.45 g (2.10 mmole) (−)-stilbenediamine was heated at 120 °C for 48 h. The brown solution was cooled to room temperature and purified by means of column chromatography (silicagel, ethyl acetate-ethanol 95-5) followed by crystallization from ethanol, affording yellow crystalline material. Yield 0.53 g (0.77 mmole, 34%). Mp 94-95 °C; $[\alpha]_D^{20}$= 99.01 (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.54 (m, *H14^{ax}*, 2H), 0.59 (d, ^{3}J= 6.96 Hz, *H9*, 6H), 0.66 (m, *H10^{ax}*, 2H), 0.71 (d, ^{3}J= 7.37 Hz, *H13*, 6H), 0.74 (m, *H11^{ax}*, 2H), 0.76 (d, ^{3}J= 6.59 Hz, *H9*, 6H), 0.88 (m, *H7*, 2H), 0.55-0.98 (s, br, *NH*, 2H), 1.01 (m, H*12*, 2H), 1.46 (m, *H10^{eq}*, 2H), 1.57 (m, *H11^{eq}*, 2H), 1.79 (m, *H8*, 2H), 2.08 (m, *H14^{eq}*, 2H), 2.19 (dd, $^2J_{AB}$= 17.94 Hz, ^{3}J= 1.47 Hz, *H3^{trans}*, 2H), 2.66 (dd, $^2J_{AB}$= 17.94 Hz, ^{3}J= 6.96 Hz, *H3^{cis}*, 2H), 3.17 (dd, ^{3}J= 6.96 Hz, ^{3}J= 1.47 Hz, *H4*, 2H), 3.20 (dt, ^{3}J= 10.62 Hz, ^{3}J= 5.86 Hz, *H6*, 2H), 3.53 (s, *H15*, 2H), 5.00 (s, *H5*, 2H), 6.89-6.99 (m, *ortho-ArH*, 2H), 7.00-7.21 (m, *ArH*, 8H); ^{13}C NMR ($CDCl_3$): δ 15.39 (CH_3), 20.64 (CH_3), 21.98 (CH_3), 22.89 (CH_2), 25.30 (CH), 31.06 (CH), 34.09 (CH_2), 34.69 (CH_2), 39.07 (CH_2), 47.36 (CH), 59.09 (CH), 67.69 (CH), 76.36 (CH), 103.94 (CH), 127.52 (CH), 127.55 (CH), 128.33 (CH), 140.05 (C), 175.39 (C); HRMS calcd 344.223 (breakdown), found 344.223; Analysis calcd for $C_{42}H_{60}N_2O_6$, C: 73.22, H: 8.78, N: 4.06. Found C: 73.01, H: 8.74, N: 3.97.

(R,R)-1,2-Ethylenediamine-N,N'-disuccinate (R,R)-(2.3)

A mixture of 0.50 g (0.93 mmole) (*R,R*)-**2.10** and 1.10 g (3.0 mmole) pyridinium dichromate in DMF (25 mL) and H_2O (0.1 mL) was stirred for 24 h at room temperature. The red solution was subsequently acidified to exactly pH 4 (using a 4 N HCl solution) and concentrated to 10 mL. The mixture was taken in 100 mL CH_2Cl_2 and washed five times with a saturated NH_4Cl solution (15 mL), three times with water (15 mL) and concentrated to dryness. The crude material was taken in water (50 mL) containing 0.23 g NaOH (5.75 mmol) and vigorously stirred for 30 min. After this period the mixture was slowly acidified to pH 3.5 with concentrated HCl (to pH 3.0-3.5) with stirring. The precipitated white needles were collected by filtration and the sequence was repeated two more times. The final crop of white needles was washed with a minimum amount of cold water and dried in vacuum at 65 °C for 6 h. Yield 0.07 g (0.24 mmole, 26%). Mp over 250 °C; ^{1}H NMR (D_2O/NaOD): δ 2.31 (dd, $^2J_{AB}$= 24.91 Hz, ^{3}J= 13.58 Hz, *H^{cis}*, 2H), 2.51 (dd, $^2J_{AB}$= 24.91 Hz, ^{3}J= 7.92 Hz, *H^{trans}*, 2H), 2.78 (d, $^2J_{AB}$= 13.60 Hz, 2H), 2.84 (d, $^2J_{AB}$= 13.60 Hz, 2H), 3.40 (dd, ^{3}J= 13.58 Hz, ^{3}J= 7.92 Hz, 2H); ^{13}C NMR (D_2O/NaOD/methanol): δ 42.12 (CH_2), 47.36 (CH_2), 62.15 (CH), 180.37 (C), 182.07 (C); HRMS calcd 292.091, found 292.090.

(S,S)-1,2-Ethylenediamine-N,N'-disuccinate (S,S)-(2.3)

Prepared from (*S,S*)-**2.10** in exactly the same way as described for (*R,R*)-**2.3**.

(R,R)-1,2-Diaminobisethylene-N,N,N',N'-disuccinate (R,R)-(2.13)

A mixture of 0.50 g (0.88 mmole) (*R,R*)-**2.11** and 1.05 g (2.86 mmole) pyridinium dichromate in acetone (25 mL) and H_2O (0.1 mL) was stirred for 24 h at room temperature. The red solution was subsequently acidified to exactly pH 5 (using a 4 N HCl solution) and concentrated to 10 mL. The mixture was taken in 100 mL of CH_2Cl_2 and washed five times with a saturated NH_4Cl solution (15 mL), three times with water (15

mL) and concentrated to dryness. Further workup as described for (R)-**2.3** afforded white crystalline material. Yield 0.06 g (0.19 mmole, 21%). Mp >250 °C; ^{1}H NMR (D_2O/Na-OD): δ 2.42 (dd, $^2J_{AB}$= 25.52 Hz, ^{3}J= 14.87 Hz, *H^{cis}*, 2H), 2.48 (dd, $^2J_{AB}$= 25.52 Hz, ^{3}J= 8.77 Hz, *H^{trans}*, 2H), 2.64 (m, 4H), 2.69 (m, 4H), 3.19 (dd, ^{3}J= 14.87 Hz, ^{3}J= 8.77 Hz, 2H); ^{13}C NMR (D_2O/NaOD/methanol): δ 41.78 (CH_2), 42.04 (CH_2), 46.12 (CH_2), 61.23 (CH), 179.51 (C), 181.34 (C); HRMS calcd 318.106, found 318.106.

(S,S)-1,2-Diaminobisethylene-N,N,N',N'-disuccinate (S,S)-(2.13)
Prepared from (*S,S*)-**2.11** in exactly the same way as described for (*R,R*)-**2.13**.

(S,S)-(S,S-1,2-Diaminodiphenylethylene)-N,N'-disuccinate (S,S)-(2.13)
Prepared from (*S,S*)-**2.12** in exactly the same way as described for (*R,R*)-**2.13**.
Yield 14%, yellow solid material. Mp >250 °C; ^{1}H NMR (D_2O/NaOD): δ 2.20 (dd, $^2J_{AB}$= 26.41 Hz, ^{3}J= 14.78 Hz, *H^{cis}*, 2H), 2.46 (dd, $^2J_{AB}$= 26.41 Hz, ^{3}J= 5.89 Hz, *H^{trans}*, 2H), 3.18 (dd, ^{3}J= 14.78 Hz, ^{3}J= 5.89 Hz, 2H), 3.42 (s, 2H), 6.83-6.89 (m, *ortho-ArH*, 2H), 6.92-7.18 (m, *ArH*, 8H); ^{13}C NMR (D_2O/NaOD/methanol): δ 39.12 (CH_2), 58.56 (CH), 101.45 (CH), 126.65 (CH), 126.98 (CH), 127.56 (CH), 138.98 (C), 178.97 (C), 180.56 (C); HRMS calcd 444.153, found 444.152.

H_2(TPP), 5,10,15,20-tetraphenylporphyrin (meso-tetraphenylporphyrin) (2.15 R= H)[31]
Purified benzaldehyde (40.0 g, 0.37 mol) and colorless pyrrole (25.3 g, 0.37 mol) were added to 300 ml of refluxing propionic acid open to air. The solution was refluxed for 30 min while a stream of oxygen was bubbled through to suppress chlorin formation. After 30 minutes the solution was cooled to 0 °C, allowed to stand overnight and filtered over a P4 glass frit. The remaining and often purple solid was washed with hot water (500 mL) and subsequently with methanol until the washings were colorless. The resulting purple crystals were dried in vacuum at 80 °C to yield 10.5 g (17.08 mmole, 18.5%) of H_2(TPP) containing some chlorin. The crude H_2(TPP)/H_2(TPC) mixture (5.0 g, 8.15 mmole) was dissolved in refluxing CH_2Cl_2 (1500 mL) and then treated with a solution of DDQ (1.25 g, 5.5 mmole) in toluene (100 mL). After the mixture was refluxed for a further 45 min, the solution was taken to dryness and dried in vacuum at 50 °C for 2 h. The purple material was chromatographed on neutral alumina (1200 g, act I-III) in $CHCl_3$. The H_2(TPP) eluates were evaporated to a volume of 750 mL and 900 mL of methanol was added; after a further co-evaporation the H_2(TPP) precipitated and was collected over a P4 glass frit and dried in vacuum at 50 °C. Yield 4.51 g (7.32 mmole, 90%) of glistening purple crystals. Mp >250 °C; ^{1}H NMR ($CDCl_3$): δ -2.65 (s, br, 2H), 7.75 (m, 4H), 7.80 (d, ^{3}J= 2.5 Hz, 8H), 8.21 (dd, ^{3}J= 2.5 Hz, ^{4}J= 0.4 Hz, 8H), 8.84 (s, 8H); ^{13}C NMR ($CDCl_3$): δ 115.01 (br, C), 117.50 (br, C), 120.00 (C), 126.55 (CH), 126.57 (CH), 127.57 (CH), 134.42 (CH), 142.04 (C); UV/VIS (CH_2Cl_2, λ/nm): 417, 486, 512, 553, 588, 645.; Analysis calcd for $C_{44}H_{30}N_4$, C: 85.97, H: 4.92, N: 9.11. Found, C: 85.95, H: 4.90, N: 9.14.

H_2T(Pyr)P, 5,10,15,20-tetra(4-pyridyl)porphyrin (2.17)[33]
Purified pyridine-4-carboxaldehyde (39.6 g, 0.37 mol) and colorless pyrrole (24.8 g, 0.37 mol) were refluxed in propionic acid (1.5 L) open to air for 45 minutes, while a stream of oxygen was bubbled through. The solvent was flashed off, and the residue was washed several times with DMF. The residue was dried in vacuum at 50 °C and three times crystallized from chloroform-methanol solutions and dried again in vacuum at 50 °C

yielding bright purple crystals, 13.3 g (21.30 mmole, 23%). Mp >250 °C; ^{1}H NMR (DMSO-d_6): δ -4.31 (s,br, 2H),2 8.53 (d, $^3J_{AB}$= 5.10 Hz, 8H), 8.99 (s, br, 8H), 9.20 (d, $^3J_{AB}$= 5.10 Hz, 8H); ^{13}C NMR (DMSO-d_6): no spectrum could be obtained due to solubility limitations; UV/VIS ($CHCl_3$, λ/nm): 420, 516, 551, 590, 647.

$H_2T(CO_2H)PP$, 5,10,15,20-tetra(4-carboxyphenyl)porphyrin (2.15 R= CO_2H)[33]

Purified 4-carboxybenzaldehyde (36.0 g, 0.24 mol) and colorless pyrrole (16.1 g, 0.24 mol) were refluxed in 1 L of propionic acid open to air for 2 h, while a stream of oxygen was bubbeld through. When the reaction mixture was cooled to room temperature $H_2T(CO_2H)PP$ precipitated as purple crystals. The crystals were recrystallized from chloroform-methanol solutions and dried in vacuum at 50 °C. Yield 14.20 g (18 mmole, 30%). Mp >250 °C; ^{1}H NMR (DMSO-d_6): δ -4.54 (s, br, 2H), 8.29 (d, $^3J_{AB}$= 8.10 Hz, 8H), 8.36 (d, $^3J_{AB}$= 8.10 Hz, 8H), 8.83 (s, br, 8H); ^{13}C NMR (DMSO-d_6): δ 119.29 (C), 127.84 (CH), 130.65 (C), 134.38 CH), 145.30 (C), 167.43 (C); UV/VIS (Pyridine, λ/nm): 515, 542, 588, 639.

H_2TPPS, 5,10,15,20-tetra(4-sulfonatophenyl)porphyrin (2.16)[34,35,45]

Tetraphenylporphyrin **2.15** (R= H)(2.0 g, 3.25 mmole) was mixed with 50 mL of concentrated (fuming) sulfuric acid and heated at 100 °C for 5 days. After this period the mixture was allowed to stand at room temperature for 2 days. The gray-green solution was filtered through a P4 glass frit and the filtrate was diluted carefully with water to 1000 mL. This solution was heated to 90 °C and $Ca(OH)_2$ was added slowly with stirring until the colour of the solution changed permanently to purple. $CaSO_4$ was filtered off and washed with a minimum quantity of hot water, which was combined with the filtrate. The filtrate was concentrated to a small volume (75 mL) and the pH of the final warm solution was regulated to 9-10 by the addition of Na_2CO_3. $CaCO_3$ was filtered off and hot 90% ethanol-water solutions were added in small quantities to the filtrate. The saturated solution was allowed to cool at room temperature and crystals of the desired adduct were obtained (together with mono-, di- and trisulfonated tetraphenylporphyrins). The crude material was purified by means of column chromatography (basic Al_2O_3, activity I-III) using a water-methanol-ethanol-acetone solvent system in the volume ratios 7:1½:½:1 respectively, changing to a water-methanol-acetone solvent system (volume ratios 7:2:1). This procedure was repeated twice and the finally obtained solution was concentrated and recrystallized twice from ethanol. The greenish-purple material was dried in vacuum at 80 °C. Yield 1.02 g (1.09 mmole, 34%). Mp >250 °C; ^{1}H NMR (D_2O): δ 6.85 (s, br, 4H), 7.28 (d, $^3J_{AB}$= 9.30 Hz, 8H), 7.88 (d, $^3J_{AB}$= 9.30 Hz, 8H), 8.41 (s, br, 4H); ^{13}C NMR (D_2O-methanol): δ 117.74 (C), 123.37 (CH), 123.42 (CH), 123.57 (CH), 134.80 (CH), 141.56 (C), 142.57 (C), 166.30 (C); UV/VIS (H_2O, λ/nm): 412, 510, 553, 574, 626.; Analysis calcd for $C_{44}H_{26}N_4S_4O_{12}Na_4.7H_2O$, C: 47.31, H: 3.61, N: 5.02, S: 11.48. Found, C: 47.09, H: 3.47, N: 4.93, S: 11.32.

Co^{III}-TPP, Co^{III}-5,10,15,20-tetraphenylporphyrin Co^{III}-(2.15 R= H)[32]

A solution of Cobalt-acetate (0.50 g, 2.0 mmole) and TPP **2.15** (R= H)(0.50 g, 0.81 mmole) was refluxed in acetic acid (2 mL) for 2 h and subsequently stirred at room temperature for 24 h. The mixture was evaporated to dryness and the remaining red solid was thoroughly washed with water (100 mL) and dried in vacuum at 100 °C. Recrystallization from pyridine (twice) afforded a red to purple solid material. Yield 0.44 g (0.65 mmol, 74%).

To a solution of the obtained material (0.41 g, 0.60 mmol) in methanol (75 mL) was added 1.5 mL HBr (48%) and the mixture was stirred for 24 h, while oxygen was bubbled through. The mixture was taken to dryness and the obtained material was recrystallized from $CHCl_3$–methanol and dried in vacuum at 50 °C for 12 h. Yield 0.21 g (0.28 mmole, 47%) of reddish purple crystals $Co^{III}(TPP)(H_2O)Br$. Mp >250 °C; ^1H NMR ($CDCl_3$): δ 1.76 (s, br, H_2O, 2H), 7.74 (m, 12 H), 8.12 (m, 8H), 9.00 (s, 8H); ^{59}Co NMR ($CDCl_3$): δ 8430 (s, br ≈ 1250 Hz); UV/VIS (CH_2Cl_2, λ/nm): 405, 412, 530, 549, 557.

Co^{III}–H_2TPPS, 5,10,15,20–tetra(4–sulfonatophenyl)porphyrin Co^{III}–(2.16)[45]

Prepared as described for Co^{III}–TPP (Co^{III}–**2.15** R= H) from H_2TPPS **2.16**. The material was purified by means of column chromatography (Al_2O_3, basic, act I–III) using methanol as eluens to remove impurities and water to elute free base TPPS (deep purple band) and finally the lanthanide TPPS complex (brigther purple band). The solvent was removed by means of several co–evaporations with methanol. The solid material was dissolved in the minimum amount of water and warmed to 65 °C. Hot 90% ethanol was added in small quantities and cooled to room temperature until small crystals of the TPPS complex were formed. After filtration the material was dried in vacuum at 100 °C for 12 h. Mp >250 °C; ^1H NMR (D_2O/NaOD): δ 6.02–8.23 (m, 16 H), 11.06 (s, 8H); ^{59}Co NMR (D_2O/NaOD): δ 8100 (s, br ≈ 260 Hz); UV/VIS (pyridine, λ/nm): 434, 508, 544, 585.

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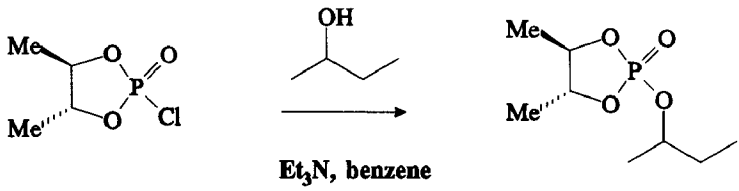
CHAPTER 3

The use of chiral diazaphospholidines as agents for the enantiomeric excess determination of amines and alcohols

3.1 *Trivalent phosphorus containing chiral derivatizing agents for the enantiomeric excess determination*

One of the most attractive nuclei to use in NMR analysis of chiral materials undoubtedly is the phosphorus-31 nucleus. The advantages of using ^{31}P NMR are clear: usually the shift dispersion is large and relatively simple spectra are obtained when broad-band decoupling is used. Furthermore, the ^{31}P nucleus is very sensitive towards (small) structural changes and can easily be measured[1]. Knowing this, it is not surprising that a number of methods have been developed in the field of enantiomeric excess determination of amines, alcohols and derivatives of amino acids using ^{31}P NMR spectroscopic techniques[#]. Nearly all known methods are based upon the use of derivatizing agents, containing a *pentavalent* phosphorus atom[2]. Typically, this means that a chiral phosphoric acid chloride is allowed to react with the substrate under well defined conditions, affording diastereomeric products which can be analyzed[3] (Scheme **3.1**).



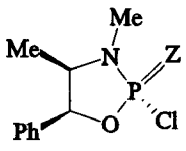
3.1 **3.2**

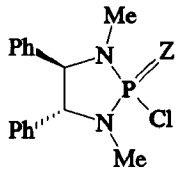
Scheme 3.1 A typical example of a chiral pentavalent phosphorus derivatizing agent

The phosphates obtained give $\Delta\delta$ values (^{31}P NMR) that are not very large, typically between 0 and 0.13 ppm. In the *C-2* symmetrical chlorodioxaphospholane **3.1**, the phosphorus atom is not chiral so that inversion or retention of configuration at phosphorus during the displacement of chloride by an enantio-pure alcohol yields the same diastereomer **3.2**. The $\Delta\delta$ values can be slightly increased by reducing the electrophilicity of the pentavalent phosphorus atom, *e.g.* by replacing one or both oxygen atoms by nitrogen atoms, as in **3.3** and **3.4** (Scheme **3.2**). It should, however, be noted that more

[#] See reference 2 and Chapter 1 for a review concerning phosphorus containing agents for the enantiomeric excess determination.

forcing conditions are required to make these agents react, for example by the use of a stronger base (NaH), higher temperatures (refluxing THF) and prolonged reaction times[4,5]. Moreover, the low reactivity towards *e.g.* tertiary alcohols and other sterically hindered systems, limits their use in the analysis of enantiomeric composition.

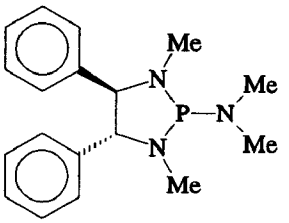


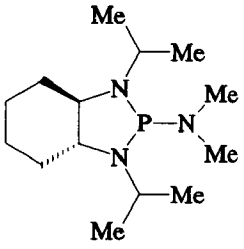


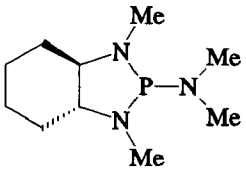
3.3 **3.4** **Z= O, S**

Scheme 3.2 Modified pentavalent phosphorus derivatizing agents 3.3 and 3.4

The highest reactivity in combination with large ^{31}P NMR $\Delta\delta$ values for the diastereomeric adducts can be obtained when chiral *trivalent* phosphorus containing reagents, as developed by Alexakis and co-workers[6], are used (Scheme **3.3**).



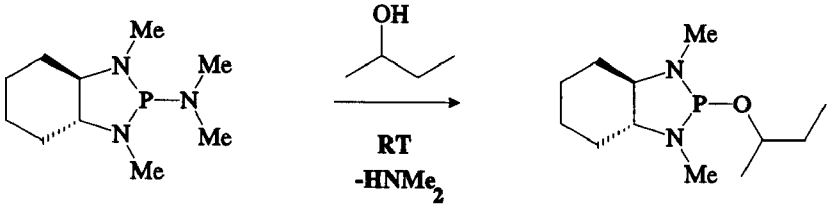




3.5 **3.6** **3.7**

Scheme 3.3 Three trivalent phosphorus containing chiral derivatizing agents

These reagents form derivatives with primary, secondary and tertiary alcohols even at room temperature, simply by stirring with the substrate of interest in toluene without the need of additional reagents. The exocylic P-N bond of these aminophosphines is easily cleaved by alcohols and amines[7,8], affording the (diastereomeric) products **3.8** as is shown for sec-butanol in Scheme **3.4**. A large variety of chiral alcohols could be analyzed with *e.g.* reagent **3.7**, to give $\Delta\delta$ values of between 0.5 and 12.1 ppm (!)[6] for the diastereomeric products using ^{31}P NMR. Furthermore, these reagents are easily prepared by an amine exchange reaction with $P(NMe_2)_3$ (*H*exa*M*ethyl*P*hosphorus*T*riamide, HMPT) and the desired chiral bisamine which is, though expensive, commercially available. These reagents are, furthermore, reported to be stable for months in an inert atmosphere, but they are very sensitive to moisture[6,9].



3.7 3.8

Scheme 3.4 The reaction of racemic sec-butanol with derivatizing agent 3.7

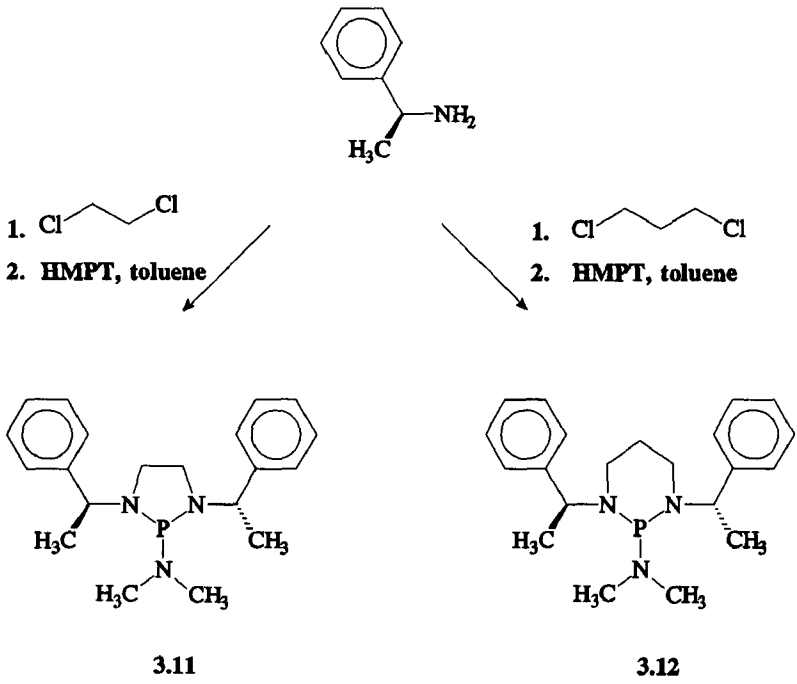
Being aware of the advantages using phosphorus containing derivatizing agents in the enantiomeric excess determination of amines, alcohols, amino alcohols and amino acids (see also Chapters 1, 4 and 5), it seemed obvious that modified, trivalent phosphorus containing compounds might be of great interest, not only for the enantiomeric excess determination itself, but also to gather more insight in the relationship between the obtained chemical shift differences $\Delta\delta$ (in the decoupled ^{31}P NMR spectra) and the structure of the phosphorus containing diastereomeric products[1,10,11].

3.2 *The synthesis of new, modified trivalent diazaphospholidines, oxazaphospholidines and dioxophospholidines.*

Although the results in the enantiomeric excess determination as obtained by the derivatizing agents **3.5**, **3.6** and **3.7** developed by Alexakis and co-workers[6] can hardly be improved, the *trivalent phosphorus* method is not tolerant towards (traces of) water, making this method in the field of enantiomeric excess determination of *amino acids* of little or no use. Besides, the bisamines as used by Alexakis are relatively expensive and only available after tedious preparation. Furthermore, reagent **3.7** is based upon a system that contains two annulated ring systems with considerable ring strain, making agent **3.7** not very stable towards reagents or solvents (like water).
Products **3.8**, obtained on reaction of **3.7** with nucleophiles, appear not to be very stable either.
Therefore, it would be of considerable interest when derivatives were available that are: (a) based upon cheaper and more easily available bisamines and (b), built in such way that the reactivity towards nucleophiles is more balanced, so that water is acceptable as (co)-solvent and amino acids still react. In this way it should be possible to study the scope and limitations as reagent for the determination of the enantiomeric excess and obtain insight into the factors that govern the diastereomeric peak separation in the decoupled ^{31}P NMR spectra.

3.2.1 *About diazaphospholidines*

An elegant way to meet both requirements described above, is the use of a bis-amine, that is based upon the very cheap and enantiomerically pure (*S*)-α-phenylethylamine in the formation of a diazaphospholidine.



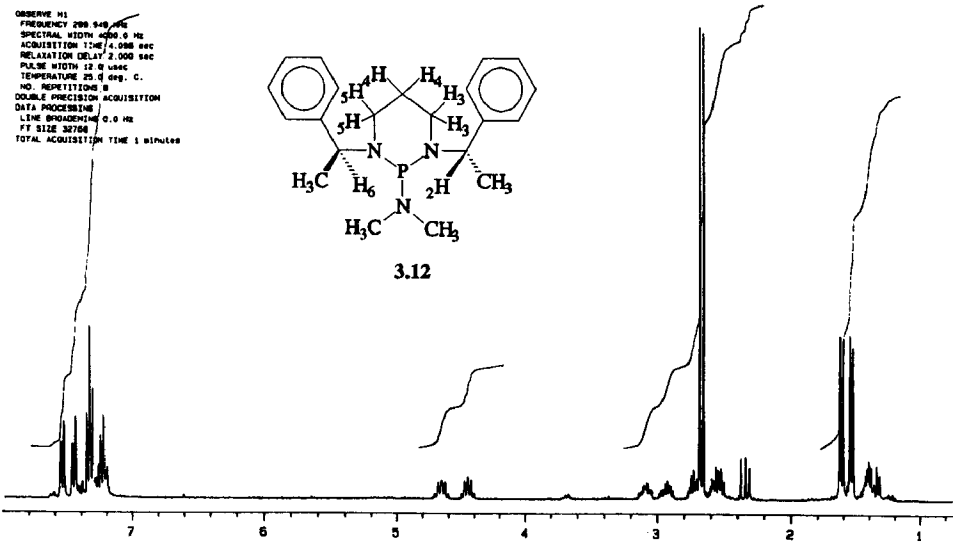
3.11 **3.12**

Scheme 3.5 Synthesis of the derivatizing agents 3.11 and 3.12

The reaction of (*S*)-α-phenylethylamine with either 1,2-dichloroethane or 1,3-dichloropropane without solvent (neat) affords bisamines **3.9** and **3.10** (not shown) in good yield (62 and 84% respectively)[12]. The subsequent amine exchange reaction with HMPT in dry toluene or benzene under acidic catalysis (dry NH_4Cl) affords diazaphospholidines **3.11** and **3.12** in nearly quantitative yield (Scheme **3.5**). *Attempts to purify these materials even on a small scale by means of distillation under reduced pressure resulted in violent explosions.* A reasonable alternative for the purification is column chromatography using a small Al_2O_3 column under nitrogen, affording the nearly pure products as determined by the ^1H NMR. These materials are sufficiently pure for use in the enantiomeric excess determination.

The ^1H NMR spectra of **3.11** and **3.12** are complex, showing large shift differences, *e.g.* for the protons of the 5 or 6 ring system. The shifts arise from the influence of the electron density located on the phosphorus nucleus (free electron pair). Due to extensive $^3J_{P-H}$ coupling and the fact that all the CH_2 protons in this system are nonequivalent, the spectra show complex coupling patterns (Figures **3.1** and **3.2**).

The ^{1}H NMR of crude **3.12** clearly shows two multiplets at δ 4.31 and δ 4.45 ppm, belonging to the exocyclic protons (2 and 6). Both are located near the free electron pairs at the ring nitrogen atoms and show a large shift compared to the free ligand (typically Δδ 0.4 ppm).



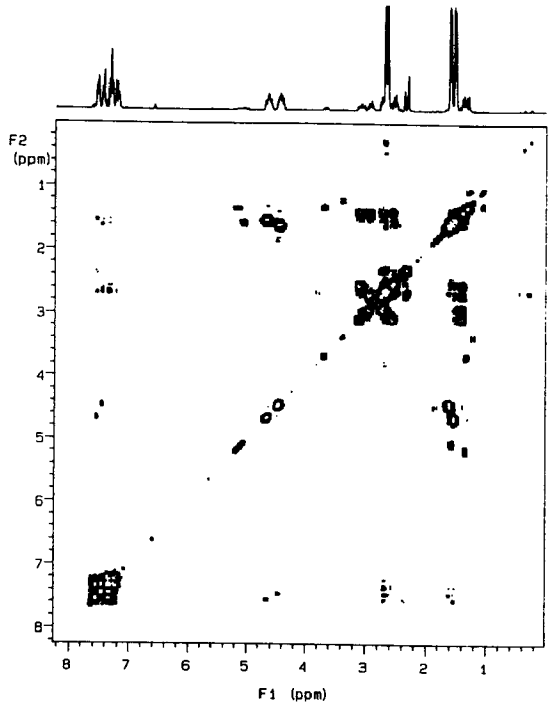
3.12

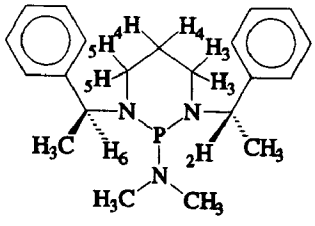
Figure 3.1 *The ^{1}H NMR spectrum of crude 3.12 recorded in $CDCl_3$ at [L]= 0.1 M*

The protons $\underline{4}^{ax}$ and $\underline{4}^{eq}$ are located around δ 1.51 ppm, and the axial protons 3 and 5 are located at δ 2.53 and δ 2.62 ppm, respectively. The equatorial protons 3 and 5 are shifted to δ 2.90 and δ 3.10 ppm, probably because they are situated in the vicinity of the electron density which is located on the phosphorus atom (free electron pair).

From the COSY spectrum (Figure **3.2**) the relationship between $\underline{3}^{ax}$ and $\underline{3}^{eq}$ as well as the relationship between $\underline{5}^{ax}$ and $\underline{5}^{eq}$ is easily revealed, although they are significantly shifted due to ring current of the aromatic ring systems. The NOESY spectrum (Figure **3.2**) is in agreement with the assignments as proposed, showing no interaction for the equatorially positioned NMe_2 group.

Surprisingly, no evidence for π–stacking of the aromatic systems was found. For further assignment, the reader is referred to the experimental section. Furthermore, the observed shifts are rather sensitive towards the solvent used. For the methyl signals (at δ 1.51 ppm), Δδ values of 0.25 ppm were found upon changing the solvent from benzene to chloroform. These shifts show no temperature dependence (in benzene) over the temperature range 20–80 °C, indicating that the conformational freedom is very limited, so that conformational changes do not contribute to the observed phenomena. The spectral behavior of reagent **3.11** is not discussed in greater detail here, but comparable shifts are found. It is important to note that **3.11** and **3.12** are stable in air and reasonably insensitive towards moisture, in contrast to the derivatizing agents used by Alexakis[5,6]. Reaction to provide the diastereomeric products nearly always proceeds quantitatively without side product





3.12

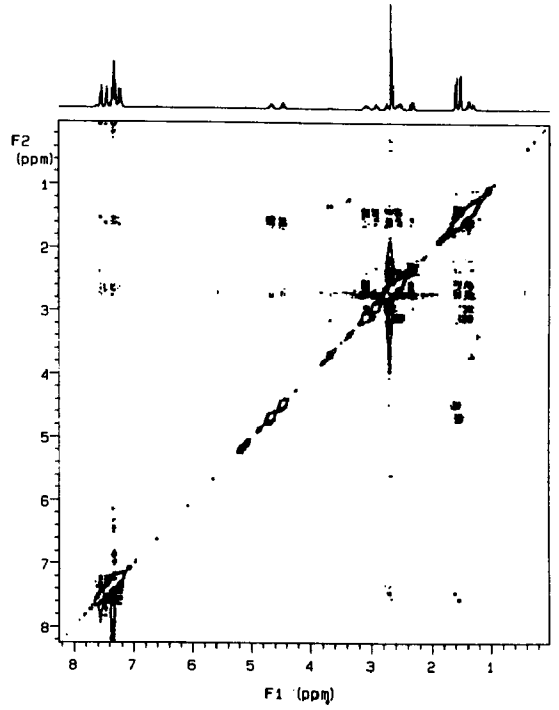
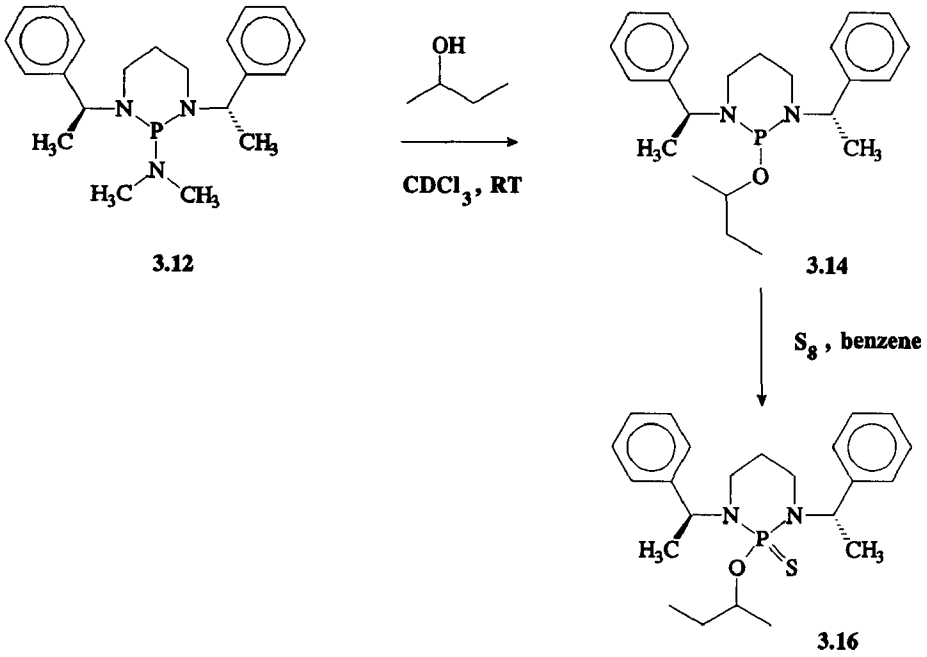


Figure 3.2 COSY and NOESY spectra of crude 3.12 recorded in C_6D_6 at [L]= 0.1 M

formation (except for certain entries that will be discussed in greater detail). The reagents **3.11** and **3.12** can be stored as such, or as a solution in benzene or chloroform for at least six months without decomposition.

3.2.2 *The use of diazaphospholidines 3.11 and 3.12 in the enantiomeric excess determination: scope and limitations*

As with reagent **3.7**, the exocyclic P–N bond of **3.11** and **3.12** is readily cleaved by various nucleophiles[9], including alcohols, amines and thiols. The derivatizing procedure involves the mixing of the substrate of interest (typically 0.1 mmole) with a slight excess (1.1 equivalent) of derivative **3.11** or **3.12** in the solvent of choice ($CDCl_3$ or C_6D_6, 1.5 mL), followed by stirring at room temperature until dimethylamine is no longer evolved (this can easily be checked with pH indicator). Normally, the reactions take about 1–8 h for completion.



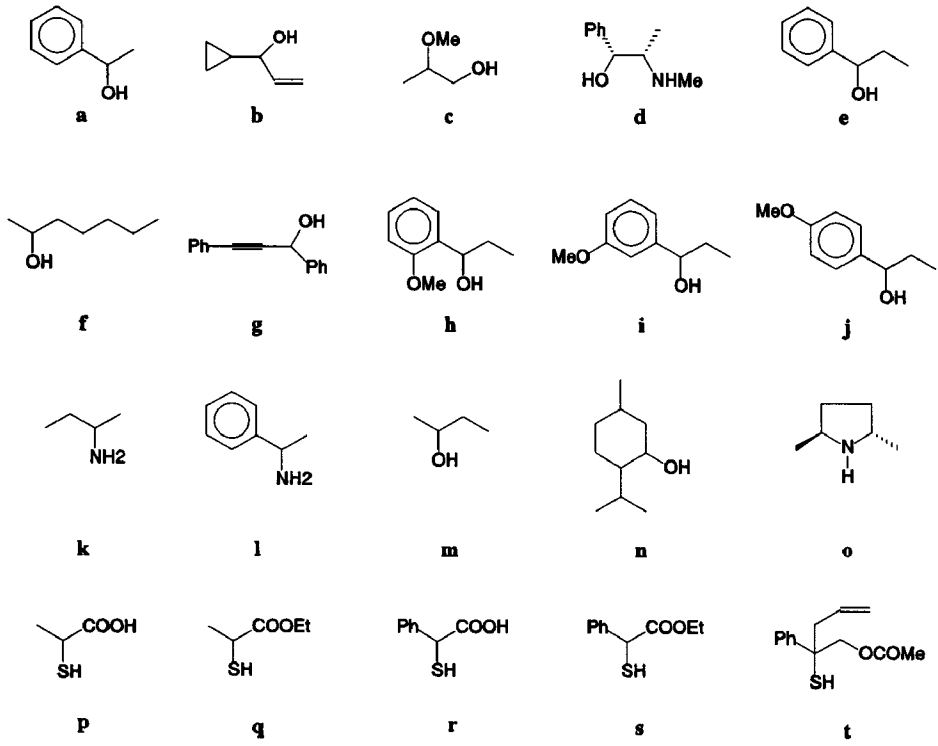
3.12 **3.14** **3.16**

Scheme 3.6 Reaction of 3.12 with racemic sec-butanol. Subsequent reaction with S_8 renders the compounds 3.14 into thiophosphoramidate 3.16

Subsequent ^{31}P or ^{1}H NMR analysis provides the diastereomeric ratios immediately. Since the diastereomeric derivatives **3.13** and **3.14** of reagents **3.11** and **3.12** (Scheme **3.6** and **3.11**) are not stable to TLC or GC analysis, the conversion was easily monitored converting **3.13** or **3.14** with sulfur (S_8) powder into the more stable thio derivatives **3.15** and **3.16**, analogous to the method described by Alexakis and co-workers[5,6]. The

thiophosphoramidates **3.15** and **3.16** are formed instantaneously and these air stable products are easily analyzed using TLC or GC techniques[6,13].

Reagents **3.11** and **3.12** were evaluated using a variety of racemic chiral alcohols, amines, amino alcohols, α–thiol acids and α–thiol acid esters and even some *free* amino acids[#] as shown in Scheme **3.7** and Tables **3.1** and **3.2**. Reactions took place with the nucleophiles tested so far within 8 h, regardless their steric bulk or structure. The obtained diastereomeric ratios for racemic substrates were all 50:50 within the experimental limits (2 %). No signs of kinetic resolution were found, not even when reactions were monitored during the complete course of the conversion. The results obtained with reagent **3.12** are collected in Table **3.1**, the indexes referring to Scheme **3.7**.



Scheme 3.7 Representative alcohols, amines and thio acid derivatives used for the e.e. determination. The indexes are also used in Table 3.1 and 3.2

The obtained Δδ values using ^{31}P NMR are typically in the order of 0.08 ppm (for **c**) to 4.52 ppm (for **d**), although larger shift differences have been observed. Usually, two nicely separated singlets are observed between δ 115–125 ppm for derivatized alcohols, between δ 90–100 ppm for derivatized amines and between δ 140–155 ppm for the thiol acid

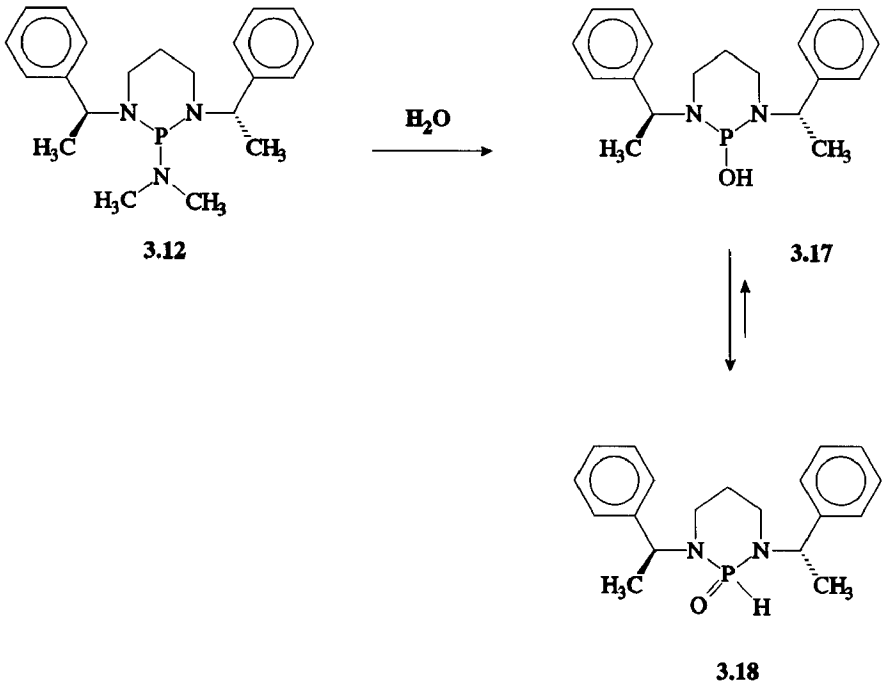
[#] Scheme **3.9** and the Tables **3.1** and **3.2** contain only a part of the analyzed substrates.

Substrate	δ (ppm)	$\Delta\delta$ (ppm)	ratio
a	121.52	1.38	50:50
b	120.60	0.15	50:50
c	116.25	0.08	49.5:50.5
d	121.70	4.52	50:50
e	118.53	3.69	50:50
f	120.60	1.10	49.4:50.6
g	118.05	4.62	49.5:50.5[a]
h	120.40	2.86	50:50
i	121.66	3.62	50:50
j	118.79	3.47	50:50
k	93.40	0.26	50:50
l	93.07	2.46	50:50
m	122.81	2.73	49.4:50.6
n	118.70	1.69	49.5:50.5
o	98.50	1.31	49.5:50.5
p	145.79	2.30	43:57[b]
q	152.47	0.23	42:58[b]
r	149.28	0.19	49.5:50.5
s	152.71	1.09	49.5:50.5
t	144.29	0.53	29.5:70.5[c]

Table 3.1 *^{31}P NMR data of derivatives of **3.12** and racemic alcohols, amines and α-thiol acids and the corresponding esters recorded in $CDCl_3$ [L]= 0.1 M.*
a) An additional signal was observed at δ 21.1 ppm, see text for explanation.
b) Enriched compounds were used, e.e. unknown.
c) The e.e. determined by GC analysis was 41%; unpublished results, Hof, R.P., Kellogg, R.M., manuscript in preparation.

adducts of **3.12** (for examples, see Chapter 3.2.3). It is clearly shown that, for the alcohols used, reagent **3.12** gives the largest shift differences ($\Delta\delta$) with alcohols that contain an aromatic group, as for **a** (1.38 ppm), **d** (4.52 ppm), **e** (3.69 ppm) and the series **h–j** (2.86–3.62 ppm). Also when bulky alcohols are used, relatively large $\Delta\delta$ values are obtained; a

typical example is menthol (**n**), showing a Δδ value of 1.69 ppm. The comparison, however, between the more bulky 2-heptanol (**f**) and smaller 2-butanol (**m**) shows that the largest Δδ is obtained when the smallest alcohol is used (1.10 and 2.73 ppm, respectively). When amines are used, the substrates possessing an aromatic ring system give the largest diastereomeric shift differences Δδ, as can be clearly seen when α-phenylethylamine (**l**) is compared with 2-aminobutane (**k**) with Δδ values of 2.46 ppm and 0.26 ppm respectively. The situation is not so clear when α-thiol acids or their ester derivatives are used. Sometimes the largest Δδ value is found for the free acid, as for **p** with Δδ 2.30 ppm compared to 0.23 ppm for the ester derivative (**q**). On the other hand, the ester derivative **3.14** (**s**) gives the largest Δδ value (1.09 ppm) as compared to the free acid **r** (0.19 ppm). Although reagent **3.12** is more reluctant towards moisture than the reagents **3.5**, **3.6** and **3.7**, described by Alexakis and co-workers[6], water can act as a nucleophile to give reaction to **3.18**.

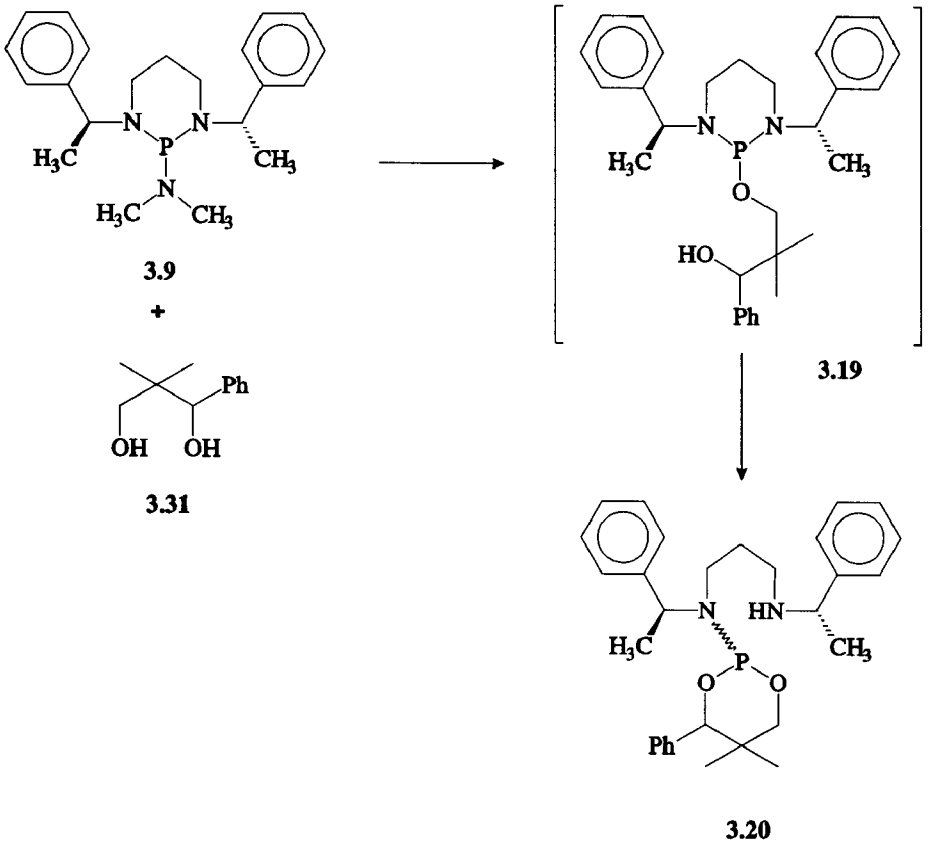


3.12 **3.17** **3.18**

Scheme 3.8 Reaction of reagent 3.12 with H_2O to 3.18

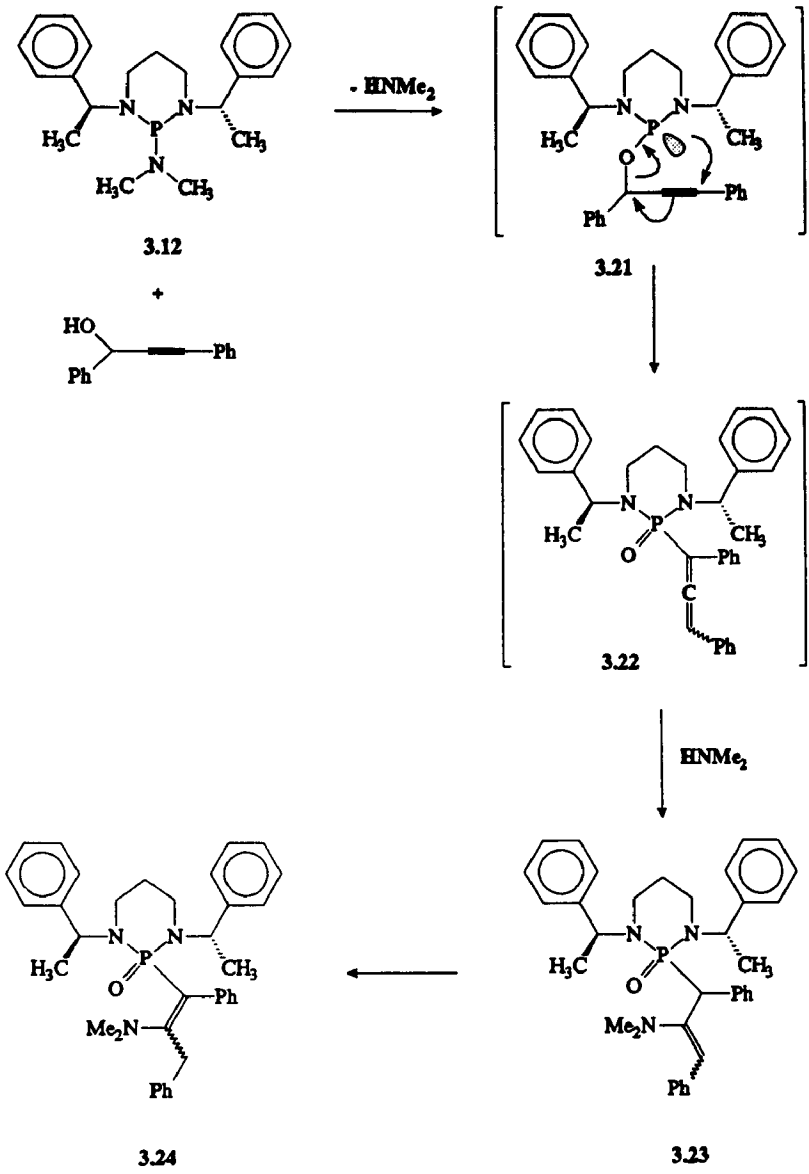
The rate of this reaction appears to be strongly dependent upon the amount of moisture present (Scheme **3.8**). When reactive alcohols or amines are used as nucleophiles in the presence of H_2O, it is possible to react them with reagent **3.12**, suppressing the side reaction with water to about 10-15%, without disturbance of the actual determination of the enantiomeric ratios.

We have also screened the applicability of the diazaphosphorinane **3.18** formed, in the enantiomeric excess determination. It appeared not to be of use because of decomposition under the conditions as needed (see Chapter 4 for the details).
Another drawback is encountered when diols are used as substrates. A cyclization reaction to the corresponding dioxaphospholane system **3.20** is observed, as depicted in Scheme **3.9** using *e.g.* phencydiol[14] **3.31** as the substrate (see also Chapter 4). Upon cleavage of the diazaphospholane ring moiety of **3.19**, the phosphorus atom in **3.20** becomes chiral and a number of signals, beside the signals belonging to the diastereomeric adducts, appear in the decoupled ^{31}P NMR spectrum.



Scheme 3.9 Reaction of 3.31 with reagent 3.12 to dioxaphospholane 3.20

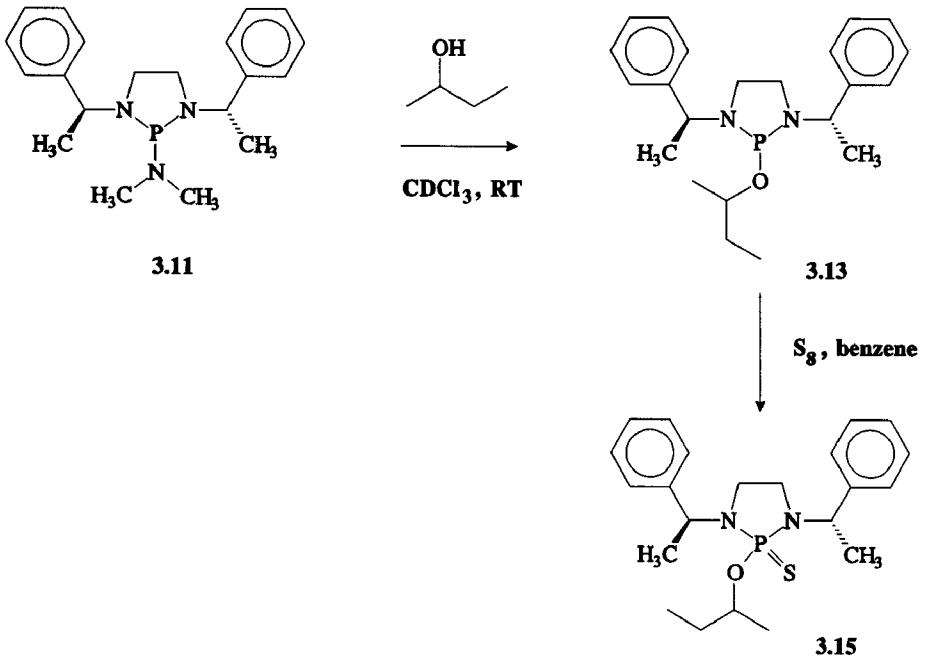
When propargylic alcohols, like in entry **g** (Table **3.1**), are used, a rapid [2,3]-sigmatropic rearrangement takes place[15,6] (Scheme **3.10**). This rearrangement appears to be so fast that the signals belonging to the initially formed products are not always observed. Although this type of rearrangement is known to occur with complete stereocontrol, the subsequent attack of the eliminated dimethylamine on the allene system of **3.22** ultimately gave enamine[16] **3.24**, therefore no information was obtained about the enantiomeric



Scheme 3.10 Proposed mechanism[6,15] for the [2,3]–sigmatropic rearrangement of propargylic alcohol and 3.12 (g in Table 3.1)

composition of propargylic alcohols by using this technique.
The results obtained with derivatizing agent **3.11** for enantiomeric excess determinations (Scheme **3.11**) are collected in Table **3.2**, the indices again referring to Scheme **3.7**.

The chemical shift differences Δδ (^{31}P NMR) as obtained using reagent **3.11** are smaller than the values as obtained using reagent **3.12**, although an interesting comparison can be made.



3.11 **3.13** **3.15**

Scheme 3.11 *Reaction of **3.11** with racemic sec-butanol. Subsequent reaction with S_8 transforms compound **3.13** into thiophosphoramidate **3.15***

When racemic alcohols are used as substrates, alcohols containing an aromatic ring (substrates **a** and **e**), typically give rather small Δδ values of 0.20 ppm and 0.39 ppm, respectively, when compared with the other alcohols, like **c** and **f**, which give much higher Δδ values, in the range from 1.05 ppm (**c**) to 1.98 ppm (**f**). This trend, however, is not so obvious as for reagent **3.12** , which shows an inversed shift dependency.

For amine containing substrates also, the observed shift differences appear to be larger for the alkyl amines when compared to the aromatic ring substituted counterparts. The largest Δδ value is found for 2-aminobutane (**k**, 5.21 ppm), while α-phenylethylamine (**l**) only gave a Δδ value of 0.15 ppm. Again, this trend seems to be the reverse of the shift behaviour of reagent **3.12**, showing the largest shift differences for α-phenylethylamine (**l**, 2.46 ppm) and the smallest for 2-aminobutane (**k**, 0.26 ppm).

Interesting is the behavior of reagent **3.11** in the analysis of free α-thiol acids (**p** and **r**), showing reaction with the thiol group as well as with the free acid group. The signals located at δ 136.51 and 134.29 ppm belong to the thiol derivatized product, whereas the acid derivatized product shows signals at δ 133.48 and 128.87 ppm. The calculated enantiomeric ratios, however, are in good agreement with each other, indicating that although two different competitive types of reaction are taking place at the same time

Substrate	δ (ppm)	Δδ (ppm)	ratio
d,l-Phe	96.91	0.49	49.5:50.5
d,l-Ala	96.99	0.45	49.5:50.5
d,l-PG[a]	122.51	0.19	49.5:50.5
e	124.08	0.39	49:51
c	125.34	1.05	49.5:50.5
f	125.00	1.98	49.5:50.5
a	124.15	0.20	49:51
g	114.20	5.21	49.5:50.5
l	94.36	0.15	50:50
k	94.61	5.21	50:50
d,l-heptylamine	93.68	0.29	49.6:50.4
t	123.89	0.08	49.1:50.9
r[c]	136.51	1.21	48.5:51.5[b]
	133.48	0.75	50:50
p[c]	134.29	0.68	43:57[b]
	128.87	0.53	44:56
s	136.84	0.56	50:50
q[c]	135.63	0.54	44:56

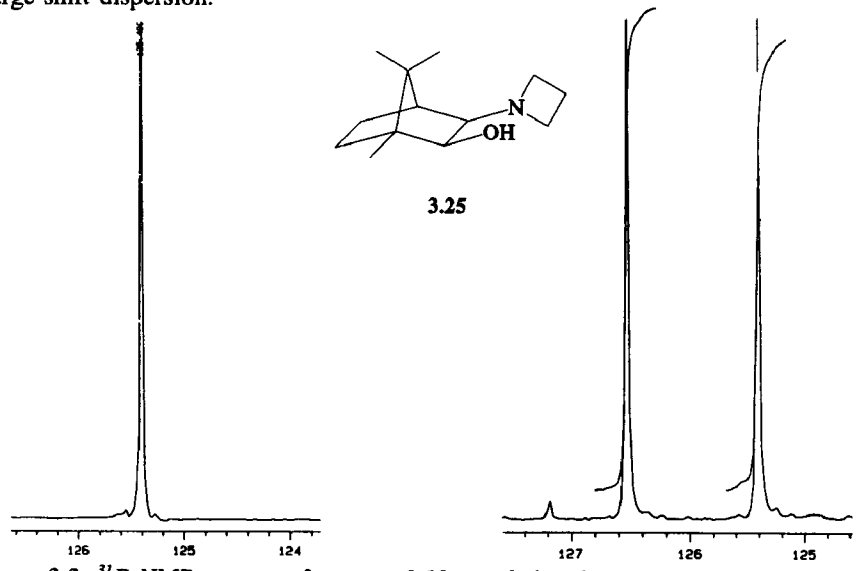
Table 3.2 *^{31}P NMR data of derivatives of **3.11** and racemic amines, alcohols, amino acids, α-thiol acids and α-thiol acid esters recorded in $CDCl_3$ [L]= 0.1 M.*
a) PG is Phenylglycine.
b) The additional absorption is due to P-O bond formation, see text for explanation.
c) Enantiomerically enriched product was used.

kinetic resolution is not likely to be of any importance.
Thus, when reagents **3.11** and **3.12** are compared, the difference in chemical shift differences ΔΔδ, appears to be sensitive not only to steric factors, but also to stereo-electronic and (ring) conformational effects. Similar behavior is also observed with a *series of substrates* and the reagents **3.11** or **3.12**.
In Chapter 6 the relationship between the induced chemical shift differences Δδ and geometry will be discussed in greater detail.

The side reactions as described for reagent **3.12** can also take place when **3.11** is used, although this reagent seems to be more sensitive towards moisture. In fact, reagent **3.11** shows a lesser selective reactivity, as compared to reagent **3.12** over the entire range of substrates used. Several free amino acids, however, were analyzed using these reagents under phase transfer conditions (solid-liquid phase). Both reagents are not reluctant towards traces of water in the reaction medium or ultimately towards water as the solvent itself, although both are more stable towards moisture than the reagents as reported by Alexakis and co-workers[5,6].

3.2.3 *Spectroscopic and conformational study of diastereomeric products of reagents 3.11 and 3.12*

As was already stated before, reagents **3.11** and **3.12**, and more so the diastereomeric products **3.13** and **3.14**, show rather complex spectral behavior in the ^{1}H NMR, due to the addopted conformations and excessive H-H and P-H coupling. Although it is possible to obtain the diastereomeric ratios from the ^{1}H NMR data, the use of ^{31}P NMR techniques is far superior, giving only two nicely separated signals for the diastereomeric products with a large shift dispersion.



3.25

Figure 3.3 ^{31}P NMR spectra of reagent 3.12 coupled with racemic and enantiomerically pure 3.25

In Figure **3.3**, the decoupled ^{31}P NMR spectra of the products of **3.12** and racemic (a) and enantiomerically pure (-)-*cis*-exo-3-(1-azetidinyl)isoborneol **3.25** (b) are given, recorded in $CDCl_3$ [L]= 0.1 M. As can be seen, the diastereomeric ratio is readily obtained from the

integration of both signals: the absence of the second signal in (*b*) indicates that the material used is enantiomerically pure[#] within the error limits of the NMR technique[##].
When racemic α-phenylethylalcohol is coupled to reagent **3.12** (**a**, Table **3.1**), the diastereomeric shift differences Δδ are not only seen in the decoupled ^{31}P NMR spectrum (Δδ 1.38 ppm), but also in the ^{1}H NMR, allowing not only the analysis of the diastereomeric ratio but also providing a tool to gather more information about the conformational behavior of the diastereomeric adducts (Figure **3.4**).

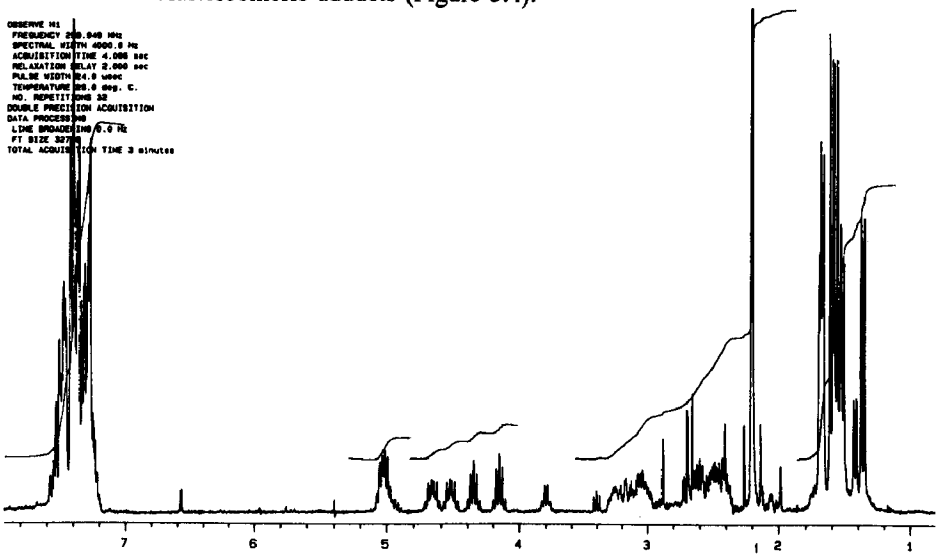


Figure 3.4 *^{1}H NMR spectrum of the diastereomeric product **3.14** using racemic α-phenylethylalcohol (**a**, Table **3.1**) recorded in $CDCl_3$ [L]= 0.1 M*

The crude spectrum of **3.14a** shows a complex pattern of six multiplets with a ^{3}J coupling, due to the α proton of the alcohol moiety, and a $^{4}J_{PH}$ coupling arising from the phosphorus nucleus, located between δ 1.37–1.69 ppm, all belonging to the three methyl groups. The protons $\underline{4}^{ax}$ and $\underline{4}^{eq}$ of both diastereomers (***A*** and ***B***) of **3.14a** are also located in this area (δ 1.80 ppm), as can be seen from the COSY spectrum (Figure **3.5**). The protons $\underline{3}^{ax}$, $\underline{3}^{eq}$, $\underline{5}^{ax}$ and $\underline{5}^{eq}$ of both diastereomers are situated in the range δ 2.40–3.35 ppm, and can only be fully assigned on the basis of the 2D COSY spectrum and the coupling constants. The axial protons $\underline{3}$ and $\underline{5}$ are located at δ 2.41 and δ 2.58 ppm for diastereomer ***A*** and at δ 2.50 and δ 2.63 ppm for diastereomer ***B***. For diastereomer ***A*** the equatorial protons $\underline{3}$ and $\underline{5}$ are located at δ 3.10 and δ 3.29 ppm, whereas the resonances belonging to diastereomer ***B*** are assigned resonating at δ 3.04 and δ 3.21 ppm, respectively.

[#] Both the racemic and the enantiomerically pure substrates were synthesized by Drs Andre de Vries, the experimental details will be part of his forthcoming thesis.

[##] For a short discussion about the error limits of NMR compared to GC and HPLC techniques see; Schurig, V., *Kontakte (Darmstadt)* **1985**, *2*, 22; **1986**, *1*, 3.

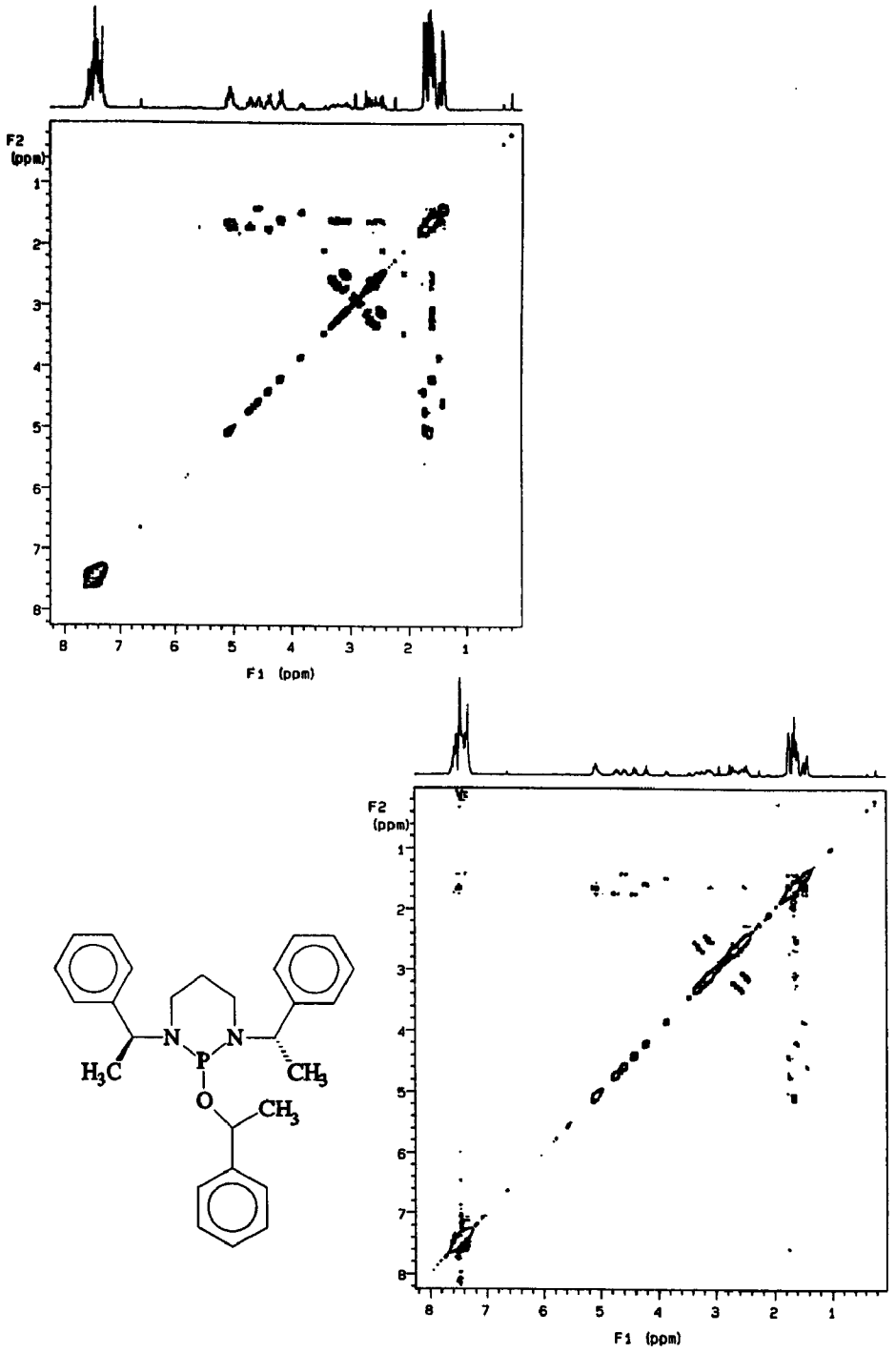


Figure 3.5 COSY and NOESY spectra of product 3.14 using racemic α-phenyl-ethanol (a) recorded in $CDCl_3$ [L]= 0.1 M

The most striking diastereomeric shift differences, however, are observed in the exocyclic part of the molecule. The protons 2 and 6 belonging to diastereomer *A* resonate at δ 4.20 and at δ 4.58 ppm respectively, whereas the same protons in diastereomer *B* are shifted to δ 4.40 and δ 4.66 ppm. Surprisingly, the α proton of the substituted alcohols hardly shows any diastereomeric shift dispersion; both show absorptions at δ 5.01 and δ 5.09 ppm. The large signal at δ 2.20 ppm belongs to the liberated dimethylamine, which does not interfere with the enantiomeric excess determination itself.

From the NOESY spectrum the interaction between the α proton of the substituted alcohol and proton 3^{ax} (diastereomer *B*) are clearly seen, probably due to the π–stacking of the three aromatic ring systems. This *normally* rather sterically hindered conformation brings the α proton in the vicinity of the axial proton at position 3. This would mean that the alcohol moiety posseses the axial position on phosphorus, since this is the only possible way for the system to give an interaction of the α proton with either one of the axial ring protons. Clearly, this substitution at the phosphorus center proceeds with inversion of configuration with respect to the NMe_2 and free electron pair on the phosphorus nucleus of reagent **3.12**. The rather large diastereomeric shift dispersion when using aromatic alcohols or amines as the substrates, could very well be the result of the π–π stacking ability. Rather severe conformational changes are necessary to bring the aromatic systems in the desired positions.

Strangely enough, no *special* NOESY signals[#] were found for diastereomer *A*. Based upon the data from the NOESY spectrum and measurements using enantiomerically pure (*S*)–α–phenylethylalcohol (not shown), it was concluded that diastereomer *B* must be the product of reagent **3.12** and (*S*)–α–phenylethylalcohol.

For diastereomeric products of reagent **3.11**, the observed chemical shift dispersion in the ^1H NMR is much smaller. When α–phenylethanol is coupled to reagent **3.11** the observed diastereomeric shift difference in the decoupled ^{31}P NMR (δ 0.20 ppm) is much smaller compared to the same adduct with reagent **3.12**. Using NOESY techniques, no signs of interaction between the coupled alcohol moiety and the five membered ring was found for either of the diastereomers. This strongly suggests that the π–π stacking as proposed for the adducts of reagent **3.12**, is of no importance here, leaving the alcohol moiety in the equatorial or at least strongly distorted axial position with no possibility to give an interaction with any of the protons belonging to the five ring system.

Also when alcohols that do not contain aromatic rings are coupled to reagents **3.11** or **3.12** interactions of interest are not observed in the NOESY spectra.

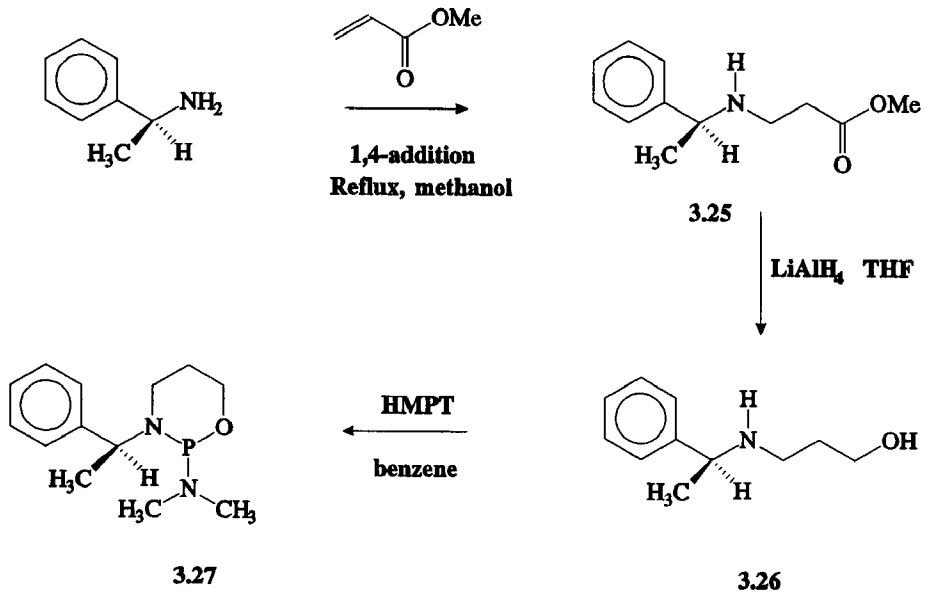
It should be noted, however, that the chemical shift is not only a function of structural variations like bond angles and lengths, but also of factors like electronegativity of the phosphorus nucleus and the substituents[11,17]. It is clear that all these factors together play a role here, the overall effect resulting in the observed chemical shift dispersion.

[#] Except for the NOESY interactions that were expected, like the interactions of the ring protons with each other very little NOE's were observed, which we not anticipated on the basis of the steric effects noted above.

In Chapter 6, the correlation between structural factors and chemical shift of penta-coordinated phosphorus will be discussed in greater detail.

3.2.4 *Other trivalent phosphorus containing reagents for the enantiomeric excess determination*

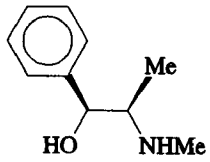
As was already mentioned in Chapter 3.2.2 and shown in Schemes **3.8** and **3.9**, phosphorus shows great preference for being substituted with oxygen (trivalent phosphorus containing compounds are in fact easily oxidized into a pentavalent moiety[18]).
We therefore developed several cyclic chiral, trivalent phosphorus derivatives which bear one or two oxygen phosphorus bonds in the ring, expecting that these systems would show a higher tolerance towards water, and show a higher selectivity towards reaction with nucleophiles. For the comparison purpose, one of the amino alcohols (**3.26**) used contains a α-phenylethylamine unit, as is shown in Scheme **3.12**. Treatment of methylacrylate with (*S*)-α-phenylethylamine easily yields the methyl-3-*N*-(1-(*S*)-phenylethyl)aminopropionate **3.25** in 93% yield, which was subsequently reduced with $LiAlH_4$ to amino alcohol **3.26** in 64% yield after a Soxhlet extraction of the lithium salts[12,19]. Amino alcohol **3.26** was also obtained by means of a nucleophilic substitution reaction using 3-chloropropanol and (*S*)-α-phenylethylamine without solvent[12].



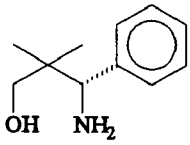
Scheme 3.12 Synthesis of product 3.27

The enantiomeric purity of **3.26** was checked using the methodology described in Chapter 4[20], which indicated that no racemization had taken place.

The reaction of **3.26** with HMPT in dry benzene, however, yielded a mixture of products, containing the two expected diastereomers of **3.27** (Note that in **3.27**, the phosphorus atom is chiral). Based upon ^{31}P NMR data of the crude mixture, it is clear that also *bis* and even *tris* oxygen substituted phosphorus derivatives and elimination products were formed, although product **3.27** is the main product (yield 65%). It appears that reaction with alcohols proceeds more readily than the desired intramolecular reaction. Attempts to separate and purify the products by distillation resulted in *violent explosions*. Column chromatography also, was not successful and gave rise to decomposition of all materials.



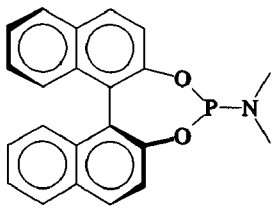
3.28



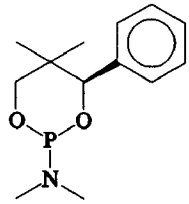
3.29

Scheme 3.13 Two amino alcohols that were not successfully derivatized with HMPT

When other amino alcohols, like ephedrine **3.28** and amino alcohol **3.29**, were used in the reaction with HMPT, the same kind of problems were faced (Scheme **3.13**).
It became obvious that, when the crude mixtures of derivatives **3.28** or **3.29** were allowed to react with racemic alcohols like *e.g.* α-phenylethanol, diastereomeric products were formed showing large shift dispersion Δδ, although the obtained results were not of direct use for analytical purposes, due to the problems already mentioned.



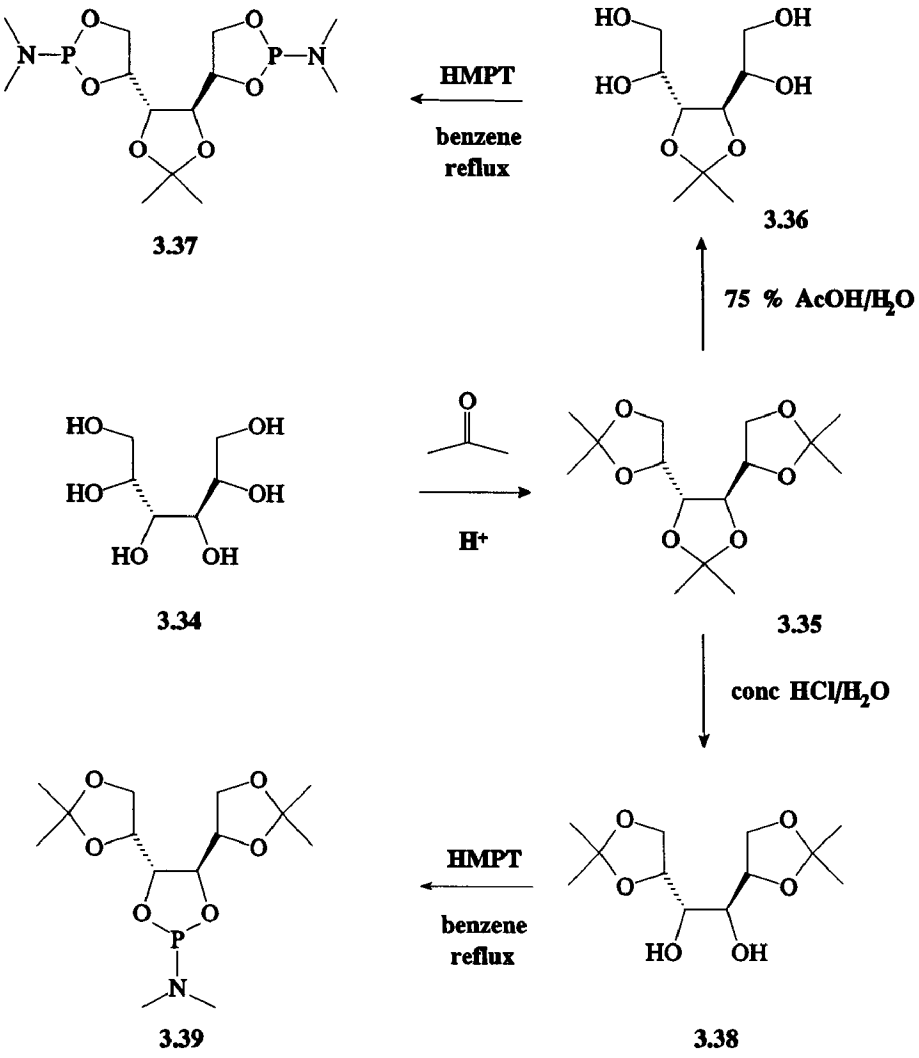
3.32



3.33

Scheme 3.14 Dioxophospholidines based upon easily available diols

Being aware of the synthetic problems that arise when trivalent phosphorus reagents like HMPT are coupled to amino alcohols, we decided to use chiral diols as ligands. Readily available diols, like (+)-bis-ß-naphthol **3.30** (not shown) and (*R*)-phencydiol **3.31** (Scheme **3.9**) were functionalized with HMPT by reflux in dry benzene, yielding the trivalent phosphoric products **3.32** and **3.33** in nearly quantitative yield (Scheme **3.14**).



Scheme 3.15 Trivalent derivatizing agents based upon mannitol

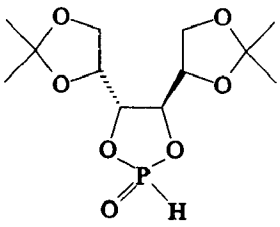
In the case of **3.33**, two diastereomers were obtained, which could not be separated, although the ^{31}P NMR signals were so far apart ($\Delta\delta$ 37.12 ppm), that use as an *e.e.* determining agent might be possible. These materials appeared to be stable towards moisture and oxygen. In fact these materials do not react with alcohols, amines or water under the employed conditions. Also, when more drastic conditions were employed, like

the use of bases (Et_3N, NaH, NaOH or n-butyllithium#) to enhance the nucleophilicity of the substrates, no reaction was achieved.

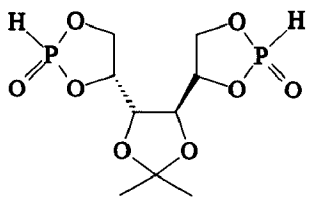
Another approach was based upon the use of trivalent phosphorus containing *sugar-like* derivatives. These ligands are capable of forming strong complexes with water when functionalized with pentavalent phosphorus, as became obvious in the course of the work described in the Chapters 4 and 5. In fact, we have synthesized these (tri or pentavalent) systems to investigate their possible behavior as cell-membrane mimics[22b], as well as possible *e.e.* determining agents. Using mannitol **3.34** as the sugar building block, trivalent phosphorus derivatives **3.37** and **3.39** were synthesized according to Scheme **3.15**.

First, *D*-mannitol was functionalized with acetone giving 1,2-3,4-5,6-O-tri-iso-propylidene-*D*-mannitol **3.35** in 70% yield[21]. Subsequent selective hydrolysis using aqueous acetic acid, yielded the tetra alcohol 3,4-O-isopropylidene-*D*-mannitol **3.36** in 80%[22]. Reaction with HMPT afforded the bis-dioxophospholidene **3.37** in 93% yield as a white, air and moisture stable, solid material.

From **3.35** the 1,2-5,6-di-O-isopropylidene-*D*-mannitol **3.38** was also easily available (61%)[23], which was subsequently converted into the desired dioxophospholidine **3.39** in 86% yield. As with **3.37**, this material was an air and moisture stable white solid. Adduct **3.37** appears to be enantiomerically pure with respect to the phosphorus nuclei, which is surprising when compared to the HMPT derivatives of the amino alcohols mentioned earlier.



3.40



3.41

Scheme 3.16 Formation of pentavalent phosphites from trivalent phospholidines

As with derivatives **3.32** and **3.33**, however, reagents **3.37** and **3.39** did not show any reactivity towards alcohols or amines, regardless the conditions used, including the use of bases (NaH, Et_3N) to enhance the nucleophilicity of the substrates. Both derivatives reacted with one or two equivalents of water, respectively, under controlled conditions, yielding pentavalent phosphorinanes **3.40** and **3.41** in 90-95% yield (Scheme **3.17**). The

When n-butyllithium is used as a base, decomposition took place resulting in the liberation of the free diols. Unpublished results, Drs Thijs Stock.

use of the phosphorinanes in the enantiomeric excess determination is described in detail in Chapter 4, although the results using them as e.e. determining agents were not hopeful. Again, as with the other oxygen containing phospholidines, the observed reactivity is too low for reaction with alcohols and amines, regardless their structure. This implies that these compounds are not useful as *e.e.* determining agents.

3.3 *Conclusions*

In Chapter 3 the successful synthesis of two, trivalent phosphorus containing chiral derivatizing agents, as well as their use as agent for the enantiomeric excess determination is described. The described compounds **3.11** and **3.12** are not the first of this type, since Alexakis and co-workers developed several trivalent phosphorus based derivatizing agents[5,6]. However, the synthetic availability and stability of the in this Chapter described compounds is striking when compared with those previously reported. The amines used by Alexakis, chiral cyclic diamines, are rather expensive and only available after much synthetic work. Furthermore, the trivalent phosphorus containing agents appear not to be stable towards moisture. The systems described in this chapter are easily available and are based upon cheap α-phenylethylamine. After derivatization with HMPT, the trivalent phosphorus containing derivatives appear to be reasonable stable towards moisture; it is even possible to react them with alcohols in the presence of traces water, provided that reactive alcohols or amines are used.
Several types of substrates are derivatized and the enantiomeric excess of alcohols, amines, amino alcohols, α-thiol acids and several amino acids can be determined easily. The diastereomeric shift differences are usually large when ^{31}P NMR techniques are used. Besides ^{31}P NMR techniques, ^{1}H NMR can also be applied sometimes. Studies based upon the use of 2D NMR techniques were performed in the hope to gather insight in the factors that govern the diastereomeric shift differences as a function of the intrinsic conformational parameters. Although not very successful, these results were useful in the modelling studies as described in Chapter 6.
Several other derivatizing agents based upon trivalent phosphorus were developed in order to reduce the reactivity towards (traces) water. This resulted in derivatives that showed in fact too little reactivity to be used as derivatizing agents in the enantiomeric excess determination. The newly synthesized compounds form, however, an interesting new class of molecules.

3.4 *Experimental*

For general remarks, see Chapter 2.5. Due to purification problems, not all the trivalent phosphorus derivatives could be fully characterized.

N,N'-bis(1-(S)-Phenylethyl)-1,2-ethylenediamine (3.9)[12]
(*S*)-α-Phenylethylamine (72.0 g, 0.59 mol) was heated to 100 °C and 1,2-dichloroethane (22.5 g, 0.23 mol) was added to the stirred solution over 2 h. Stirring of the mixture was continued for 16 h at 100 °C. The mixture was cooled to 60 °C and 150 mL of a saturated

KOH solution in water was added with continued stirring. After cooling to room temperature, the mixture was extracted three times with 150 mL of CH_2Cl_2. The combined organic layers were washed with brine and dried (Na_2SO_4). After removal of the solvent unreacted (*S*)–α–phenylethylamine was removed by vacuum distillation. The product was distilled (149–150 °C, 0.5 mm Hg)(lit[12] 110 °C, 0.02 mm Hg), yielding a colorless oil. Yield 37.5 g (0.15 mol, 62%). $[\alpha]_D^{20}$= –69.4° (c 1.10, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.35 (d, ^{3}J= 7.45 Hz, 6H), 1.50 (s, br, 2H), 2.54 (m, 4H), 3.65 (q, ^{3}J= 7.45 Hz, 2H), 7.20–7.41 (m, 10H); ^{13}C NMR ($CDCl_3$): δ 24.31 (CH_3), 47.22 (CH_2) 58.01 (CH), 126.40 (CH), 126.58 (CH), 127.99 (CH), 145.71 (C); HRMS calcd 268.194, found 268.193.

N,N'–bis(1–(S)–Phenylethyl)–1,3–propylenediamine (3.10)[12]

This compound was prepared using the procedure as described for **3.9** from 43.6 g (0.36 mol) (*S*)–α–phenylethylamine and 25.0 g (0.12 mol) of 1,3–dibromopropane. The product was distilled at 148–151 °C, 0.01 mm Hg (lit[12] 115 °C, 0.02 mm Hg) to yield a colorless oil (28.6 g, 0.10 mol, 84%). $[\alpha]_D^{20}$= –66.3° (c 0.55, $CHCl_3$)(lit[12] $[\alpha]_D^{20}$= –66.4°); ^{1}H NMR ($CDCl_3$): δ 1.38 (d, ^{3}J= 6.65 Hz, 6 H), 1.43 (s, br, 2H), 1.62 (m, 2H), 2.52 (m, 2H), 3.78 (q, ^{3}J= 6.65 Hz, 2H), 7.21–7.40 (m, 10H); ^{13}C NMR ($CDCl_3$): δ 24.28 (CH_3), 30.28 (CH_2), 46.32 (CH_2), 58.30 (CH), 126,42 (CH), 126.66 (CH), 128.31 (CH), 145.68 (C); HRMS calcd 282.210, found 282.210.

N,N'–bis(1–(S)–Phenylethyl)–1,2–ethylenediamino–N,N'–diaza–N'',N''–dimethyl–phospholidine (3.11)

A mixture of bisamine **3.9** (2.50 g, 9.33 mmole), hexamethylphosphorus triamide (3.05 g, 18.7 mmole) and a catalytic amount of dry NH_4Cl was gently refluxed in 50 mL of dry benzene for 96 h. During the reaction a stream of N_2 was passed through the flask in order to remove the formed dimethylamine. Benzene and excess $P(NMe_2)_3$ were removed under vacuum (0.01 mm) at 50 °C. The resulting oil was purified by chromatography over Al_2O_3 (benzene) and used as such. *Attempts to distill the products were not successful, and sometimes resulted in violent explosions.* The product appeared to be over 98% pure, based upon ^{31}P and ^{1}H NMR data. Yield 2.86 g (8.40 mmole, 90 %), colorless oil.
^{1}H NMR (C_6D_6): δ 1.55 (d, ^{3}J= 7.20 Hz, 3H), 1.58 (d, ^{3}J= 7.18 Hz, 3H), 2.54 (d, $^3J_{PH}$= 9.00 Hz, 6H), 2.62 (m, 1H), 2.76 (m, 1H), 3.02 (m, 1H), 3.09 (m, 1H), 4.18 (dq, $^3J_{PH}$= 7.15 Hz, ^{3}J= 7.20 Hz, 1H), 4.23 (dq, $^3J_{PH}$= 7.15 Hz, ^{3}J= 7.18 Hz, 1H), 7.18–7.39 (m, 10H); ^{13}C NMR (C_6D_6): δ 21.34 (d, $^3J_{PC}$= 13.10 Hz, CH_3), 21.91 (d, $^3J_{PC}$= 12.09 Hz, CH_3), 37.13 (d, $^3J_{PC}$= 17.21 Hz, CH_3), 46.82 (d, $^2J_{PC}$= 7.05 Hz, CH_2), 47.13 (d, $^2J_{PC}$= 8.18 Hz, CH_2), 56.76 (d, $^2J_{PC}$= 22.16 Hz, CH), 57.12 (d, $^2J_{PC}$= 18.13 Hz, CH), 126.65 (CH), 126.69 (CH), 127.42 (CH), 127.93 (CH), 128.03 (CH), 128.03 (CH), 128.33 (CH), 145.29 (d, $^3J_{PC}$= 5.02 Hz, C), 145.43 (d, $^3J_{PC}$= 5.03 Hz, C); ^{31}P NMR (C_6D_6): δ 105.62; HRMS calcd 341.202, found 341.202.

N,N'–bis(1–(S)–Phenylethyl)–1,3–propylenediamino–N,N'–diaza–N'',N''–dimethyl–phospholidine (3.12)

Prepared as described for **3.11**, using bisamine **3.10** (2.50 g, 8.86 mmole), yield 2.99 g (8.42 mmole, 95%). ^{1}H NMR (C_6D_6): δ 1.48 (d, ^{3}J= 6.05 Hz, 3H), 1.49 (m, *H4^{ax}*, 1H), 1.50 (d, ^{3}J= 5.90 Hz, 3H), 1.51 (m, *H4^{eq}*, 1H), 2.48 (d, $^3J_{PH}$= 9.05 Hz, 6H), 2.53 (m, *H3^{ax}*, 1H), 2.62 (m, *H5^{ax}*, 1H), 2.90 (m, *H3^{eq}*, 1H), 3.10 (m, *H5^{eq}*, 1H), 4.31 (dq, $^3J_{PH}$= 3.00 Hz, ^{3}J= 6.05 Hz, *H2*, 1H), 4.45 (dq, $^3J_{PH}$= 4.10 Hz, ^{3}J= 5.90 Hz, *H6*, 1H), 7.18–7.42 (m, *ArH*,

10H); ^{13}C NMR (C_6D_6): δ 17.87 (d, $^3J_{PC}$= 6.05 Hz, CH_3), 19.75 (d, $^3J_{PC}$= 13.09 Hz, CH_3), 26.66 (CH_2), 38.48 (d, $^2J_{PC}$= 18.13 Hz, CH_3), 38.97 (d, $^2J_{PC}$= 5.03 Hz, CH_2), 40.81 (d, $^2J_{PC}$= 3.02 Hz, CH_2), 58.14 (d, $^2J_{PC}$= 38.27 Hz, CH), 59.82 (d, $^2J_{PC}$= 35.26 Hz, CH), 126.36 (CH), 126.44 (CH), 127.24 (CH), 127.92 (CH), 127.96 (CH), 128.00 (CH), 144.63 (d, $^3J_{PC}$= 9.06 Hz, C), 145.15 (d, $^3J_{PC}$= 5.04 Hz, C); ^{31}P NMR (C_6D_6): δ 107.41; HRMS calcd 355.218, found 355.217.

Methyl-3-N-(1-(S)-phenylethyl)aminopropionate (3.25)

A solution of methylacrylate (50.0 g, 0.58 mol), (*S*)-α-phenylethylamine (54.5 g, 0.45 mol) and 250 mL of absolute methanol was heated to reflux for 12 h, and then concentrated. The remaining oil was directly distilled (bp 114–116 °C, 0.09 mm Hg), affording a colorless oil. Yield 80.7 g (0.39 mol, 86%). $[\alpha]_D^{20}$= −35.11° (c 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.26 (d, ^3J= 6.00 Hz, 3H), 1.62 (s, br, 1H), 2.40 (d, ^3J= 6.00 Hz, 1H), 2.41 (d, ^3J= 6.00 Hz, 1H), 2.64 (m, 2H), 3.57 (s, 3H), 3.71 (q, ^3J= 6.00 Hz, 1H), 7.18 (m, 1H), 7.22–7.27 (m, 4H); 24.15 (CH_3), 34.27 (CH_2), 42.51 (CH_2), 51.04 (CH), 57.77 (CH_3), 126.16 (CH), 126.50 (CH), 128.02 (CH), 145.16 (C), 172.80 (C); Analysis calcd for $C_{12}H_{17}NO_2$, C: 69.54, N: 6.76, H: 8.27. Found, C: 69.38, N: 6.71, H: 8.22; HRMS calcd 207.126, found 207.126

N-(1-(S)-Phenylethyl)aminopropanol (3.26)

A solution of **3.25** (25.0 g, 0.12 mol) in dry ether (100 mL) was added to a suspension of $LiAlH_4$ (9.61 g, 0.25 mol) in dry ether (100 mL). After heating under reflux for 6 h followed by stirring overnight at room temperature, water was added (10 mL), followed by 10 M NaOH solution (50 mL) and 5.0 g silicagel. The suspension was filtered over a P2 glass frit and the filtrate layers were separated. The ether layer was washed with water (50 mL) and brine (50 mL), dried over Na_2SO_4 and concentrated to an oil which was distilled (105–106 °C, 0.05 mm Hg) to give **3.26**. Yield 16.7 g (93.02 mmole, 77%).
$[\alpha]_D^{20}$= −53.02° (c 0.19, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.30 (d, 3H), 1.61 (m, 2H), 2.57 (m, 1H), 2.63 (m, 1H), 3.61 (m, 2H), 3.63 (m, 1H), 7.10–7.30 (m, 5H); ^{13}C NMR ($CDCl_3$): δ 23.80 (CH_3), 31.17 (CH_2), 46.86 (CH_2), 58.20 (CH), 63.09 (CH_2), 126.13 (CH), 126.68 (CH), 128.17 (CH), 144.61 (CH);; Analysis calcd for $C_{11}H_{17}NO$, C: 73.70, N: 7.81, H: 9.56. Found C: 73.55, N: 7.66, H: 9.48; HRMS calcd 179.131, found 179.131.

N-(1-(S)-Phenylethyl)aminopropanol (3.26)

Prepared as described for **3.9** from 3-chloropropanol (20.0 g, 213 mmole) and (*S*)-α-phenylethylamine (25.7 g, 213 mmole). Yield 41.4 g (200 mmole, 94%). Spectral data and purification as described above.

2,2'-O,O-(1,1'-Binaphthyl)-O,O'-dioxo-N,N-dimethyl-phospholidine (3.32)

(+)-bis-ß-Naphtol (2.00 g, 7.50 mmole), HMPT (1.40 g, 9.50 mmole), 0.01 g NH_4Cl and 10 mL of dry benzene were heated to reflux for 12 h. The mixture was concentrated under reduced pressure affording an oil. The oil was stirred with 25 mL of dry ether, upon which crystals were formed spontaneously. The crystals were recrystallized from dry ether. Yield 2.65 g (7.38 mmole, 98%). $[\alpha]_D^{20}$= 578.95° (c 0.06, $CHCl_3$); Mp 190–191 °C;
^1H NMR ($CDCl_3$): δ 2.75 (d, $^3J_{PH}$= 9.20 Hz, 6H), 7.25–7.55 (m, 8H), 7.91–8.00 (m, 4H); ^{13}C NMR ($CDCl_3$): δ 35.81 (d, $^3J_{PC}$= 22.00 Hz, CH_3), 121.83 (d, $^3J_{PC}$= 1.01 Hz, CH), 123.02 (d, $^3J_{PC}$= 84.00 Hz, C), 124.53 (d, $^4J_{PC}$= 15.13 Hz, CH), 125.94 (s, CH), 126.78 (d, $^4J_{PC}$= 6.04 Hz, CH), 128.14 (d, $^7J_{PC}$= 6.01 Hz, CH), 130.01 (d, $^6J_{PC}$= 36.03 Hz, CH),

130.80 (d, $^4J_{PC}$= 47.38 Hz, C), 132.67 (d, $^3J_{PC}$= 2.01 Hz, C), 149.51 (d, $^2J_{PC}$= 38.31 Hz, C): ^{31}P NMR ($CDCl_3$): δ 148.72; Analysis calcd for $C_{22}H_{18}NO_2P$, C: 73.53, N: 3.90, P: 8.62, H: 5.05. Found C: 73.39, N: 3.74, P: 8.39, H: 4.97; HRMS calcd 359.107, found 359.108.

1,2-3,4-5,6-Tri-O-isopropylidene-D-mannitol (3.35)[21]

A suspension of *D*-mannitol (50.0 g, 0.27 mol), acetone (685 mL) and concentrated H_2SO_4 (6 mL) was stirred at room temperature. After stirring for 2 h the solution became clear and was stirred for another 8 h. Subsequently, the solution was diluted with H_2O (750 mL) upon which crystals formed spontaneously. The crystals were dried in vacuum at 50 °C and recrystallized from ethanol–H_2O, affording tiny white needles. Yield 57.38 g (0.19 mol, 70%). $[\alpha]_D^{20}$= 12.5° (c= 1.0, ethanol)(lit[21] $[\alpha]_D^{20}$= 12.5°, c= 1.0, ethanol);
Mp 69–70 °C (lit[21] 67–68 °C); ^{1}H NMR ($CDCl_3$): δ 1.26 (s, 6H), 1.31 (s, 6H), 1.32 (s, 6H), 3.98 (m, 2H), 4.11 (m, 2H), 4.2 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 25.16 (CH_3), 26.32 (CH_3), 27.26 (CH_3), 65.33 (C), 63.82 (CH_2), 69.61 (CH), 71.26 (CH), 108.70 (C); HRMS calcd 302.173, found 302.172.

3,4-O-Isopropylidene-D-mannitol (3.36)[21,22]

1,2–3,4–5,6–Tri–O–isopropylidene–*D*–mannitol **3.35**, (30.0 g, 99.3 mmole) was dissolved in 75% aqueous acetic acid (650 mL) and kept at 45 °C for 2 h. The solvent was removed at reduced pressure and twice co–evaporated with benzene. The obtained light yellowish oil was dissolved in acetone (1 L) and stirred with K_2CO_3 (30.0 g). Excess K_2CO_3 and mannitol were removed by filtration over a P4 glass frit. The filtrate was evaporated to yield an oil. The oil was dissolved in chloroform (250 mL) and stirred with 10.0 g of siligagel. After filtration, petroleum–ether 40–60 was added until **3.29** crystallized as white, very tiny crystals. Yield 17.32 g (79.44 mmole, 80%). $[\alpha]_D^{20}$= 29.61° (c 0.5, methanol); Mp 84–85 °C (lit[21,22] 85 °C); ^{1}H NMR (DMSO d_6): δ 1.28 (s, 6H), 3.31–3.40 (m, 2H), 3.41–3.62 (m, 4H), 3.79–3.91 (m, 2H), 4.50 (s, br, 2H), 5.15 (s, br, 2H); ^{13}C NMR (DMSO d_6): δ 27.24 (CH_3), 62.98 (CH_2), 72.88 (CH), 79.07 (CH), 108.28 (C); HRMS calcd 222.110, found 207.087 (– CH_3).

1,2-5,6-Di-O-isopropylidene-D-mannitol (3.38)[22]

A suspension of *D*–mannitol (200.0 g, 1.10 mol), acetone (4 L) and concentrated HCl (80 mL) was stirred for 4 h. The solution was neutralized with K_2CO_3 and concentrated to an yellowish oil. Subsequently, the oil was dissolved in 150 mL of boiling ethanol and filtered over a P4 glass frit. Upon cooling, tri–isopropylidene–*D*–mannitol **3.35** crystallized and was purified as described above. The filtrate was concentrated to a volume of 50 mL and stirred with hot water (150 mL) for 30 min. The remaining solution was filtered from traces of tri–isopropylidene–*D*–mannitol and refluxed in petroleum–ether 40–60 for 3 h. After the solution was cooled to room temperature, large lumps of crystalline material were obtained, which were crystallized from petroleum ether 40–60. The procedure was repeated twice, affording large needles.
Yield 16.6 g (63.7 mmole, 5.8%). $[\alpha]_D^{20}$= –0.6° !(c 0.2, water)(lit[22] $[\alpha]_D^{20}$= –0.5°, c 0.2, water); Mp 123–124 °C; ^{1}H NMR ($CDCl_3$): δ 1.45 (s, 6H), 1.51 (s, 6H), 2.78 (s, br, 2H), 3.85 (d, ^{3}J= 6.0 Hz, 2H), 3.92 (dd, $^2J_{AB}$= 8.4 Hz, ^{3}J= 4.6 Hz, 2H), 4.21 (dd, $^2J_{AB}$= 8.4 Hz, ^{3}J= 5.7 Hz, 2H), 4.24 (ddd, ^{3}J= 5.7 Hz, ^{3}J= 4.6 Hz, ^{3}J= 6.0 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 25.31 (CH_3), 27.51 (CH_3), 67.97 (CH_2), 72.00 (CH), 76.02 (CH), 110.01 (C); HRMS calcd 262.142, found 247.118 (– CH_3).

O,O-(1,2-5,6-Di-O',O''-isopropylidene-D-mannitol)-dioxo-N,N-dimethyl-phospholidine (3.39)

Prepared as described for **3.32** from **3.38** (2.00 g, 7.62 mmole) and HMPT (1.46 g, 9.00 mmole). Yield 2.49 g (7.45 mmole, 98%) white crystalline material. $[\alpha]_D^{20}$= 26.23° (c 0.04, $CHCl_3$); Mp 53-54 °C; ^{1}H NMR ($CDCl_3$): δ 1.36 (s, 6H), 1.40 (s, 3H), 1.42 (s, 3H), 2.59 (d, $^3J_{PH}$= 12.03 Hz, 6H), 3.70 (m, 1H), 3.88-4.02 (m's, 3H), 4.04-4.18 (m's, 3H), 4.20 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 25.62 (d, $^6J_{PC}$= 4.21 Hz, CH_3), 26.32 (d, $^6J_{PC}$= 2.33 Hz, CH_3), 32.43 (d, $^3J_{PC}$= 19.34 Hz, CH_3), 66.45 (d, $^4J_{PC}$= 105.06 Hz, CH_2), 74.39 (d, $^2J_{PC}$= 1.67 Hz, CH), 76.54 (d, $^3J_{PC}$= 33.12 Hz, CH), 118.56 (s, C); ^{31}P NMR ($CDCl_3$): δ 150.78; Analysis calcd for $C_{14}H_{26}NO_6P$, C: 50.14, N: 4.18, P: 9.24, H: 7.81. Found, C: 49.92, N: 4.06, P: 9.08, H: 7.76; HRMS calcd 335.150, found 335.150.

O,O'-(3,4-O''-Isopropylidene-D-mannitol)-bis-dioxo-bis(N,N-dimethyl)-phospholidine (3.37)

Prepared as described for **3.32** from **3.36** (2.50 g, 11.26 mmole) and HMPT (4.31 g, 26.60 mmole). Yield 2.63 g (7.09 mmole, 63%) white crystalline material. $[\alpha]_D^{20}$= 5.67° (c 0.05, $CHCl_3$); Mp 43-47 °C; ^{1}H NMR ($CDCl_3$): δ 1.14 (d, $^7J_{PH}$= 3.45 Hz, 3H), 1.16 (d, $^7J_{PH}$= 2.32 Hz, 3H), 2.24 (d, $^3J_{PH}$= 11.02 Hz, 6H), 2.83 (m, 2H), 2.45 (m, 4H), 2.78 (m, 2H); ^{13}C NMR ($CDCl_3$): due to excessive P-C coupling and relatively low stability in chloroform no good ^{13}C NMR spectrum could be obtained ; ^{31}P NMR ($CDCl_3$): δ 146.43; Analysis calcd for $C_{13}H_{26}N_2O_6P_2$, C: 42.39, N: 7.61, P: 16.82, H: 7.12. Found C: 42.27, N: 7.48, P: 16.68, H: 7.08; HRMS calcd 368.127, found 368.127

Typical procedure for the determination of the enantiomeric excess of alcohols, amines and thiols by the use of reagents 3.11 and 3.12

A solution of the substrate (0.1 mmole) and a slight excess (1.1 equivalent) reagent **3.11** or **3.12** in 1.5 mL of $CDCl_3$ or C_6D_6 is stirred at room temperature until dimethylamine is no longer evolved. This was checked with a pH indicator. Normally the reactions take about 1-8 h to be completed. Subsequent ^{31}P or ^{1}H NMR analysis affords the diastereomeric ratios immediately. The products can sometimes be purified by means of column chromatography over Al_2O_3 under a nitrogen atmosphere.

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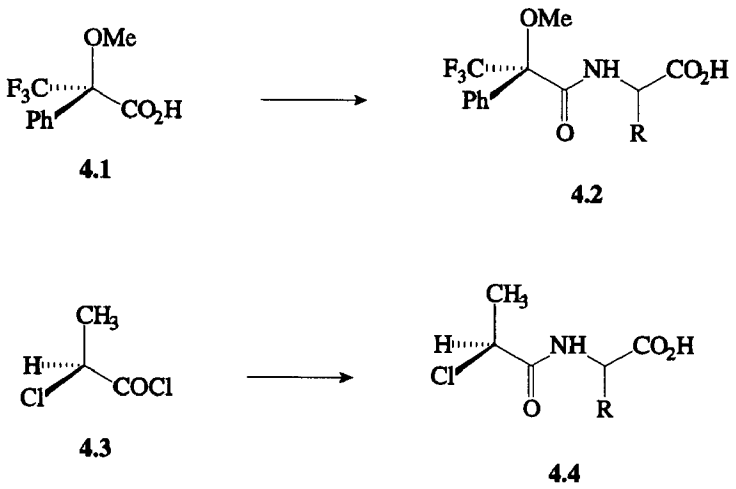
CHAPTER 4

Chiral dioxophosphonates; synthesis and application in the enantiomeric excess determination of amino acids in aqueous solutions

4.1 *Chiral derivatizing agents for unprotected amino acids applicable in aqueous solvent systems: a short story*

Probably the most elegant way to determine the enantiomeric excess of the widest number of chiral substrates by means of NMR techniques is the formation of discrete diastereomers by means of a chiral derivatizing agent. As is described in Chapter 1, a large number of derivatizing agents has been developed over the years for this purpose. The available number of derivatizing techniques for unprotected amino acids, however, is limited, mainly due to solubility restrictions, since water by neccessity is the solvent of choice. Methods that are based upon the derivatization of unprotected amino acids therefore all have to be tolerating water as (co)-solvent.



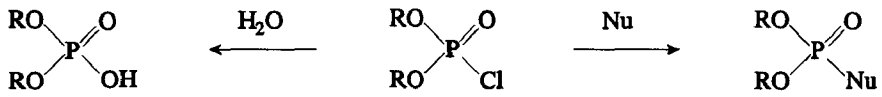
Scheme 4.1 *Two reported derivatizing methods for the enantiomeric excess determination of unprotected amino acids*[1,2,3]

The enantiomeric excess of unprotected α-amino acids has been determined after derivatization with enantiomerically pure α-methoxy-α-(trifluoromethyl)phenyl-acetic acid **4.1**, known as *Mosher's* reagent[1,2] (Scheme **4.1**). The enantiomeric composition of the formed amides **4.2** is usually analyzed by means of ^{1}H or ^{19}F NMR techniques.

Unprotected α-amino acids, but sterically hindered α-alkylated amino acids too, have been analyzed by means of (*S*)-2-chloropropionyl chloride **4.3**, using phase transfer conditions for the actual coupling reaction. This procedure, known as the *Kruizinga* method[3], readily affords the diastereomeric amides **4.4**, showing excellent peak separation in the ^{1}H NMR spectra.
Although several other chiral derivatizing methods for the enantiomeric excess determination of amino acids are known[#], they are all based upon the formation of esters or amides prior to the actual derivatization reaction and subsequent analysis. Moreover, these methods normally do not tolerate water as (co-)solvent.
Furthermore, the methods are based upon the use of ^{1}H or ^{19}F NMR techniques, the former showing a rather poor diastereomeric shift dispersion (compared to other nuclei) as well as complex coupling patterns, the latter having certain technical disadvantages.
Being aware of the great potential of ^{31}P NMR based methods in the enantiomeric excess determination of alcohols, amines and esters of α-amino acids, it became obvious that a method based upon the use of ^{31}P NMR for *unprotected* amino acids would be of importance.

4.2 *The design, synthesis and use of phosphorus reagents for the enantiomeric excess determination of unprotected amino acids in aqueous solutions*

Phosphorus containing derivatizing agents, which nearly always are of the type phosphoryl or thiophosphoryl chloride, are most conveniently coupled to the substrate by means of a nucleophilic substitution reaction on the pentavalent phosphorus atom[4].
This approach, however, prevents the use as derivatizing agent in water containing solutions, because water can act as a nucleophile itself, affording phosphoric acids and degradation products (Scheme 4.2).



4.6 **4.5** **4.7**

Nu = RNH_2, ROH, RSH

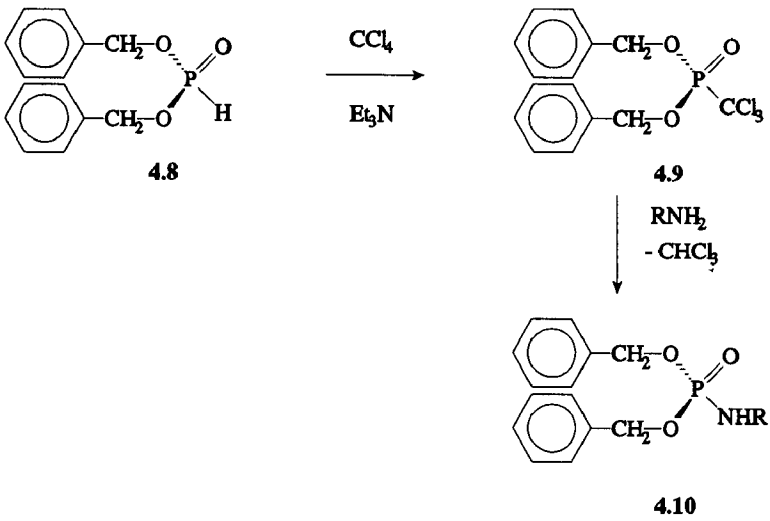
Scheme 4.2 Pentavalent phosphorus containing reagent 4.5 and possible competing reaction with water

[#] For a review of these methods, see Chapter 1.

Therefore, the development of suitable derivatizing agents for water-containing solutions is closely related to the search for coupling techniques that can be used in water. This probably means that the reaction cannot be performed using phosphoryl chloride type reagents, and a different approach has to be found.
Fortunately, such a method is available. This is the *Atherton-Openshaw-Todd* reaction.

4.2.1 *Phosphorylation reactions with dialkylphosphonates using polyhalogens: the Atherton-Openshaw-Todd reaction*

In 1945, Atherton, Openshaw and Todd[5] reported the use of *O,O*-dibenzylphosphonate **4.8** to functionalize α-phenylethylamine and other amine containing materials using CCl_4 and strong bases, like gaseous ammonia or Et_3N, as reagents. This rather peculiar reaction probably proceeds in two stages[5,6]. In the first step the deprotonated phosphonate **4.8** attacks CCl_4 to give trichloromethylphosphonate **4.9**. Next a substitution reaction of **4.9** with the amine substrate easily affords chloroform and the phosphonic amide **4.10**.

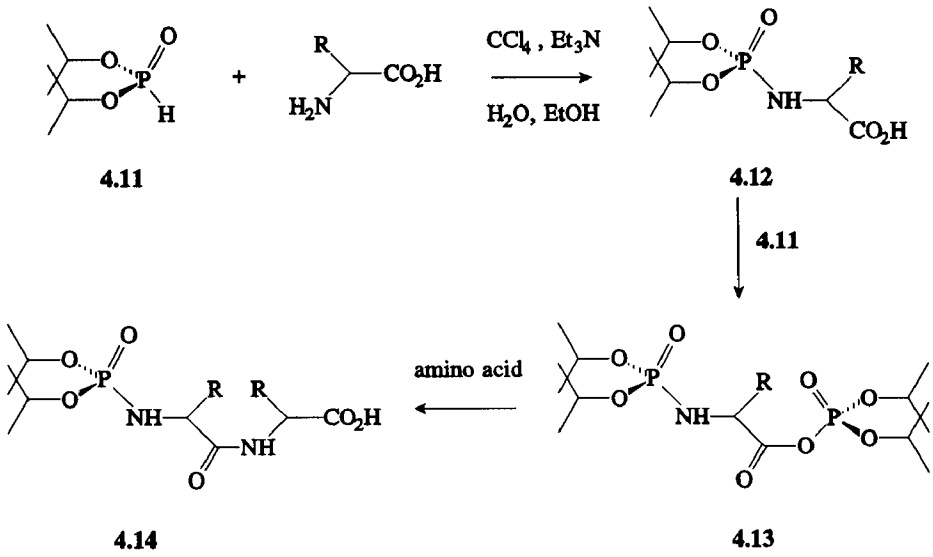


4.8 **4.9** **4.10**

Scheme 4.3 *The Atherton-Openshaw-Todd reaction and the proposed reaction sequence*[5,6]

The P-CCl_3 bond appears to be relatively strong[7] and strongly basic conditions are needed to induce the breaking of such a bond. Zhao and co-workers[8], however, found that using mild, weakly basic, anhydrous conditions, *N*-(dialkylphosphoryl)amino acids are readily available using the same procedure, although the low solubility of the amino acid limits the scope. Four years later, the same authors[9] reported that, using the procedure as described above, α-amino acids are easily transferred into *N*-(diisopropylphosphoryl)-amino acids **4.12**, using *O,O*-diisopropylphosphonate **4.11** under weakly basic conditions and in *aqueous* media, in which the amino acids are readily soluble (Scheme **4.4**).

A second reaction with *O,O*-diisopropylphosphonate **4.11** affords the mixed phosphorus–carboxylic acid anhydride **4.13** which, being a very reactive intermediate[9], immediately reacts with the solvent or unreacted substrate, to yield small dipeptides such as **4.14**.

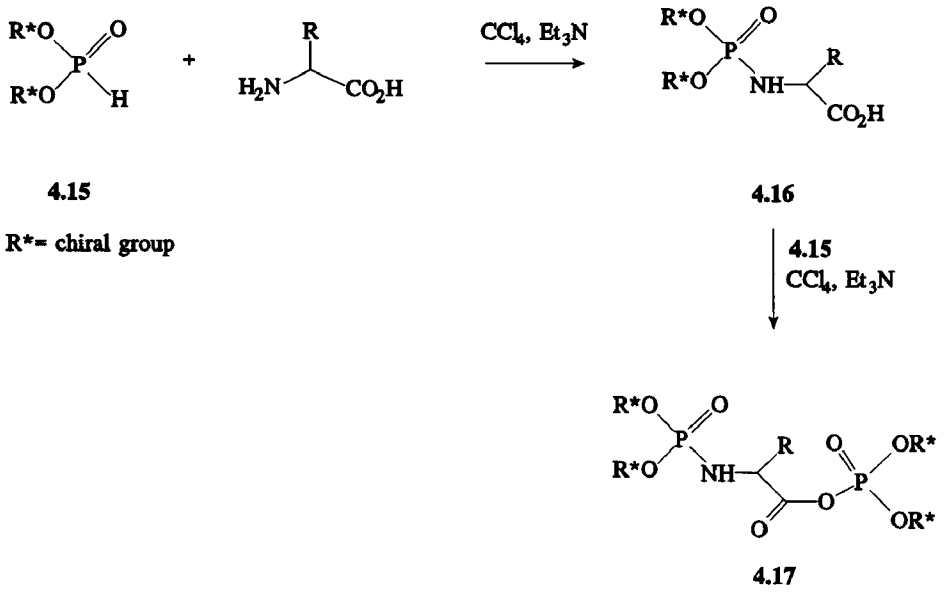


Scheme 4.4 Formation of N-substituted phosphoryl amino acids in aqueous solutions

Modified *chiral* phosphonates, like **4.15** (Scheme **4.5**), should afford, via the same methodology, the diastereomeric *N*-substituted, chiral phosphorylamino acids **4.16** when using racemic amino acids as substrate. Subsequent analysis of the products **4.16** using ^{1}H or ^{31}P NMR techniques would allow the exact determination of the enantiomeric ratio of the amino acids.

Reactions at the phosphorus center may proceed with inversion or retention of configuration, however, reaction with the *C–2* symmetrical reagent **4.15** yields the same diastereomeric products **4.16**, regardless whether the reaction proceeds with inversion or retention of configuration at the phosphorus center. Since the exact mechanism of the Atherton–Openshaw–Todd reaction is not known with certainty[5] and several factors are known to influence the ratios of retention and inversion products[10], the *C–2* symmetry can be seen as an advantage.

Thus, when other, not *C–2* symmetrical chiral phosphonates as is described in Chapter 4.2.3 are used, the stereochemistry at the phosphorus atom has to be carefully controlled, to make sure that the diastereomeric ratios as obtained reflect the enantiomeric composition of the sample and that no kinetic resolution is taking place.

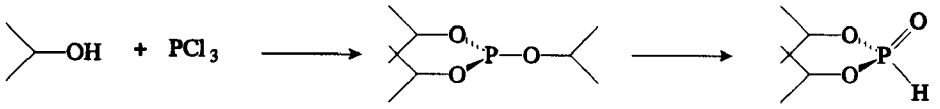


Scheme 4.5 Chiral phosphonates 4.15 and the possible use as derivatizing agent for unprotected amino acids

4.2.2 *(S,S)-O,O-Di-2-butylphosphonate 4.20 as reagent for the enantiomeric excess determination of unprotected amino acids: the aqueous solution ?*

O,O-Dialkylphosphonates, being the diesters of phosphoric acid, are described in the literature as early as 1854[11], but impressive improvements have been made in the synthetic availability[12] over the years. The most common and simple synthesis of *O,O*-dialkylphosphonates probably is the reaction of PCl_3 with three equivalents of the alcohol leading, via an Arbuzov rearrangement[13] of the usually not isolated trialkylphosphites **4.18**, to the corresponding *O,O*-dialkylphosphonates **4.19** (Scheme **4.6**). Chiral *O,O*-dialkyl-phosphonates, needed for enantiomeric excess determination purposes, can be prepared using this methodology also. The desired chiral *O,O*-dialkylphosphonates must, however, meet a few requirements; (a) the ready availability, (b) preferentially low cost of the chiral alcohol, and more important (c), the phosphonate must be resistant towards the basic conditions that are employed in the reaction with amino acids. This means that no acidic proton must be attached at the stereogenic center or at the α-position to it.
A suitable chiral alcohol is (*S*)-2-butanol, which gives phosphonate **4.20**[14] upon treatment with PCl_3 (Scheme **4.7**). Its structure strongly resembles that of *O,O*-diisopropylphosphonate **4.19**, described by Zhao and coworkers[9], which gave good results in the coupling reaction with unprotected amino acids. A nice coincidential advantage of the route chosen is the fact that the reaction of alcohols with PCl_3 into the phosphonates is

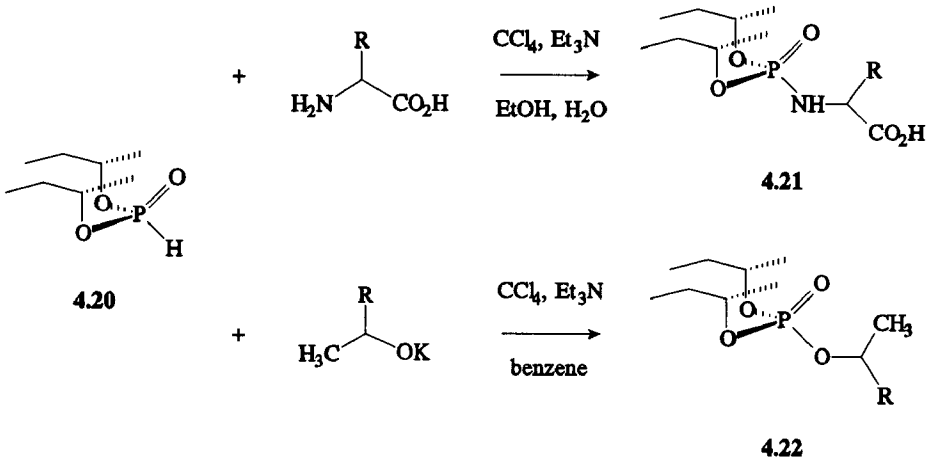
also used as a method for the determination of the enantiomeric excess of alcohols[15]. Therefore, the *enantiomeric purity* of the derivatizing agent can be easily checked.



4.18 **4.19**

Scheme 4.6 Synthesis of O,O–dialkylphosphonate 4.19 from PCl_3 and isopropanol

(S,S)–O,O–Di–2–butylphosphonate **4.20** reacts readily with several nucleophiles including alcohols, amines, amino alcohols, thiols, amino acid esters and unprotected amino acids in the presence of CCl_4 and Et_3N.



4.20 **4.21** **4.22**

*Scheme 4.7 Derivatization of agent **4.20** with amino acids and alcohols, affording diastereomeric products **4.21** and **4.22***

A typical derivatizing procedure for *unprotected amino acids* involves the mixing of the substrate of interest (0.1 mmole) with Et_3N (1 mL), ethanol (1 mL) and water (1 mL) and cooling the resulting suspension to 0 °C with stirring. Phosphonate **4.20** in 1 mL of CCl_4 is added dropwise to the suspension, and the solution turns clear within 15 min. The mixture is stirred for 8 h at room temperature, and becomes a suspension again due to the formation of the product and $Et_3N.HCl$. The mixture is subsequently acidified with dilute HCl solution to pH 3 and extracted with ethyl acetate providing the products as yellowish oils. These can be analyzed directly (^{31}P or ^{1}H NMR, recorded in $CDCl_3$)(*vide infra*), affording the diastereomeric ratio's. In several cases, the products can be purified by crystallization from ethyl acetate–petroleumether mixtures or by column chromatography on silica gel, providing the products **4.21** as white solids or colorless oils.

Alternatively, when *alcohols*, *amines*, *amino alcohols*, *thiols* or *amino acid esters* are used as substrates, water as co-solvent may be omitted. In fact, it is possible to perform the derivatization reaction in $CDCl_3$ or C_6D_6 as the solvent, so that further workup of the crude reaction mixture is not neccessary and the enantiomeric excess can be determined directly. When amines are used as the substrates, this procedure gives the possibility to follow the reaction by ^{31}P or ^{1}H NMR, in order to examine kinetic resolution processes during the course of the reaction.

The results of the enantiomeric excess determinations in *aqueous mixtures* using racemic α-amino acids and derivatives as substrates and reagent **4.20** as derivatizing agent are collected in Table **4.1**. The Δδ values using ^{31}P NMR are typically in the order of between 0.025 and 0.487 ppm, although sometimes larger shift differences have been obtained. Usually, two baseline separated, nonsymmetrical singlets are observed, which allow easy integration. The shift differences are not that large (with *d,l*-tyrosine showing the largest Δδ value (0.191 ppm, entry **g**), when compared to *d,l*-tryptophane (entry **d**), having a Δδ value of 0.091 ppm. The α-alkylated amino acids sometimes show a remarkably large diastereomeric shift difference, like for *d,l*-α-methylphenylglycine (Δδ 0.487 ppm, entry **j**), whereas at the other hand small shift differences have also been observed (Δδ 0.035 ppm for the amide of *d,l*-α-methyl-phenylglycine, entry **l**).

The situation appears to be even more complex when the free acid derivatives are compared with primary amides. For instance, *d,l*-α-methyl-phenylalanine and the corresponding primary amide (entries **k** and **l**), give shift differences of 0.051 and 0.035 ppm, respectively. When, however, the methyl-ester of *d,l*-α-allylphenylglycine (entry **n**) is compared with the corresponding primary amide (entry **m**), the shift differences are 0.037 and 0.185 ppm, respectively. Unfortunately, it was impossible to find a relation between the intrinsic structure of the diastereomeric products and the observed chemical shift differences when using derivatizing agent **4.20**#.

When products **4.21** and **4.22** are isolated without special precautions to completely remove the solvents, exactly one mole equivalent of ethanol and/or half a mole equivalent of water are complexed to the products. It became clear that both solvents have a large influence upon the diastereomeric shift differences Δδ, as can be seen in Table **4.1**. Removal of these solvents is difficult, even after careful crystallization of the products from ethyl acetate-petroleumether, the crystals have to be dried in vacuum for 12 h, to remove the ethanol or water completely (see Chapter 4.3). The removal of solvents always has a positive influence upon the diastereomeric shift differences, although the increase in ΔΔδ is not always large. Using *e.g. d,l*-alanine, the observed Δδ is 0.099 ppm compared with Δδ 0.116 ppm for the solvent-free diastereomers of **4.21**.

In Figure **4.1** the ^{1}H spectrum of the crude product **4.21** from *d,l*-alanine is given, ($CDCl_3$ [L]= 0.1 M), clearly showing complexed ethanol and water (at δ 3.12 and 5.08 ppm, respectively). Interesting is the NH resonance at δ 4.61 ppm, showing a splitting pattern due to coupling with the α-proton of the alanine residue and the phosphorus nucleus

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It is clear, however, that the conformational freedom of the adduct is of great importance, see Chapter 4.2.4 for a discussion.

Entry	Substrate	δ (ppm)[a]	$\Delta\delta$ (ppm)[a]	ratio
a	*d,l*–Ala	6.51	0.099	49.5:50.5
		6.47	0.116	49.5:50.5
b	*d,l*–Phe	5.93	0.025	50:50
		5.91	0.106	50:50
c	*d,l*–Val	6.93	0.069	49.5:50.5
		6.92	0.127	49.5:50.5
d	*d,l*–Try	6.03	0.038	49:51
		6.00	0.091	49:51
e	*d,l*–Ser	6.11	0.079	49.5:50.5
		6.03	0.117	49.5:50.5
f	*d,l*–PG	5.71	0.098	49.5:50.5
		5.69	0.172	49.5:50.5
g	*d,l*–Tyr	5.65	0.093	49.5:50.5
		5.59	0.191	49.5:50.5
h	*d,l*–Cys	5.98	0.087	50:50
		5.98	0.156	50:50
i	*d,l*–Pro	4.63	0.127	49:51
j	*d,l*–α–Me–PG	2.20	0.487	49.5:50.5
k	*d,l*–α–Me–Phe	4.32	0.051	49.5:50.5
l	*d,l*–α–Me–Phe–amide	5.85	0.035	49.5:50.5
m	*d,l*–α–allyl–PG–amide	5.50	0.185	49.5:50.5
n	*d,l*–α–allyl–PG–methylester	5.48	0.037	49.5:50.5

Tabel 4.1 *^{31}P NMR data of derivatives **4.21** of racemic amino acids, esters and amides of amino acids using **4.20**, recorded in $CDCl_3$ [L]= 0.1 M.*
*a) In each of the entries **a–h** the first row applies to the diastereomeric shift differences of the product with half a molar equivalent of bound water, the second to the diastereomeric separations of the products after removal of the bound water.*

phosphorus nucleus. Also the ^{1}H NMR analysis of **4.21** can sometimes be used for the *e.e.* determination. For instance, using *d,l*–phenylalanine, ^{1}H NMR allows the determination of the enantiomeric composition of the product **4.21**, as is shown in Figure **4.2** (only a part of the spectra is given).

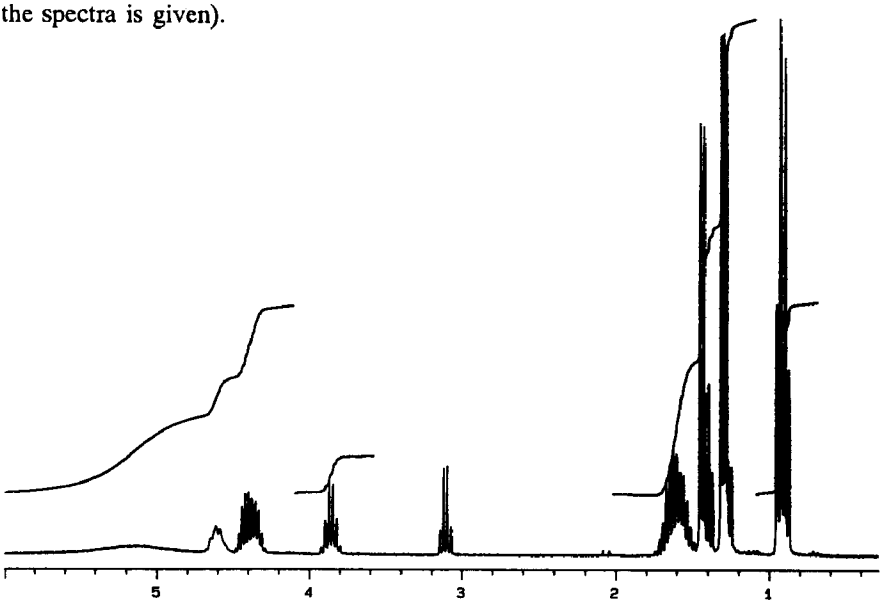


Figure 4.1 *^{1}H NMR of 4.20 coupled with d,l–Ala, recorded in $CDCl_3$ [L]=0.1 M*

The complex pattern situated around δ 3.08 ppm is due to the CH_2 group of the phenylalanine moiety, showing two double AB patterns split again due to a phosphorus coupling. The signal at δ 3.72 ppm arises from the NH group, and has a coupling with the α–proton of the amino acid; furthermore a phosphorus coupling is present. When compared with the spectrum of the *d*–phenylalanine product, showing only a double AB system for the CH_2 group and a double doublet structure for the NH resonance, the differences are obvious, though not large. In contrast, the simplicity of ^{31}P NMR spectra is striking. The decoupled ^{31}P NMR spectrum of this product shows two nearly baseline–separated singlet signals, the spectral data allowing adequate integration. As can also be seen, the ^{1}H NMR spectrum of the product **4.21** of *d*–phenylalanine contains some complexed ethanol (quartet at δ 4.11 ppm), which was not removed before recording the spectrum.

As was already mentioned, not only amino acids, but also *alcohols*, *amines*, *amino alcohols* and *thiols* can be coupled to reagent **4.20**. When alcohols are coupled to this reagent, a catalytic amount of *N,N*–dimethylaminopyridine or, alternatively, the potassium salts of the alcohols can be used to enhance the nucleophilicity. The potassium salts may be prepared just prior to the derivatization using the same reaction medium without further effects on the obtained enantiomeric ratios.

In Table **4.2** the results of the analyses using reagent **4.20** are collected, recorded in $CDCl_3$ at [L]= 0.1 M.

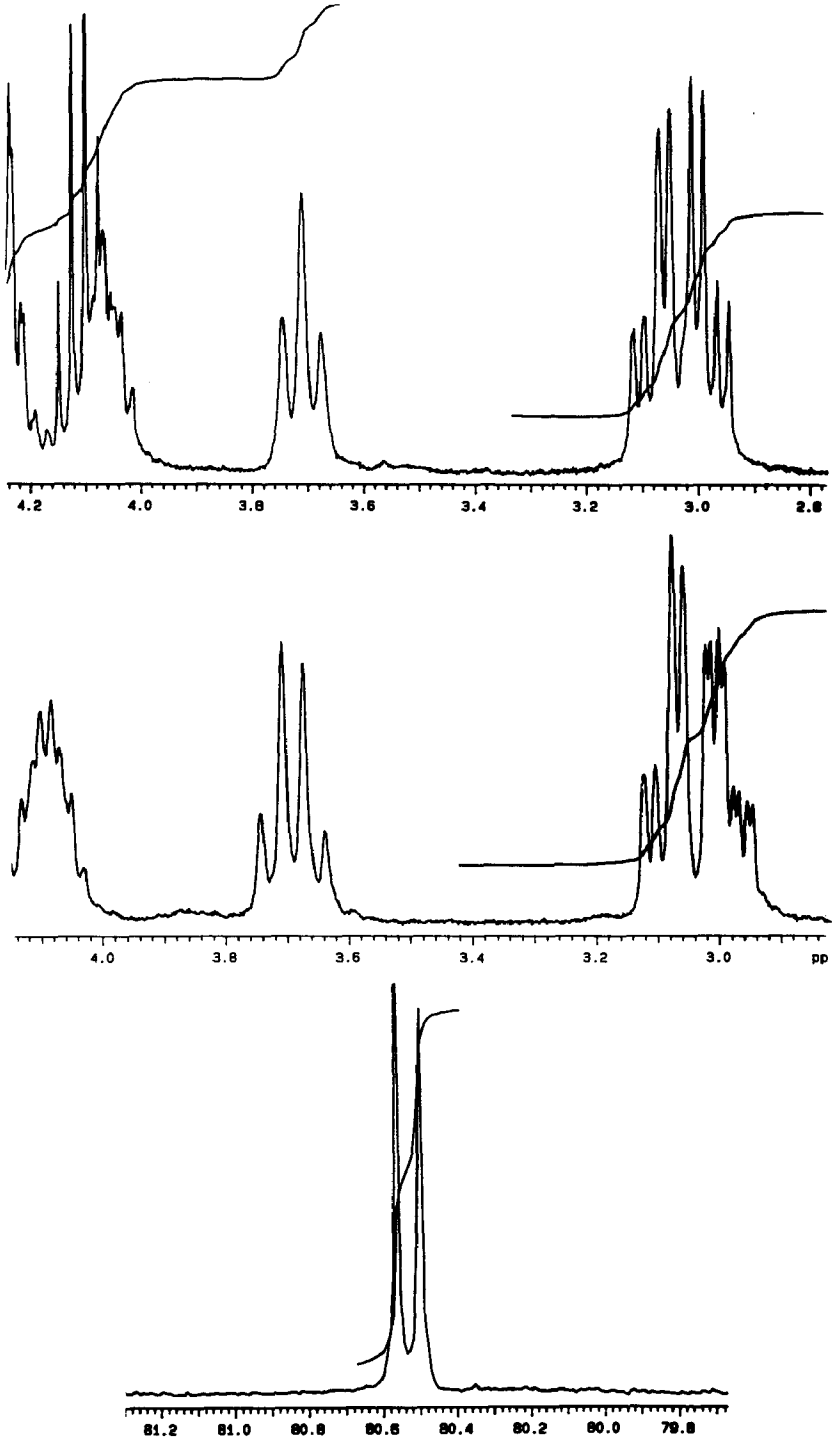


Figure 4.2 ^{1}H *NMR data of phosphoramide* ***4.21*** *with d–Phe (a) d,l–Phe (b),* ^{31}P *NMR data of* ***4.21*** *with d,l–Phe (c) recorded in* $CDCl_3$ *[L]= 0.1 M*

Entry	Substrate	δ (ppm)	Δδ (ppm)	ratio
a	*d,l*–phenylethyl–alcohol	5.23	0.103	49.5:50.5
b	*d,l*–2–butyl–alcohol	5.66	0.124	49.5:50.5
c	*d,l*–menthol	5.63	0.127	50:50
d	*d,l*–phenylethyl–amine	5.94	0.185	50:50
e	*d,l*–heptylamine	6.32	0.098	49.5:50.5
f	*d,l*–2–butylamine	6.67	0.102	49.5:50.5
g	*d,l*–α–thioacetic–acid	3.21	0.127	49:51

Table 4.2 *^{31}P NMR data of **4.21** prepared from amines and **4.22** prepared from alcohols and thiols, recorded in $CDCl_3$ [L]= 0.1 M*

As for the amino acid products **4.21**, no relationship was found between the observed diastereomeric shift differences of products **4.22** and the intrinsic structure of the products. When *e.g.* *d,l*–α–phenylethylamine (Δδ 0.185 ppm) is compared with *d,l*–α–phenyl–ethanol (Δδ 0.103 ppm), the amine shows the largest shift difference, whereas at the other hand the comparison between *d,l*–2–butylamine and *d,l*–2–butylalcohol shows shift differences of Δδ 0.102 and Δδ 0.124 ppm, respectively. It turned out to be possible to cause reagent **4.20** to react with thio–acids twice; first at the thiol moiety and then at the carboxyl group, affording a mixed anhydride.

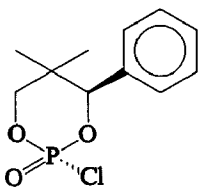
Substrate	polarimetry	α–chloropropionyl method	reagent 4.20
d,l–Ala	76.4:23.6	76.4:23.6	76.4:23.6
d,l–Phe	73.5:26.5	74.1:25.9	74.1:25.9
d,l–menthol	71.2:28.8	70.8:29.2	70.9:29.1
d,l–α–phenylethyl–amine	70.8:29.2	70.1:29.9	70.5:29.5

Table 4.3 *Comparison of the enantiomeric composition as determined using agent **4.20** and by means of optical rotation and the α–chloropropionyl chloride method[3]*

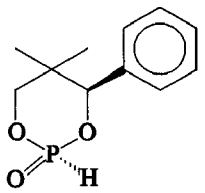
All diastereomeric shift differences were obtained without purification of the products.
In order to ensure the correctness of the method using reagent **4.20** and to exclude kinetic resolution, a comparison between the enantiomeric compositions of several enriched samples as determined by means of optical rotation, the α-chloropropionyl chloride method[3] and the ^{31}P NMR method described here, was made. Data are given in Table **4.3**. The results of the three methods appeared to be in excellent agreement, showing only small differences[16].

4.2.3 *A chiral phosphonate reagent based upon cyclic dioxaphosphorinanes: synthesis and the application in the enantiomeric excess determination*

Although reagent **4.20** is readily available and has been successful in the enantiomeric excess determination of several nucleophiles, including unprotected amino acids in aqueous solvent systems, the diastereomeric shift dispersion is usually not large.
We found, however, (as is described in Chapter 5) that the cyclic (*R*)-(-)-2-chloro-2-oxo-5,5-dimethyl-4-(*R*)-phenyl-1,3,2-dioxaphosphorinane **4.23** is readily coupled with alcohols and amines, affording diastereomeric products which show large shift dispersion[17] in the decoupled ^{31}P NMR spectra (Scheme **4.8**).



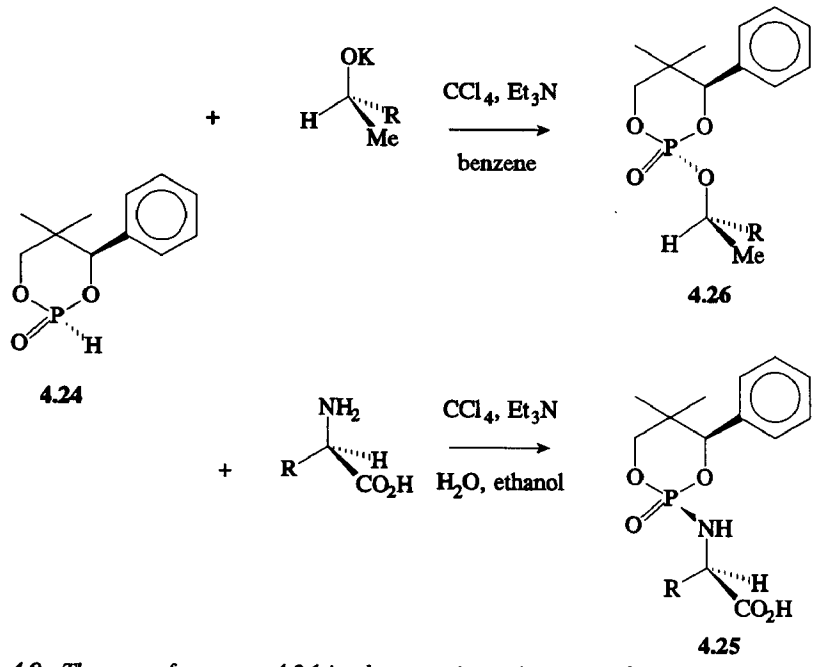
4.23



4.24

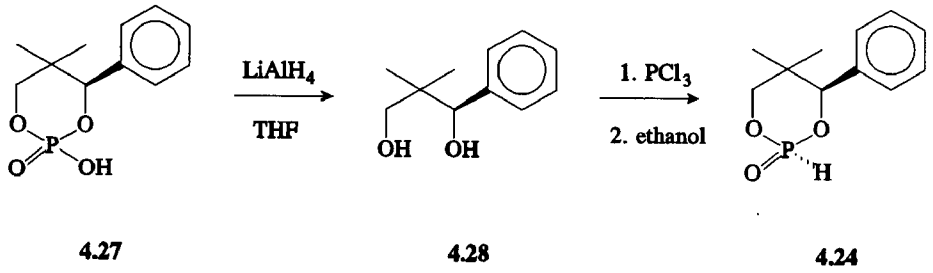
Scheme 4.8 Two phosphorus containing derivatizing reagents for enantiomeric excess determination

Derivatizing agent **4.23**, which cannot be used in water containing solutions, serves as the model reagent for a modified derivatizing agent **4.24** of the dioxophosphorinane type, that could be resistant to aqueous solvent systems. The diastereomeric products **4.25** and **4.26** that are formed from **4.24** and amino acids or amino alcohols are the same as obtained with reagent **4.23** (Scheme **4.9**).
Reagent **4.24** is easily obtained in 65% yield from (-)-phencyphos **4.27**[18] by reduction to the free diol **4.28** using $LiALH_4$ in THF (or ether)(Scheme **4.10**). Subsequent reaction with PCl_3, followed by an Arbuzov rearrangement[13] using ethanol, yields (*S*)-(-)-2H-2-oxo-5,5-dimethyl-4-(*R*)-phenyl-1,3,2-dioxaphosphorinane **4.24** in 83% yield.
In contrast to **4.20**, the phosphorus atom in reagent **4.24** is chiral, and derivatization reactions in principle can proceed with retention or inversion of configuration at the phosphorus center. The ^1H NMR and NOESY analysis (not shown) clearly indicated that reagent **4.24** is diastereomerically pure, with the phosphorus atom having the *S* configu-



Scheme 4.9 The use of reagent 4.24 in the enantiomeric excess determination of alcohols and amino acids

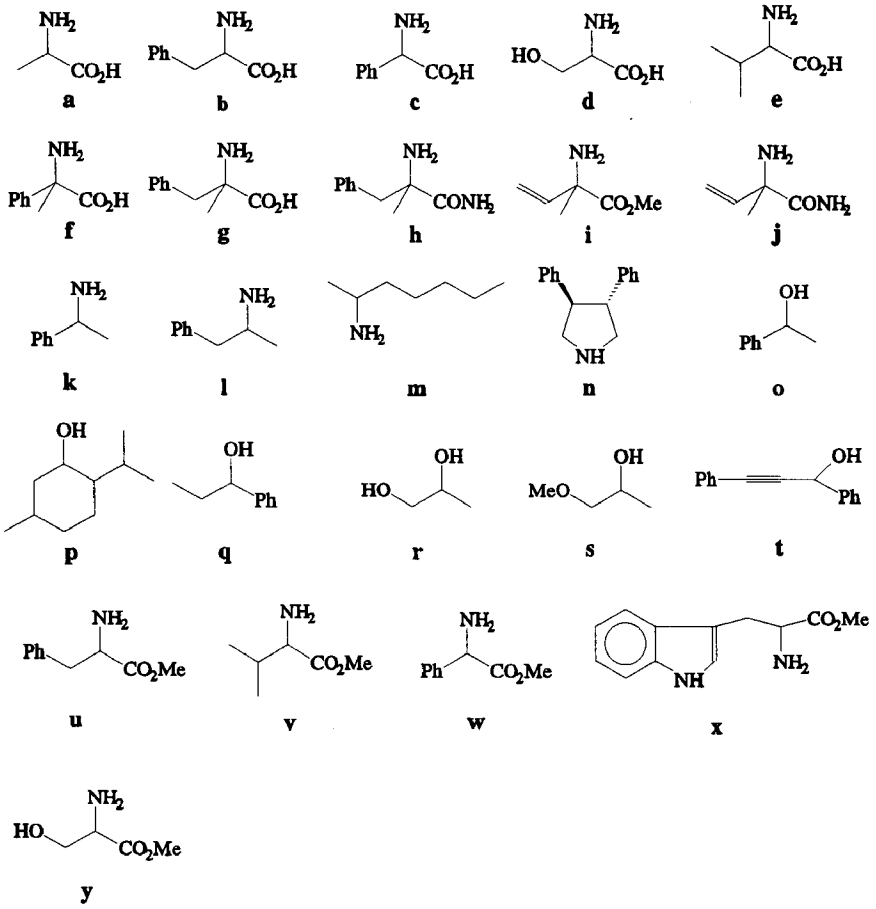
ration when prepared from (–)–phencyphos. As with reagent **4.23**, reaction of **4.24** with amines proceeds with inversion and with alcohols with retention of configuration at the phosphorus center (Scheme **4.9**). In Chapter 5 and 6, a discussion of the stereochemistry of these reactions will be given.



Scheme 4.10 The synthesis of reagent 4.24 from phencyphos 4.27

Phosphorinane **4.24** reacts with a variety of nucleophiles including alcohols, amines, amino acid esters and unprotected amino acids, using CCl_4 and Et_3N as reagent–cocktail and ethanol (or chloroform) as solvent. Furthermore, water is acceptable as (co)–solvent (for

practical details, see Chapter 4.2.2). Some of the results are collected in Table **4.4**, the indices refer to Scheme **4.11**.



Scheme 4.11 *Representative chiral alcohols, amines and amino acids used in the ^{31}P NMR analysis with reagent **4.24**. The indices are also used in Table **4.4***

As can be seen from the data in Table **4.4**, all diastereomeric derivatives showed sufficient chemical shift dispersion to allow accurate *e.e.* determination. It appears that large substituents, such as a phenyl group attached to the substrate, mostly have a positive influence upon the diastereomeric shift dispersion. Using *amino acids* as substrates, the diastereomeric shift differences Δδ were the largest for *d,l*- phenylglycine (**c**, Δδ 1.218 ppm) and relatively small for *d,l*-alanine (**a**, Δδ 0.256 ppm). The other amino acids gave comparable Δδ values, all situated in this chemical shift range. For *d,l*-serine (**d**) a ratio different from the expected 50:50 value was found (47:53). We assume that a competetive attack of the alcohol moiety also takes place. Also, *α-alkylated amino acids* can be analyzed with reagent **4.24**, which gave diastereomeric shift differences between Δδ 0.786

Substrate[a]	δ (ppm)	$\Delta\delta$ (ppm)	ratio
a	1.01	0.256	49.5:50.5
b	0.56	1.019	49.5:50.5
c	0.92	1.218	49.5:50.5
d	0.78	0.931	47:53
e	0.89	0.871	49.5:50.5
f	-2.09	1.653	49.5:50.5
g	-1.20	0.931	49.5:50.5
h	0.32	0.786	49.5:50.5
i	0.56	1.354	49:51
j	0.12	0.596	50:50
k	5.05	0.631	49.5:50.5
l	1.86	0.543	49.5:50.5
m	2.25	0.112	50:50
n	3.35	0.476	49.5:50.5
o	-7.34	0.073	49:51
p	-1.25	0.048	49.5:50.5
q	-7.91	0.257	50:50
r	-7.41	0.912	49.5:50.5
s	-5.23	0.071	49.5:50.5
t	-11.65	0.168	49:51
u	4.73	0.510	49.5:50.5
v	2.28	0.209	49.5:50.5
w	0.82	0.297	49:51
x	11.51	0.442	49.5:50.5
y	-7.45	0.201	49.5:50.5

Table 4.4 *^{31}P NMR data of products **4.25** and **4.26** and several substrates, recorded in $CDCl_3$ [L]= 0.1 M, with 1 mole eq of H_2O complexed*

(**h**) and Δδ 1.653 ppm with *d,l*–α–methyl–phenylglycine (**f**). These products, however, are less easily formed when compared to the α–amino acids, probably due to steric hindrance by the α–alkyl group.

When *amines and alcohols* are used as substrates, water may be omitted in the reagent–cocktail. Alcohols that are reacted with reagent **4.24** can best be transferred into the potassium salts prior to the actual reaction to enhance the nucleophilicity.

For amines, the obtained diastereomeric differences typically are between the Δδ 0.110 ppm (for *d,l*–2–heptylamine, **m**) and Δδ 0.631 ppm (for *d,l*–α–phenylethylamine, **k**).

Again, it looks as if a phenyl group attached directly to the chiral center has a positive influence upon the diastereomeric shift dispersion, whereas the difference between *e.g.* a methyl or pentyl group (as for *d,l*–2–heptylamine, **m**), hardly gives any diastereomeric shift differentiation. The ester protected α–amino acids (**u–y**) only show little differentiation in the diastereomeric chemical shift differences, except for *d,l*–methylserine (**y**), which appears to be coupled through the alcohol group rather than the amine functionality (Δδ 0.201 ppm). *d,l*–Methyltryptophan (**x**) probably is coupled through the ring nitrogen, and gives a Δδ value of 0.441 ppm. For the alcohol substrates, no relation between the observed diastereomeric shift differences and structure was found, the shift differences being between the Δδ 0.053 (**p**) and Δδ 0.912 ppm (**r**).

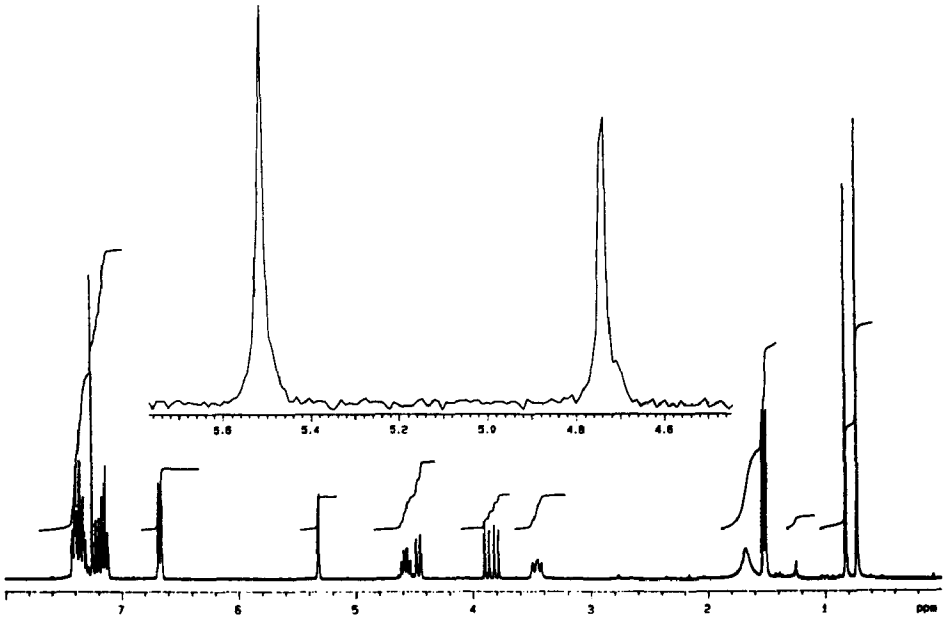
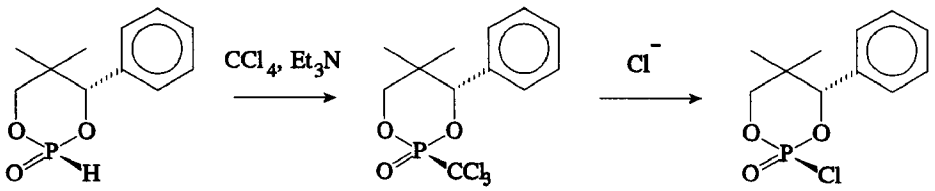


Figure 4.3 ^{1}H *and* ^{31}P *NMR spectra (inset) of product* ***4.26*** *coupled to d– and d,l–α–phenylethylamine, respectively, recorded in* $CDCl_3$ *[L]= 0.1 M*

In Figure **4.3** the ^{1}H and ^{31}P NMR spectra of product **4.25** using *d,l*-α-phenylethylamine is shown (**k**, Table **4.4**). Again, the great advantages of the ^{31}P NMR over the ^{1}H NMR technique in enantiomeric excess determinations becomes clear; only two singlets are observed in the former case, whereas the latter shows a very complex pattern, that is not readily analyzed.

It is important to note that, although the reaction conditions and the derivatizing reagents are different when comparing reagents **4.23** and **4.24**#, the products are the same with alcohols or amines as substrates[17]. The absolute stereochemistry of the phosphorus atom in reagent **4.23** is *S*, based upon the X-Ray structure (see Chapter 5 for details) and the use of (+)-phencyphos as starting material[18]. For **4.24**, the *S* configuration at the phosphorus atom is found when using the (-)-phencyphos. Based upon the fact that reagent **4.23** does not tolerate water as solvent, contrary to reagent **4.24**, it is concluded that the reactive intermediates are probably not the same. This is surprising, because it is possible to synthesize chloro-dioxaphosphorinane **4.23** from reagent **4.24**, using the same reagent cocktail CCl_4 and Et_3N, but without a substrate, in nearly quantitative yield#. Similar to the reaction with amine or alcohol nucleophiles, the liberated chloride probably attacks the initially formed trichloromethylester **4.29**, yielding chloroform and the chloro-dioxa-phosphorinane **4.23** (see Scheme **4.3** and **4.12**) with overall retention of configuration on phosphorus.



4.24 **4.29** **4.23**

Scheme 4.12 *Proposed reaction sequence of the Atherton-Openshaw-Todd reaction when using reagent 4.24, based upon the stereochemistry of the products*

It is known that *large* substituents at phosphorus[19] preferentially assume the axial position, leaving the double bonded oxygen in the equatorial position. Although there is no evidence, the most likely route to **4.23** would involve two subsequent retentions of configuration on the phosphorus center. In Chapters 5 and 6, a detailed mechanistic study concerning these stereochemical phenomena is undertaken.

The reaction normally proceeds without the formation of side products, although sometimes small amounts of the *pyrophosphate* of phencyphos are formed. The formation of this pyrophosphate, however, does not influence the actual *e.e.* determination. In Chapter 7, a detailed study of the mechanism of this reaction is described.

See Chapter 5 and 6 for a discussion about the reactivity of reagent **4.23**.

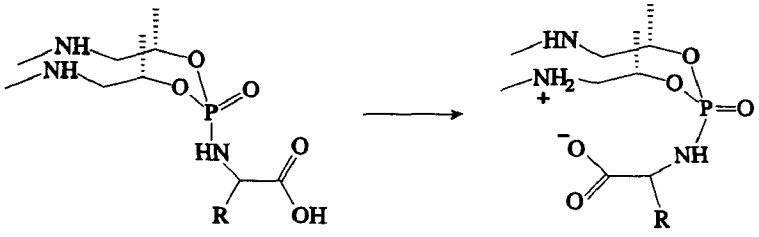
The enantiomeric excesses of some enriched samples as determined with reagent **4.24** was checked by comparison with the enantiomeric purity determined by the optical rotation and the α-chloropropionyl chloride method[3] (Table **4.5**). The results are in excellent agreement.

Substrate	polarimetry	α-chloropropionyl method	reagent 4.24
d,l-Ala	76.4:23.6	76.3:23.7	76.1:23.9
d,l-Phe	73.4:26.6	74.1:25.9	74.5:25.5
d,l-menthol	71.2:28.8	70.8:29.2	70.8:29.2
d,l-α-phenylethyl-amine	70.8:29.2	70.1:29.9	70.9:29.1.

*Table 4.5 Comparison of the enantiomeric composition as determined using reagent **4.24** and by means of optical rotation and the α-chloropropionyl chloride method[3]*

4.2.4 *Other phosphonate systems for enantiomeric excess determination: how to influence diastereomeric shift dispersion*

Regardless of the fact that the enantiomeric excess of several substrates, including unprotected amino acids, is readily determined by the use of reagents **4.20** and **4.24**, the obtained diastereomeric shift dispersion usually is not large. Enhancement of the Δδ values would therefore be highly desirable. It is clear that with the design of other derivatizing agents considerations of several of the factors that might govern the relationship between the intrinsic structural variations and the chemical shifts have to be taken into account.



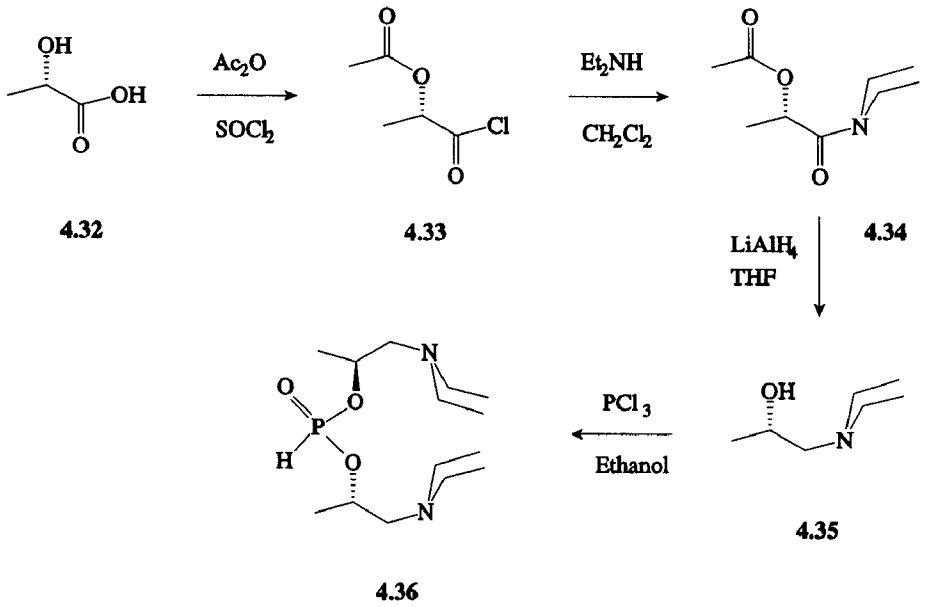
4.30 unlocked **4.31** locked

Scheme 4.13 Proposed conformational locking of amino acids adducts

Probably one of the most important factors in this respect is the restriction of the conformational freedom[19], yielding two distinct diastereomers with more or less *locked* conformations. Hopefully, these *locked* conformations would give rise to different shifts in the ^{1}H or ^{31}P NMR#, with a larger diastereomeric shift dispersion compared with the diastereomers that are not conformationally locked.

A system in which an amino acid residue would possess a limited flexibility after the derivatization, should be ideal for this approach. A system that meets these requirements, is drawn in Scheme **4.13**. The protonated amine moiety in the derivatizing agent could possibly *lock* the deprotonated acid part of the molecule by means of a intramolecular tight ion pair formation.

A system of this kind is available from optically pure lactic acid. Alternatively, mandelic acid was used to enhance the observed shift differences by the anisotropic effect of the aromatic ring systems.



Scheme 4.14 Synthesis of derivatizing agent 4.36 from S-lactic acid

When *S*-lactic acid **4.32** (Scheme **4.14**) is treated with acetic anhydride followed by thionyl chloride, the acetyl protected acid chloride **4.33** is obtained in 60% yield after distillation[20]. The enantiomeric composition of this material was checked by a reaction with *l*-α-phenylethylamine. For racemic **4.33**, two distinct diastereomers were obtained, which could be analyzed by means of ^{1}H NMR. One diastereomer was apparent when

In Chapter 6, the factors that govern the shift as a function of intrinsic variations in the structure will be discussed in detail.

enantiomerically pure **4.32** was used, and it was concluded that the conversion to the acid chloride did not influence the enantiomeric composition. The protected acid chloride **4.33** was subsequently transferred into amide **4.34** in 91% yield, by reaction with diethylamine. Subsequent reduction ($LiAlH_4$ in THF) afforded amino alcohol **4.35** in 69% yield by Soxhlet extraction from the lithium- and aluminium salts, which tend to form strong complexes with product **4.35**.

The enantiomeric composition of amino alcohol **4.35** was checked with the methods described in Chapters 3 and 4.2.2, and was over 99%. The reaction of **4.35** (as HCl salt) with PCl_3 yielded several products, including the desired product **4.36** (37% yield), but also other phosphonates were found besides several elimination products (accordimg to the ^1H NMR of the crude mixtures). Product **4.36** was shown by the method of Feringa and co-workers[15] to consist of one enantiomer only. Although more amines were used in the amide formation reaction with **4.33**, like benzylamine, aniline and *S*-α-phenylethyl-amine, these are not presented here because they turned out to be less easy to handle. During subsequent reduction into the corresponding amino alcohols partial racemization took place and the subsequent functionalization with PCl_3 gave several by- and elimination products.

The use of reagent **4.36** in the enantiomeric excess determination of amines and unprotected amino acids, is more troublesome than that of reagents **4.20** and **4.24**, primarily due to the rather low stability of **4.36**. It also became clear that reagent **4.36** could not be properly coupled to alcohols, because of severe decomposition.

In Table **4.6** several results are collected using derivatizing agent **4.36** and amines and unprotected amino acids. The analysis of the amino acid products took place at pH 7-8 and the observed diastereomeric shift differences Δδ are, as expected, pH dependent (see below for a short discussion).

Substrate	δ (ppm)	Δδ (ppm)	ratio
d,l-2-butylamine	4.51	0.151	49:51
d,l-α-phenylethyl-amine	2.67	0.213	49.5:50.5
d,l-Ala	-1.42	0.273	49:51
d,l-Phe	-2.33	0.243	49.5:50.5
d,l-Val	-1.99	0.252	49.5:50.5
d,l-PG	-4.56	0.347	49.5:50.5
d,l-Try	-3.01	0.189	49:51

Table 4.6 *^{31}P NMR data of derivatives **4.30** using racemic amines and amino acids, recorded in $CDCl_3$ [L]= 0.01 M*

The structure of reagent **4.36** resembles that of reagent **4.20** most, and for this reason a comparison is made between these two reagents. When the product of *d,l*–2–butylamine and **4.36** (Δδ 0.151 ppm) is compared with the product of reagent **4.20** (Δδ 0.102 ppm) the difference in Δδ is not large, although somewhat larger for reagent **4.36**. Also when *d,l*–α–phenylethylamine is used, the difference in Δδ is larger using reagent **4.36** (Δδ 0.213 ppm) compared with **4.20** (Δδ 0.185 ppm). Probably, the bulk of reagent **4.36** has a positive influence upon the diastereomeric shift dispersion of the products **4.30** compared to the products formed using reagent **4.20**.

When *d,l*–alanine is coupled to reagent **4.36** a diastereomeric shift difference Δδ of 0.273 ppm is observed in comparison with 0.099 ppm for the product using reagent **4.20**. For *d,l*–phenylalanine (Δδ 0.243 ppm) the product **4.30** also showed a significantly larger diastereomeric shift dispersion than the corresponding product using reagent **4.20**, being only Δδ 0.025 ppm. The amino acids *d,l*–valine, *d,l*–phenylglycine and *d,l*–tryptophan, with a Δδ of 0.252, 0.347 and 0.189 ppm, respectively, when coupled to reagent **4.36**, also show larger diastereomeric shift differences when compared with the shifts obtained with reagent **4.20**, being 0.069, 0.098 and 0.038 ppm. All data are recorded with a half equivalent of water complexed to the products; it was not possible to remove this water from the products **4.30** without inducing decomposition processes.

Furthermore, the enantiomeric compositions as determined using reagent **4.36** were compared with the ratios as obtained using reagent **4.20**, and were in perfect agreement.

If the larger diastereomeric shift differences using reagent **4.36** indeed arise from the restricted conformational freedom due to intramolecular tight ion pair formation (in **4.31**) rather than from small, structural differences in the vicinity of the phosphorus nucleus, compared with reagent **4.20**, the observed shift differences should show a large pH dependency.

Using *d,l*–alanine as substrate coupled to reagent **4.36**, a dependence of the diastereomeric shift differences upon the pH was observed (Figure **4.4**). The shift differences appear to be significantly higher in the pH range from 4.5 to 8.0, reaching a maximal Δδ value at pH 7. Furthermore, decomposition of the products above pH 10.0 was observed, as expected. In the pH domain of increased shift differences itself, the differences are relatively small. At lower pH, the protonation of the carboxylic acid group probably gives rise to a situation in which the intramolecular tight ion pair no longer exists. This results in a situation in which the product more strongly resembles the structure of the analoque product of reagent **4.20**, which indeed shows a smaller diastereomeric shift dispersion. At higher pH, the total deprotonation of the product **4.30** also yields a situation in which the tight ion pair contribution to the restricted conformation is of little importance. As expected, the diastereomeric shift dispersion is smaller in the higher pH domain, when compared with the pH 4.5 to 8.0 domain. Using *d,l*–phenylglycine, strongly analoqous behavior is found, as can be seen in Figure **4.4**. Again, the diastereomeric shift dispersion is largest in the domain of pH 4–8. Although not extensively studied, the other amino acid products of reagent **4.36** showed the same type of behavior. The products **4.30** with *d,l*–2–butylamine and *d,l*–α–phenylethylamine do not show this pH dependency.

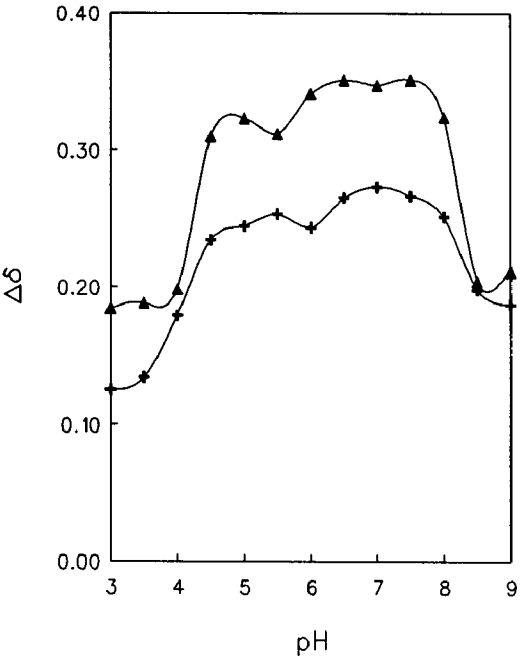
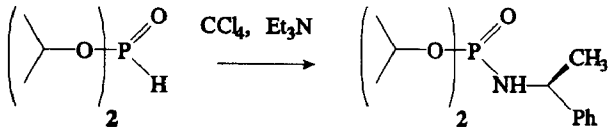


Figure 4.4 Diastereomeric shift differences vs pH of 4.30 with d,l-Ala (+) and d,l-PG (▲) recorded in D_2O [L]= 0.01 M

It should be emphasized that in control experiments where reagent **4.20** is coupled to amino acids, the shifts and ΔΔδ values also appear to be pH dependent, although the differences are very small (about 0.02 ppm maximally) and probably arise from the normal dependency of the ^{31}P nucleus to solvent and solvent polarity effects[19].
The observed phenomena strongly suggest (but are not the ultimate proof) that a type of conformational locking must be in operation in the pH 4.5 to 8.0 domain. Therefore, the use of products **4.30**, result in larger diastereomeric shift differences than observed outside this domain.

4.3 *Dioxophosphonates as complexing agents*

Derivatives of dioxophosphonates **4.20** or **4.36** tend to give complexes with water and ethanol[21,22]. These solvents are bound very tightly, since it is difficult (or even impossible) to remove the solvent completely. We therefore studied the complexing behavior of amide **4.37** towards water in greater detail. Compound **4.37** (Scheme **4.15**) is easily prepared from diisopropylphosphite and (*S*)-α-phenylethylamine using CCl_4 and Et_3N as reagents in a chloroform or benzene solution on a multigram scale (100 g), although the reaction temperature can hardly be kept under control (*this is a very exothermic reaction*).



4.11 **4.37**

Scheme 4.15 Synthesis of 4.37

In Figure **4.6** the ^{1}H NMR spectrum of **4.37** is shown in $CDCl_3$ at very low concentration ([L]= 0.001 M), which is necessary to keep the added water dissolved in the $CDCl_3$. Upon addition of water a signal at δ 2.10 ppm is formed, which upon further addition of water is shifted to δ 2.80 ppm, were the position is stabilized. After the addition of more than 0.6 mole equivalents of water a new signal starts to form at δ 4.65 ppm, as can be seen in Figure **4.6**. The NH resonance shows only a small dependence (!) upon the amounts of water added; the position is shifted from δ 3.0 ppm to δ 3.4 ppm. The decoupled ^{31}P NMR spectrum did not show a large shift dependence upon the addition of water, which is surprising when compared to the diastereomeric products of reagent **4.20** (Table **4.1**), in which the removal of complexed water has a positive influence upon the diastereomeric shift differences.

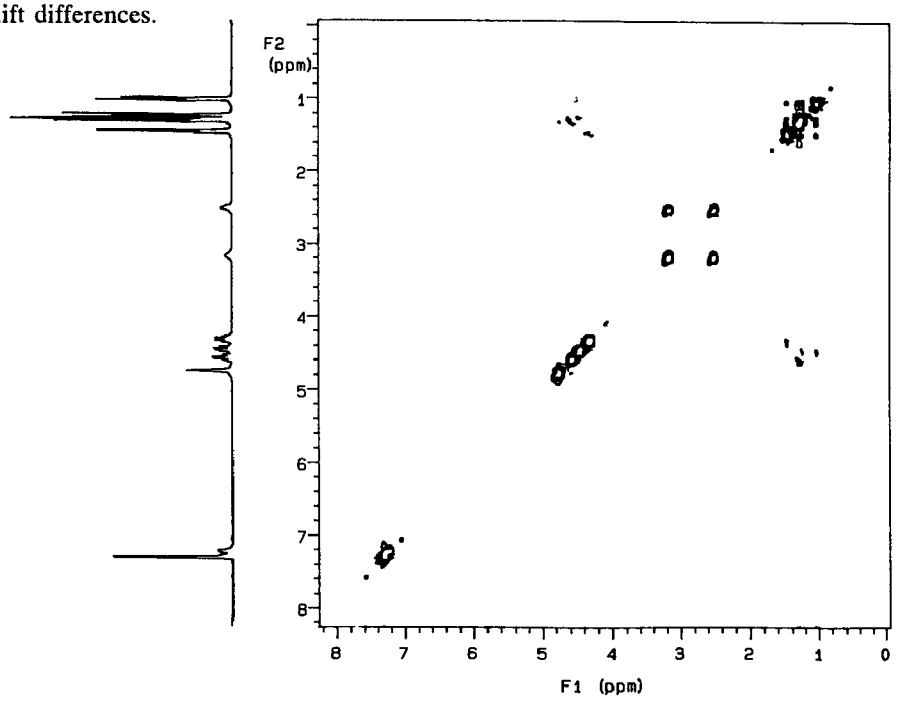


Figure 4.5 NOESY NMR data of product 4.37 complexed to water, recorded in $CDCl_3$ [L]= 0.001 M

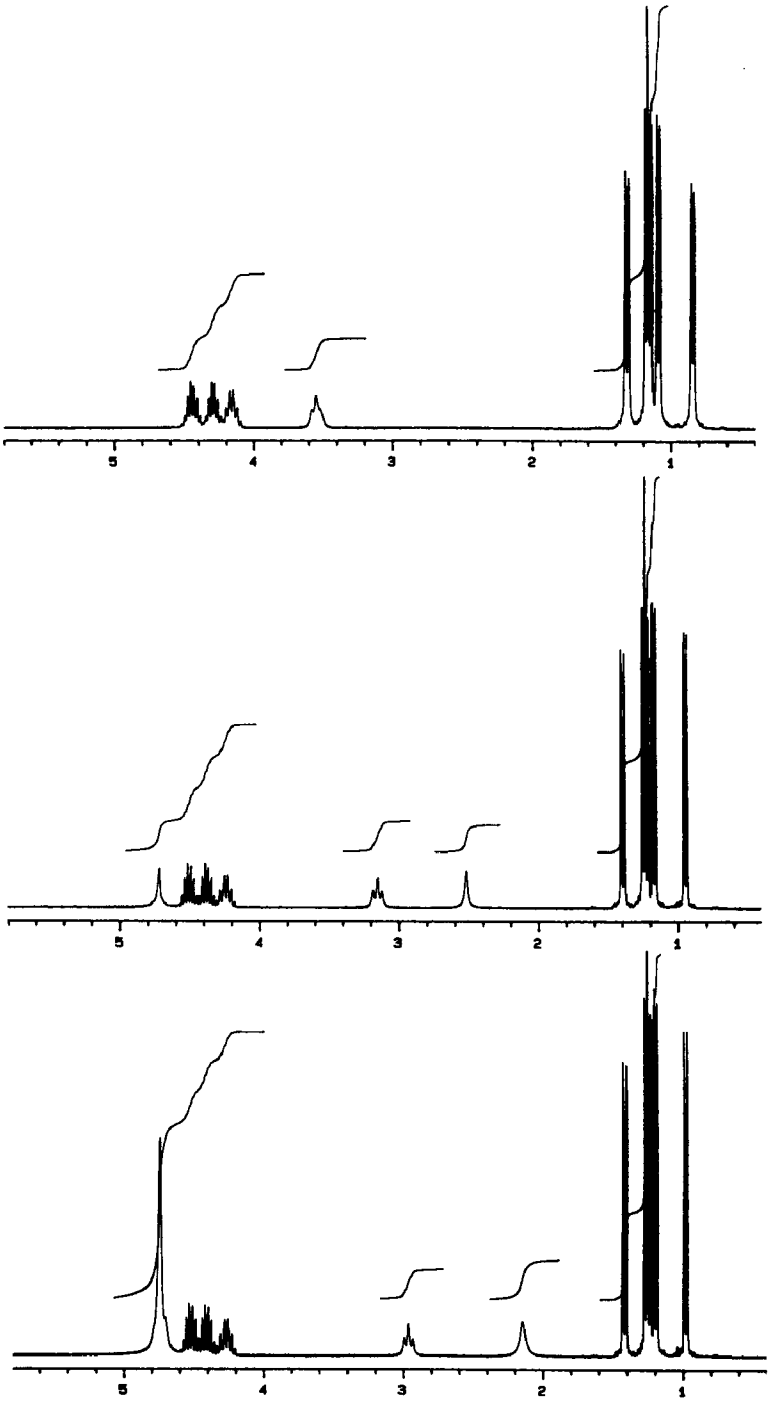
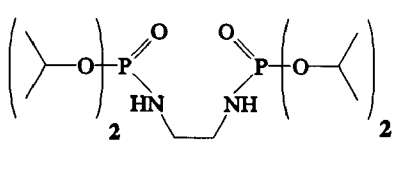
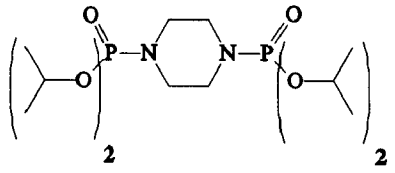


Figure 4.6 ^{1}H *NMR data using product 4.37 upon the addition of water recorded in* $CDCl_3$ *[L]= 0.001 M*

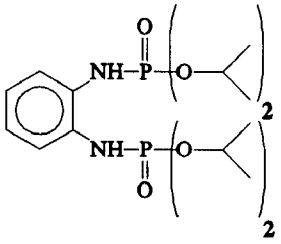
Strangely enough, the 2D NOESY NMR spectrum (Figure **4.5**) only showed the interaction of the *NH* proton with the complexed water, which in turn also has only one interaction. Both the complexed water and the NH proton seem not to have any through space interaction of importance. This strongly suggests the formation of systems in which the apolar methyl groups point outward with the polar O–P–NH moiety pointed inward, complexed with half a mole equivalent of water. The extra (this means more than the half mole equivalent) water is situated outside this region, and is not able to give interaction or exchange with the other polar groups.
Being aware of the possibilities when complexing molecules water or ethanol to this type of phosphorus products, we developed model systems of type **4.38**, **4.39** and **4.40** (scheme **4.16**), using the same reaction conditions as used for compound **4.37**, employing ethylenediamine, piperazine and *o*–aminoaniline, respectively.



4.38



4.39



4.40

Scheme 4.16 *Three potential complexating agents for water or ethanol*

Unfortunately, in our hands, these systems showed no complexation with water, ethanol or ureum. It is, however, possible to prepare air and moisture stable complexes of these reagents with BH_3.THF. This work is under current investigation, so further results are not presented here.

4.4 *Conclusions*

The chiral reagents **4.20** and **4.24** described in this Chapter appear to be excellent derivatizing reagents for the enantiomeric excess determination of alcohols, amines, amino alcohols and unprotected α–amino acids and α–alkylated amino acids, tolerating water as solvent or co–solvent. The observed diastereomeric shift differences Δδ using ^{31}P NMR techniques are large enough to guarantee rapid and adequate analysis. Moreover, the reagents are readily available and stable for at least a year in (optically) pure form.

Furthermore, these reagents are very easy to use and can be applied and purified on a multigram scale if desired.
An attempt was made to develop reagents that, after the derivatization, are able to lock the conformation of the diastereomers formed through an intramolecular ionic bond as in product **4.30**. A study was undertaken to examine the dependence of the NMR shift differences of these products on the pH of the solvent system. Although the observed diastereomeric shift differences are indeed a function of the pH of the solution, the difficult synthesis of **4.36** and low stability of this reagent limits the use as chiral derivatizing reagent for the enantiomeric excess determination to an academic one.
The described systems **4.37**, **4.38**, **4.39** and **4.40** form stable complexes with water and ethanol, but the study of this phenomenon using the phosphorus containing complexes is still in progress. Only on completion it will be posible to provide a more detailed analysis of this phenomenon.

4.5 *Experimental*

For general remarks see Chapter 2.5.

O,O-Di-sec-(S)-butylphosphonate (4.20)[12]
To a solution of 5.00 g (67.56 mmole) (*S*)-2-butanol in 25 mL of dry diethyl ether under nitrogen was added dropwise a solution of 3.06 g (22.52 mmole) of PCl_3 in 10 mL of diethyl ether. The mixture was stirred for one hour, while a stream N_2 was led through the solution, to remove the formed HCl. The solvent was removed under reduced pressure affording a nearly colorless oil. The residue was distilled at 110-115 °C (12 mm Hg) yielding 3.81 g of **4.20** as a colorless oil (19.60 mmole, 87%). $[\alpha]_D^{20}$=17.7° (c 1.0, $CHCl_3$)(lit[12] $[\alpha]_D^{20}$= 17.6°, c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.89 (dd, ^{3}J= 7.26 Hz, $^4J_{PH}$= 7.26 Hz, 6H), 1.27 (ddd, ^{3}J= 5.14 Hz, ^{3}J= 1.23 Hz, $^5J_{PH}$= 0.75 Hz, 6H), 1.56 (m, 4H), 4.36 (m, 2H), 6.81 (d, $^1J_{PH}$= 688.51 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 9.46 (d, $^4J_{PC}$= 5.31 Hz, CH_3), 21.23 (d, $^3J_{PC}$= 15.90 Hz, CH_3), 30.37 (d, $^3J_{PC}$= 5.22 Hz, CH_2), 75.58 (d, $^2J_{PC}$= 5.70 Hz, CH); ^{31}P NMR ($CDCl_3$): δ 5.17; HRMS calcd 194.107, found 194.106.
The enantiomeric purity of this material was checked by means of the method as described by Feringa and co-workers[15].

(R)-1-Phenyl-2,2-dimethyl-1,3-propanediol (4.28)
A solution of 7.00 g (180 mmole) of $LiAlH_4$ in dry ether (150 mL) was brought to reflux. (*R*)-Phencyphos# (20.0 g, 80 mmole) was slowly added to this solution as a solid which led to a violent reaction. The mixture was subsequently refluxed for 5 h, followed by stirring for 12 h at room temperature. The excess $LiAlH_4$ was destroyed by the slow addition of 7.0 mL 1 N KOH solution (*Caution: this reaction yields PH_3 gas, which is very poisonous*). The mixture was stirred with Celite (15.0 g) for 30 min and filtered. The ether layer was dried over Na_2SO_4 and concentrated, yielding a yellow oil. The oil was stirred, and upon slow addition of ether a white solid material crystallized. This material was recrystallized from hexane and dried in vacuum at 40 °C to provide pure **4.28**. Yield

The synthesis and resolution of phencyphos is described in Chapter 5[16].

9.36 g (52.10 mmole, 65%). Mp 65–66 °C; $[\alpha]_D^{20}$= −49.9° (c 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.84 (s, 3H), 0.88 (s, 3H), 2.80 (s, br, 1H), 3.00 (s, br, 1H), 3.51 (d, $^2J_{AB}$= 10.75 Hz, 1H), 3.53 (d, $^2J_{AB}$=10.75 Hz, 1H), 4.65 (s, 1H), 7.32 (m, 5H); ^{13}C NMR ($CDCl_3$): δ 18.90 (CH_3), 22.57 (CH_3), 39.05 (C), 71.95 (CH_2), 82.03 (CH), 127.43 (CH), 127.64 (CH), 141.33 (C); Elemental analysis calcd for $C_{11}H_{16}O_2$, C: 73.30, H: 8.95. Found C: 73.18, H: 8.66; HRMS ($M^+ - H_2O$) calcd 162.104, found 162.105.

(S)-2H-2-Oxo-5,5-dimethyl-4-(R)-phenyl-1,3,2-dioxaphosphorinane (4.24)

A solution of 5.00 g (27.80 mmole) diol **4.28** in benzene (25 mL) under nitrogen was cooled to 0 °C. Over a 15 min period, 4.06 g (30.00 mmole) of PCl_3 was added carefully, while the solution was degassed regularly. After this addition, the solution was stirred at room temperature for 1 h. Subsequently, ethanol (2.60 mL) was added slowly to the mixture which was stirred for another hour at room temperature. Evaporation of the solvent yielded an oil, which crystallized upon addition of ether while the mixture was stirred vigorously. The obtained white solid material was recrystallized from ether and dried in vacuum at 50 °C to afford pure **4.14** as a white solid. Yield 5.21 g (23.07 mmole, 83%). Mp 151–153 °C; $[\alpha]_D^{20}$= −73.02° (c 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.81 (s, 3H), 1.08 (s, 3H), 4.01 (dd, $^2J_{AB}$= 11.53 Hz, $^3J_{PH}$= 25.85 Hz, 1H), 4.20 (dd, $^2J_{AB}$= 11.53 Hz, $^3J_{PH}$= 3.85 Hz, 1H), 5.16 (d, $^3J_{PH}$= 3.20 Hz, 1H), 7.03 (d, $^1J_{PH}$= 688.29 Hz, 1H), 7.20–7.42 (m, 5H); ^{13}C NMR ($CDCl_3$): δ 17.45 (CH_3), 21.21 (CH_3), 36.27 (CH_2), 77.15 (d, $^3J_{PC}$= 4.78 Hz, C), 86.82 (d, $^2J_{PC}$= 4.52 Hz, CH), 127.34 (CH), 127.99 (CH), 128.75 (CH), 134.89 (C); ^{31}P NMR ($CDCl_3$): δ 3.69; Elemental analysis calcd for $C_{11}H_{15}O_3P$, C: 58.41, H: 6.68, P: 13.69. Found C: 58.32, H: 6.59, P: 13.10; HRMS calcd 226.076, found 226.076.

2-(S)-O-Acetylpropionylchloride (4.33)

To a solution of 180.0 g (1.80 mol) of a *S*-lactic acid in water (90%) was added slowly 310.0 g (3.39 mol) acetic anhydride over a 2 h period. Subsequently 30.00 g (0.38 mol) acetylchloride was added and the mixture was brought to reflux. After 2 h reflux, 100.0 g (0.99 mol) of acetic anhydride was added and the mixture was refluxed for another 12 h. Subsequently the mixture was concentrated, and the resulting yellowish oil was used as such in the formation of the acid chloride. To this oil was added 160 mL $SOCl_2$ over a 15 min period. The mixture was stirred at room temperature for 1 h, and then brought to reflux for 5 h. The crude reaction mixture was distilled, first some fractions of unreacted $SOCl_2$ were collected, followed by the desired product **4.33** at 67–68 °C (18 mm Hg). Yield 136.5 g (1.09 mol, 60%) of a colorless oil. $[\alpha]_D^{20}$= −14.92° (c 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.56 (d, ^3J= 6.63 Hz, 3H), 2.11 (s, 3H), 5.14 (q, ^3J= 6.63 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 16.03 (CH_3), 20.16 (CH_3), 74.90 (CH), 169.81 (C), 172.71 (C); Elemental analysis calcd for $C_5H_7O_3Cl$, C: 39.89, H: 4.69, Cl: 23.55. Found C: 39.48, H: 4.61, Cl: 23.10; HRMS calcd 150.008, found 150.007.

2-(S)-O-Acetylpropionyl-N,N-diethyl amide (4.34)

A solution of 48.6 g (0.65 mol) of diethylamine and 67.4 g (0.65 mol) Et_3N in CH_2Cl_2 (500 mL) was cooled to 0 °C. A solution of 100.0 g **4.33** in CH_2Cl_2 (125 mL) was added slowly, while the temperature was maintained at 0 °C. After the addition the temperature was allowed to reach room temperature again, and the mixture was stirred for 12 h. Subsequently, the mixture was washed three times with a saturated NH_4Cl solution (150 mL) and once with water (150 mL). The CH_2Cl_2 layer was dried over Na_2SO_4 and concentrated. The resulting slightly yellow oil was distilled at 126–127 °C (13 mm Hg) to

afford **4.34** as a colorless oil. Yield 110.33 g (0.59 mol, 91%). $[\alpha]_D^{20}$= −26.44° (c 0.55, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.95 (dd, $^3J_1$=$^3J_2$= 7.08 Hz, 3H), 1.09 (dd, $^3J_1$=$^3J_2$= 7.32 Hz, 3H), 1.27 (d, ^3J= 6.83 Hz, 3H), 1.94 (s, 3H), 3.17 (m, 2H), 3.31 (m, 2H), 5.13 (q, ^3J= 6.83 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 12.34 (CH_3), 13.74 (CH_3), 16.76 (CH_3), 20.33 (CH_3), 40.08 (CH_2), 41.17 (CH_2), 66.54 (CH), 169.11 (C), 170.08 (C); Elemental analysis calcd for $C_9H_{17}O_3N$, C: 57.73, H: 9.15, N: 7.48. Found C: 57.22, H: 8.89, N: 7.29; HRMS calcd 187.121, found 187.121.

2-(S)-N,N-Diethyl-1-amino-2-hydroxypropane (4.35)

A suspension of 25.1 g (0.64 mol) of $LiAlH_4$ in dry THF (500 mL) under nitrogen was cooled to 0 °C. A solution of 75.0 g (0.43 mol) amide **4.34** in 50 mL of THF was added slowly, while the temperature was not allowed to exceed 5 °C. After the addition was completed (1 h), the mixture was brought to reflux for 12 h. Subsequently, water was added (25 mL) followed by 25 mL of a 1 N KOH solution. The solution was stirred with 15.0 g of Celite, followed by a Soxhlet extraction of the Celite–salt mixture with THF. The THF layers were dried over Na_2SO_4 and concentrated. The yellow residue was purified by means of column chromatography over silica gel, using ethyl acetate–hexane as eluens. Yield 39.30 g (0.30 mol, 69%) of **4.35** as a colorless oil. $[\alpha]_D^{20}$= −34.21°; ^1H NMR ($CDCl_3$): δ 1.00 (dd, $^3J_1$=$^3J_2$= 4.80 Hz, 6H), 1.11 (d, ^{3}= 6.00 Hz, 3H), 2.19 (dd, $^2J_{AB}$= 11.40 Hz, ^3J= 10.80 Hz, 1H), 2.38 (dd, $^2J_{AB}$= 11.40 Hz, ^3J= 1.20 Hz, 1H), 2.44 (m, 2H), 2.63 (m, 2H), 3.52 (s, br, 1H), 3.71 (ddq, ^3J= 10.80 Hz, ^3J= 6.00 Hz, ^3J= 1.20 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 11.57 (CH_3), 15.52 (CH_3), 28.59 (CH_2), 41.54 (CH_2), 48.91 (CH_2), 70.12 (CH_3), 84.01 (CH); Elemental analysis calcd for $C_7H_{17}ON$, C: 64.07, H: 13.06, N: 10.67. Found C: 63.88, H: 12.96, N: 10.39; HRMS calcd 131.131, found 131.131.

The enantiomeric composition of this product was checked by the method as described by Feringa and co-workers[13].

2-(S)-1-Amino-N,N-diethyl-2-hydroxypropane.HCl, HCl salt of (4.35)

Free **4.35** was dissolved in 250 mL of dry ether and upon treatment with HCl gas the product crystallized spontaneously. The solid material was collected, washed with hexane and dried in vacuum at 40 °C. Mp 138–140 °C. ^1H NMR ($CDCl_3$): δ 1.24 (d, ^3J= 4.76 Hz, 3H), 1.42 (dd, $^3J_1$=$^3J_2$= 5.31 Hz, 6H), 2.98 (m, 2H), 3.01 (s, br, 1H), 3.22 (m, 2H), 4.38 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 8.63 (CH_3), 20.47 (CH_3), 48.65 (CH_2), 61.27 (CH_2), 61.94 (CH_3), 98.36 (CH).

O,O-Di-(2-(S)-(N,N-diethyl-2-hydroxypropyl)phosphonate (4.36)

A solution of 1.00 g (5.98 mmol) of **4.35**.HCl in 75 mL of CH_2Cl_2 under nitrogen was cooled to 0 °C. To this solution was added dropwise a solution of 0.41 g (3.00 mmol) of PCl_3 in CH_2Cl_2 (10 mL) while the mixture was degassed regularly. After 1 h of stirring at 0 °C, 0.14 g (3.00 mmol) of ethanol was added with vigorous stirring. The reaction mixture was allowed to reach room temperature and the mixture was stirred for 1 h at this temperature. The reaction mixture was concentrated to dryness yielding a white solid material, which was purified by means of chromatography over silicagel (under a nitrogen atmosphere) using $CHCl_3$ as eluens. Yield 0.34 g (1.11 mmol, 37%) of white solid material, being **4.36**.HCl. It was not possible to obtain a proper, reproducable rotation for this product. ^1H NMR ($CDCl_3$): δ 1.40 (dd, $^3J_1$=$^3J_2$= 9.01 Hz, 12H), 1.54 (dd, $^4J_{PH}$= 15.60 Hz, ^3J= 5.48 Hz, 6H), 2.98 (m, 4H), 3.22 (m, 4H), 4.31 (m, 2H), 7.41 (d, $^1J_{PH}$= 723 Hz,

1H), 10.82 (d, br, J= 52.94 Hz, 1H), 11.38 (d, br, J= 42.36 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 9.32 (CH_3), 22.23 (CH_3), 50.45 (d, $^3J_{PC}$= 5.40 Hz, CH_2), 64.78 (d, $^5J_{PC}$= 5.21 Hz, CH_2), 67.54 (d, $^3J_{PC}$= 6.98 Hz, CH_3), 101.34 (d, $^2J_{PC}$= 6.89 Hz, CH); ^{31}P NMR ($CDCl_3$): δ 5.81; No proper HRMS or elemental analysis could be obtained, due to decomposition reactions. The enantiomeric composition of this product was checked by the method as described by Feringa and co-workers[15].

Typical procedure for the enantiomeric excess determination of alcohols, amines and amino acids using phosphonates 4.20, 4.24 and 4.36

For alcohols and amines; only when using reagents 4.20 and 4.24:

A suspension of the alcohol or amine (1.0 mmol) and Et_3N (0.4 mL)# in $CDCl_3$ or C_6D_6 (3.0 mL) was cooled to 0 °C and treated dropwise with a solution of phosphonate **4.20** or **4.24** (1.15 mmol) in CCl_4 (0.5 mL) and the mixture was stirred at room temperature for 2 h. After this period, for analyses purposes, a decoupled ^{31}P or ^1H NMR spectrum is recorded directly. Alternatively, the mixture was taken to dryness and the crude mixture was purified by crystallization from ethyl acetate petroleum-ether mixtures or column chromatography on silica gel providing white solids or colorless oils.

For amino acids, using reagents 4.20, 4.24 and 4.36:

A suspension of the amino acid (1.0 mmol), Et_3N (0.4 mL), H_2O (0.2 mL) and ethanol (0.2 mL) was cooled to 0 °C and treated dropwise with a solution of the phosphonates **4.20**, **4.24** or **4.36** in CCl_4 (0.5 mL). The mixture was subsequently stirred at room temperature for 2 h. The reaction was quenched by acidifying to pH 2.0 with 10 % HCl solution. After extraction of the mixture with ethyl acetate (3 x 5.0 mL) the combined ethyl acetate phases were washed with water (5.0 mL) and dried (Na_2SO_4). The solvent was then removed by evaporation and the oily residue used as such for the enantiomeric excess determination (by taking the residue in $CDCl_3$, C_6D_6 or D_2O). The phosphonic amides were purified by crystallization from ethyl acetate petroleum-ether mixtures or column chromatography on silica gel, providing white solids or colorless oils.

N-(Diisopropylphosphonamido)-2-(R)-phenylethylamine (4.37)

A solution of 10.0 g (82.65 mmol) (*R*)-α-phenylethylamine, 10.0 mL of Et_3N and ethanol (35.0 mL) was cooled to 0 °C. A solution of 15.77 g (95.0 mmol) diisopropyl-phosphite in 12.5 mL of CCl_4 was added to this solution with vigorous stirring. During the addition, the temperature was not allowed to exceed 0 °C. The mixture was stirred at room temperature for 2 h and subsequently quenched with 1 N HCl solution (100 mL). This mixture was extracted with three portions of $CHCl_3$ (75 mL) and the $CHCl_3$ layers were dried over Na_2SO_4 and concentrated. The resulting colorless oil was distilled (83-84 °C, 0.7 mm Hg). Yield 21.54 g (78.92 mmol, 95%). $[\alpha]_D^{20}$= 39.7° (c 1.0, methanol); ^1H NMR ($CDCl_3$): δ 0.96 (d, ^3J= 6.23 Hz, 3H), 1.17 (d, ^3J= 6.23 Hz, 3H), 1.22 (d, ^3J= 7.32 Hz, 3H), 1.24 (d, ^3J= 6.59 Hz, 3H), 1.39 (d, ^3J= 6.96 Hz, 3H), 2.93 (dd, $^2J_{PH}$=^3J= 5.49 Hz, 1H), 4.22 (m, 1H), 4.40 (m, 1H), 4.51 (m, 1H), 7.10-7.20 (m, 1H), 7.22-7.25 (m, 4H); ^{13}C NMR ($CDCl_3$): δ 23.82 (d, $^3J_{PC}$= 2.39 Hz, CH_3), 25.40 (d, $^3J_{PC}$= 6.38 Hz, CH_3), 51.38 (CH), 70.67 (d, $^2J_{PC}$= 5.58 Hz, CH), 125.80 (CH), 126.93 (CH), 128.36 (CH), 145.25 (C);

When alcohols are used, a catalytic amount of *N,N*-dimethylaminopyridine is recommended or alternatively the potassium alkoxides can be used.

^{31}P NMR ($CDCl_3$): 5.48; Elemental analysis calcd for $C_{14}H_{24}O_3NP$, C: 58.93, H: 8.48, N: 4.91, P: 10.86. Found C: 58.58, H: 8.25, N: 4.84, P: 10.57; HRMS calcd 285.149, found 285.149.
When *d,l*–α–phenylethylamine was used the product crystallized upon standing (after a few weeks had passed), yielding white crystals, Mp 83–84 °C.

Bis–(diisopropylphosphonamido)–N,N'–diaminoethylene (4.38)
Using the same procedure as for **4.37** from diisopropylphosphite and ethylenediamine. Crystallization from ether afforded white needles in 87% yield. Mp 74–75 °C.
^{1}H NMR ($CDCl_3$): δ 1.12 (d, ^{3}J= 6.00 Hz, 24H), 2.91 (m, 4H), 3.30 (s, br, 2H), 4.57 (m, 4H); ^{13}C NMR ($CDCl_3$): δ 23.54 (d, $^3J_{PC}$= 5.25 Hz, CH_3), 42.52 (d, $^2J_{PC}$= 6.02 Hz, CH_2), 70.38 (d, $^2J_{PC}$= 6.03 Hz, CH); ^{31}P NMR ($CDCl_3$): δ 7.31; Elemental analysis calcd for $C_{14}H_{34}O_6N_2P_2$, C: 43.30, H: 8.82, N: 7.21, P: 15.95. Found C: 43.12, H: 8.77, N: 7.13, P: 15.76; HRMS calcd 388.189, found 388.188.

Bis–(diisopropylphosphonamido)–N,N'–piperazine (4.39)
Using the same procedure as for **4.37** from diisopropylphosphite and piperazine. Crystallization from ether afforded white needles in 95% yield. Mp 66–67 °C.
^{1}H NMR ($CDCl_3$): δ 1.27 (dd, $^4J_{PH}$=^{3}J= 6.30 Hz, 24H), 3.04 (m, 8H), 4.53 (m, 4H);
^{13}C NMR ($CDCl_3$): δ 23.62 (d, $^3J_{PC}$= 4.50 Hz, CH_3), 44.44 (d, $^2J_{PC}$= 6.02 Hz, CH_2), 70.58 (d, $^2J_{PC}$= 6.00 Hz, CH); ^{31}P NMR ($CDCl_3$): δ 6.13; Elemental analysis calcd for $C_{16}H_{36}O_6N_2P_2$, C: 46.37, H: 8.76, N: 6.76, P: 14.95. Found C: 46.12, H: 8.68, N: 6.71, P: 14.63; HRMS calcd 414.205, found 414.205.

Bis–(diisopropylphosphonamido)–N,N'–diaminophenylene (4.40)
Using the same procedure as for **4.37** from diisopropylphosphite and *O*–phenylenediamine. Crystallized from ether–hexane mixtures to afford white solid material in 95 % yield.
Mp 123–124 °C. ^{1}H NMR ($CDCl_3$): δ 1.22 (d, ^{3}J= 6.19 Hz, 6H), 1.35 (d, ^{3}J= 6.19 Hz, 6H), 4.72 (m, 4H), 6.88 (d, br, $^2J_{PH}$= 31.41 Hz, 2H), 6.82–7.02 (m, 2H), 7.20–7.37 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 23.65 (d, $^3J_{PC}$= 12.01 Hz, CH_3), 71.61 (d, $^2J_{PC}$= 8.32 Hz, CH), 120.32 (CH), 120.37 (CH), 122.42 (CH), 130.20 (d, $^2J_{PC}$= 12.75 Hz, C); ^{31}P NMR ($CDCl_3$): δ 0.82; Elemental analysis calcd for $C_{18}H_{34}O_6N_2P_2$, C: 49.54, H: 7.85, N: 6.42, P: 14.19. Found C: 49.31, H: 7.75, N: 6.37, P: 13.97; HRMS calcd 436.189, found 436.188.

4.6 *References*

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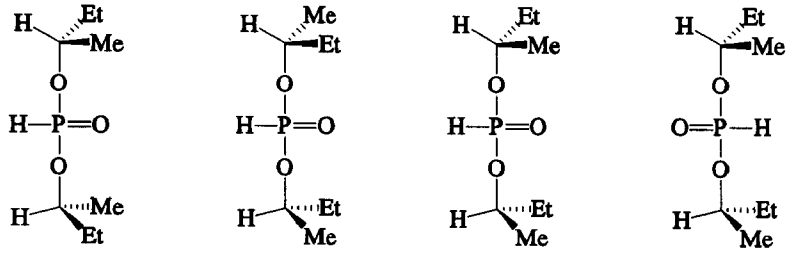
CHAPTER 5

Synthesis and application of 2-chloro-2-oxo-1,3,2-dioxaphosphorinanes and derivatives as *e.e.* determining agents

5.1 *The use of phosphoryl chlorides in the enantiomeric excess determination: an introduction*

As discussed in the previous Chapters, ^{31}P NMR techniques are, analogously to ^{1}H and ^{19}F based NMR methods, highly suitable for the determination of the enantiomeric excess of many different organic substrates. Due to several reasons, like the high reactivity of phosphorus derivatizing agents towards a broad range of substrates, the remarkable sensivity of the phosphorus nucleus towards small environmental changes, the rather large chemical shift range and the simple spectra that are obtained when broad band decoupling is used, the phosphorus nucleus is of growing importance in enantiomeric excess determination[1].

The most popular classes of phosphorus containing reagents for enantiomeric excess determination are achiral or chiral phosphoryl chlorides.



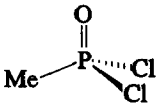
5.1a(SS) **5.1b(RR)** **5.1c(SR)** **5.1d (SR)**

Scheme 5.1 Diastereomeric products 5.1 formed from PCl_3 and chiral 2-butanol

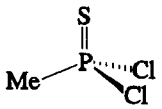
The most remarkable of the derivatizing agents developed so far probably is *achiral* PCl_3, introduced by Wynberg and co-workers[2]. The actual enantiomeric excess determination is based upon the principle first introduced by Horeau[3]. This states that when a *R,S* mixture of a chiral substrate is allowed to react with an achiral derivatizing agent and two equivalents of the chiral compound are coupled, this leads to the formation of distinct isomers which consist of a racemate and two meso compounds: the *R*-X-*R*, *S*-X-*S*, *S*-X-*R* and *R*-X-*S* (Scheme **5.1**).

Because the phosphorus atom is *pseudochiral* in the products **5.1**, this leads to the detection of three signals (1:1:2 ratio) in the decoupled ^{31}P NMR spectrum. Two of these

arise from the meso forms (*R,S* and *S,R*) and one from the racemic *R,R* and *S,S* pair. The closely related *achiral* reagents $MePOCl_2$ and $MePSCl_2$ (**5.2** and **5.3**) also belong to the same category of derivatizing agents[4].



5.2



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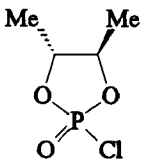

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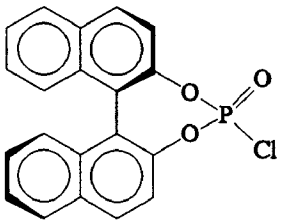
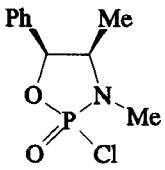


5.5



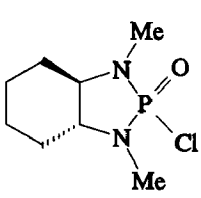
5.6

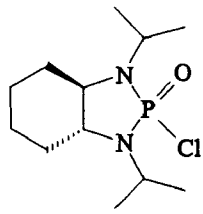
Scheme 5.2 Some typical pentavalent phosphorus containing derivatizing agents for the enantiomeric excess determination

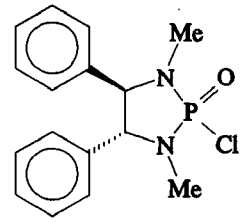
Anderson and Shapiro[5] introduced chlorodioxaphospholane **5.4** (Scheme **5.2**), based upon enantiomerically pure 2,3-butanediol, which gave after reaction with primary and secondary alcohols diastereomers showing chemical shift differences up to $\Delta\delta$ 0.13 ppm. A distinct improvement was the introduction of the phosphoric chloride based upon optically pure bis-ß-naphthol **5.5** that, although described as unstable by Johnson and co-workers[6], was reintroduced by Kato[7].

Both reagents **5.4** and **5.5** are unique as the phosphorus atom is not chiral because of the C_2 axis of symmetry of the chiral diol. Therefore, either retention or inversion of configuration at phosphorus during the derivatization of an enantiomerically pure alcohol yields a single diastereomer. Needles to say, the stereochemistry at the phosphorus atom in the reactions with several nucleophiles is of utmost importance when the phosphorus atom is a stereogenic center. This is the case with reagent **5.6**, introduced by Johnson[6], where the phosphorus atom is a stereogenic center. Usually nucleophilic substitution reactions at phosphorus proceed with retention of configuration[8], although Cullis and co-workers[9] found that stereochemical scrambling also can take place.

For this reason, the reagents recently developed by Alexakis and co-workers[10] are all based upon the use of suitable C_2 symmetrical phosphorus-amides **5.7-5.9** (Scheme **5.3**) which, after derivatization with a racemic chiral substrate, yield diastereomeric pairs that show $\Delta\delta$ values up to 0.936 ppm.







5.7 **5.8** **5.9**

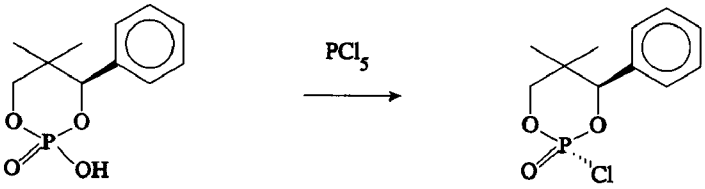
Scheme 5.3 *Some C–2 symmetrical phosphoramide derivatizing agents*[10]

Because of the enormous potential of phosphorus containing reagents in the enantiomeric excess determination, the development of a chiral pentavalent phosphorus reagent which structure can be readily modified leading to higher diastereomeric shift differences and showing a larger tolerance towards water, would be highly desired.

5.2 *Chiral cyclic chlorodioxaphosphorinanes: a readily available solution to enantiomeric excess determination problems*

5.2.1 *Synthesis of chlorophosphorinane 5.13*

As described in Chapter 4.2.3, chiral reagents based upon cyclic phosphoric acids appear to be excellent derivatizing agents for the enantiomeric excess determination of alcohols, amines and amino acids and derivatives thereof. The reagents **5.13** (Scheme **5.4**) and **5.12** (Scheme **5.5**) are easily obtained from (–)–phencyphos[11] **5.10**.

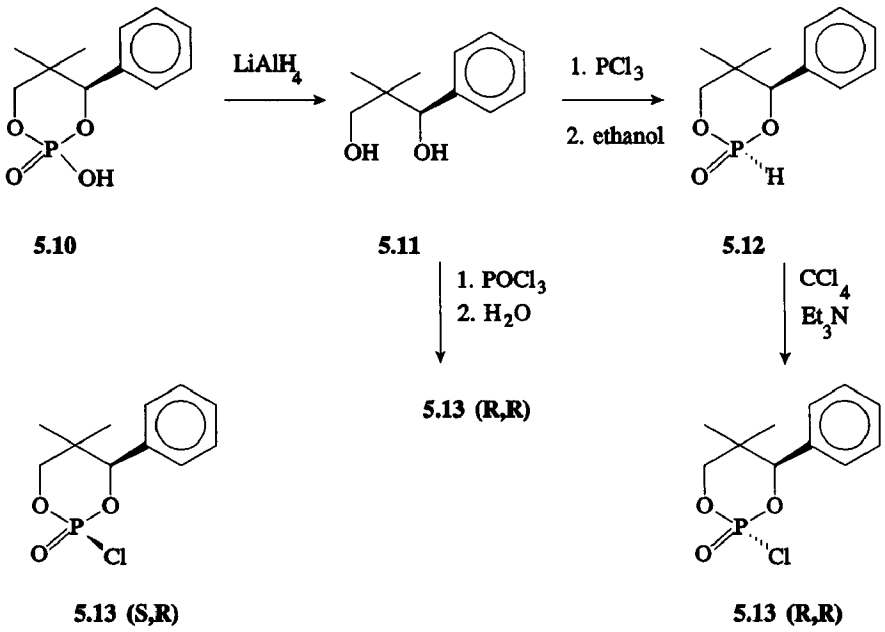


5.10 **5.13**

Scheme 5.4 *Synthesis of derivatizing agent 5.13 from (–)–phencyphos 5.10*

Reagent **5.13** can be prepared by reaction of (–)–phencyphos **5.10** with PCl_5 in CH_2Cl_2 in only 15% isolated yield (Scheme **5.4**). This seems a rather straightforward method although the yield is low. Furthermore, the reaction is very sensitive to solvent effects.

When chloroform is used instead of dichloromethane the conversion does not proceed at all[12]. We therefore developed two alternative routes to prepare agent **5.13**.

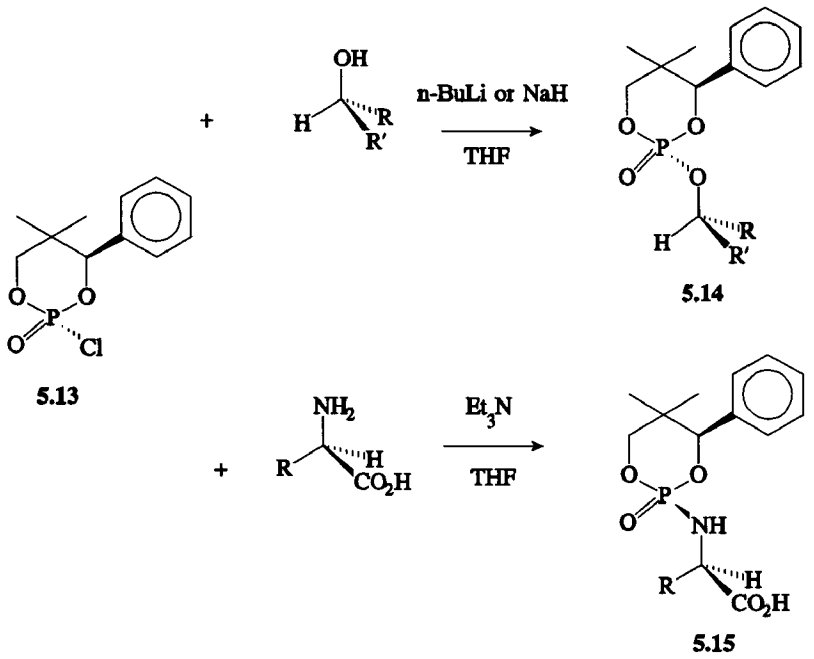


Scheme 5.5 An alternative synthesis for reagent 5.13 from (-)-phencyphos 5.10

In both routes shown in Scheme **5.5**, the enantiomerically pure phosphoric acid **5.10** is treated with $LiAlH_4$ to give optically pure diol **5.11** (65% yield). Reaction with $POCl_3$, analogously to the synthesis of the racemic chlorophosphorinane[11] (see Scheme **5.11**), affords a 80:20 mixture of two diasteromeric chlorophosphorinanes **5.13**. These can, however, be separated (though troublesome) by means of crystallization or column chromatography, affording enantiomerically pure **5.13** in 35% isolated yield.
In the second route the first step is the formation of the *S*-(-)-2H-2-oxo-5,5-dimethyl-4-(*R*)-phenyl-1,3,2-dioxaphosphorinane **5.12** by means of a reaction with PCl_3 followed by a treatment with ethanol (83% yield). The phosphorinane **5.12** was subsequently transferred into the desired diastereomerically pure chlorophosphorinane **5.13** by means of a reaction with CCl_4 and Et_3N in benzene or chloroform[13] in nearly quantitative yield (Scheme **5.5**).

5.2.2 *The use of chlorophosphorinane 5.13 in the enantiomeric excess determination of alcohols and amines*

Ten Hoeve and Wynberg[11] were the first to report the use of chlorophosphorinane **5.13** as a possible derivatizing agent for enantiomeric excess determination purposes. These findings were based upon a small number of test reactions, which, however, were not



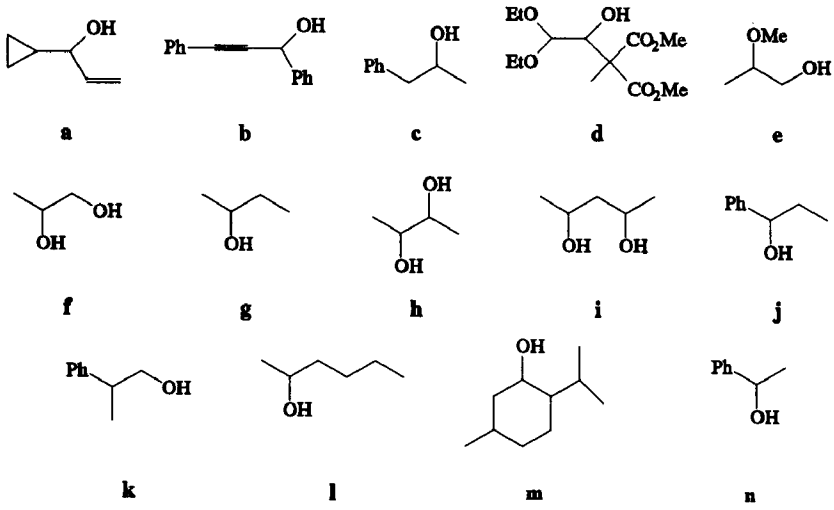
Scheme 5.6 Formation of diastereomeric phosphonic esters 5.14 and amides 5.15 using reagent 5.13

completely optimized. For obvious reasons we have compared reagent **5.13** with other known pentavalent phosphoric acid chlorides[5,6], using 2-butanol as the test substrate. It appeared that reagent **5.13** has a poor reactivity towards alcohols. Reagent **5.13** does not react with alcohols in THF in the presence of two equivalents of triethylamine. Several byproducts are formed, probably arising from ring opening processes[5,6]. The use of other bases, such as DBU, DMAP and KOtBu or other solvents (CH_2Cl_2, $CHCl_3$, DMF or benzene) did not improve these results.

Therefore, more forcing conditions were required, like the use of strong bases like NaH or n-butyllithium (Scheme **5.6**). The use of strong bases like n-butyllithium clearly limits the scope of this reagent when using base-sensitive alcohols. The subsequent reaction with reagent **5.13** fortunately is very fast: the formation of all the phosphonic esters is completed within one hour. As with alcohols, the reaction of **5.13** with most of the secondary amines can only be achieved when strong bases like n-butyllithium or NaH are used. The coupling of reagent **5.13** with primary amines can be performed at elevated temperatures, *e.g.* refluxing solvents like CH_2Cl_2, having the advantage that less reactive bases (Et_3N) are needed (Scheme **5.6**).

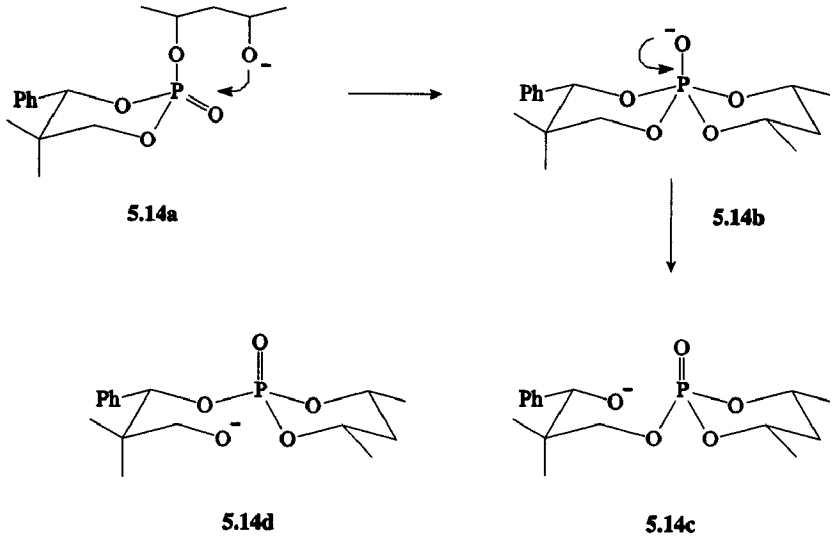
In Scheme **5.7** several of the alcohols are collected that were used for enantiomeric excess analysis; the indices used refer to Table **5.1**.

As can be seen, a large variation in the observed diastereomeric chemical shift differences is obtained, ranging from $\Delta\delta$ 0 ppm (for **d** and **g**) to $\Delta\delta$ 0.905 ppm when a closely similar



Scheme 5.7 Several chiral alcohols coupled to derivatizing agent 5.13; The indices refer to Table 5.1

alcohol is used (**f**). Small aliphatic alcohols, like substrate **e**, **l** and even **m**, give a rather disappointing diastereomeric shift dispersion, typically in the order of less than $\Delta\delta$ 0.07 ppm in the decoupled ^{31}P NMR spectra. In some of these cases, however, it is possible to use ^{1}H NMR techniques to determine the enantiomeric excess, although these spectra tend to be rather complex.



5.14a **5.14b** **5.14d** **5.14c**

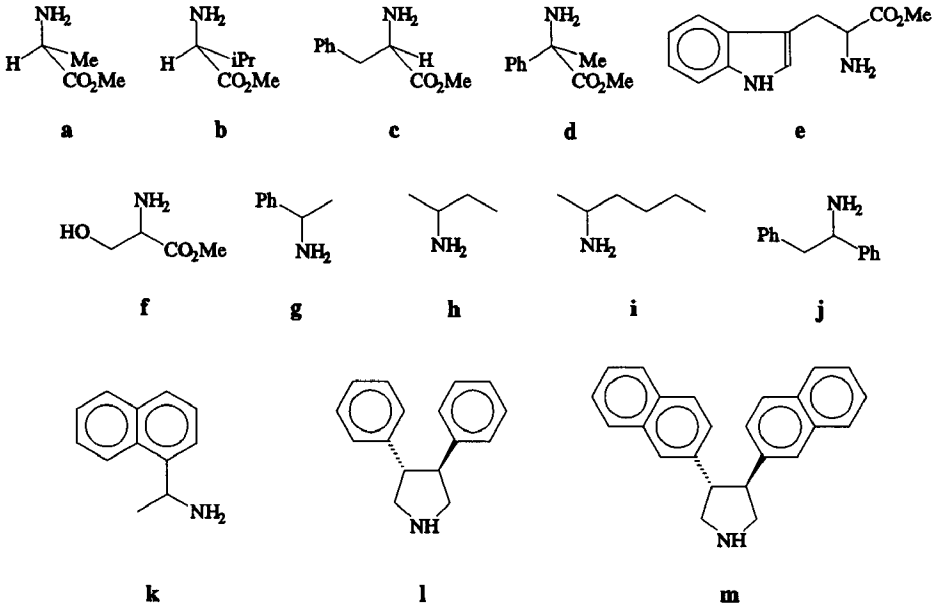
Scheme 5.8 Possible side reactions in the addition of diols to reagent (S)-5.13

Substrate	δ (ppm)	$\Delta\delta$ (ppm)	ratio
a	decomposition	-	-
b	-11.65[a]	0.171	49:51
c	-6.93	0.237	52:48
d	-5.90	0	-
e	-5.23	0.071	49.5:50.5
f	-7.41	0.910	49.5:50.5
g	-7.23	0	-
h	-7.56	0.135	50:50
i	-6.78[a]	-	-
j	-7.91	0.263	49.5:50.5
k	-7.50	0.151	49.5:50.5
l	-7.26	0.013	49:51
m	-1.25	0.051	49.5:50.5
n	-7.34	0.213	50:50

Table 5.1 *^{31}P NMR data of products **5.14** using various alcohols ($CDCl_3$ [L]= 0.01 M)*
a) See text for explanation

The best results are obtained with alcohols containing aromatic groups; these give nicely separated signals in the decoupled ^{31}P NMR spectra with large diastereomeric shift dispersion (typically in the order of $\Delta\delta$ 0.2 to 0.3 ppm). Using for example α or ß-phenylpropanols (**j** and **k**), shift differences are found of $\Delta\delta$ 0.262 and 0.213 ppm, respectively. The most remarkable results, however, are encountered when reagent **5.13** is coupled with diols, like racemic 1,2-propanediol (**f**). Regardless whether one or two equivalents of base are used in the reaction, four signals are observed in the decoupled ^{31}P NMR spectrum, indicating that reaction takes place with the primary as well as with the secondary alcohol moiety[14]. The different intensities of the groups of absorptions can be explained by the different reactivities of the primary and the secondary alcohol moieties, leading to non-equal amounts of primary and secondary coupled products. It is, however, possible to use both signal groups for the enantiomeric excess determination. The situation is even more complex when *d,l*-2,4-pentanediol (**i**) is coupled to reagent **5.13**, as the hydroxyl groups are no longer attached to adjacent carbons, like in the case of *d,l*-1,2-propanediol (**f**). A transesterification reaction takes place leading to a new, six-membered ring system, as is shown in Scheme **5.8**.

Several of the racemic amines and amino acid esters used in the derivatizing reactions with reagent **5.13** are collected in Scheme **5.9**; the indices refer to Table **5.2**.



Scheme 5.9 Amines and esters of amino acids derivatized using reagent 5.13; the indices refer to Table 5.2

As for the alcohol derivatives, ^{31}P NMR diastereomeric shift differences ranging from $\Delta\delta$ 0 ppm to 1.04 ppm are found for the derivatives of these chiral amines with reagent **5.13**. When unfunctionalized amines are used as the substrates, it appeared that chiral amines with a phenyl group attached directly to the stereocenter give the largest diastereomeric shift differences $\Delta\delta$ in the decoupled ^{31}P NMR spectrum. For derivatives **5.15** using *d,l*–α–phenylethylamine (**g**) or the more crowded naphthyl analog (**k**), $\Delta\delta$ values of 0.636 ppm and 0.951 ppm, respectively, are obtained.

The aliphatic amines (**h** and **i**) show no or hardly any diastereomeric shift dispersion, the $\Delta\delta$ values being 0 and 0.111 ppm, respectively. Also for α–amino acid esters, a large range of diastereomeric shift differences was found, although the diastereomeric shift differences tend to be somewhat larger than those obtained for the amines. Again, the presence of a phenyl group has a positive influence upon the observed diastereomeric shift differences, with $\Delta\delta$ values ranging from 0.297 to 0.510 ppm (for entries **d** and **c**).

For *d,l*–alanine methyl ester (**a**) the smallest $\Delta\delta$ value was found ($\Delta\delta$ 0.060 ppm), while the methyl ester of *d,l*–phenylalanine (**c**) gave the largest diastereomeric shift dispersion, being 0.510 ppm. Using *d,l*–tryptophan methyl ester (entry **e**), only the product coupled with the secondary amine group was found, this contrary to the results described in Chapter 4. The coupling of *d,l*–serine methyl ester took place at the alcohol moiety instead of the amine functionality.

Substrate	δ (ppm)	$\Delta\delta$ (ppm)	ratio
a	3.15	0.060	50:50
b	2.28	0.212	49.5:50.5
c	4.73	0.510	49.5:50.5
d	0.82	0.297	49:51
e	11.51	0.437	49.5:50.5
f	-7.45	0.196	49.5:50.5
g	5.05	0.636	49.5:50.5
h	1.26	0	-
i	2.23	0.111	50:50
j	1.86	0.540	49.5:50.5
k	4.89	0.951	49:51
l	3.35	0.480	95.5:4.5[a]
m	2.67	1.040	76:24[a]

Table 5.2 ^{31}P NMR data if derivatives 5.14, recorded in $CDCl_3$ [L]= 0.01 M
a) enriched samples used

The same products **5.14** and **5.15** are obtained regardless whether reagent **5.12** (**4.24** in Chapter 4) or **5.13** is used for the derivatization reaction (see Chapter 4). As is shown in Scheme **5.6**, the reactions proceed with retention of configuration when alcohols are coupled and with inversion of configuration when amines are coupled with respect to the chlorine at the phosphorus atom. The stereochemistry with respect to the phosphorus atom in the derivatization reactions will be discussed in greater detail in Chapter 6.
The reaction with alcohols or amines (nearly) always gives one side product, the *pyrophosphate*, although this does not disturb the actual enantiomeric excess determination. The formation of *pyrophosphate* will be discussed in more detail in Chapter 7.
The decoupled ^{31}P NMR spectrum of product **5.14**, using racemic α-phenylethanol, is shown in Figure **5.1** (next page). Two baseline separated singlet signals are observed, which allow easy integration and subsequent analysis.
In Table **5.3** the enantiomeric ratios of a selected number of chiral substrates as obtained by means of reagent **5.13** are compared with the enantiomeric ratios as determined by means of the optical rotation and the α-chloropropionyl chloride method[15]. The different methods appeared to be in perfect agreement.
In some of the cases discussed above, small differentiations from the expected enantiomeric ratio, as determined using other methods, were found. Although these

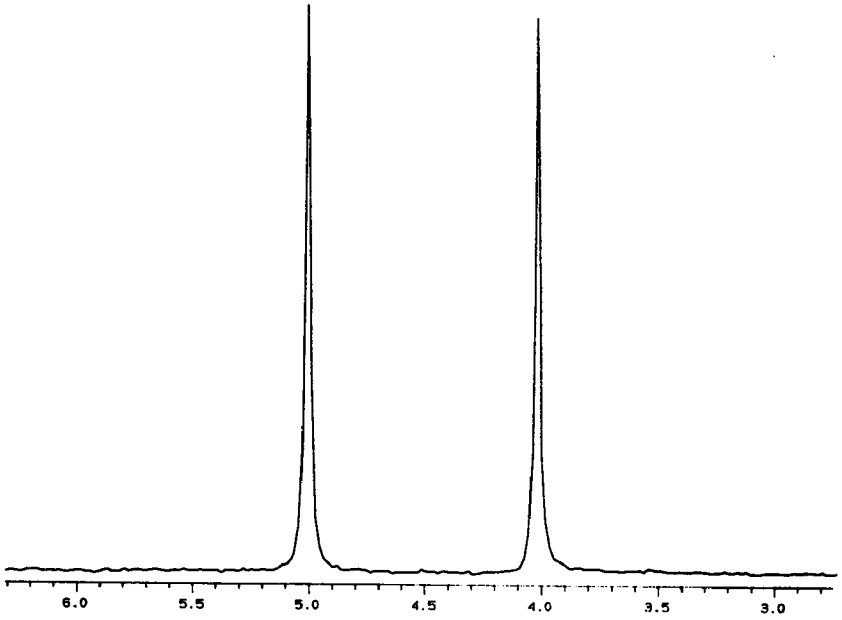


Figure 5.1 ^{31}P *NMR spectrum of product* ***5.14*** *with racemic α–phenylethanol* (C_6D_6)

situations gave rise to some concern, they do not influence the significance of reagent **5.13** in the enantiomeric excess determination[16].

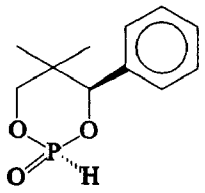
Substrate	polarimetry	α–chloropropionyl method	reagent 5.13
d,l–AlaOMe	75.5:24.5	75.4:24.6	75.2:23.8
d,l–PheOMe	75.0:25.0	74.5:25.5	74.6:25.4
d,l–menthol	70.4:29.6	70.8:29.2	70.3:29.7
d,l–α–phenylethyl–amine	71.3:28.7	71.0:29.0	71.1:28.9

Table 5.3 The enantiomeric compositions as determined by means of reagent ***5.13****, via optical rotation and using the α–chloropropionyl chloride method*[15]

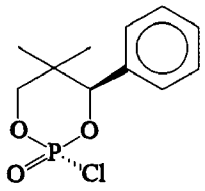
5.2.3 *Two derivatizing reagents* ***5.12*** *and* ***5.13****, yielding the same diastereomeric products: a brief comparison*

As already stated in Chapter 4, reagents **5.12** and **5.13** (Scheme **5.10** and Chapter 4) appear to be closely related. Regardless of the nucleophile used, the products are the same,

although the (probable) reactive intermediates as well as the reaction conditions differ strongly.
Milder reaction conditions can be used with reagent **5.12** as compared to reagent **5.13**. In the coupling with alcohols, the use of a catalytic amount of *N,N*-dimethylaminopyridine and one equivalent of Et_3N guarantees the facile and quantitative reaction of the alcohol with reagent **5.12**, whereas reagent **5.13** can only be coupled after the use of strong bases like n-butyllithium to enhance the nucleophilicity of the substrate by deprotonation of the alcohol. Needless to say, this is a great advantage of reagent **5.12** over **5.13**. Furthermore, side reactions with diols as described for reagent **5.13** are not or less frequently appearing with reagent **5.12**, probably because of the less drastic reaction conditions used.



5.12



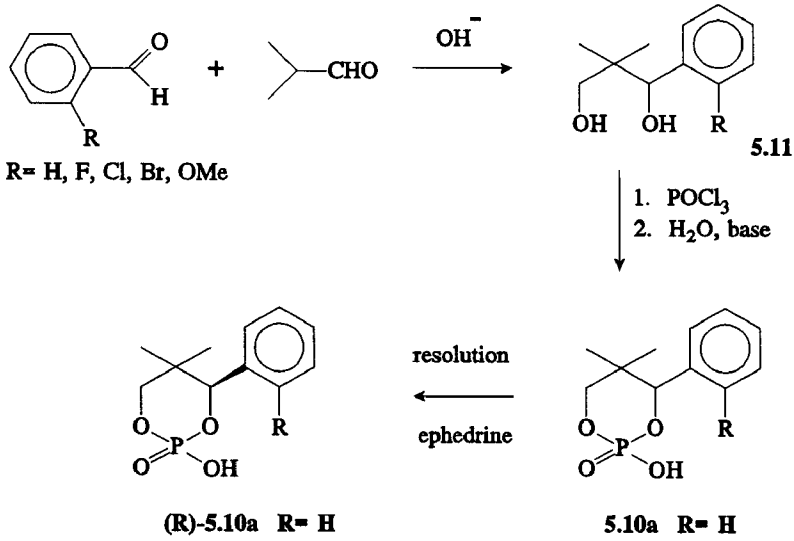
5.13

Scheme 5.10 The reagents 5.12 and 5.13

Moreover, reagent **5.12** can be used in water-containing solvent systems, making it suitable for the coupling of unprotected amino acids (for a discussion, see Chapter 4). This is not possible when using reagent **5.13**, as it reacts readily to the free phoshoric acid **5.10**. The side reaction to the pyrophosphate, as described in Chapter 7, occurs for both reagents, although when using reagent **5.12**, it is possible to prevent the pyrophosphate formation by working under dry conditions. This contrary to the derivatization with reagent **5.13**, where pyrophosphate formation always takes place.
Although both reagents probably are converted into different reactive intermediates during the derivatization reaction, the products are the same with respect to the stereochemistry at the phosphorus atom. When alcohols are allowed to react with reagent **5.12** or **5.13**, the reaction proceeds with retention of configuration on the phosphorus atom with respect to the hydrogen or chlorine atom respectively, whereas amines react with inversion of configuration (Scheme **5.6**). The basis for this phenomenon is discussed in more detail in Chapter 6.
In conclusion we might say that particulary reagent **5.12** shows excellent reactivity towards a large variety of nucleophiles, yielding nearly quantitatively the desired diastereomeric products.

5.3 *Other cyclic phosphorinane systems and their possible use in the enantiomeric excess determination: a first approach*

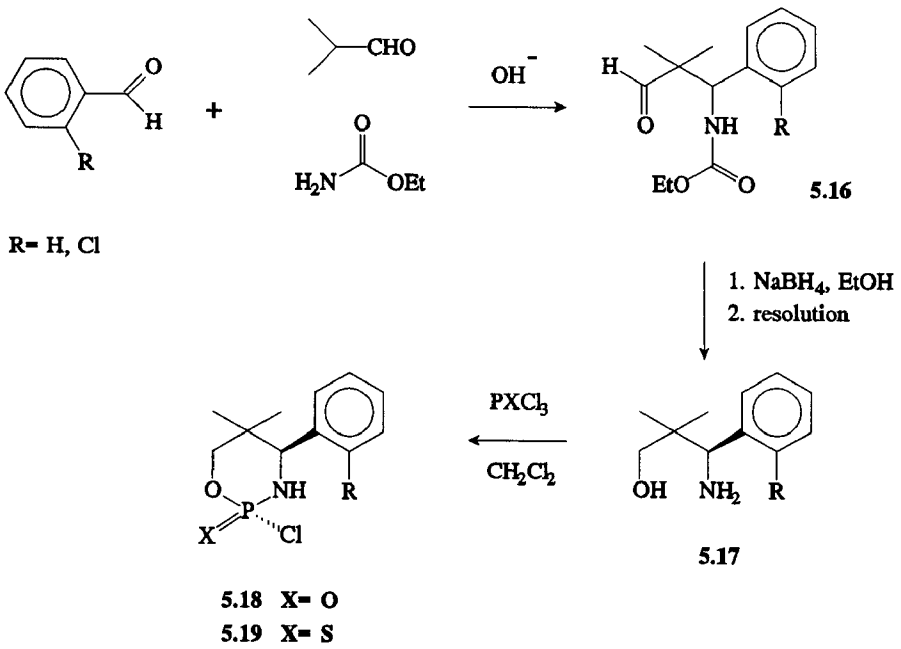
The development of cyclic phosphoric acids and derivatives thereof has been an important area of research, not only in our laboratory[11,17], but also in other groups[18]. As already mentioned before, cyclic phosphoric acids of the type **5.10** are readily available in both enantiomeric forms from substituted benzaldehydes and two equivalents of isobutyraldehyde in an aldol condensation followed by a Cannizzaro reaction in 70% isolated yield (Scheme **5.11**). Diol **5.11** is subsequently converted into racemic phosphoric acid **5.10** in 90% yield. Classical resolution by means of crystallization of the diastereomeric salts of the racemic phosphoric acid with optically pure 1-phenyl-2-amino-1,4-butanediol or ephedrine, affords the enantiomerically pure phosphoric acid **5.10**.



Scheme 5.11 Synthesis of cyclic phosphoric acids 5.10 with different aryl substituents

This methodology allows the synthesis of optically pure cyclic phosphoric acids with a broad structural variation[11]. Subsequent reduction or basic hydrolysis of (*S*)- or (*R*)-**5.10** readily affords the free, optically pure diols, which were transferred into phosphoric acid chlorides, like reagent **5.13**. Besides the use of substituted benzaldehydes, structural variation can be obtained using the so obtained enantiomerically pure diols as a building block. An example of this approach is the synthesis of diastereomerically pure *thio*-phosphoric acid chloride **5.25** (not shown) from **5.13** using $PSCl_3$ in benzene in 70% yield, after purification by column chromatography.

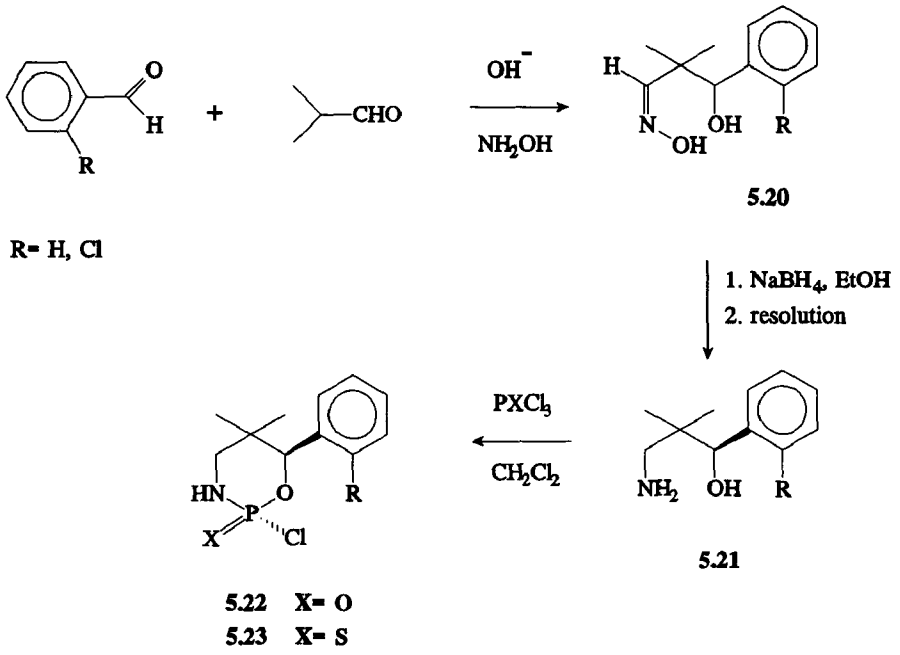
Closely analogous structures can be obtained by means of a slightly modified reaction, using a substituted benzaldehyde, isobutyraldehyde and ethyl carbamate, yielding derivative **5.16**. Subsequent reduction with $NaBH_4$ afforded racemic amino alcohols of the type **5.17**[19]. These are subsequently resolved into the enantiomers by crystallization with optically pure cyclic phosphoric acids[11] ((+)-chlocyphos **5.10** R= Cl) in an overall yield of 35%. Reaction of **5.17** with $POCl_3$ or $PSCl_3$ afforded the diastereomerically pure phosphoric or thiophosphoric acid chloride **5.18** and **5.19**, in 70 and 50% yield, respectively (Scheme **5.12**).



Scheme 5.12 Synthesis of reagents 5.18 and 5.19

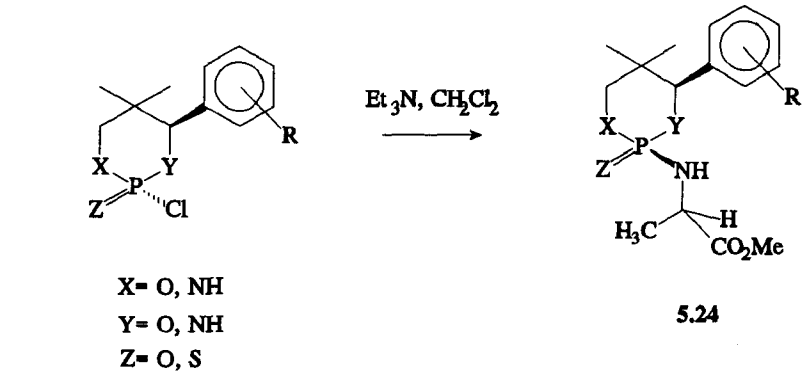
The final structural variation was achieved by reaction of a substituted benzaldehyde with one equivalent of isobutyraldehyde, preventing the Cannizzaro reaction from taking place by quenching initially formed aldehyde with hydroxylamine, to provide the racemic hydroxyloxime **5.20** in 54% overall yield. Subsequent reduction ($NaBH_4$ ethanol, 65% yield) of **5.20**, followed by a resolution by crystallization with optically pure phosphoric acid ((+)-chlocyphos, **5.10** R= Cl) yields the *nearly* enantiomerically pure (*e.e.* around 95%) amino alcohol **5.21** in 42% yield. Functionalization with $POCl_3$ or $PSCl_3$ readily afforded the cyclic phosphoric and thiophosphoric acid chlorides **5.22** and **5.23** in moderate yields (56 and 41%). It appeared, however, to be impossible to purify these products completely (Scheme **5.13**).

So far, only a few analyses were performed with these new derivatizing agents **5.13**, **5.18**, **5.19**, **5.22**, **5.23** and **5.25** using *d,l*-alanine methyl ester, as is shown in Scheme **5.14**.



Scheme 5.13 Synthesis of reagents 5.22 and 5.23

The *d,l*-alanine methyl ester was coupled with these reagents using Et_3N as a base and CH_2Cl_2 as solvent at reflux temperature. When thiophosphoric acid chlorides **5.19** and **5.23** were used, a catalytic amount of *N,N*-dimethylaminopyridine was added, due to the lower reactivity of the thiophosphoric acid chloride in comparison with the chloro-phosphorinane.



Scheme 5.14 Diastereomeric product formation using phosphoric acid chloride derivatizing agents

Reagent	δ (ppm)	$\Delta\delta$ (ppm)	ratio
5.13	3.15	0.066	49.5:50.5
5.25	-18.34	0.053	49:51
5.18	24.12	0.487	49:51
5.19	62.15	2.011	48.5:51.5
5.22	18.91	0.365	49:51
5.23	58.12	1.897	48:52

Table 5.4 ^{31}P *NMR data of diastereomeric products 5.24 and d,l-AlaOme, recorded in* $CDCl_3$ *[L]= 0.01 M*

Using reagent **5.13**, the observed diastereomeric shift difference $\Delta\delta$ is 0.066 ppm in comparison with $\Delta\delta$ 0.053 ppm when using thio analoque **5.25**. When reagent **5.18** and **5.19** are compared, derivatives of the latter showed a large diastereomeric shift difference of $\Delta\delta$ 2.01 ppm whereas a rather poor $\Delta\delta$ 0.487 ppm for the former was observed.

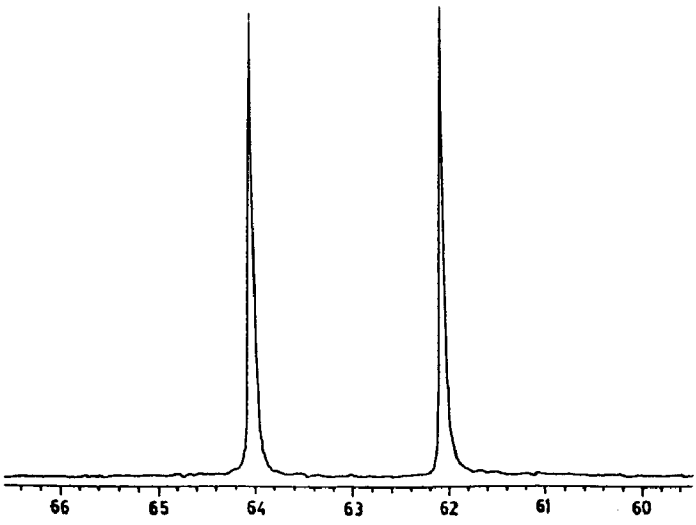


Figure 5.2 ^{31}P *NMR spectrum of d,l-alanine methylester coupled to 5.19 (R= 2-Cl), recorded in* $CDCl_3$ *[L]= 0.01 M*

The same tendency is observed with reagents **5.22** and **5.23**, showing $\Delta\delta$ values of 0.365 and 1.897 ppm, respectively.
The reactions with the thiophosphoric acid chlorides proved to be more troublesome compared to those with the oxygen analogues. These problems probably arise from the addition of stronger bases, like *N,N*-dimethylaminopyridine, inducing unfavourable side reactions to take place. Furthermore, using reagents **5.22** and **5.23**, considerable amounts of ring opened product were found under the required reaction conditions, based upon ^{31}P NMR analysis.
Although for amine containing thiophosphorus agents, like **5.19** and **5.23**, larger diastereomeric shift dispersion was observed compared to the oxygen analogues, the troublesome reactivity as well as synthetic availablity limits their use as a derivatizing agent.

5.4 *Conclusions*

In this Chapter a new phosphoric acid chloride **5.13** for the enantiomeric excess determination of alcohols, amines and amino acid esters is presented, based upon the readily accessible cyclic phencyphos **5.10**. The use of this derivatizing agent compares favourably with the other known ^{31}P NMR e.e. determining methods because of the ease in handling and the good accessibility of the reagent, the rather large shift differences of the derivatives and the broad structural variation allowed.
Other reagents (**5.17**, **5.18**, **5.21**, **5.22** and **5.25**), having the same basic structure (a six-membered ring system with a reactive phosphorus acid chloride moiety) are, although not always readily available, interesting derivatizing agents as they show a large diastereomeric shift dispersion in comparison with reagent **5.13**. Problems were, however, encountered with respect to the reactivity and the appearence of side reactions when these reagents were applied.
From a synthetic point of view, the number of possible structural variations are, however, so large, that a restriction based upon some knowledge about the expected behaviour of the reagent as an enantiomeric excess determining agent would be very welcome. A more rational approach could then be made in the synthesis of new derivatizing agents. Preliminary attempts towards such an approach are presented in Chapter 6.

5.5 *Experimental*

For general remarks, see Chapter 2.5. The synthesis of enantiomerically pure cyclic phosphoric acids has been described by ten Hoeve and Wynberg[11], the enantiomeric composition of the phosphoric acids was determined by means of diastereomeric salt formation[20]. The synthesis of reagents **5.11** and **5.12** is given in Chapter 4.

(R)-2-Chloro-2-oxo-5,5-dimethyl-4-(R)-phenyl-1,3,2-dioxaphosphorinane (5.13)[11]
Using the PCl_5 method
R-(−)-Phencyphos **5.10** (40.0 g, 0.17 mol) was suspended in dry CH_2Cl_2 (250 mL). To this stirred suspension was added 40.0 g of PCl_5 (0.19 mol) over a 10 min period, while

the temperature was kept below 10 °C. Subsequently, the mixture was stirred for 3 h at room temperature. The solution was concentrated to dryness and the residue dissolved in ether (250 mL). The insoluble excess PCl_5 was removed by filtration and the filtrate stored at −20 °C for 7 days. After this period, small white needles were isolated and dried carefully in vacuum at 35 °C for 2 h. Yield of **5.13**, 7.02 g (0.027 mol, 16%). Mp 156–158 °C (lit[11] 160–164.5 °C); $[\alpha]_D^{20}$= −81.9° (c 0.5, $CHCl_3$)(lit[11] −82.4°, c 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.82 (s, 3H), 1.03 (s, 3H), 4.03 (dd, $^2J_{AB}$= 9.0 Hz, $^3J_{PH}$= 30.01 Hz, 1H), 4.18 (dd, $^2J_{AB}$= 9.0 Hz, $^3J_{PH}$= 4.05 Hz, 1H), 5.21 (d, $^3J_{PH}$= 2.04 Hz, 1H), 7.20–7.40 (m, 5H); ^{13}C NMR ($CDCl_3$): δ 17.14 (CH_3), 20.64 (CH_3), 36.36 (d, $^3J_{PC}$= 4.61 Hz, C), 79.51 (d, $^2J_{PC}$= 8.06 Hz, CH_2), 89.78 (d, $^2J_{PC}$= 6.91 Hz, CH), 127.16 (CH), 127.93 (CH), 128.86 (CH), 134.67 (C); ^{31}P NMR ($CDCl_3$): δ −4.49 ppm; Elemental analysis calcd for $C_{11}H_{14}O_3PCl$, C: 50.69, H: 5.41, P: 11.88, Cl: 13.60. Found C: 50.34, H: 5.22, P: 11.48, Cl: 13.47; HRMS calcd 260.037, found 260.037.

Using the $POCl_3$ method

A solution of 8.0 g (0.052 mol) of $POCl_3$ in CH_2Cl_2 (500 mL) was cooled to −20 °C. To the stirred solution was slowly added a solution of 10.0 g (0.052 mol) (*R*)-(−)-phencydiol **5.11** (see Chapter 4) and Et_3N (12 mL) in CH_2Cl_2 (200 mL). After the addition was completed (30 min) another 12 mL of Et_3N in CH_2Cl_2 (50 mL) was added. The mixture was stirred for 5 h, concentrated and the residue dissolved in ethyl acetate. The solution was filtered and evaporated to dryness and the residue was dissolved in ether and stored at −20 °C to allow the chlorophosphorinane **5.13** to crystallize. After 7 days, small white needles were collected and treated as described above. Yield 3.81 g (0.0146 mol, 28%). Spectroscopic data were found to be identical compared to the material as obtained by the PCl_5 method.

Using the CCl_4–Et_3N method

To a cooled (0 °C) mixture of 5.0 g (0.022 mol) of cyclic (*S*)-(−)-phosphorinane **5.12** and 3.4 g (0.022 mol) CCl_4 in dry benzene (150 mL) was added slowly 5 mL of Et_3N over 15 min. Subsequently, the mixture was allowed to stir at room temperature for another hour. After stirring, the mixture was concentrated to dryness and the residue dissolved in ethyl acetate, filtered and concentrated again to dryness. The residue was dissolved in ether (75 mL) and stored for 7 days at −20 °C. After this period, white needles were formed that were treated as described above. Yield 5.45 g (0.021 mol, 95%). Spectroscopic data were found to be identical compared to the material as obtained by the PCl_5 method.

(R)-2-Chloro-2-thio-5,5-dimethyl-4-(R)-phenyl-1,3,2-dioxaphosphorinane (5.25)

A solution of 2.0 g (11.11 mmol) of (*R*)-(−)-phencydiol **5.11**, 5.05 g (50.00 mmol) of Et_3N and a catalytic amount of DMAP in benzene (150 mL) was cooled to 0 °C. To this mixture was added a solution of 1.93 g (11.50 mmol) $PSCl_3$ in 100 mL of benzene over a 60 min period. The yellowish solution was subsequently stirred for 12 h at room temperature, filtered and concentrated to dryness. Crystallization from ethyl acetate–acetone (5:1) afforded a epimeric mixture of **5.25**, ratio 85:15, as indicated by ^{1}H and ^{31}P NMR. Column chromatography (silicagel, ethyl acetate–hexane) yielded the epimerically pure thiophosphoric acid chloride as bright yellow needles. Yield 2.15 g (7.77 mmol, 70%). Mp 154–156 °C; $[\alpha]_D^{20}$= −72.27° (c 0.1, $CHCl_3$); ^{1}H NMR ($CDCL_3$): δ 0.85 (s, 3H), 1.03 (s, 3H), 4.01 (dd, $^2J_{AB}$= 12.50 Hz, $^3J_{PH}$= 28.13 Hz, 1H), 4.53 (dd, $^2J_{AB}$= 12.50 Hz, $^3J_{PH}$= 14.37 Hz, 1H), 5.53 (d, $^3J_{PH}$= 5.00 Hz, 1H), 7.22–7.42 (m, 5H); ^{13}C NMR ($CDCl_3$): δ

18.15 (CH_3), 21.69 (CH_3), 37.50 (C), 78.83 (d, $^2J_{PC}$= 6.90 Hz, CH_2), 87.92 (d, $^2J_{PC}$= 6.90 Hz, CH), 127.12 (CH), 127.82 (CH), 128.58 (CH), 134.55 (C); ^{31}P NMR ($CDCl_3$): δ -10.80 ppm; Elemental analysis calcd for $C_{11}H_{14}O_2SPCl$, C: 47.75, H: 5.10, P: 11.20, S: 11.59, Cl: 12.81. Found C: 47.56, H: 4.99, P: 11.01, S: 11.34, Cl: 12.52; HRMS calcd 276.014, found 276.013.

3-Amino-3-phenyl-2,2-dimethyl-1-propanol (5.16)

A solution of 50.0 g (0.69 mol) of isobutyraldehyde, 71.0 g (0.66 mol) of benzaldehyde, 59.6 g (0.67 mol) of urethane and 0.1 g of NH_4Cl in 250 mL benzene were brought to reflux under azeotropic water separation for 5 h. After cooling, the mixture was poured into diluted NaOH solution and after adding CH_2Cl_2 (300 mL) the layers separated. The CH_2Cl_2 layer was dried over Na_2SO_4 and evaporated to yield a yellowish oil, which is a mixture of benzaldehyde and 3-amino-2,2-dimethyl-*N*-carbethoxy-3-phenyl-1-propanal. (The benzaldehyde was removed by means of an extraction with $NaHSO_3$, yielding only the crude product). The residue was dissolved in 500 mL of 96% ethanol and cooled to 0 °C. $NaBH_4$ (12.37 g, 0.33 mol) in 100 mL water was added portionwise, while the temperature was kept beneath 10 °C. After stirring for 12 h at room temperature, additional 3.00 g (0.079 mol) of $NaBH_4$ was added and the mixture was stirred for 2 h. Then, 95.0 g (1.44 mol) of KOH and water (100 mL) were added, while the mixture was refluxed with vigorous stirring for 6 h. The mixture was concentrated in vacuo, acidified with diluted HCl solution (to pH 6) and subsequently extracted with ethyl acetate. The combined ethyl acetate layers were dried over Na_2SO_4 and concentrated to yield a sticky oil, that solidifies upon standing. Yield 28.50 g (0.16 mol, 24%). ^{1}H NMR ($CDCl_3$): δ 0.73 (s, 3H), 0.97 (s, 3H), 3.10 (s, br, 3H), 3.34 (d, $^2J_{AB}$= 10.98 Hz, 1H), 3.66 (d, $^2J_{AB}$= 10.98 Hz, 1H), 3.88 (s, 1H), 7.20-7.35 (m, 5H); ^{13}C NMR ($CDCl_3$): δ 20.54 (CH_3), 23.67 (CH_3), 37.69 (C), 64.90 (CH), 71.80 (CH_2), 126.99 (CH), 127.54 (CH), 127.69 (CH), 142.15 (C); Elemental analysis calcd for $C_{11}H_{17}NO$, C: 73.70, H 9.56, N: 7.81. Found C: 73.58, H: 9.44, N: 7.75; HRMS calcd 179.131, found 179.131.

A solution of 30.0 g (0.16 mol) of racemic **5.16** and 46.50 g (0.17 mol) (+)-chlocyphos **5.10** (R= 2-Cl)[11] was refluxed in 325 mL ethanol (96%) for 1 h. After stirring for 5 h whilst slowly cooling to room temperature the precipitation was collected and washed with ethanol-ether 1:1 (50 mL). Yield 19.21 g (26%), $[\alpha]_D^{20}$= +17.9 °C (c 0.5, methanol). The salt was subsequently hydrolyzed by stirring with 5% of aqueous NaOH in CH_2Cl_2 3:1 (200 mL) for 2 h followed by an extraction with CH_2Cl_2. The combined CH_2Cl_2 layers were dried over Na_2SO_4 and evaporated to dryness, yielding an oil. The oil solidified after a while, and was recrystallized from ethyl acetate-hexane mixtures, yielding white lumbs of material. Yield 8.01 g (0.044 mol, 26%). Mp 64-65 °C; $[\alpha]_D^{20}$= -6.9° (c 0.5, methanol). Further spectroscopic data as described above for the racemate. The enantiomeric composition was 98%, as determined with the method described in Chapter 4, using (*S*)-di-sec-butylphosphonate.

(-)-2-Chloro-2-oxo-5,5-dimethyl-4-phenyl-3H-1,3,2-oxazaphosphorinane (5.18)

Prepared as for reagent **5.13** using (-)-**5.16** and $POCl_3$. Crystallized from ethyl acetate-acetone mixtures, affording **5.17** as a white powder. Mp 165-166 °C, contains traces of epimeric material (3%). $[\alpha]_D^{20}$= -102.51° (c 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.71 (s, 3H), 0.95 (s, 3H), 3.96 (dd, $^2J_{AB}$= 12.00 Hz, $^3J_{PH}$= 30.68 Hz, 1H), 4.17 (d, $^3J_{PH}$= 4.51 Hz, 1H), 4.24 (dd, $^2J_{AB}$= 12.00 Hz, $^3J_{PH}$= 13.20 Hz, 1H), 4.34 (d, $^2J_{PH}$= 15.60 Hz, 1H), 7.21-7.39 (m, 5H); ^{13}C NMR ($CDCl_3$): δ 17.30 (CH_3), 20.96 (CH_3), 30.12 (C), 59.98 (d, $^2J_{PC}$=

3.78 Hz, CH), 76.90 (d, $^2J_{PC}$= 9.56 Hz, CH_2), 127.64 (CH), 127.78 (CH), 127.96 (CH), 136.68 (d, $^3J_{PC}$= 15.20 Hz, C); ^{31}P NMR ($CDCl_3$): δ 6.81 ppm; Elemental analysis calcd for $C_{11}H_{15}NO_2PCl$, C: 50.88, H: 5.82, N: 5.39, P: 11.93, Cl: 13.65. Found C: 50.52, H 5.67, N: 5.23, P: 11.84, Cl: 13.56; HRMS calcd 259.053, found 259.052.

(-)-2-Chloro-2-thio-5,5-dimethyl-4-phenyl-3H-1,3,2-oxazaphosphorinane (5.19)
This compound was prepared as described for reagent **5.14**, using (-)-**5.16** and $PSCl_3$. Crystallization from ethyl acetate-hexane afforded **5.18** as white powder. Mp 172-175 °C; $[\alpha]_D^{20}$= -94.59° (c 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.74 (s, 3H), 1.05 (s, 3H), 3.76 (d, $^2J_{PH}$= 6.95 Hz, 1H), 3.93 (dd, $^2J_{AB}$= 11.35 Hz, $^3J_{PH}$= 31.85 Hz, 1H), 4.24 (d, $^3J_{PH}$= 3.66 Hz, 1H), 4.34 (dd, $^2J_{AB}$= 11.35 Hz, $^3J_{PH}$= 5.13 Hz, 1H), 7.19-7.40 (m, 5H); ^{13}C NMR ($CDCl_3$): δ 17.95 (CH_3), 21.58 (CH_3), 35.35 (C), 66.18 (d, $^2J_{PC}$= 6.11 Hz, CH), 80.75 (d, $^2J_{PC}$= 10.99 Hz, CH_2), 128.04 (CH), 128.27 (CH), 128.62 (CH), 136.97 (C); ^{31}P NMR ($CDCl_3$): δ -17.86 ppm; Analysis calcd for $C_{11}H_{14}NOSPCl$, C: 47.92, H: 5.48, N: 5.08, P: 11.23, Cl: 12.86. Found C: 47.82, H: 5.39, P: 11.06, Cl: 12.75; HRMS calcd 275.030, found 275.031.

3-Amino-2,2-dimethyl-1-phenyl-1-propanol (5.21)
A solution of 25.0 g (0.34 mol) of isobutyraldehyde, 35.0 (0.33 mol) of benzaldehyde and 1.0 g of KOH (catalytic amount) in water (250 mL) was refluxed for 5 h. The mixture was concentrated to half its volume. Subsequently, a mixture of 23.7 g (0.34 mol) $NH_2OH.HCl$ and 18.20 g (0.17 mol) of Na_2CO_3 in water (50 mL) was added slowly to this solution. The mixture was refluxed for 3 h and stirred for 5 h at room temperature. The mixture was extracted with ether, dried over Na_2SO_4 and taken to dryness. Further reduction as described for reagent **5.16** afforded a yellow oil, that was purified by means of column chromatography (silicagel, ethyl acetate with a little Et_3N), affording a sticky oil. Yield 65% based upon the amount of crude reagent used for chromatography. This material was resolved following the same method as used for reagent **5.16**, although several crystallizations were necessary for complete resolution. Yield 42% of **5.20**, white solid material, having a low melting point. Contains some traces of water and Et_3N that could not be removed.
^{1}H NMR ($CDCl_3$): δ 0.81 (s, 3H), 0.92 (s, 3H), 1.88 (s, br, 3H), 2.62 (d, $^2J_{AB}$= 14.40 Hz, 1H), 2.81 (d, $^2J_{AB}$= 14.40 Hz, 1H), 4.56 (s, 1H), 7.15-7.34 (m, 5H); ^{13}C NMR ($CDCl_3$): δ 20.61 (CH_3), 22.01 (CH_3), 39.66 (C), 49.78 (CH_2), 72.76 (CH), 126.17 (CH), 127.54 (CH), 129.10 (CH), 141.37 (C).
The enantiomeric composition (*e.e.* 94%) was determined by means of the method described in Chapter 4, using (*S*)-di-sec-butylphosphonate as derivatizing agent.

(-)-2-Chloro-2-oxo-5,5-dimethyl-6-phenyl-3H-1,3,2-oxazaphosphorinane (5.22)
Prepared as described for reagent **5.13** using (-)-**5.20** and $POCl_3$. Crystallization from ethyl acetate-hexane mixtures afforded a white powder, still containing traces of Et_3N and water. Mp 145-148 °C. ^{1}H NMR ($CDCl_3$): δ 0.78 (s, 3H), 0.93 (s, 3H), 3.41 (dd, $^2J_{AB}$= 13.65 Hz, $^3J_{PH}$= 28.78 Hz, 1H), 3.65 (d, $^2J_{PH}$= 16.90 Hz, 1H), 3.85 (dd, $^2J_{AB}$= 13.65 Hz, $^3J_{PH}$= 10.25 Hz, 1H), 4.85 (d, $^3J_{PH}$= 4.65 Hz, 1H), 7.18-7.38 (m, 5H); ^{31}P NMR ($CDCl_3$): δ 13.67 ppm; HRMS calcd 259.053, found 259.052

(-)-2-Chloro-2-thio-5,5-dimethyl-6-phenyl-3H-1,3,2-oxazaphosphorinane (5.23)
Prepared as for reagent **5.14** using (-)-**5.20** and $PSCl_3$. Crystallization from ethyl acetate-hexane mixtures afforded a white powder, still containing traces of Et_3N and water. Mp

151–154 °C. ^{1}H NMR ($CDCl_3$): δ 0.81 (s, 3H), 0.97 (s, 3H), 3.36 (dd, $^2J_{AB}$= 12.52 Hz, $^3J_{PH}$= 29.98 Hz, 1H), 3.52 (d, $^2J_{PH}$= 9.32 Hz, 1H), 3.98 (dd, $_2J_{AB}$= 12.52 Hz, $^3J_{PH}$= 8.76 Hz, 1H), 4.56 (d, $^3J_{PH}$= 3.87 Hz, 1H), 7.19–7.40 (m, 5H); ^{31}P NMR ($CDCl_3$): δ –13.89 ppm; HRMS calcd 275.030, found 275.029.

Typical e.e. determination procedure for alcohols and secondary amines using chlorophosphorinanes

To a solution of 0.1 mmol alcohol or secondary amine in 3 mL of THF was added 1 equivalent of n–butylllithium solution in hexane. After stirring the mixture for 15 min at room temperature, 0.1 mmol of derivatizing agent was added. The reaction was allowed to stir for another 30 min. Next, the mixture was taken to dryness, and the residue dissolved in 2 mL $CDCl_3$ an filtered. The solution was directly analyzed using ^{1}H or ^{31}P NMR.
The diastereomerically pure derivatives can be purified if wanted by recrystallization from ethyl acetate–ether–hexane mixtures, see Chapter 6.

Typical e.e. determination procedure for primary amines and amino acid esters using chlorophosphorinanes

To a solution of 0.1 mmol of amine or amino acid ester and one equivalent of Et_3N in $CHCl_3$ or THF 0.1 mmol of derivatizing agent was added. The mixture was refuxed for 8 h and subsequently taken to dryness. The residue was dissolved in 2 mL $CDCl_3$, filtered and a ^{1}H or ^{31}P NMR spectrum was recorded. Alternatively, the derivatization reaction can be performed in $CDCL_3$ and the spectra were recorded, without prior workup procedure.
Diastereomerically pure derivatives can be purified by crystallization from ethyl acetate–ether–hexane mixtures, see Chapter 6.

5.6 *References*

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CHAPTER 6

Conformational analysis of 2-oxo-1,3,2-dioxaphosphorinanes: A remarkable correlation between ^{31}P NMR chemical shift and geometry

6.1 *Conformational analysis: an introduction*[#]

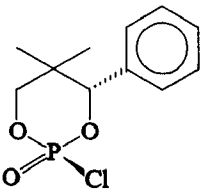
Conformational analysis, *i.e.* the elucidation of the spatial orientation of a particular configuration, can provide important information about dynamic structural related behavior. The most straightforward approach to the analysis of a preferred conformation is by the use of spectroscopic techniques, among which Röntgen diffraction and multi-dimensional NMR techniques are the most important. Since reactions are usually carried out in solution, the latter technique is preferred over the former. Also, conformational orientations in a crystal lattice may be significantly different from the preferred orientations in solution, and great care must be taken when these data are *translated* into conformational behavior of reactive intermediates in solution[1].

The use of multidimensional NMR techniques, however, also faces certain problems. Nuclei with a through space dependent relaxation often show a scalar J coupling as well, which leads, in an unfiltered NOESY experiment, to interference with the observed NOE signal. This causes disturbance of the integration of the signal in question[2]. In such cases, only qualitative information can be obtained, which is sometimes not sufficient. The greatest problem, however, probably arises from the fact that with either of these spectroscopic techniques, conformational analysis can only be performed after the desired materials have been synthesized (with a high degree of purity).

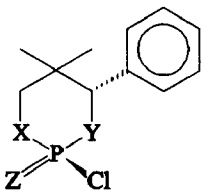
Fortunately, the development of powerful computer systems has provided the modern chemist with a complete new approach of conformational analysis in the form of computational simulations. The structural behavior of a molecule or a set of molecules can be predicted (within certain limits) instead of determined. In this way, a possibility of a *rational design* of target molecules is opened.

In Chapter 5 we described how a relatively simple cyclic phosphoric acid chloride **6.1**, (Scheme **6.1**), can be modified in several ways. The total number of target molecules **6.2**, however, is very large. Thus examination of all the possible phosphorinane systems by actual synthesis would be highly time consuming. It would be of great value if we could predict which of these molecules, based upon their expected success as a derivatizing agent for the enantiomeric excess determination, should be prepared and tried out in the actual analyses. An important aspect of the use of derivatizing agents **6.1** and **6.2** is the stereochemistry (inversion or retention) at the phosphorus atom during the coupling reactions (Scheme **6.2**).

[#] The modelling work described in this Chapter was performed by Drs Rob Zijlstra.



6.1



6.2

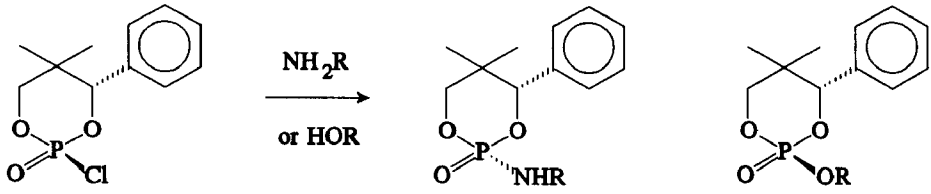
X= O, NH

Y= O, NH

Z= O, S

Scheme 6.1 Derivatizing agent 6.1 and possible structural variations shown in 6.2

Furthermore, it is important to gain insight in the factors that govern the chemical shifts and, moreover, the diastereomeric shift differences in the decoupled ^{31}P NMR spectra. Unfortunately, the factors inducing the chemical shifts of the diastereomers of **6.3** and **6.4** are only poorly understood[3], which makes the development of the new chiral derivatizing agents mainly a matter of trial and error.



6.1 6.3 6.4

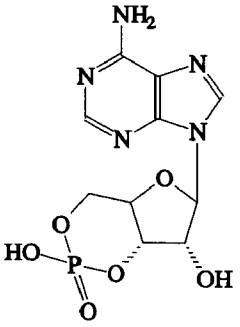
Scheme 6.2 Reagent 6.1 as a chiral derivatizing agent for alcohols and amines

Within the scope of our research, the use of computational methods for conformational analysis might lead to a more rational design of derivatizing agents of the 1,3,2–dioxaphosphorinane type **6.2**, if we are able to reveal the effects of variables such as bond length and bond and/or torsional angles, which determine the induced chemical shift differences in the ^{31}P NMR. Furthermore, the stereochemical course at the phosphorus nucleus in these coupling reactions might be unraffled and predicted.

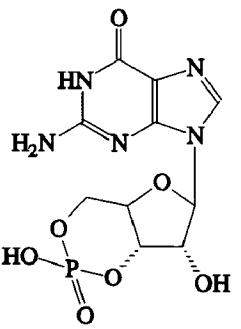
6.2 *Conformational analysis of 1,3,2–dioxophosphorinanes: a historical perspective*

From a biological point of view, the conformational analysis of 1,3,2–dioxaphosphorinanes is of great interest, since several cyclic phosphates play an important role in the animal and human physiology. In Scheme **6.3**, two examples are given of such phosphorinanes:

the cyclic nucleotide adenosine 3',5'-monophosphate (cAMP, **6.5**)[4] which plays an important role as a hormonal messenger whereas the related guanosine 3',5'-phosphate (cGMP, **6.6**) is also of biological importance[5].



6.5



6.6

Scheme 6.3 *Two 1,3,2-dioxaphosphorinane systems occurring in nature*

Besides the conformation of the phosphorinane ring, the axial versus the equatorial orientation of the substituents at the phosphorus atom itself has also been an important subject of study.

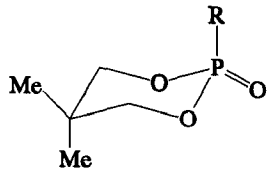
For the conformational analysis of functionalized 5,5-dimethyl-4-phenyl-2-oxo-1,3,2-dioxaphosphorinanes, it is of great interest to obtain information about the orientation of the chlorine atom before the derivatization has taken place (Scheme **6.2**). After the derivatization has taken place, the orientation of the nitrogen and oxygen substituents at phosphorus is important from a stereochemical as well as conformational point of view. This means, that insight in the required energy barriers for a successive chair-chair ring inversion during the nucleophilic substitution reaction is of utmost importance.

In general, *OR* substituents (*R* being alkyl, aryl, aryloxy, alkylamino or halogens[6]) at the phosphorus atom of 2-*OR*-2-oxo-1,3,2-dioxaphosphorinanes show a preference for the axial position, as shown in earlier studies with **6.7**[6] (Scheme **6.4**).

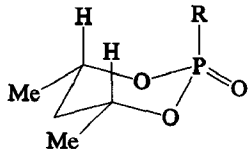
6.7

Scheme 6.4 *Chair-chair interconversion of (2R)-2-oxo-1,3,2-dioxaphosphorinane **6.7**, R= alkyl, aryl, aryloxy, alkylamine or halogen*

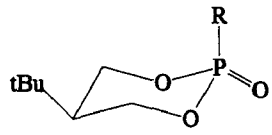
The same studies, however, showed that in solution an equilibrium exists between the both chair conformers **6.7**^{eq} and **6.7**^{ax}, which makes the assignment of the preferred stereochemistry not unambiguous. This is also the case with a slightly modified system[7] **6.8**, bearing two methyl groups in the 5 position (Scheme **6.5**).



6.8



6.9



6.10

Scheme 6.5 Three phosphorus containing agents, showing higher conformational flip barriers

The introduction of two equatorially oriented methyl groups at the 4 and 6 position, shown in **6.9** (Scheme **6.5**), however, led to a significantly higher energy barrier for the ring inversion, making the conformational analysis of a number of 1,3,2-dioxaphosphorinanes functionalized at phosphorus (*R*= OR) in solution possible[8]. These studies were based upon the use of IR and NMR techniques#, and showed that all products possessed a chair conformation with the OR group oriented axially, except for amino groups, which seem to favour the equatorial position at the phosphorus atom. The same results were found in a number of dipole measurements[9], and were confirmed by means of CNDO calculations[10].

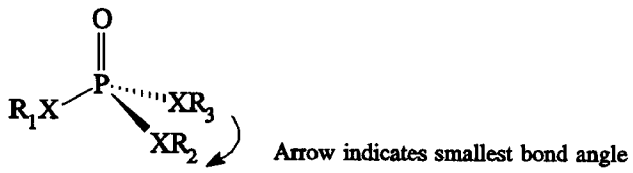
The differences in stability are, however, only small, and some exceptions have been reported[11] with regard to the larger stability of the chair conformers. For 2-alkoxy or 2-aryloxy substituted 5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinanes **6.8** (Scheme **6.5**) a ring flip was observed, placing the substituents in both equatorial and axial positions at the phosphorus atom. Again, the amino substituents showed a strong preference for the equatorial position, causing a larger energy difference between the two chair conformers. This exceptional behaviour of amino groups has been explained using the results on trivalent phosphorinanes[12], which showed that the lone pair positioned on nitrogen gave the largest p_π–d_π stabilisation when the amine is placed in an equatorial position at the phosphorus atom. The conformational stability of the phosphorinane ring system was enhanced by the introduction of a sterically demanding group at the 5-position of the ring system, as is shown in Scheme **6.5**. The conformational analysis of a number of *2-R* substituted compounds bearing a 5-t-butyl group, as in **6.10**, showed a strong

NMR analyses were based upon the $^3J_{PH}$ coupling constants in **6.9**, which differ significantly for equatorial versus axial hydrogens, being typically in the range of 20 Hz for the former and 0–4 Hz for the latter.

predominance of the chair conformation, placing the bulky t-butyl group in the energetically more favourable equatorial position[13,14].
In the case of 4-phenyl substituted 1,3,2-dioxaphosphorinanes, which are the subject of the present analysis, the assumption can be made (*vide infra*) that, in analogy with the t-butyl substituted phosphorinanes, the phenyl group will act as a lock for only one chair conformation.
Furthermore, since (almost) no exceptions have been reported for the presented preference of the various substituents at phosphorus, the orientation of alkoxy substituents is expected to be axial at phosphorus, where the amino substituents should adopt an equatorial orientation. Whether this actually is the situation for the functionalized derivatives of phosphoric acid chloride **6.1** will be discussed in Chapter 6.4.

6.3 *^{31}P NMR chemical shifts: about the correlations between structure and induced chemical shift*

With the development of high field, multinuclear, Fourier transform NMR spectrometers, ^{31}P NMR has taken its own, important place in conformational analysis. Starting in the mid 1950's, the quantity of data generated using ^{31}P spectroscopy grew very rapidly, and in 1967 Van Wazer and co-workers were able to produce a book that is still widely used[15]. In the years following, the enormous progress made in this area has led to the publication of several review articles[16] and specialized books[3].
Since that time, several attempts have been made to establish some kind of correlation between the intrinsic structural variations at the phosphorus nucleus and the ^{31}P NMR chemical shift of the phosphorus containing compounds.
One of the more successful contributions in this field has been made by Letcher and Van Wazer[17,18], who showed by quantum mechanical calculations on tri- and penta- coordinated phosphorus compounds of type PX_3, PX_4 and OPX_3 (X= H, alkyl, phenyl) that the ^{31}P chemical shifts mainly depend on three factors; (i) the difference in electronegativity between phosphorus and X, leading to polarization in the $P-X$ bond; (ii) changes in the overlap of π orbitals of heteroatoms (X) with the empty d-orbitals of the phosphorus atom, *e.g.* backbonding; and (iii) changes in the ($X-P-X$) σ-bond angle, although the latter contribution should have a negligible effect on the chemical shifts of phosphoryl compounds (Scheme **6.6**).



Scheme 6.6 X-P-X bond angle in phosphorus containing compounds. See text for explanation

Other studies[19,20], however, have shown that the ^{31}P NMR chemical shifts correlates with the *X–P–X* bond angle of phosphorus containing compounds, which is inconsistent with the results obtained by Letcher and Van Wazer[17,18]. In 1975, Gorenstein proposed an empirical correlation for the ^{31}P NMR chemical shifts of phosphate esters, based upon various available X-ray structures of this particular class of compounds. The ^{31}P NMR shifts show a strong dependency upon the smallest *O–P–O* bond angle[21] (Figure **6.1**) and a less pronounced dependency upon torsional angles[22] in these esters. In Figure **6.1**, several phosphates are compiled: five membered cyclic esters (■), monoester mono anions (△), acyclic diester mono anion (o), acyclic diester free acids (●), six membered cyclic esters (□) and Li_3PO_4 (▲). This empirical relationship provides a simple tool for correlating chemical shifts with conformational as well as chemical changes in all kinds of phosphates and phosphonates. Although no physical significance is claimed for this correlation, it is still considered useful to analyze differences in ^{31}P NMR chemical shifts[23].

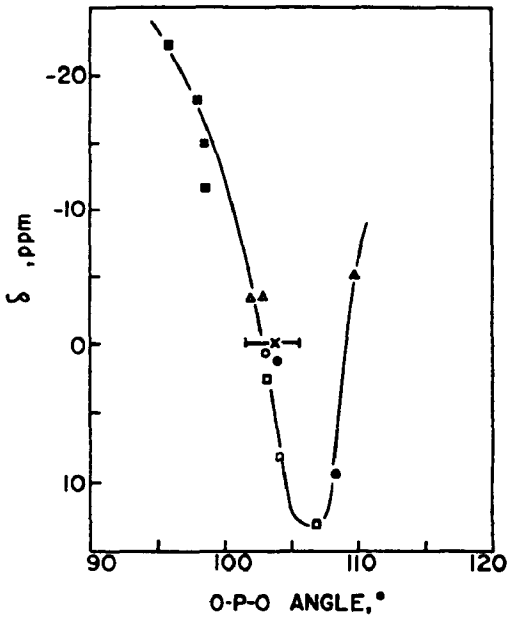
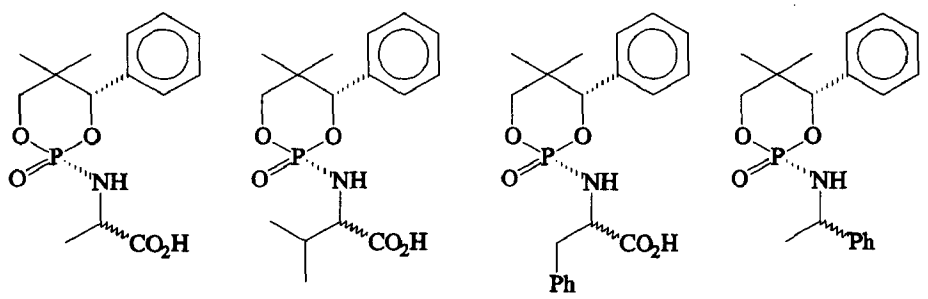


Figure 6.1 Emperical relationship as found by Gorenstein[21] between the smallest bond angle O–P–O and observed chemical shift. See text for explanation

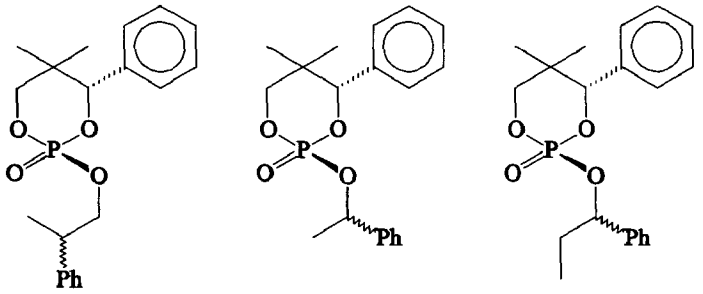
Insight into the possible physical effects is being provided by more recent investigations using ^{31}P CP-MAS NMR techniques, which showed a dependence of the *O–P–O* bond angle on the variation of the *P–O* bond length, caused by changes in the bond order of this bond[24,25], resulting in linear dependence of chemical shifts on both bond angle and bond length.

6.4 *Conformational analysis of functionalized 1,3,2-dioxaphosphorinanes*

Since functionalized 1,3,2-dioxaphosphorinanes can be considered as large molecules in terms of *QM* (Quantum Mechanical) approaches and since available facilities were limited, we have chosen to calculate the conformations of derivatives **6.3** and **6.4** by means of semi-empirical methods. In this field, the *QCPE*[26] packages *AMPAC* and *MOPAC* are most widely used, mostly as a vectorized combination of these two packages (*VAMP*).
These packages contain a number of programs based on variable Hamiltonians. In *VAMP*, two programs are suitable and *parametrized* for the optimization of phosphorus containing compounds, namely *MNDO*[27] and *PM3*[28]. *MNDO* (*m*odified *n*eglect of *d*ifferential *o*verlap) is based on the *NDDO* (*n*eglect of *d*iatomic *d*ifferential *o*verlap) approximation, which was the first method to take directionality of atomic orbitals into account, and gives a significant improvement in cases where repulsions between lone pairs play an important role. However, *MNDO* has a large number of optimizable parameters where experimental parametrization has a part in the formation of the final solution, like for instance in the calculation of heats of formation. *PM3* (*p*arametric *m*ethod *3*) is based upon *MNDO*, but also uses a *AM1*-like description[29] of the core-core repulsions, in which corrections are provided for excessive long range repulsions. Furthermore, *MNDO* parameters for phosphorus were optimized in *PM3*, so better results could be expected from this method. However, since the *PM3* method in some cases has led to ambigious results, the outcome of the *PM3* calculations must be treated with care.
The analysis of the amide- **6.3** and ester- **6.4** derivatives of cyclic 1,3,2-dioxaphosphorinane (Scheme **6.7**), serves a twofold purpose.
First, the derivatives were prepared from the enantiomerically pure 2-(*S*)-chloro-2-oxo-5,5-dimethyl-4-(*S*)-phenyl-1,3,2-dioxaphosphorinane **6.1**, by means of a nucleophilic substitution reaction on the phosphorus atom. A conformational analysis of the products could provide information about the stereochemistry in the coupling reactions, which can in principle proceed with retention or inversion of configuration.
Comparison of the results using experimental techniques, like X-ray diffraction and 2D NMR techniques (NOESY), with the results as obtained by means of the computational conformational analysis can provide, within certain limits, useful information about the reliability of the used computational methods.
Secondly, the conformational analysis of the diastereomeric pairs of amide or ester 1,3,2-dioxaphosphorinanes **6.3** and **6.4** might provide insight in the factors that govern the chemical shift (differences) in the ^{31}P NMR spectrum. If the chemical shifts, and hence the diastereomeric shift differences, can be correlated with a geometric parameter (*vide supra*) of these compounds, in principle a tool exists to design derivatizing agents for the enantiomeric excess determination with the aid of molecular modeling.
In the preparation of the diastereomeric derivatives **6.3** and **6.4** using chiral amines and alcohols, respectively, 2-(*S*)-chloro-2-oxo-5,5-dimethyl-4-(*S*)-phenyl-1,3,2-dioxaphosphorinane **6.1** was used as the chiral derivatizing agent. Alternatively, the corresponding (*R*)-2H-2-oxo-5,5-dimethyl-4-(*S*)-1,3,2-phosphorinane has been used. The products of the derivatizing reactions are the same (see Chapters 4 and 5).



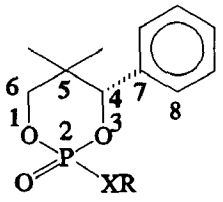
6.3[a] **6.3**[b] **6.3**[c] **6.3**[d]



6.4[a] **6.4**[b] **6.4**[c]

Scheme 6.7 The phosphorus amides and esters used for conformational analysis

The diastereomeric pairs of amides **6.3**[a] to **6.3**[d] and esters **6.4**[a] to **6.4**[c] were chosen as the target structures used in the analysis (Scheme **6.7**).



Scheme 6.8 Numbering of atoms used in the conformational analysis of amides 6.3 and esters 6.4

In these diastereomers, the stereochemistry at the exocyclic stereogenic center was varied, while the stereogenic center of the phosphorinane ring was left unchanged (*S*–isomer used in the calculations). Conformations of **6.3** and **6.4** were built up and generated within the *CHEM–X* molecular modelling program[30]. In the conformational analysis of **6.3** and **6.4** the phosphorinane ring was considered to have no rotational degree of freedom, since studies by Leusen et al[31] showed that the phenyl group is locked in the equatorial 4–position by the 5,5–dimethyl groups. Although earlier conformational analyses by means of ^{1}H NMR of 5,5–disubstituted phosphorinanes showed that in some cases an equilibrium between the two chair conformers exists even at room temperature[32,33], most of the studies on the conformational behaviour of 2–oxo–1,3,2–dioxaphosphorinanes showed that this equilibrium must be considered as exceptional[34,35]. Furthermore, the introduction of a sterically demanding group at the 4–position of the ring in combination with the introduction of the 5,5–dimethyl groups will lead to a significantly higher chair–chair inversion energy barrier in the phosphorinane ring due to steric hindrance between the phenyl and methyl groups. Therefore, the investigation of the rotational freedom of **6.3** and **6.4**, could mainly be focussed on the exocyclic amide or ester residues.

The diastereomeric pairs of **6.3** and **6.4** were optimized using the *PM3* package, since the available versions of the *MM* packages like *MM2P*[36] and *MMX*[37] lacked parametrization for the *P–N* single bond in the amides of type **6.3**.

Conformations of the *amides* **6.3** were generated within *CHEM–X* by rotating the *P–N* and *N–C* bond in steps of ten degrees using a *PM3* optimized starting geometry, which led to 1296 different conformations. For each of the 1296 different conformations the *MME* (*m*olecular *m*echanics *e*nergy) was calculated. The other (rotational) degrees of freedom in the exocyclic part of the structures were also analyzed, but only the specified bonds (Scheme **6.8**) showed to have a significant effect on the change in *MME* of the molecule. The selected (local) minimal energy conformations were further optimized using the *PM3* package within the *VAMP 4.3* version of *MOPAC*[38].

The same procedure was followed for the diastereomeric pairs of *esters* **6.4**, in which the exocyclic *P–O* and *O–C* bond was rotated in steps of ten degrees. Again, investigation of the flexibility in the rest of the ester part of the molecule, by rotating the other bonds in the ester moiety, showed to have a negligible effect upon the energy of the molecule. Furthermore, the axial versus the equatorial preference of the exocyclic oxygen on the phosphorus atom (the *oxo*) has been studied by performing the above described conformational analysis of **6.3** and **6.4** for both orientations of the phosphoroxo moiety.

Besides the analyses performed with the products of **6.1**, the phosphoric acid chloride itself was analyzed with regard to the stereochemistry at the phosphorus atom in order to reveal stereochemical changes in the nucleophilic substitution reactions.

6.4.1 *Conformational analysis of the products 6.3*

From the *PM3* optimized conformations of the diastereomeric pairs of **6.3**[a] to **6.3**[d] some interesting features regarding similarities and differences between the two diastereomers of each of these amides appeared. First of all, the survey of the axial versus the equatorial orientation for the exocyclic phosphoroxo moiety in **6.3**[d] showed a strong preference for the former by ca. 13.4 kJ/mole. This is in agreement with earlier analyses[11,39], in which the interference of the lone pair electrons situated on nitrogen with the nonbonding electrons of the endocyclic oxygen atoms was shown to be minimized with the amine at the equatorial site of the phosphorus atom.

	6.3[a]		**6.3**[b]	
	SS	***RS***	***SS***	***RS***
ΔH_{form} **(PM3)** **(kJ.mole^{-1})**	−1076.1	−1064.0	−1101.6	−1102.5
Bond Lengths (Å)				
P_2–O_3	1.709	1.696	1.708	1.708
P_2–O_1	1.698	1.705	1.706	1.703
P_2=O	1.448	1.448	1.445	1.448
P_2–N	1.758	1.765	1.765	1.754
Bond Angles (Å)				
O_1–P_2–O_3	**101.7**	**101.9**	**100.7**	**101.1**
N–P_2–O_3	101.0	101.9	102.4	101.6
O=P_2–O_3	115.7	117.5	115.9	116.8
Dihedral Angles (Å)				
H–N–C–H	145.4	−169.2	−168.5	−149.4
C–N–P_2–O_3	−55.3	−49.7	−39.0	−54.5
H–N–P_2=O	36.3	−32.5	−16.9	−33.2
C_8–C_7–C_4–C_5	95.8	92.3	95.8	93.1

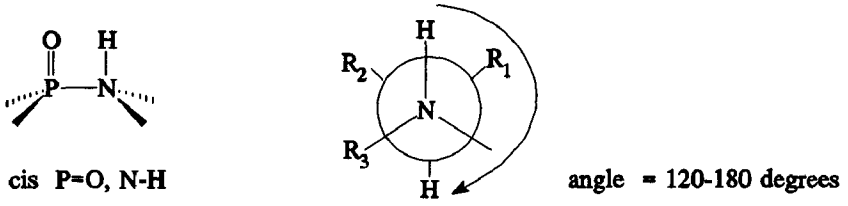
Table 6.1 (part 1) Geometrical data of phosphorus amides 6.3[a] and 6.3[b]

	6.3[c]		6.3[d]		X-ray
	SS	*RS*	*SS*	*RS*	*3[d]-SR*
ΔH_{form} (PM3) (kJ.mole^{-1})	-959.8	-954.4	-590.8	-589.9	-
Bond Lengths (Å)					
P_2-O_3	1.710	1.704	1.712	1.706	1.596
P_2-O_1	1.699	1.709	1.703	1.710	1.577
$P_2=O$	1.448	1.446	1.451	1.451	1.473
P_2-N	1.755	1.762	1.750	1.745	1.597
Bond Angles (Å)					
$O_1-P_2-O_3$	**101.6**	**100.5**	**101.5**	**100.9**	**101.9**
$N-P_2-O_3$	101.9	103.6	101.5	101.2	106.3
$O=P_2-O_3$	115.7	116.4	115.2	116.7	114.8
Dihedral Angles (Å)					
$H-N-C-H$	176.1	-165.7	160.7	-169.2	168.0
$C-N-P_2-O_3$	-14.6	-44.8	-55.0	-50.4	-52.6
$H-N-P_2=O$	75.5	-24.6	26.7	-32.9	-35.1
$C_8-C_7-C_4-C_5$	94.7	95.4	94.3	92.1	90.9

Table 6.1 (part 2) Geometrical data of phosphorus amides 6.3[c] and 6.3[d]

Furthermore, all diastereomeric pairs of **6.3**[a] to **6.3**[d] have an energetically most favourable conformation with a cis-like orientation of the *N-H* bond towards the phosphoroxo bond. The dihedral angle *H-N-C-H* shows a strong preference for a *gauche* to *trans*-like orientation of the two protons, with the *H-N-C-H* angle being typically around 120-180° (Scheme **6.9**). These specific *PM3* calculated global minimal energy conformations appeared to be significantly more stable than the next local energy conformation, which lacked at least one of these two conformational features.

The energy differences between global and next local minimal energy conformation varied from 2.1 (for **6.3**[a]) up to 36.4 (for **6.3**[c]) kJ/mole. Relevant geometrical data of the *global* minimal energy conformations of the diastereomeric pairs **6.3**[a] to **6.3**[d] are summarized in Table **6.1**.



Scheme 6.9 Proposed orientation of the H–N–C–H moiety

To determine the significance of these calculations, both diastereomers of **6.3**[d] were synthesized according to the procedures described in the Chapters 4 and 5, and the COSY and NOESY spectra were recorded. The $^3J_{PH}$ coupling constants in the ^1H NMR spectra#, together with the observed NOE interactions of the axial 4 and 6 protons with each other, showed that the phosphorinane ring adapts a chair conformation for both diastereomers with the phenyl group in an equatorial orientation (Figures **6.2** and **6.3**).
For both diastereomers, the benzylic proton at the exocyclic stereogenic centre of the α-phenylethylamino group showed a NOE interaction with the axial methyl group of the phosphorinane ring (Figure **6.2**), which proved to be in excellent agreement with the *PM3* calculated *global* minimal energy conformation. Furthermore, all other minima of regardless which diastereomer of **6.3**[d] have interatomic distances between these two protons of over 5 Å, ruling out the possibility of an observable NOE interaction for these conformations. Also, the shielding effect on the ortho protons of the phosphorinane phenyl group, as is observed in the ^1H NMR spectrum of the *R,S* diastereomer of **6.3**[d] (an upfield shift of about δ 0.6 ppm is observed), can be explained with the *PM3* calculated global minimal energy conformation in which both phenyl groups are oriented in a stacked orientation at a minimum distance of ca. 2.5 Å. This effectively leads to an intramolecular anisotropy effect on one of the ortho protons, which is pointing towards the center of the opposite phenyl group, and is most clearly seen in the crystal structure (Figure **6.3**).
Moreover, the earlier mentioned NOE interaction is also a clear indication for an equatorial orientation of the amide group, since this interaction is spationally impossible for any conformation having an axial orientation of the amide moiety positioned at the phosphorus atom. The same interactions were found in the *PM3* calculated global minimal energy conformations of **6.3**[a] to **6.3**[c], but unfortunately, instability of these compounds has

$^3J_{PH}$ coupling constants for these benzylic protons are 2 Hz typically, whereas the equatorial and axial protons at the 6-position of the dioxaphosphorinane ring have $^3J_{PH}$ coupling constants of 25 Hz for the former and 2 Hz for the latter (see also page 148). This is in agreement with the earlier reported coupling constants for the chair conformation of 2 substituted 5-t-Butyl-1,3,2-dioxaphosphorinanes. Bentrude, W.G., Hargis, J.H., *J. Chem. Soc., Chem. Commun.* **1969**, 1113.

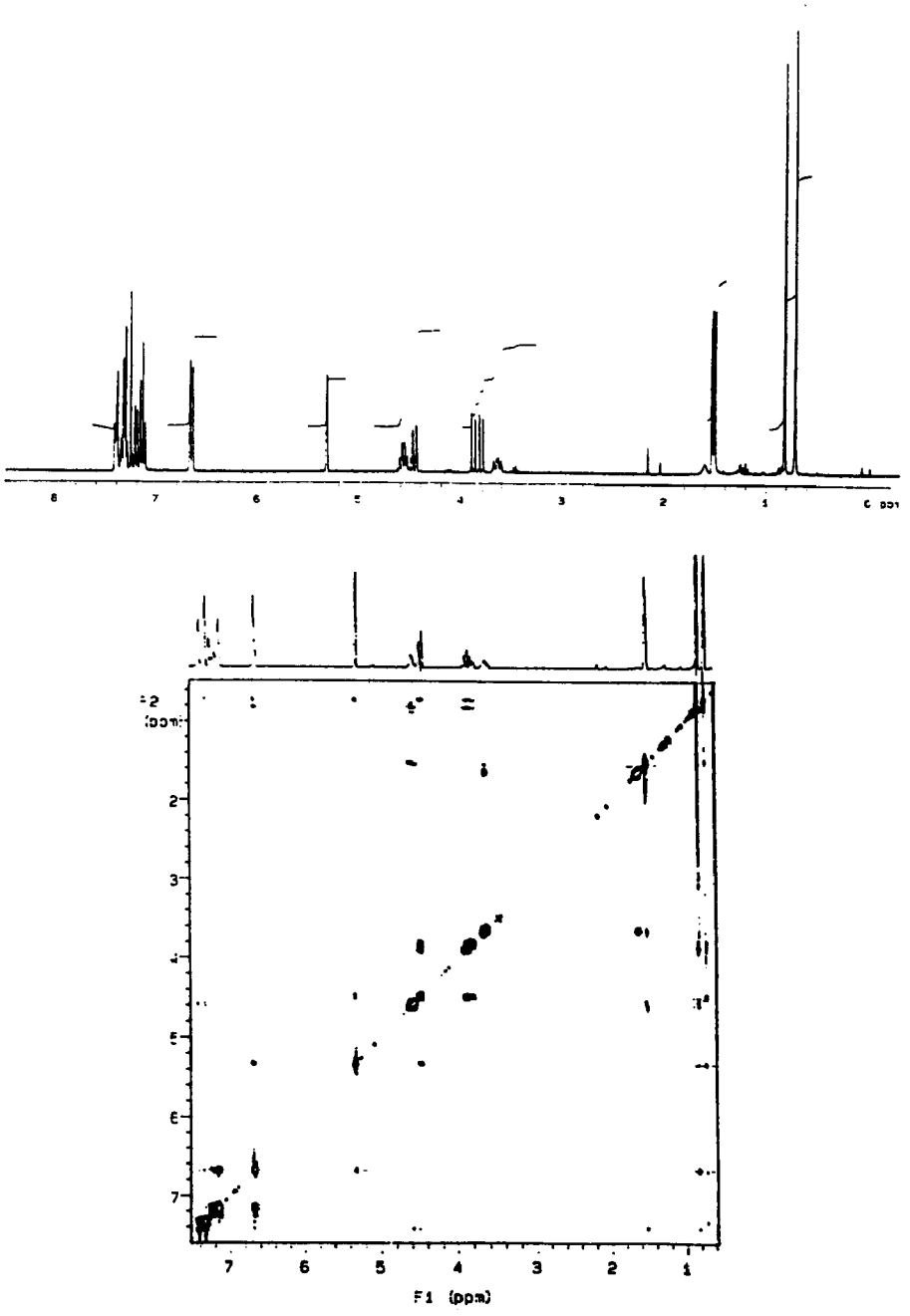


Figure 6.2 ^{1}H *and NOESY NMR spectra of the R,S product of* **6.3**[d]*, recorded in* $CDCl_3$

prevented us from producing further experimental data for these diastereomers.
In order to confirm the configuration at phosphorus and to obtain information on the conformation and possible interactions, an X-ray analysis of (*S,R*)-**6.3**[d] was undertaken, as is shown in Figure **6.3**. This clearly shows, that the calculated *H–N–C–H* trans-like orientation (Scheme **6.9**) appeared in the crystal structure, and it confirms the calculated minimal energy conformation. Also, the exocyclic phenyl group possesses an orientation, in which one of the ortho protons of the phosphorinane phenyl ring system is directed towards the center of the opposite phenyl group, leading to the observed anisotropic effects in the ^{1}H NMR spectrum. Clear is also the possible interaction of the axial methyl group placed at *C4* with the proton situated at the stereogenic centre of the exocyclic α-phenylethyl moiety, leading to the observed NOE interactions.

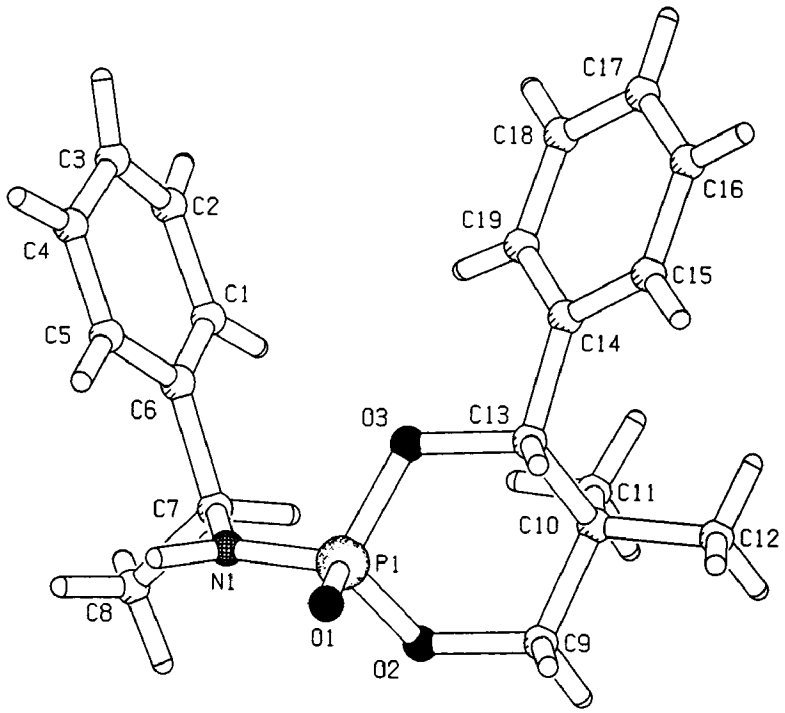


Figure 6.3 *Molecular structure of the S,R product of* ***6.3***[d]

6.4.2 *Conformational analysis of the products 6.4*

In the conformational analysis of the phosphorinane esters **6.4** the same procedure was used as described for the phosphorinane amides **6.3**. The *PM3* optimized minimal *MME* conformations of **6.4**[a] to **6.4**[c] show that no stable minimal energy conformations could be optimized using the *PM3* package with an equatorially oriented exocyclic ester group of regardless which diastereomer of **6.3**[a] to **6.3**[c] (Table **6.2**).

	6.4[a]		**X-Ray**
	RS	***SS***	***4*[a]*-RS***
ΔH_{form} **(PM3)** **(kJ.mole^{-1})**	−815.5	−808.3	–
Bond Lengths (Å)			
P_2-O_3	1.695	1.697	1.570
P_2-O_1	1.687	1.687	1.577
$P_2=O$	1.445	1.444	1.446
P_2-O_{exo}	1.699	1.701	1.566
Bond Angles (Å)			
$O_1-P_2-O_3$	**100.8**	**101.0**	**105.5**
$O_{exo}-P_2-O_3$	103.1	102.8	101.1
$O=P_2-O_3$	115.1	114.7	113.7
$O=P-O_{exo}$	117.4	118.0	116.8
Dihedral Angles (Å)			
$H-C-O_{exo}-P_2$	49.4	131.0	−54.0
$C-O_{exo}-P_2-O_3$	172.8	−109.6	158.3
$C-O_{exo}-P_2=O$	51.6	8.0	−77.8
$C_8-C_7-C_4-C_5$	94.8	94.8	90.8

Table 6.2 (part 1) Geometrical data of phosphorus ester 6.4[a]

The optimized conformations of **6.4**[a] to **6.4**[c], with an equatorially oriented ester moiety in the starting conformations, all show a twisted or even pure boat conformation of the

	6.4[b]		6.4[c]	
	RS	*SS*	*RS*	*SS*
ΔH_{form} (PM3) (kJ.mole^{-1})	-784.1	-782.0	-804.6	-191.8
Bond Lengths (Å)				
P_2–O_3	1.693	1.689	1.693	1.697
P_2–O_1	1.689	1.692	1.688	1.687
P_2=O	1.446	1.444	1.444	1.444
P_2–O_{exo}	1.700	1.703	1.703	1.701
Bond Angles (Å)				
O_1–P_2–O_3	**100.8**	**101.0**	**100.6**	**100.9**
O_{exo}–P_2–O_3	103.2	101.7	103.0	102.8
O=P_2–O_3	115.5	116.4	115.7	114.7
O=P–O_{exo}	117.7	117.6	117.7	117.9
Dihedral Angles (Å)				
H–C–O_{exo}–P_2	-9.6	-8.3	-6.7	1.0
C–O_{exo}–P_2–O_3	-101.5	-158.6	-99.1	-92.1
C–O_{exo}–P_2=O	26.9	-31.0	29.5	35.2
C_8–C_7–C_4–C_5	94.9	95.1	96.2	94.8

Table 6.2 (part 2) Geometrical data of phosphorus esters 6.4[b] and 6.4[c]

phosphorinane ring, with a preference of about 29.3 kJ/mole for the former in all cases. In the twisted as well as the pure boat conformations, the orientation of the ester group at phosphorus had changed from the equatorial to the axial position.

After altering the stereochemistry of the phosphorus atom by interchanging the phosphoroxy moiety with the exocyclic ester group, the *PM3* minimized geometries of the diastereomers of **6.4[a]** to **6.4[c]** all resulted in chair conformations with the ester group at phosphorus oriented axially. These chair conformations, however, appeared to be only about 4.2 kJ/mole more stable than the twisted boat conformations with an equatorially-oriented ester group. At this point, it must be emphasized that the twisted boat geometries with axially oriented ester groups at the phosphorus atom originated from a chair

conformer with an equatorially oriented ester moiety. This means that the stereochemistry at phosphorus of the twisted boat geometries is inverted in comparison with the chair conformations with axially oriented ester groups positioned at the phosphorus atom. This indicates that, unless scrambling at the phosphorus atom is involved in these processes (for which, however, no evidence has been found), no comparison can be made between the (twisted) boat and the chair conformers.

In contrast to **6.3**[d] no further experimental evidence for the calculated conformations of **6.4** was found by NMR techniques. The 2D NOESY NMR spectra of both diastereomers of **6.4**[a] showed no unambiguous interactions between the exocyclic ester moieties and the phosphorinane ring part of the system itself. The configuration at the phosphorus atom and the conformation of the *R,S*-diastereomer of **6.4**[a] was supported by an X-ray analysis (Figure **6.4**).

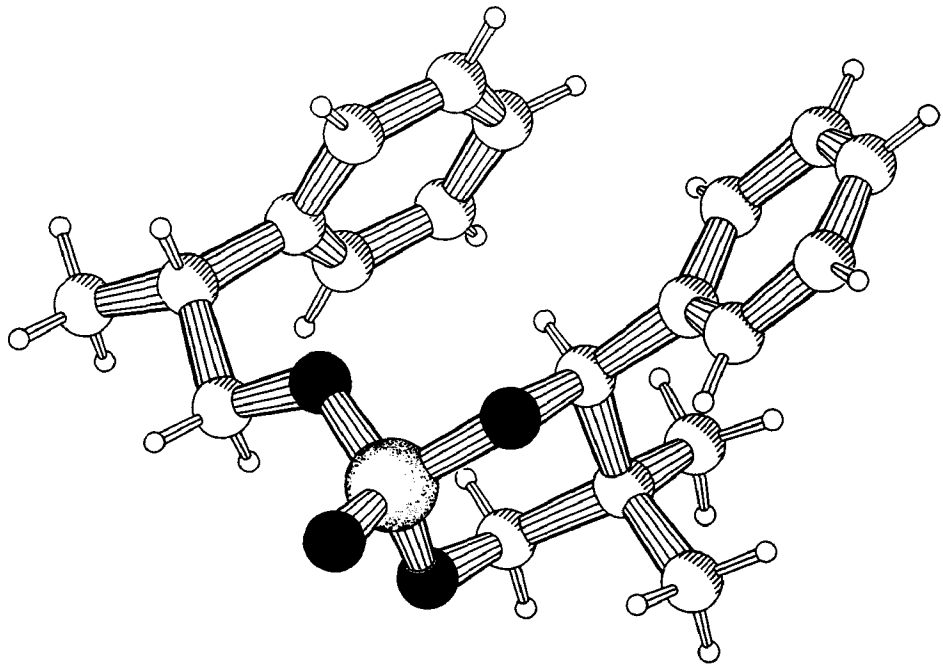


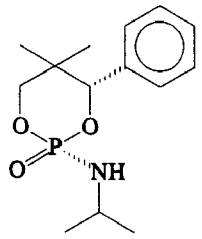
Figure 6.4 *Molecular structure of R,S* ***6.4***[a]

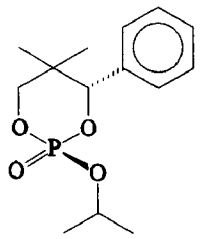
The analysis showed the phosphorinane ring to adapt a *pure* chair conformation with an axial orientation of the ester group on phosphorus. Furthermore, the analysis suggests that

the stereochemically distinct (twisted) boat conformations of **6.4**[a]–**6.4**[c] can be excluded from the conformational analysis.
The X-ray analysis of (*R,S*)-**6.4**[a] showed the conformation in the crystal lattice to be significantly different from the minimal energy conformation found by calculation. Nevertheless, the conformation obtained by X-ray also appeared as a *local minimum* in the conformational analysis, which was found to be ca. 5.0 kJ/mole higher in energy than the *global* minimum. Since, due to crystal lattice effects, solid state conformations often differ from the conformations in solution, no definite conclusions can be drawn from this discrepancy.
The appearance of a number of different minima within a small energy range does suggest that no pronounced conformational preference exists for the esters **6.4**. This might imply that a large degree of rotational freedom for the ester unit exists. This is in fact confirmed, by temperature dependent NMR experiments (not shown, the experiments were carried out from −50 ° to +50 °C, with 10 °C intervals). A decrease in the chemical shift difference for the two exocyclic diastereotopic protons at the α-carbon with increasing temperature was observed for both diastereomers of **6.4**[a]. In the same temperature dependent ^{1}H NMR studies, both diastereomers of **6.4**[a] also showed a collaps of the AB system of the two protons at the 6-position of the phosphorinane ring, even at room temperature, suggesting that at least some degree of conformational freedom of the ring system must exist. Similar temperature dependent NMR experiments on the diastereomers of the phosphorinane amide **6.3**[d], however, showed no temperature dependence at all.

6.4.3 *The correlation between intrinsic structure and chemical shift: a first approach*

The significantly different results of comparable NMR studies performed on **6.3**[d] and **6.4**[a] indicate a large difference in the conformational freedom of the amides **6.3** and esters **6.4**. Obviously, the phosphorinane esters **6.4** are much more flexible than the more rigid amides **6.3**.
To compare the freedom of rotation of the *P–N* versus *P–O* bond, rotational energy barriers of the exocyclic *P–X* bond (*X= O, N*) were calculated for the model compounds with isopropyl amide **6.11** and isopropyl ester **6.12** (Scheme **6.10**) using the *PM3* package. These calculations showed a rotational energy barrier for the *P–N* bond of about 16.7 kJ/mole versus 6.3 kJ/mole for the *P–O* bond, thus indicating more conformational flexibility for the isopropyl ester in comparison with the isopropyl amide group. This larger degree of rotational flexibility was also indicated by the calculated energy profiles of the *P–N* and *P–O* bond upon variation of the O_3–P–X–C_α dihedral angle.
In the case of the isopropyl amide **6.11**, a distinct *global* minimum exists. Deviations from this 'ideal' orientation lead to a large increase in energy, as demonstrated by the steep well in which the minimum lies. The isopropyl ester **6.12**, however, shows a much less pronounced *global* minimum as well as the appearance of a number of *local* minima. The small energy barrier between the *local* and *global* minima of **6.11** indicates the large degree of freedom of the isopropyl ester tail.





6.11 **6.12**

Scheme 6.10 Phosphorus amide 6.11 and ester 6.12

The differences in the flexibility between the model systems **6.11** and **6.12** are in good agreement with both calculated and experimentally observed differences in conformational behaviour between the amides **6.3** and esters **6.4**.

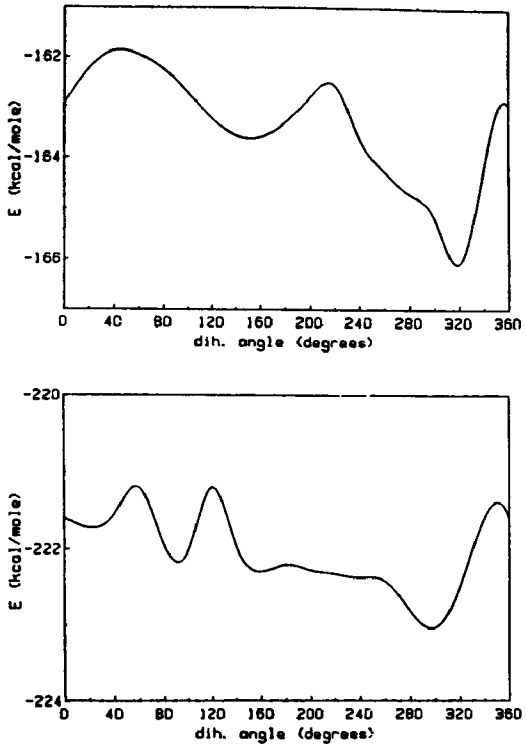


Figure 6.5 P–N and P–O dihedral angle (°) vs energy (kcal/mole)

The restricted conformational freedom of the examined phosphonic amides **6.3** may well contribute to the significantly larger diastereomeric chemical shift differences when the products **6.3** (Δδ 0.256–1.019 ppm) are compared with the ester products **6.4** (Δδ 0.151–0.262 ppm). An attempt has been made to establish some kind of correlation between the calculated geometrical parameters and the observed diastereomeric differences in the observed chemical shift using ^{31}P NMR.

Compound	$\Delta\delta$ ^{31}P NMR shift (ppm)	$\Delta\alpha$ O_1–P–O_3
6.3[a]	0.256	0.27
6.3[b]	0.314	0.32
6.3[c]	1.019	1.10
6.3[d]	0.626	0.62
6.4[a]	0.151	0.18
6.4[b]	0.215	0.21
6.4[c]	0.262	0.35

*Table 6.3 Correlation between Δδ (=δ(R,S)–δ(R,R)) ^{31}P NMR chemical shift and O_1–P–O_3 angle differences of diastereomers **6.3** and **6.4***

As is shown in Table **6.3**, a remarkable correlation is found between the calculated differences in the smallest *O–P–O* angle in diastereomers of the amide and ester adducts of **6.3** and **6.4**, and the measured diastereomeric differences in the chemical shift dispersion of these compounds measured with ^{31}P NMR techniques. A linear correlation was found between the O_1–P–O_3 angle of the diastereomeric adducts **6.3** and **6.4** and the observed shifts[#]. Although it appears that a Gorenstein–type relationship exists between diastereomeric shift dispersion and the smallest O_1–P–O_3 bond angle, care has to be taken with regard to the accuracy of the calculated angles. Nevertheless, diastereomeric discrimination is correlated to a geometrical parameter and this finding should make it possible to *design* a derivatizing agent *giving* predictable and large diastereomeric shift differences in the ^{31}P NMR spectrum. If this is actually the case, a rational design of the derivatizing agents should in fact be possible, and the development of new derivatizing agents for the enantiomeric excess determination therefore is no longer purely a matter a trial and error. In Chapter 5, some examples are given of the synthesis of structurally modified derivatizing reagents. In combination with the results described here, a more rational design of these reagents should be possible.

[#] The 1:1 ratio of Δδ (ppm) and Δα (°) is a coincidence.

6.4.4 *Conformational analysis of (R)-2-chloro-5,5-dimethyl-2-oxo-4-(R)-phenyl-1,3,2-dioxaphosphorinane 6.1*

The orientation of the chlorine atom at the phosphorus atom in **6.1** has been examined by means of the earlier mentioned methodology. Again, the starting conformations were generated with the *MM* package *CHEM-X*. In these starting conformations, the phenyl group was placed in the equatorial position of the chair conformation of the phosphorinane ring system. The chlorine atom was placed in both possible configurations at phosphorus. Both *MNDO* and *PM3* predict the conformation with the axially oriented chlorine to be more stable: *MNDO* suggests an energy difference of 8.6 kJ.mole^{-1} in favour of this orientation, whereas the *PM3* calculated energy difference is somewhat higher, being 14.1 kJ.mole^{-1}, also in favour of the axial position of the chlorine. This is in agreement with earlier reported phosphorus acid chlorides of 1,3,2-dioxaphosphorinanes (see Chapter 6.2 and 6.3). The axial preference can be explained by the anomeric effect of the 1,3 adjacent oxygen atoms.
In order to confirm the actual stereochemistry at the phosphorus atom, an X-ray analysis was undertaken.

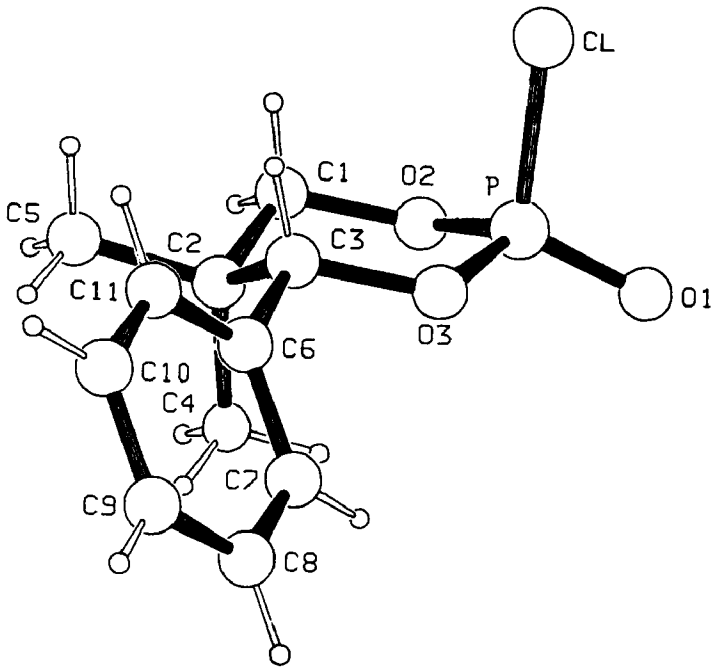


Figure 6.6 Molecular structure of R-6.1

Cl_{ax} configuration	MNDO	PM3	X-Ray
Bond Lengths (Å)			
P_2–Cl	2.044	2.072	1.992
P_2–O_1	1.603	1.676	1.557
P_2–O_3	1.601	1.677	1.557
P_2–O_{exo}	1.481	1.429	1.449
O_3–C_4	1.405	1.414	1.475
O_1–C_6	1.396	1.400	1.452
Bond Angles (°)			
Cl–P_2–O_3	106.5	101.5	104.9
O_{exo}–P_2–O_3	112.5	117.0	114.1
O_{exo}–P_2–Cl	114.8	115.5	111.7
O_1–P_2–O_3	103.9	101.5	106.2
C_6–O_1–P_2	123.3	118.8	119.1
C_4–O_3–P_2	124.2	119.0	121.4
Dihedral angles (Å)			
Cl–P_2–O_3–C_4	75.25	58.78	72.54
O_{exo}–P_2–O_3–C_4	–158.59	–174.58	–164.9
O_1–P_{exo}–O_3–C_4	–36.53	–45.29	–37.18
C_6–O_1–P_2–O_3	33.81	43.21	36.75
P_2–O_1–C_4–C_5	50.21	56.21	51.09
C_5–C_4–C_7–C_8	–99.12	–95.80	–96.76

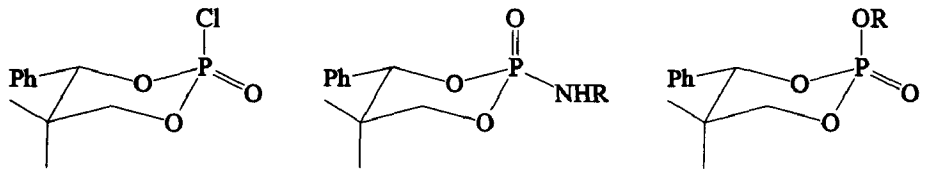
Table 6.4 Geometrical data of phosphoric acid chloride 6.1

Comparison of the X-ray structure of ***R*–6.1** (Figure **6.6**) with the calculated geometries (Table **6.4**) for the axial orientation of the chlorine showed a satisfactory correlation. However, some deviations using either *MNDO* or *PM3* were observed. Both packages tend to overestimate the bond lengths of lone pair bearing hetero atoms with phosphorus. A possible reason for this increased discrepancy for *PM3* is the fact that the phosphorus parametrization is largely based on tricoordinated phosphorus compounds as in phosphines, in which phosphorus bears a lone pair in the 3d orbital. However, for the compounds used

here, the 3d orbital is empty, which allows adjacent hetero atoms with nonbonding electron pairs to transfer some electron density towards the phosphorus atom[40]. This interaction, sometimes referred to as backdonation, causes a contraction in the bond length between phosphorus and the back donating hetero atoms, which can be seen as a partial double bond character in these bonds. For tricoordinated phosphorus, which possesses a lone pair, this backdonation is much smaller (or even absent), which might explain the poor performance of the *PM3* in the calculation of these bond lengths.
Clearly, the stereochemistry on the phosphorus atom is *R* in *R*–**6.1** (Scheme **6.1**), placing the chlorine atom in the axial position.

6.5 *Conclusions*

Related to the stereochemistry at the phosphorus atom in phosphoric acid chloride **6.1**, conformational analysis has shown that the formation of phosphorinane amides **6.3** proceeds with inversion of configuration, wheras the formation of the phosphorinane esters **6.4** proceeds with retention of configuration on the phosphorus atom (Scheme **6.11**).



6.1 **6.3** **6.4**

Scheme 6.11 The stereochemistry at the phosphorus atom of 6.1, 6.3 and 6.4

The combination of *PM3* calculations with NOESY NMR techniques and the crystallographic data analysis of product **6.3**[d] and **6.4**[a] provides unambiguous evidence for the stereochemistry at the phosphorus atom.
The much larger diastereomeric shift differences of the phosphorinane amides **6.3** in comparison with the esters **6.4** are most likely explained by the differences in conformational freedom between the two types of derivatives. The more rigid amides give rise to more pronounced differences in steric interactions between the exocyclic amide moiety and the phosphorinane ring, which can give rise to larger differences in ^{31}P NMR chemical shift between the diastereomers. In the case of the esters, the larger degree of conformational freedom can lead to strain relaxation, thus minimizing the diastereomeric differences.
Furthermore, the axial orientation of the OR group in the esters leads to a much smaller steric interaction with the bulky phenyl group at the equatorial site of the phosphorinane ring in comparison with the equatorially oriented amide moieties, again leading to less pronounced diastereomeric differences due to steric interactions for the former compounds.

The much larger degree of conformational freedom of the phosphorinane esters **6.4**, as shown by temperature dependent NMR measurements and *PM3* reaction path calculations are a clear indication of these effects.
As shown in Figure **6.1**, the observation of a Gorenstein type correlation between the observed differences in chemical shift and the difference in the smallest (endocyclic) *O–P–O* angle (calculated by *PM3*) of the diastereomeric pairs **6.3** and **6.4** is striking. However, the observed differences in bond lengths between calculated and experimentally obtained geometries show the *PM3* Hamiltonian to give a somewhat poor performance in establishing single bond lengths between four coordinated phosphorus and hetero atoms with nonbonding electrons in the valence shell. This is caused by the use of s and p orbitals only in *PM3*, which implies that the *PM3* neglects p–d orbital overlap between atoms with nonbonding π orbitals and four coordinated phosphorus (*e.g.* backbonding), leading to somewhat overestimated bond lengths. Therefore, these results, although matching experimental values, must be interpreted with caution.
However, the establishment of a reproducible correlation between geometries and chemical shift differences with *PM3*, despite of underestimated (σ) bond orders by the *MO* package might imply that in the case of functionalized phosphorinanes of type **6.3** and **6.4**, the σ bond order plays no role of importance in the determination of the magnitude of the chemical shifts of these compounds. Therefore, although *PM3* calculations for some bond lengths lead to significant deviations from the experimental values, the observed correlation between the mentioned *O–P–O* bond angle and ^{31}P NMR chemical shift (Table **6.3**) still is remarkable and appears to be valid.
Unfortunately, no acceptable physical explanation for the established correlation could be obtained from the calculations performed. This is mainly due to the highly empirical nature of this type of calculations. The results, however, confirm the Gorenstein relation, without providing a physically sound explanation for it.
In addition, the performed calculations show that the Gorenstein relation also holds for phosphonic amides, which to the best of our knowledge has not been reported so far.
In principle, the calculations might lead to a more rational design of derivatizing agents, by tuning the *O–P–O* bond angles in the chiral derivatizing agents.

6.6 *Experimental*

For the *MOPAC* calculations, the use of the services of the Dutch CAOS–CAMM centre under grant numbers SON–11–20–700 and STW–NCH–440703 is gratefully ackowledged. The useful comments of Dr G.M. Visser (PET centre of the University Hospital, University of Groningen) and Prof. Dr. C. Altona (Department of Organic Chemistry, University of Leiden) are gratefully ackowledged. All NMR measurements were performed using a Varian VXR–300 spectrometer, see for experimental details Chapter 2.
The phosphorinane acid chloride **6.1** was prepared as described in Chapter 5, and was recrystallized several times from ether at –20 °C. The compound crystallized in the orthorombic space group $P2_12_12_1$ with unit cell dimensions a= 7.123 (1), b= 10.723 (1), c= 16.123 (1) Å, α= 90.0°, β= 90.0°, λ= 90.0°.

The phosphoric amide **6.3**[d] was prepared as described in Chapter 5, and was recrystallized several times from ethyl acetate/ether mixtures at -20 °C. The *R,R* acid chloride **6.1** was used. The compound crystallized in the trigonal space group P_{31} with unit cell dimensions a= 12.4172 (15), b= 12.4172 (15), c= 10.9016 (10) Å, α= 90.0°, ß= 90.0°, λ= 120°. The other phosphoric amides **6.3** were prepared by the same methodology, however, no crystals suitable for X-ray analysis were obtained.
The phosphoric ester **6.4**[a] prepared as described in Chapter 5 was recrystallized several times from ethyl acetate/ether mixtures at -20 °C. The compound crystallized in the orthorhombic space group, with unit cell dimensions a= 6.4799 (10), b= 17.1893 (10), c=17.1893 (10) Å, α= 90.0°, ß= 90.0°, λ= 90.0°. The other phosphoric esters were prepared using the same methodolgy, although it appeared not to be possible to obtain crystals suitable for an X-ray analysis.
The obtained X-ray analyses and this Chapter will be published separately[41,42,43,44].

6.7 *References*

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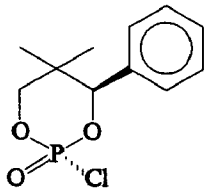
CHAPTER 7

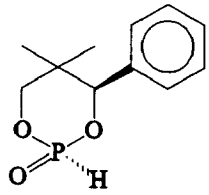
The formation of dioxaphosphorinane pyrophosphates: About a remarkable side reaction

7.1 *The observation of a remarkable side reaction during the use of the acid chloride of phencyphos in the enantiomeric excess determination*

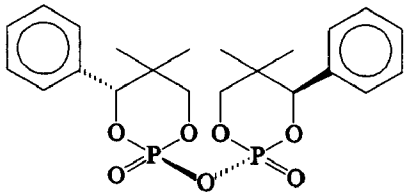
During the use of derivatizing agents **7.1** and **7.2** (Scheme **7.1**) in the enantiomeric excess determination as described in the Chapters 4 and 5, minor traces of a remarkable side reaction product **7.3** were observed. From the reactions performed it was concluded that no racemization or kinetic resolution had occurred, it thus appears that the derivatization reaction itself is not affected. Moreover, the ^{31}P NMR signals of the side products, which appear in the range from δ −15.0 to −22.0 ppm, are situated in a different part of the spectrum than the derivatizing agents themselves (at δ 3.70 and δ −4.50 ppm respectively), and the diastereomeric amide and ester products, which gave absorptions typically in the range δ −12.0 to 5.0 ppm in the decoupled ^{31}P NMR spectra.



7.1



7.2



7.3

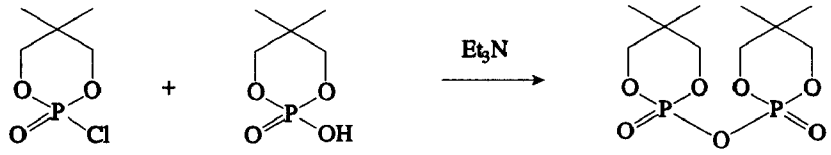
Scheme 7.1 Derivatizing agents 7.1, 7.2 and the side product pyrophosphate 7.3

When the reactions are performed using reagent **7.2**, however, side product formation could be prevented, if desired, by using completely dry conditions. The derivatization reaction with amines or alcohols has to proceed quickly, otherwise substantial amounts of phosphoric acid chloride **7.1** are formed. Although **7.1** reacts with amines and alcohols to

yield the same products as **7.2**, this reaction also appears to give rise to the formation of the side reaction products. Furthermore, for all the reactions performed the signals of the products formed in the side reaction showed the same chemical shifts. This strongly suggests that the side products are formed from the phosphoric acid chloride **7.1**.
A careful analysis of the reaction mixture showed that the side products are in fact the *pyrophosphate* **7.3** (Scheme **7.1**) and intermediate structures leading to its formation. It should be noted that the thiophosphoric acid chloride as described in Chapter 5 shows similar behaviour, although the reaction tends to proceed much more slowly.
From an analytical as well as a mechanistic point of view, the reaction leading to pyrophosphate **7.3** is of particular interest. The elucidation of the reaction mechanism and the factors playing an important role in the formation of **7.3** are described in this Chapter.

7.2 *Pyrophosphates and their formation: a true debate*

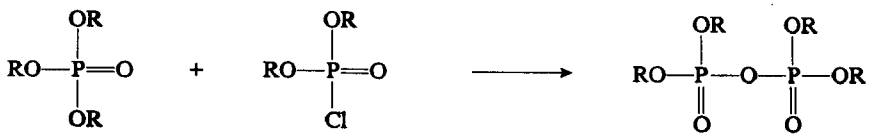
The stereochemical course of exocyclic displacement reactions on phosphorus containing five and six membered ring systems in general, and the formation of pyrophosphates of the 2-oxo-1,3,2-dioxaphosphorinanes in particular has been subject of a large number of mechanistic studies, which have in common that the different authors do not agree on the interpretations.
One of the first reports concerning the mechanism was by Simpson and Zwierzak[1], who described the formation of the pyrophosphate **7.6** from 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinane **7.4** and 2-hydroxy-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinane (**7.5**, Scheme **7.2**), in a straightforward nucleophilic substitution reaction.



7.4 **7.5** **7.6**

Scheme 7.2 The formation of pyrophosphate 7.6 from phosphoric acid chloride 7.4 and acid 7.5

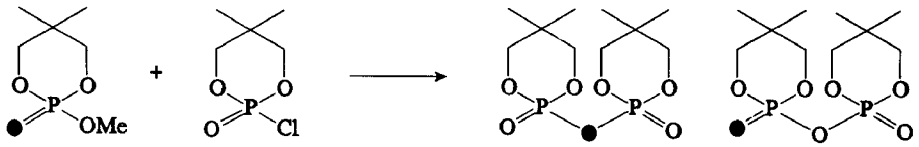
Zwierzak[2] was interested in the mechanism of the reaction of trialkyl phosphates **7.7** and dialkyl phosphorochloridates **7.8**, to form condensation products **7.9** (Scheme **7.3**)[3]. Therefore, Simpson and Zwierzak used the ^{18}O labelled phosphoric methyl ester **7.10**, with the label situated in the phosphoroxy bond instead of the acid itself, in the condensation reaction. They established that the labelled oxygen was distributed between the bridged oxygen and one of the phosphoroxy oxygens in pyrophosphate **7.6**, although in unequal amounts (Scheme **7.4**).



7.7 7.8 7.9

Scheme 7.3 Reaction of trialkyl phosphates 7.7 and dialkylphosphochloridates 7.8

To account for this observation, two mechanisms were proposed for the pyrophosphate formation. The first mechanism involved nucleophilic attack of the labelled phosphoryl oxygen in **7.10** on the phosphoric acid chloride **7.4**, leading to a product with the labelled oxygen positioned in the bridge (Scheme **7.5**, Route a).



7.10 **7.4** **7.6a** **7.6b**

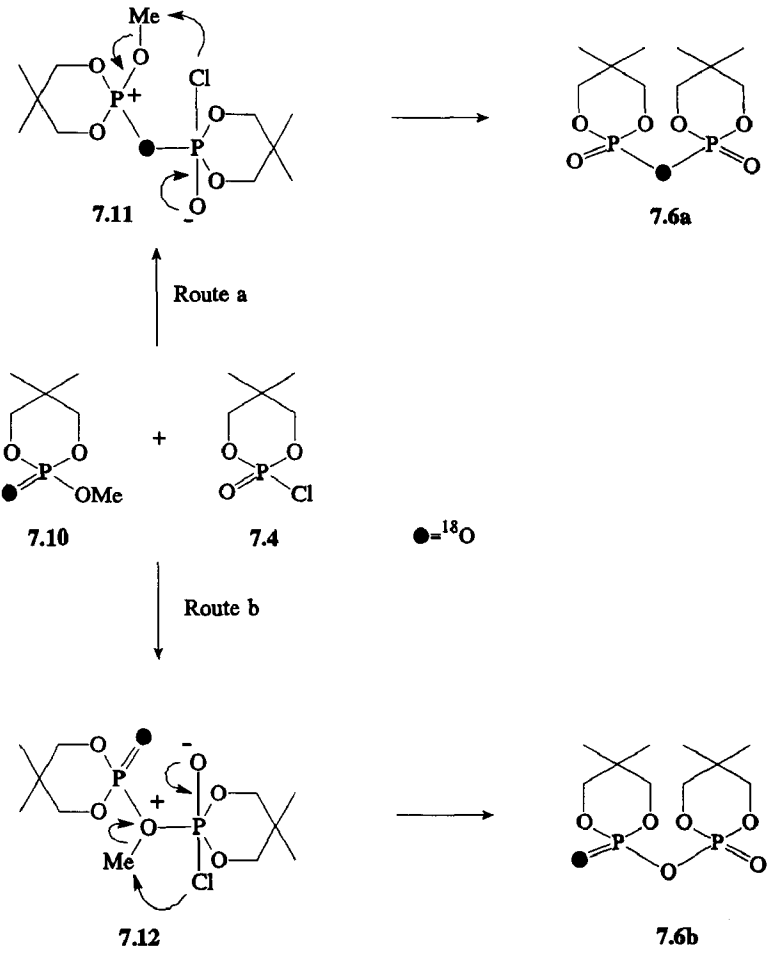
●=^{18}O

Scheme 7.4 The use of ^{18}O labelled phosphorus reagents in the pyrophosphate formation

The second mechanism accounts for the formation of product **7.6b**, in which the label is situated in the phosphoroxy moiety; this product could be formed by initial attack of the unlabelled alkoxy oxygen of **7.10** at the phosphoric acid chloride **7.4**, as is depicted in Route b. These interpretations were based upon IR measurements of the deprotonated phosphoric acid and phosphoric methyl ester, which were obtained after degradation of the synthesized pyrophosphate with NaOMe (Scheme **7.6**). In the degradation products, the bridge-labelled oxygens will be found exclusively in the phosphate anion, since the nucleophilic attack of the methoxide nucleophile forces the phosphate anion, with a labelled oxygen, to act as a leaving group to yield the phosphoric acid methyl ester. From the distribution of the labelled oxygen it was concluded that Route b (Scheme **7.5**) affords the largest contribution to the formation of the pyrophosphate **7.6**.

Alternative mechanisms, based upon ring opening of the phosphorinane ring system were ruled out, since earlier studies showed that 1,3,2-dioxaphosphorinane systems are very reluctant towards ring opening processes[4].

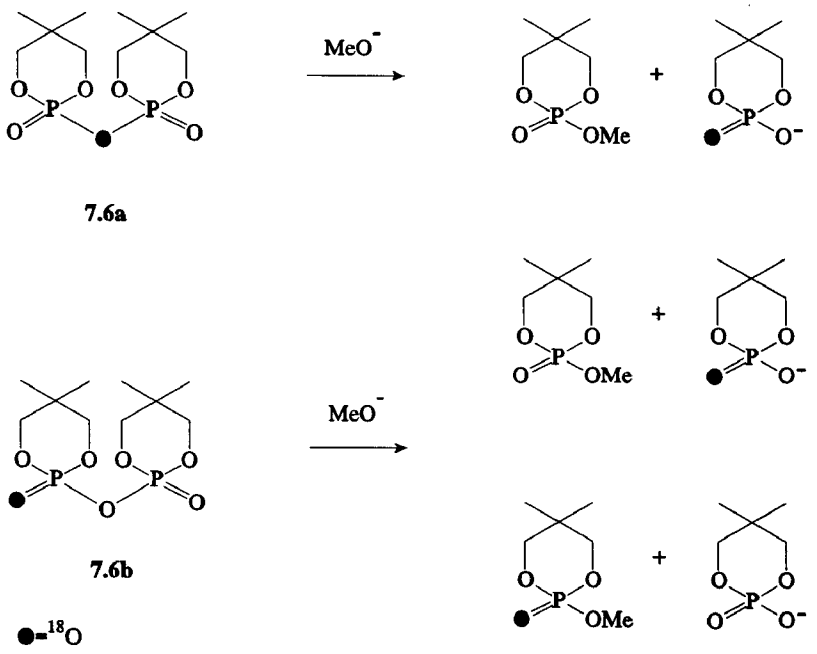
The introduction of high field NMR techniques led to a reinvestigation of the Zwierzak-Simpson experiment by Cullis and co-workers[5]. They were able to make use of ^{31}P NMR techniques and starting materials that were labelled to a much higher degree. If the



Scheme 7.5 Reaction pathways of labelled phosphoric ester 7.10 with acid chloride 7.4

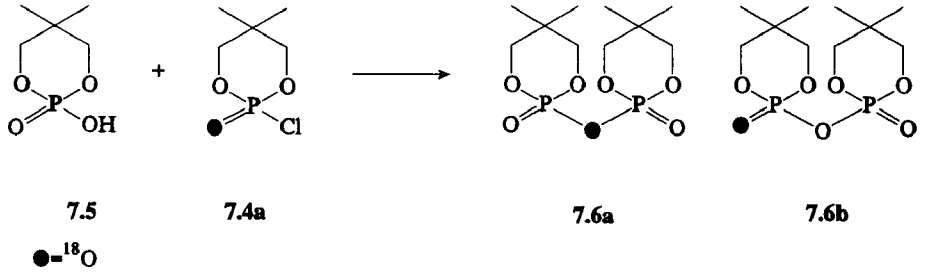
interpretations of Zwierzak were correct, a reaction of unlabelled phosphate anion **7.5** with ^{18}O labelled phosphoric acid chloride **7.4a** would lead to the formation of a product in which the oxygen label would be exclusively situated in the *P=O* oxygen of pyrophosphate **7.6b** (Scheme 7.7). However, complete scrambling of the labelled oxygen between the phosphoroxy and the bridging oxygen in a 1:1 ratio was found, an observation for which the Zwierzak model cannot account.

For this reason, Cullis and co-workers[5] proposed an alternative mechanism for the formation of the pyrophosphate from phosphoric acid chloride and phosphoric acid (Scheme **7.8**), in which the formation of four membered ring systems **7.14** and **7.15**, so called dioxadiphosphetanes, play an important role (Scheme **7.8**).



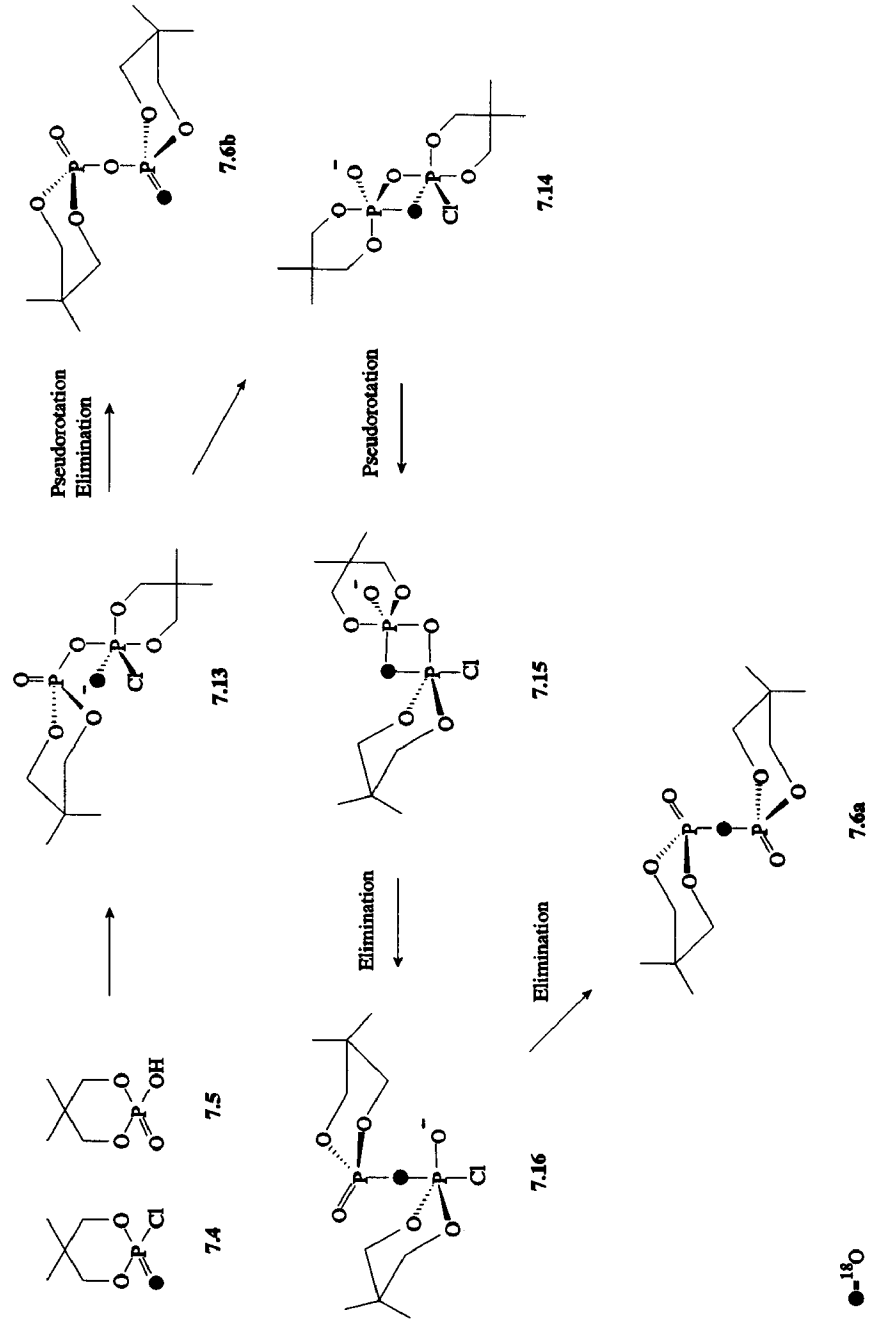
Scheme 7.6 Degradation of labelled pyrophosphates 7.6 using NaOMe[1,2]

In this process, a Berry pseudorotation[6] will eventually lead to the bridge-labelled pyrophosphate **7.6a**. If the proposed mechanism is correct, both the direct route from **7.13** to the *P=O* labelled pyrophosphate **7.6b** as well as the route via the dioxaphosphetanes must have the same Gibbs energy barrier to yield the 1:1 ratio of the labelled products. This would be a remarkable coincidence, and other options should be taken into consideration.



Scheme 7.7 Formation of pyrophosphate 7.6a from labelled phosphoric acid chloride 7.4a and unlabelled 7.5

The stereochemistry on the pentavalent phosphorus of intermediate **7.13** might not be correct. Instead of the direct pseudorotation and chlorine elimination in the formation of the *P=O* labelled pyrophosphate **7.6b**, the *P=O* labelled pyrophosphate was proposed to

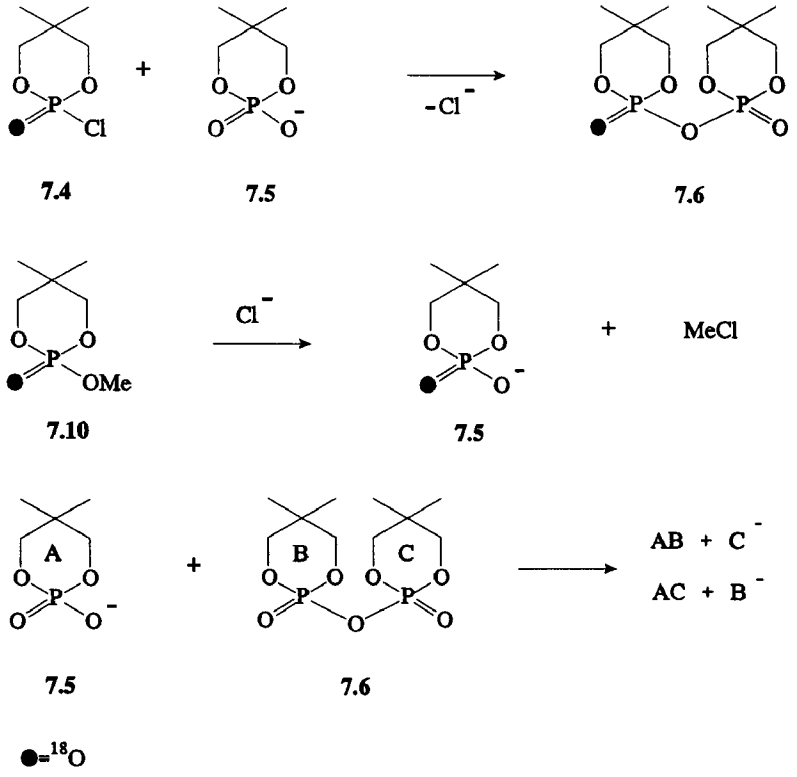


Scheme 7.8 Formation of pyrophosphates via dioxaphosphetanes

be formed also via dioxaphosphetane intermediates **7.14** and **7.15**, which would give a more satisfactory explanation for the observed 1:1 ratio. Furthermore, if labelled phosphoric acid **7.5** is allowed to react with unlabelled phosphoric acid chloride **7.4**, a ratio of 3:1 (**7.6b**:**7.6a**) throughout the course of the reaction is found, so clearly at some stage in the reaction the attacking oxygen atoms of **7.5** must become nonequivalent.

Also, the reaction of the phosphorinane methyl ester **7.10** with the acid chloride **7.4**, as carried out by Zwierzak[3], was proven to proceed via a different mechanism than originally proposed.

Cullis and co-workers[5] found that scrambling of the labelled oxygen took place over all exocyclic oxygens, which is impossible in the Zwierzak-mechanism. Furthermore, in the initial stages of the reaction, the formation of the same ratio of ^{18}O *P=O* and *P-O-P* labelled products as in an experiment with labelled phosphate anion and unlabelled phosphoric chloride was found. This observation was explained by the assumption that traces of phosphate anion **7.5** (which is very likely when the phosphoric acid chloride is prepared from the corresponding acid) are present. The presence of this phosphate anion can lead to the catalytic cycle involving nucleophilic attack of phosphate on the pyrophosphate **7.6**, as proposed in Scheme **7.9**.

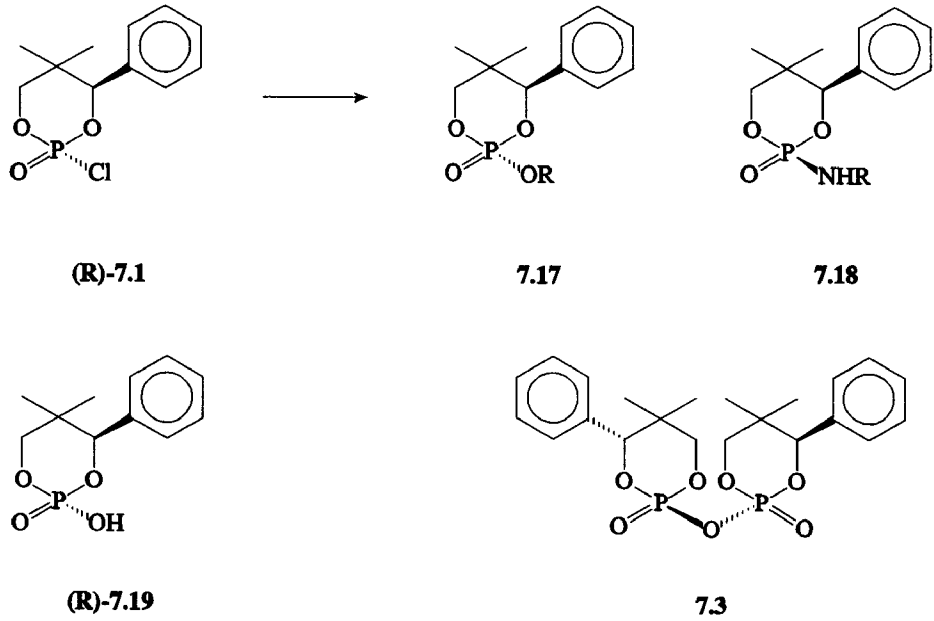


***Scheme 7.9** Scrambling of labelled oxygen in the formation of 7.6 involving 7.5 and 7.10*

It is easily understood that the observed scrambling of labelled oxygen over all exocyclic oxygens takes place. Some important questions are, however, not answered by Cullis and co-workers. First, using their system, *it was not possible to detect any of the intermediates directly*. Furthermore, esters of 5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinanes are known to give a rapid chair-chair inversion at room temperature[7], *which rules out the possibility to analyze the stereoselectivity (axial vs. equatorial orientation of the bridged oxygen at both rings in 7.6) at the phosphorus atom for the compounds used by Cullis and co-workers*. It would therefore be of interest when analogues providing the stereochemical information were available. Such a system, fortunately, is known: it is the phencyphos acid **7.19** and phencyphos acid chloride **7.1** system.

7.3 *The formation of phencyphos pyrophosphate*

The use of reagent **7.1** in the enantiomeric excess determination of alcohols and amines not only leads to the observation of signals belonging to the desired products **7.17** or **7.18** in the decoupled ^{31}P NMR, but often gives rise to the formation of a side product that was characterized as being the phencyphos pyrophosphate **7.3** (Scheme **7.10**).



(R)-7.1 **7.17** **7.18**

(R)-7.19 **7.3**

Scheme 7.10 *Reagents and products obtained using reagent 7.1 in the enantiomeric excess determination of alcohols and amines*

Pyrophosphate **7.3** was synthesized independently and characterized for comparison purposes. When compared to the systems used by Zwierzak[2] and Cullis[5], **7.1** could provide us with an additional source of information. The chair-chair inversion, which ruled out the

possibility to analyze the stereoselectivity (axial vs. equatorial orientations of the bridged oxygens at both ring systems in **7.6**) probably does not play an important role in the phencyphos system, since the equatorial oriented phenyl group at the 4–position of the phosphorinane ring system *locks* the chair conformer[8] (see also Chapter 6).
The equatorial–axial displacement of the phenyl group not only will lead to a much higher chair–chair inversion energy barrier, but also will cause a significant energy difference between both chair conformations, resulting in the existence of only one of the conformers. Conformations with the axially oriented phenyl group are not observed. Moreover, the stereocenter provides an additional mechanistic tool to obtain information about the formation of pyrophosphate **7.3**.
For the sake of clearity, the analysis of the formation of pyrophosphate **7.3** will be treated in separate Sections 7.3.1 to 7.3.5, while Section 7.4 contains the conclusions.

7.3.1 *Possible pathways for nucleophilic attack of 7.1 by phosphates*

The attack of deprotonated phosphoric acid **7.19** (Scheme **7.10**) at phosphoric acid chloride **7.1** is assumed to proceed in the same manner as the addition of other nucleophiles, resulting in the formation of a five–coordinated phosphorus (P^V) intermediate. Commonly, P^V structures have a trigonal bipyramidal geometry[9] (*TBP*), although distortions towards a square–pyramidal (*SP*) geometry have been reported[10]. In the trigonal bipyramid, the two axially oriented ligands have slightly longer and weaker bonds than the three equatorial ligands, resulting in slightly different chemical behavior. The differences can be explained in terms of hybridization; phosphorus has *pd*–hybridization along the *z*–axis, and sp^2–hybridization in the equatorial plane. According to the theory introduced by Westheimer[9], nucleophiles attacking the phosphate substrate initially occupy an axial site in the P^V trigonal bipyramid. Furthermore, it is always an axial oriented ligand that is expelled upon conversion of the P^V system to the phosphate stage.
P^V–*TBP* systems show a highly fluctional behavior, usually designated as pseudorotation (*BPR*, *B*erry *P*seudo*R*otation)[6], in which the five ligands are rapidly distributed over the five sites in the trigonal bipyramid. In this process, two equatorial and two axial ligands interchange their positions via an intermediate *SP* structure, whereas one (equatorial) ligand, (called the *pivot*, retains its position (Figure **7.1**).

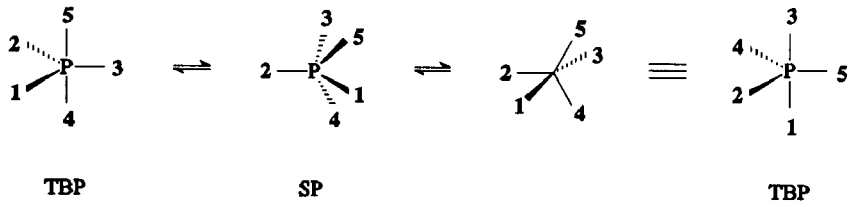


Figure 7.1 The Berry pseudorotation[6]; in this example ligand 2 is called the pivot

Ramirez and Ugi[11] have proposed an alternative mechanism, the so-called turnstile rotation (*TR*). In this mechanism, two equatorial ligands move towards each other in the equatorial plane until the angle has become 90°. The third equatorial and one axial ligand are moved by about 9°, followed by an internal rotation of the ligands 3 and 4 and of the three ligands 1, 2 and 5. The results of the *BPR* and *TR* are exactly the same. Calculations, however, have shown that the transition state for *TR* is about 2–6 times higher in energy compared to the *BPR*[6]. Therefore, *BPR* is the most plausible ligand exchange mechanism in phosphoranes, and the *TR* is not taken further into consideration during the following discussion.

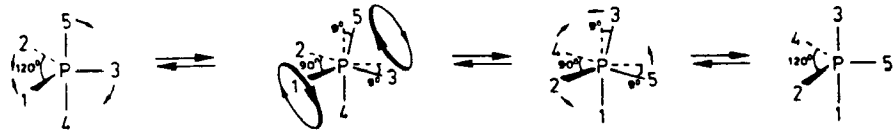
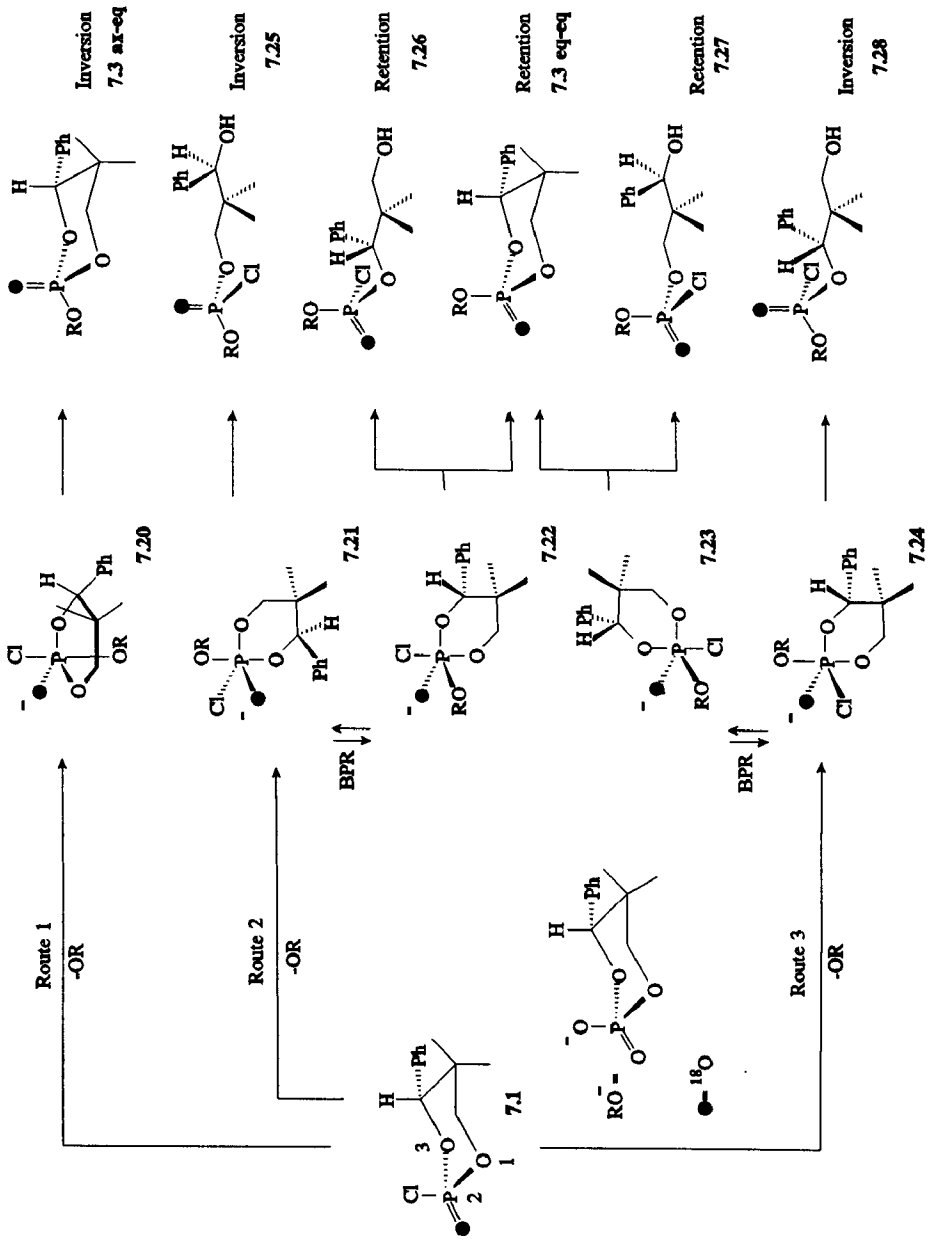


Figure 7.2 The Turnstile rotation

The possible modes of addition of (*R*)-**7.19** to (*R*)-**7.1** are depicted in Scheme **7.11**.
The nucleophile, in this case the deprotonated phosphoric acid (^{-}OR), enters the P^{V}-*TBP* at one axial site, inducing an axial cleavage of the leaving group[9]. It is generally accepted that negatively charged oxygens can only adopt equatorial sites in the P^{V}-*TBP*[12] and that a Berry pseudorotation[6] can occur prior to the actual bond breaking, placing the proper leaving groups in the axial positions. This way, the nucleophile can approach from four different sites, although the attack of ^{-}OR on the phosphorus atom opposite the *P=O* bond is not taken into consideration, because the P^{V}-*TBP* formed will possess a negatively charged oxygen in an axial position, which is energetically not favourable.
In *Route 1*, the attack of the nucleophile takes place opposite the *P–Cl* bond; the dioxaphosphorinane formed this way spans diequatorially (**7.20**), which is regarded as being energetically unfavourable[13]. Subsequent elimination of chloride results in the formation of pyrophosphate **7.3** with inversion of configuration with respect to the phosphorus atom in the former phosphoric acid chloride part of the molecule. This leads to an axial–equatorial orientation of the bridging oxygen with respect to the phosphorus atoms in **7.3** (**7.3**$^{ax-eq}$).
Attack of ^{-}OR opposite the $P–O_1$ bond (*Route 2*), resulting in the P^{V}-*TBP* intermediate **7.21**, gives by cleavage of the axial $P–O_1$ bond the acyclic phosphate diester **7.25** which is formed with inversion of configuration with regard to the phosphorus atom. Berry pseudorotation of the P^{V}-*TBP* **7.21** intermediate leads to the formation of another P^{V}-*TBP* intermediate **7.22** which, by elimination of the axially placed leaving groups, leads to the acyclic phosphate diester **7.26** or the cyclic product **7.3**$^{eq-eq}$. Both products are formed with retention of configuration on phosphorus. In pyrophosphate **7.3** formed via this route, the bridging oxygen adopts an axial–equatorial position.



Scheme 7.11 Possible pathways for the nucleophilic attack of phencyphos 7.19 at acid chloride 7.1

In *Route 3* attack of ^{-}OR takes place opposite the $P-O_3$ bond. The chloride leaving group is placed equatorially in the P^V*-TBP* intermediate **7.24** and can only be eliminated after Berry pseudorotation to **7.23**, placing the chloride in the axial position. Subsequent cleavage of the axial $P-Cl$ or $P-O_3$ bond leads to the formation of the cyclic pyrophosphate $\mathbf{7.3}^{eq-eq}$ or the acyclic phosphate ester **7.27**, respectively. Both reactions proceed with retention of configuration on the phosphorus atom. Alternatively, a direct cleavage of the axially oriented $P-O_1$ in **7.24** leads to the formation of the acyclic phosphate ester **7.28** with retention of configuration#.
Although not all the routes are energetically favourable, the research described by Buck and co-workers[14], using closely analogous systems, showed that all products can be formed. *At this point, it has to be emphasized that the intermediate structures have not been observed. Their existance, however, is concluded from the products and the distribution of the oxygen label over the oxygens in the pyrophosphate.*

7.3.2 *The formation of pyrophosphate using phencyphos acid chloride 7.1*

As already stated, the formation of pyrophosphate **7.3** from (*R*)-phencyphos acid chloride **7.1**, appears to be sensitive to several factors. The addition of a catalytic amount of the corresponding (*R*)-phencyphos acid **7.19** has a positive influence upon the rate of formation of the pyrophosphate. Also, when water is not completely excluded, the formation of pyrophosphate **7.3** is more rapid compared to perfectly dry conditions. Since the processes, and in particular the amount of **7.3** formed, showed complex dependencies upon several other factors as well, like solvent, the concentration and the temperature, the following analysis will focus on the qualitative description of the formation of pyrophosphate **7.3**. Furthermore, on using reagent **7.2** under perfectly dry conditions, the formation of pyrophosphate **7.3** can be prevented, although the reaction (when no substrate molecule is available) with Et_3N and CCl_4 readily affords phosphoric acid chloride **7.1**[15]. For this reason, reagent **7.2**, which is converted into reagent **7.1** prior to the pyrophosphate formation, has been used for mechanistic studies unless stated otherwise.
The formation of the pyrophosphate could easily be monitored, using (*R*)-**7.2**, resulting in (*R*)-**7.1**, and (*R*)-phencyphos **7.19** (both 1.0 mmol), 0.1 mL of Et_3N and CCl_4 (0.1 mL) dissolved in $CDCl_3$ (2 mL) or C_6D_6 (carefully dried glaswork was used, all solvents were dried just prior to use) and following the reaction by means of ^{31}P NMR (Figure **7.3**).
A total of five signals, other than the signals belonging to the starting materials, are observed. The pairs of signals at δ -16.29 ppm and δ -22.20 ppm, which have an AB coupling ($^2J_{PP}$= 33.01 Hz), can be assigned to intermediate **7.29** (Scheme **7.12**), having one of the geometries **7.20**, **7.21**, **7.22**, **7.23** or **7.24** (Scheme **7.11**). The disappearance of the pair of signals leads to an increase in intensity of the signal at δ -20.56 ppm, which is assigned to pyrophosphate **7.3** (Scheme **7.12**).
The reaction shows a large temperature dependency, and is usually completed between 1 h (C_6D_6 at 65 °C) and 12 h (C_6D_6 at 30 °C). The only product is the symmetrical pyrophos-

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The equatorial or axial description refers to the orientation of the $P=O$ moiety.

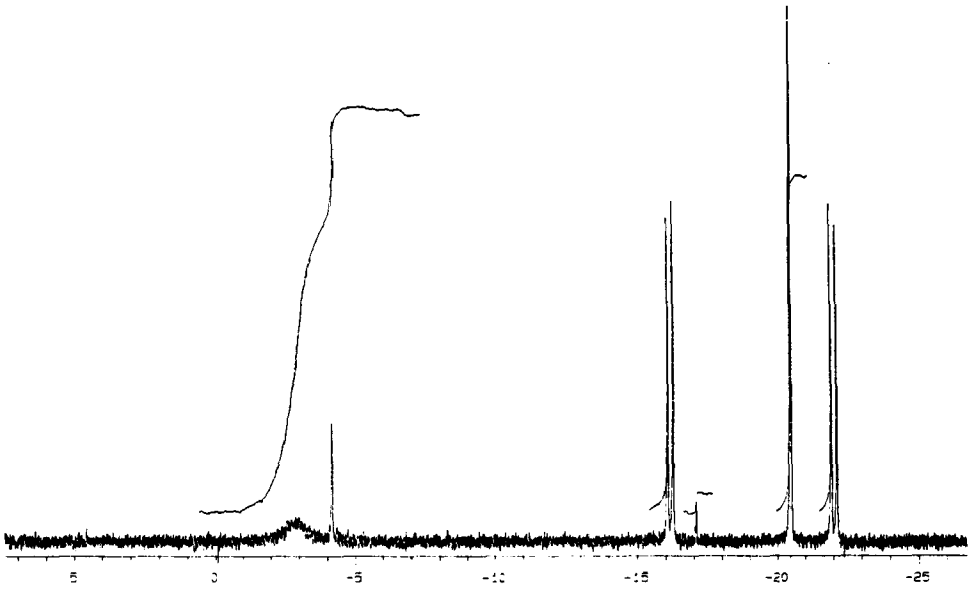
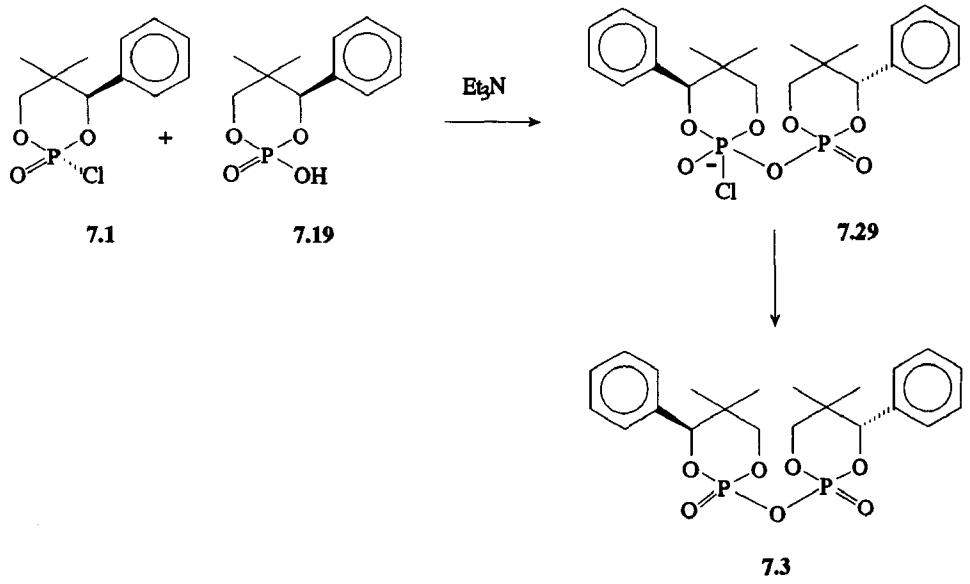


Figure 7.3 ^{31}P *NMR spectrum of 7.3 and 7.29 recorded in* $CDCl_3$ *[L]= 0.01 M*

phate **7.3**. It is obvious that the formation of **7.3** proceeds completely *stereospecifically*; otherwise a total of six signals would be expected: (*1*) the axial–axial coupled product **7.3**,



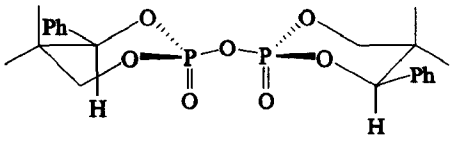
7.1 **7.19** **7.29** **7.3**

Scheme 7.12 The formation of 7.29 and 7.3 from 7.1 and 7.19

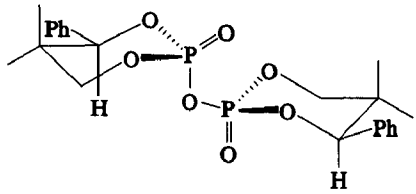
which being symmetrically gives only one ^{31}P NMR absorption; (*2*) the equatorial–equatorial coupled product **7.3**, which also gives one signal; and (*3*), the axial–equatorial pyro–

phosphate, which gives two signals with different chemical shifts and a coupling ($^2J_{PP}$ AB coupling), yielding four absorptions in total[16].

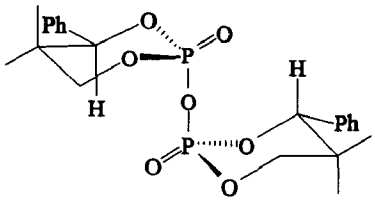
The stereogenic center at position 4 of the phosphorinane ring in **7.1** provides an extra source of information, since the formation of the pyrophosphate **7.3** from racemic starting material will lead to the observation of diastereomers; two *meso* compounds and a *d,l* pair, provided the coupling reaction is stereospecific with regard to phosphorus. Therefore, the pyrophosphate would give two signals in the decoupled ^{31}P NMR spectrum; the *R,R* and *S,S* pyrophosphates, both being symmetrical and mirror images, giving one signal only, and the *R,S* and *S,R* diastereomers, also being symmetrical, yielding one signal. However, when the reaction is not stereospecific with regard to phosphorus, the pyrophosphate can be formed with a total of 12 different configurations (6 pairs). A *d,l* or *meso* pair with an equatorially oriented *P=O* in both ring systems, a *d,l* or *meso* pair with both *P=O* groups axially oriented or a *d,l* or *meso* pair with one *P=O* equatorially and one *P=O* group axially positioned (Scheme **7.13**).



7.3 ax-ax



7.3 eq-ax



7.3 eq-eq

Scheme 7.13 The three possible pyrophosphates 7.3

If the coupling reaction is, however, stereospecific as is the case with enantiomerically pure **7.1**, only the symmetrical *meso* and the *d,l* configurations are formed and this would lead to the formation of two signals in the decoupled ^{31}P NMR spectrum.

Therefore, the pyrophosphate formation with racemic **7.1** and racemic **7.19** was investigated. All the signals mentioned before are doubled, which can be explained by the formation of the *meso* and the *d,l* compounds in both intermediate **7.29** and the pyrophosphate **7.3** itself (Figure **7.4**). The signals at δ −16.29 ppm and δ −22.20 ppm, which show a ^{31}P–^{31}P coupling ($^2J_{PP}$= 33.01 Hz), belong to the *d,l* form of intermediate **7.29** and lead to the formation of the *d,l* pyrophosphate **7.3** (δ −20.56 ppm). The *meso* form of

7.29, gives rise to the signals at δ −16.44 and δ −22.32 ppm with a $^2J_{PP}$= 27.82 Hz, leading to the formation of *meso* **7.3** (δ −20.62 ppm). The isomers of **7.29** are cleanly and quantitatively converted into *d,l* and *meso* **7.3** (Table **7.1**).

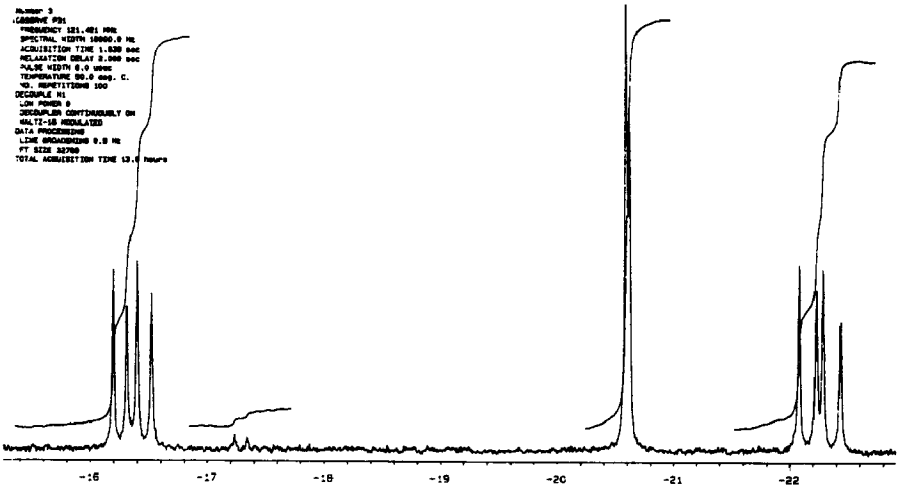


Figure 7.4 *^{31}P NMR spectrum of the reaction product of racemic 7.1 and racemic 7.19 recorded in $CDCl_3$ [L]= 0.01 M.*

Since pyrophosphate 7.3 only gives one signal when using enantiomerically pure 7.1 and 7.19, and only two signals when using racemic 7.1 and 7.19, it can be concluded that the formation of the pyrophosphate is completely stereospecifically at the phosphorus center, regardless the enantiomeric composition of the phosphoric acid and phosphoric acid chloride used. Moreover, 2D NOESY NMR and ^{31}P NMR studies (not shown) clearly showed the product 7.3 to be the axial-axial coupled pyrophosphate 7.3^{eq-eq}.

compound	δ ^{31}P NMR (ppm)	δ ^{31}P NMR (ppm)	J_{PP} (Hz)
d,l 7.3	−20.56	–	–
meso 7.3	−20.62	–	–
d,l 7.29	−16.29	−22.20	33.01
meso 7.29	−16.44	−22.32	27.82

Table 7.1 *^{31}P NMR chemical shifts and coupling constants for the intermediates and product in the formation of (racemic) pyrophosphate 7.3*

At this point, the mechanism as proposed by Cullis[5] still provides an acceptable and unambigious explanation for the observations made. Less easily understood, however, are the findings that pyrophosphate **7.3** is formed nearly quantitatively when only a catalytic amount or even no acid **7.19** is available. This strongly points to the involvement of (traces) of water. Therefore, experiments with racemic and enantiomerically pure **7.1** and **7.19** in various ratios were performed to obtain further information about the mechanism of the pyrophosphate formation (Table **7.2**).

Entry	7.1	7.19	products
1	**R** (1 eq)	**R** (1 eq)	**R,R-7.3**, 98%
2	**R** (1 eq)	**R** (cat)	**R,R-7.3**, 36%
3	**R** (1 eq)	**RS** (1 eq)	**R,R** and **S,S-7.3**, 67%, **R,S-7.3**, 26%
4	**R** (1 eq)	**RS** (cat)	**R,R-7.3**, 42%
5	**RS** (1 eq)	**R** (1 eq)	**R,R** and **S,S-7.3**, 63%, **R,S-7.3**, 29%
6	**RS** (1 eq)	**R** (cat)	**R,R** and **S,S-7.3**, 16%, **R,S-7.3**, 5%
7	**RS** (1 eq)	**RS** (1 eq)	**R,R** and **S,S-7.3**, 78%, **R,S-7.3**, 17%
8	**RS** (1 eq)	**RS** (cat)	**R,R** and **S,S-7.3**, 23%, **R,S-7.3**, 13%
9	**R**	-	see text for results
10	**R** (1 eq)	**S** (0.5 eq)	**R,R** and **S,S-7.3**, 51%, **R,S-7.3**, 48%

Table 7.2 Several combinations of 7.1 and 7.19 used in the pyrophosphate formation, see text for explanation

The formation of free acid **7.19** from acid chloride **7.1** is not likely to take place, since phosphoric acid chloride **7.1** is very reluctant towards hydrolysis as was shown by Ten Hoeve and Wynberg[17]. Should this, however, be the case an experiment as is shown in entry *10* should give important information. Hydrolysis of (*R*)-**7.1** to (*R*)-**7.19** would allow the formation of both *R,S*-**7.3** (from (*S*)-**7.19** with (*R*)-**7.1**) and *R,R*-**7.3** (from hydrolyzed (*R*)-**7.1**, yielding (*R*)-**7.19**, and (*R*)-**7.1**) pyrophosphates and intermediates, which would give the same doubling of signals in the decoupled ^{31}P NMR spectrum as in the case (one) of the starting materials was used as racemate. Both the *meso* and *d,l* pair of the pyrophosphate **7.3** are formed, although not all **7.1** has reacted, not even after 24 h of reaction time at 50 °C (C_6D_6). This strongly suggests that the presence of water in the reaction medium must play some role in the conversion of **7.1** into **7.3**. The results are collected in Table **7.2**.

At this point, it might be of interest to take another interesting aspect into consideration. The conversion of (*R*)-**7.1** was followed with acid chloride obtained from different sources (entry *9*). It makes a considerable difference whether acid chloride **7.1** is prepared from

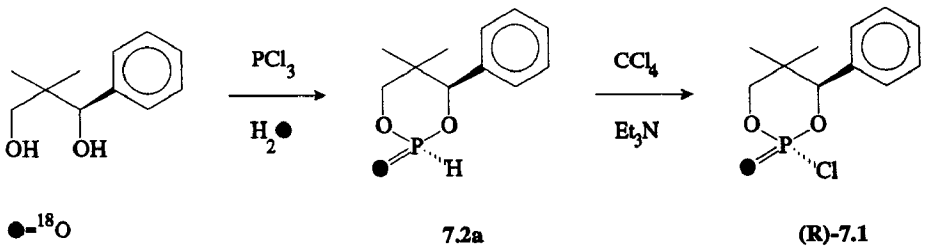
reagent **7.2** (as described above) or from the corresponding acid **7.19** (as described in Chapter 5). In the former case, almost no conversion to the pyrophosphate **7.3** takes place. In the latter case, however, all acid chloride **7.1** is converted into pyrophosphate **7.3**. This can be explained by traces of **7.19** still present in the acid chloride, which initiate the formation of pyrophosphate **7.3**. These observations imply that not acid chloride **7.1**, but pyrophosphate **7.3** formed is hydrolyzed, yielding two equivalents of free acid **7.19**. This indicates that a catalytic amount of free acid, combined with traces of (crystal) water, effect the complete conversion of acid chloride **7.1** into pyrophosphate **7.3**. For entry *10*, the ratio of *d,l*:*meso* products formed provides the desired information. The observed ratio is close to 1:1 (small enrichment in the *meso* compound), which cannot solely be explained by simple (partial) hydrolysis of (*R*)–acid chloride **7.1**. This would lead to a 2:1 ratio in favour of the *meso* pyrophosphate **7.3**, assuming the hydrolysis reaction to proceed without stereoselectivity. Four explanations are possible to account for these observations. The *first* possibility is the hydrolysis of the phosphoric acid chloride (*R*)–**7.1** into the free acid (*R*)–**7.19**, which means that both (*R*)–**7.19** and (*S*)–**7.19** are available to react with (*R*)–**7.1** and yield *meso* and *d,l*–pyrophosphate **7.3**. *Secondly*, the reaction of one equivalent of (*R,S*)–**7.3** with one equivalent of anion (*S*)–**7.19** would lead to the formation of 0.5 eq. of (*R,S*)–**7.3** and 0.5 eq. of (*S,S*)–**7.3** with the release of 0.5 eq. of (*R*)–**7.19** and 0.5 eq. of (*S*)–**7.19**. After reaction with the phosphoric acid chloride (*R*)–**7.1** still present, both *meso* and *d,l* pyrophosphate **7.3** can be formed. A disadvantage of this mechanism is the assumption that the phosphate anion **7.19** reacts more easily with pyrophosphate **7.3** than with phosphoric acid chloride **7.1**. This is considered not to be very likely. A *third* mechanism would be the attack of liberated chloride ion at (*R,S*)–pyrophosphate **7.3**, yielding (*R*)–**7.19** and (*S*)–**7.19** as well as (*R*)–**7.1** and (*S*)–**7.1**. After recombination, *meso* and *d,l*–pyrophosphate **7.3** are formed again. Unfortunately, the reaction of *e.g.* (*S*)–**7.1** with (*S*)–**7.19** yields (*S,S*)–pyrophosphate **7.3**, which is the enantiomer of (*R,R*)–**7.3**, and therefore could not be detected separately by the NMR techniques used. It is not possible, at present, to assess with certainty a possible contribution of this route to the formation of pyrophosphate **7.3**. The *fourth* possibility, the *hydrolysis* of pyrophosphate **7.3**, provides a more reasonable explanation for the observed (though small) enrichment in favour of the formation of the *racemic (meso)* pyrophosphate **7.3**. The hydrolysis of pyrophosphate (*R,S*)–**7.3** leads to the formation of both enantiomers of the phosphate anion **7.19**, which accounts for the formation of both the *meso* and *d,l* pyrophosphate **7.3**. After formation, (*R,R*)–pyrophosphate **7.3** may hydrolyse, leading to the formation of 2 eq. of (*R*)–**7.19**. If this, however, should be the case, excess of water and base would lead to the formation of phosphate anion **7.19** from hydrolysis of pyrophosphate **7.3** after the phosphoric acid chloride **7.1** has reacted. This is exactly what is found in all the experiments carried out under *normal*, not completely dry conditions. Therefore, the explanation by Cullis and co-workers[5] for the complete scrambling of labelled oxygen over all exocyclic oxygens of the pyrophosphate formed in the reaction of the phosphate methyl ester **7.10** with the phosphoric acid chloride **7.4** is not necessarily correct. When a small amount of pyrophosphate is hydrolyzed, there is no need for the proposed attack of the phosphate anion on the pyrophosphate to provide the exchange of labelled and unlabelled material. In this

case, the observation of the complete scrambling of labelled and unlabelled oxygen does not exclude the mechanism proposed by Zwierzak[1,2].

7.3.3 *Oxygen-labelled phencyphos derivatives in the formation of pyrophosphates*

The presence of ^{18}O induces a small upfield shift in the ^{31}P NMR resonance frequency of phosphates (*ca.* 0.02 ppm for each ^{18}O bond present), the magnitude of which is related to the *P–O* bond order[18]. Cullis and co-workers[5] used oxygen-labelled phosphoric acid chloride to follow the scrambling process of the oxygens in the formation of pyrophosphates. In their system a $P^{18}O$ single bond produces an upfield shift of 0.023 ppm in the ^{31}P NMR spectrum and a $P^{18}O$ double bond results in a shift of 0.035 ppm; the effects are additive.

The use of oxygen-labelled (*R*)-**7.2a** and unlabelled (*R*)-**7.19** could provide us with information about the oxygen scrambling and, subsequently, the mechanism of this reaction. Oxygen labelled (*R*)-**7.2a** was easily prepared following the *normal* synthetic route, using $H_2{}^{18}O$ to perform the Arbuzov rearrangement. This yields (*R*)-**7.2a** with 85% labelled and 15% unlabelled oxygen (Scheme **7.14**), as can be seen in the decoupled ^{31}P NMR spectrum, showing two signals with $\Delta\delta$ of 0.034 ppm[19].



Scheme 7.14 Synthesis of ^{18}O labelled phosphorinane 7.2a

The reaction of ^{18}O-labelled (*R*)-**7.2a**, and therefore (*R*)-**7.1**, with (*R*)-**7.19** (both 1.0 mmol), Et_3N (0.1 mL) and CCl_4 (0.1 mL) in C_6D_6 (2.0 mL) at 30 °C, according to Scheme **7.12**, yields pyrophosphate **7.3** with extensively, though not completely, scrambled oxygens (Figure **7.5**).

A total of six signals is observed in the ^{31}P NMR spectrum of the pyrophosphate, which accounts for the formation of all possible combinations of unlabelled and ^{18}O-labelled pyrophosphate **7.3**, as is depicted in Scheme **7.15**. At this stage it has to be noted that the symmetrical pyrophosphates **7.3a**, **7.3c**, **7.3d** and **7.3f** give one signal in the ^{1}H decoupled ^{31}P NMR spectrum, whereas the unsymmetrically labelled molecules **7.3b** and **7.3e** show an extreme AB system, leading to one apparent signal halfway between the two expected absorptions[19,20]. The ^{31}P NMR chemical shift difference in this type of labelled molecules typically resembles the coupling constant $^{2}J_{PP}$, giving rise to three signals instead of four. An extreme AB system under these conditions gives rise to the observation of only one signal in the ^{31}P NMR spectrum[20].

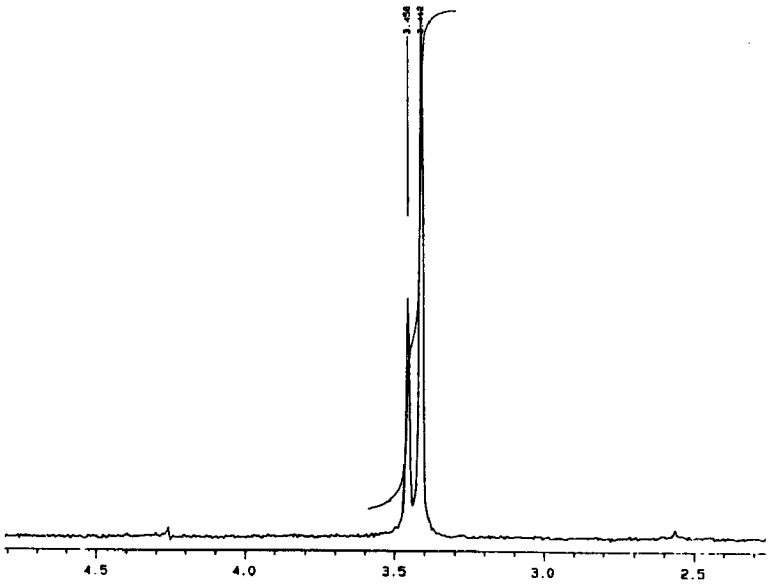
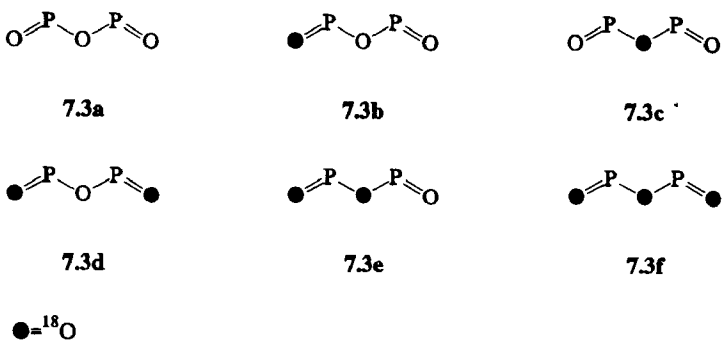


Figure 7.5 ^{31}P *NMR spectrum of labelled 7.3, recorded in* $CDCl_3$ *[L]= 0.01 M, T= 30°*



Scheme 7.15 Possible scrambled combinations of ^{18}O *labelled 7.3, other substituents at phosphorus omitted for clarity*

As expected, the main product (40%) resembles mono *P=O* labelled pyrophosphate **7.3b**, which is formed from labelled (*R*)-**7.1** and (*R*)-**7.19**, giving rise to a signal in the ^{31}P NMR spectrum at δ -20.60 ppm. Bis *P=O* labelled pyrophosphate **7.3d** and the *P=O* and *P-O* labelled pyrophosphate **7.3e** are formed also in reasonable amounts (25 and 20%, respectively), whereas the other three pyrophosphates **7.3a**, **7.3c** and **7.3f** are formed in small amounts. Another interesting observation emerges from the ^{31}P NMR spectrum as shown in Figure **7.6**. Because of incomplete degree of labelling (85% labelled **7.2a** was converted into 85% labelled **7.1**), it can be concluded that the doublet at δ -16.32 ppm,

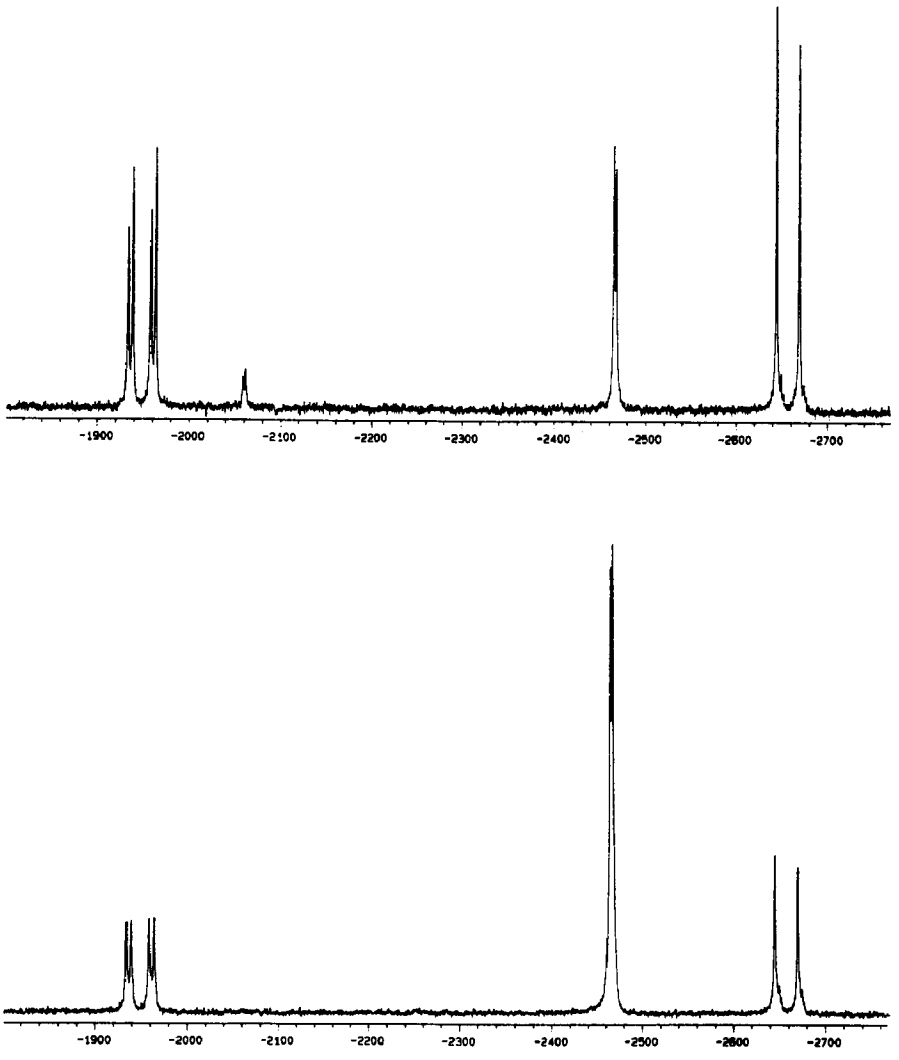


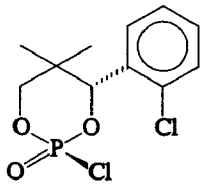
Figure 7.6 *^{31}P NMR spectra of the formation of ^{18}O labelled 7.3 and intermediates leading to it, recorded in $CDCl_3$ [L]= 0.01 M, T= 30 °C (axis in Hz)*

which is assigned to intermediate **7.29** (Scheme **7.12**), arises from the phosphorus atom bearing the chlorine. These signals double in the initial stages of the reaction, showing the 85:15 ratio that was present in **7.1**. The other doublet, at δ -22.20 ppm, belongs to the other part of intermediate **7.29**, arising from unlabelled phosphoric acid **7.19**. The labelled

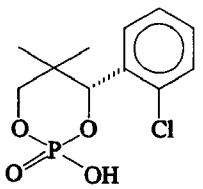
and unlabelled intermediate **7.29** show, however, the same $^2J_{PP}$ coupling of 33.01 Hz. The situation becomes more complex as the reaction proceeds, which leads to the formation of several doublets from intermediates that have one or more scrambled oxygens. This leads to the formation of various ^{18}O scrambled pyrophosphates **7.3**, in accordance with observations made by Cullis and co-workers[5] for closely analogous systems. These observations can be explained by hydrolysis of the initially formed pyrophosphate **7.3b**, resulting in unlabelled and labelled phosphate anions **7.19**, of which the latter reacts with labelled **7.1** to give double labelled pyrophosphates. Following the same reaction sequence, all the combinations of labelling can be obtained, leading to complex spectra, due to low resolution and partly overlapping signal groups. The other possibility, the attack of phosphate anion **7.19** on pyrophosphate **7.3b**, with subsequent release of unlabelled or labelled phosphate anion **7.19**, leads to the same type of intermediate signals. Therefore, definitive conclusions concerning these aspects of the reaction mechanism cannot be drawn at this stage.

7.3.4 *The use of combinations of substituted phencyphos acids and acid chlorides in the formation of pyrophosphates*

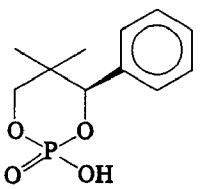
Although the experiments described above provide us with a rich source of information, several questions remain unanswered. For example, it is not clear whether the anion of (*R*)-**7.19** (Table **7.2**, entry *10*) is formed by hydrolysis of pyrophosphate (*R,S*)-**7.3** into two equivalents phosphate anion **7.19** (*R* and *S* form) or that pyrophosphate **7.3** is attacked by a phosphate anion **7.19**. This yields phosphate anion with *R* or *S* configuration. Also, chloride attack (or hydrolysis) of (*R*)-**7.1** can possibly lead to the observed phenomena (see discussion above). An experiment using two different phosphorinane reagents **7.30** and **7.31** (Scheme **7.16**) should provide us with experimental data concerning this problem.



7.30



7.31



7.19

Scheme 7.16 Reagents 7.19, 7.30 and 7.31

When (*R*)-chlocyphos acid chloride **7.30**[17] is allowed to react with (*R*)-**7.19** (both 1.0 mmol) and Et_3N (0.1 mL) in $CDCl_3$ (2.0 mL) at 30 °C, the ^{31}P NMR spectrum clearly indicates the formation of the mixed pyrophosphate **7.32** (Scheme **7.17**). The pyrophosphate gives two doublets at δ −20.37 and δ −20.84 ppm, with a $^2J_{PP}$ coupling of 23.19 Hz. Furthermore, pyrophosphate **7.3** (δ −20.55 ppm) and **7.33** (δ −21.19 ppm) are formed also

(Figure 7.7 and Scheme **7.17**). The formation of the three pyrophosphates can be explained by either of the four proposed mechanisms (see Section 7.3.1).
The observation, however, that *four* different intermediates are formed during the course of the reaction, leading to the formation of *three* different pyrophosphates (see Figure **7.7**), is unique, and offers the possibility to investigate this reaction at the level of the intermediate structures themselves. Because of the low resolution in the 1D ^{31}P NMR spectrum, this is most clearly seen in the 2D phosphorus COSY NMR spectrum[21], as is shown in Figure **7.7**.

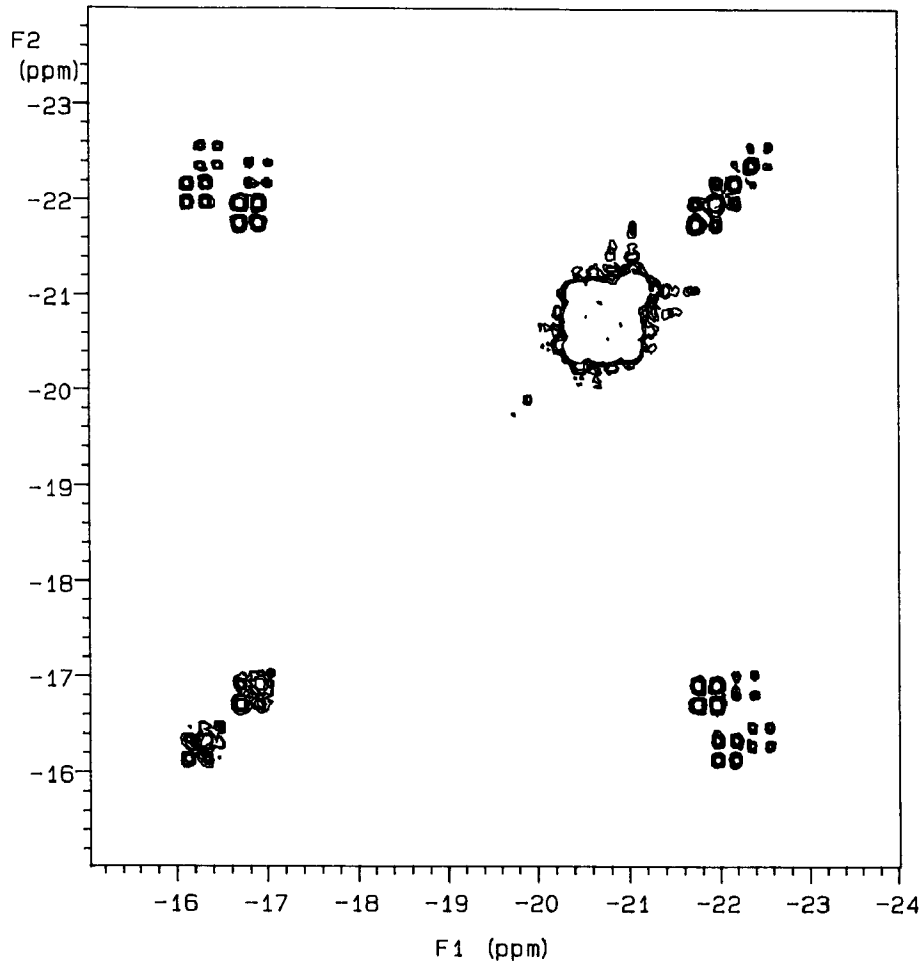
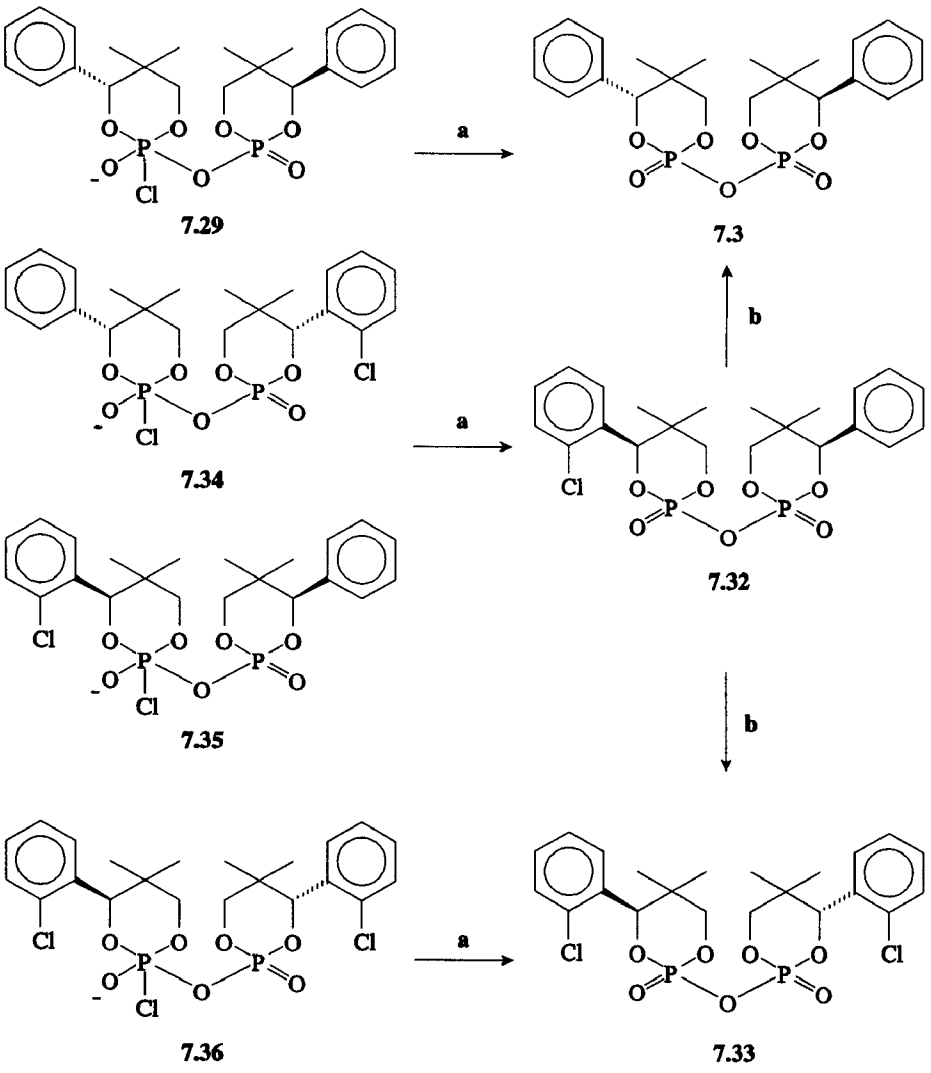


Figure 7.7 *^{31}P COSY spectrum of the reaction products of (R)-7.19 and (R)-7.30, recorded in $CDCl_3$ [L]= 0.01 M*

The four different intermediates, for which we propose structures **7.29** and **7.34** to **7.36**,

are given in Scheme **7.17**. Intermediate **7.35**, giving signals in the ^{31}P NMR spectrum at δ −17.60 ppm and δ −22.76 ppm ($^2J_{PP}$= 29.9 Hz) is formed by the reaction of acid chloride (*R*)-**7.30** with (*R*)-**7.19** and leads to the formation of pyrophosphate **7.32**.



Scheme 7.17 Intermediates and pyrophosphates formed in the reaction of (R)-7.19 and (R)-7.30, see text for explanation

Knowing, however, that the signals situated around δ −16 to −17 ppm belong to the chloride substituted part of the intermediates 7.29, 7.34, 7.35 and 7.36, it is obvious that at some stage in the reaction leading to the pyrophosphates 7.3, 7.32 and 7.33, phosphoric

acid chloride (R)–7.1 is also formed. Alternatively, chloride attack at 7.32 could also give a chloride substituted (R)–7.1 part.

The other intermediate signals be can assigned as follows: intermediate **7.29** gives signals at δ –16.29 and δ –22.20 ppm ($^2J_{PP}$= 33.01 Hz); intermediate **7.34** yields signals in the ^{31}P NMR spectrum at δ –16.40 and δ –22.58 ppm ($^2J_{PP}$= 27.03 Hz). The signals belonging to intermediate **7.36** are located at δ –17.02 and δ –22.17 ppm, showing a $^2J_{PP}$ coupling of 27.74 Hz. These assignments were made because of corresponding coupling constants and integration of the absorptions that belong to a single isomer group. The results are collected in Table **7.3**.

compound	δ ^{31}P NMR (ppm)	δ ^{31}P NMR (ppm)	J_{PP} (Hz)
d,l 7.3	–20.56	–	–
7.29	–16.29	–22.20	33.01
7.32	–20.37	–20.84	23.19
7.33	–21.19	–	–
7.34	–16.40	–22.58	27.03
7.35	–17.60	–22.76	29.90
7.36	–17.02	–22.17	27.74

Table 7.3 *^{31}P NMR chemical shifts and coupling constants of intermediates leading to the formation of mixed pyrophosphates 7.3, 7.32 and 7.33*

Moreover, experiments using (*R*)–**7.1** and (*R*)–**7.31** in initial stages, showed the groups of signals as discussed belonging to the four intermediates, as well as (*R*)–**7.30** and (*R*)–**7.31**. Furthermore, the three pyrophosphates formed are converted after some time (about 24 h) into the symmetrical pyrophosphates **7.3** and **7.33**, which demonstrated a large preference of the phencyphos units for the identical counterparts (Figure **7.8**, Scheme **7.17**).

The only acceptable explanation for this observation is based on the assumption that the initially formed pyrophosphate **7.32** is attacked by the liberated chloride ion, yielding either acid chloride (*R*)–**7.1** or (*R*)–**7.30**, or free acid (*R*)–**7.19** and (*R*)–**7.31**. It should be noted that pyrophosphate **7.32** can be attacked also by anion (*R*)–**7.19**, yielding pyrophosphates **7.3**, **7.33** and **7.32** and also the anions (*R*)–**7.31** and (*R*)–**7.19.**

The observation that the chloride ion (see also Section 7.3.4) acts as a nucleophile attacking the pyrophosphate **7.3** is not accounted for by Zwierzak[2] or Cullis[5], although it forms an explanation for the scrambling of the labelled oxygens without the need of dioxaphosphetane **7.14** and **7.15** formation as proposed by Cullis (Scheme **7.8**). At this point it is not clear whether one of the three earlier discussed mechanisms are also operating, or that the proposed attack of the chloride ion actually is the major route followed (see page 187, Route three).

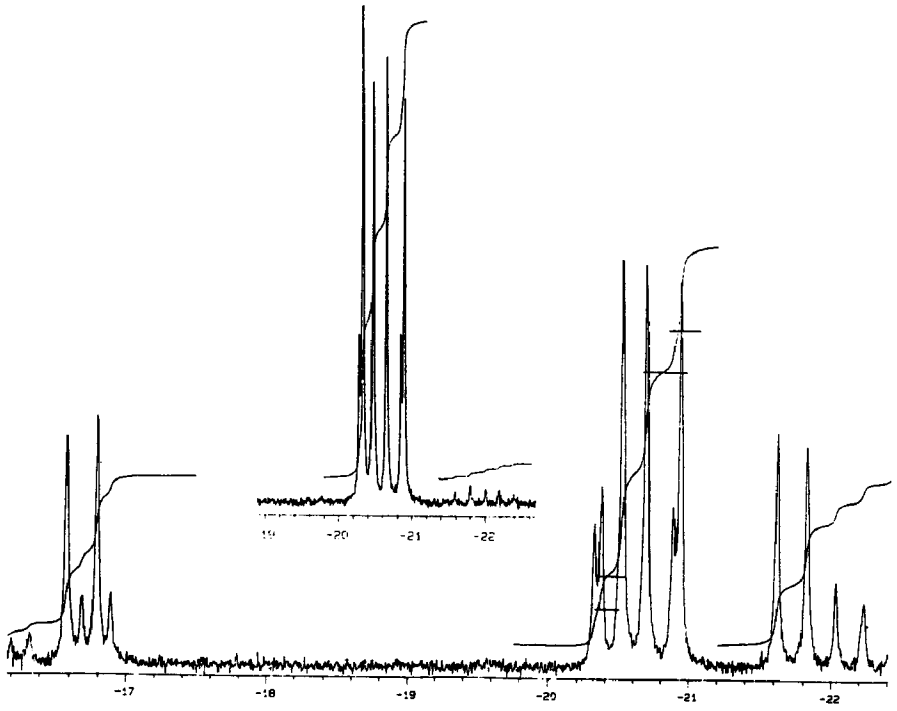


Figure 7.8 ^{31}P *NMR spectrum of the reaction products of (R)-7.19 and (R)-7.30 after 1 and 24 h (inset), recorded in* $CDCl_3$

7.3.5 *The use of phosphorus COSY techniques in the elucidation of the formation of pyrophosphate*

During the course of the research described in the previous Chapters, it became obvious that the rate of the reaction of (*R*)-**7.1** and (*R*)-**7.19** yielding pyrophosphate **7.3** showed a large dependence upon the temperature. Normally, experiments were performed at 30 °C, unless stated otherwise. To our surprise, however, experiments carried out using (*R*)-**7.1** and (*R*)-**7.19** at temperatures between the 45 and 50 °C showed an even more complex behaviour than expected on the basis of previous ^{31}P NMR analysis, as is shown in Figure **7.9**. The (only) final product again appeared to be pyrophosphate **7.3**.
The 1D spectrum shows the signals of intermediate **7.29**, at δ -16.29 and δ -22.20 ppm ($^2J_{PP}$= 33.01 Hz), and pyrophosphate **7.3** (δ -20.56 ppm). Besides these signals, several absorptions are observed that show extensive phosphorus coupling, which is a strong indication that these intermediates contain more than one, nonequivalent phosphorus atoms, connected with each other through a bridging oxygen atom.

The signal at δ −17.23 ppm (singlet), which is observed regardless the phosphoric acids used, cannot be assigned definitively. It is likely, that small amounts of degrated (poly)-phosphorus components give rise to this absorption.

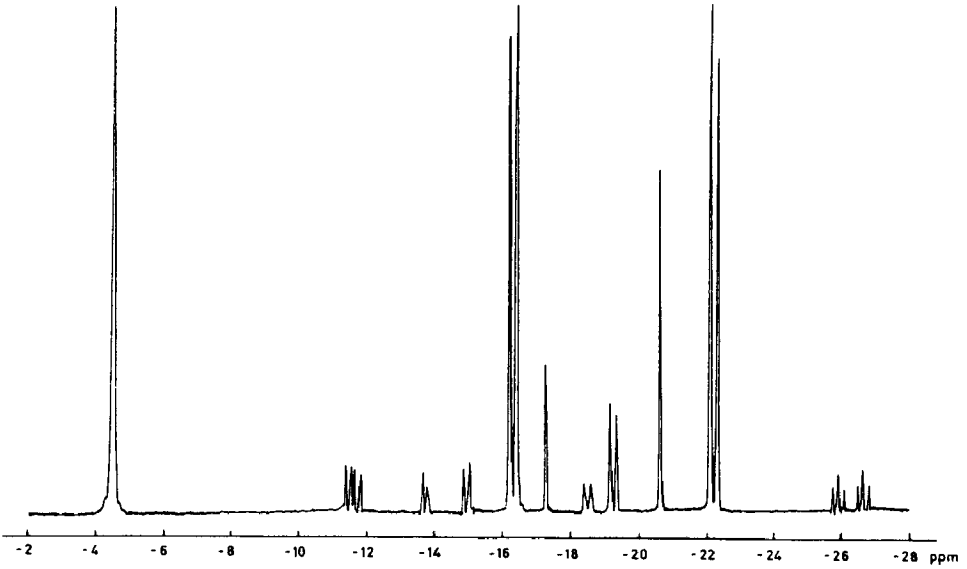


Figure 7.9 ^{31}P *NMR spectrum during the formation of (R)−7.3 at 47.5 °C, recorded at −50 °C, solvent* $CDCl_3/C_6D_6$ *[L]= 0.01 M*

The other signals, all showing nearly the same coupling constants, are less easily assigned by the use of 1D decoupled ^{31}P NMR spectra, although the integration is of great help for the full assessment of the 2D phosphorus COSY spectrum that is shown in Figure **7.10**. The recording of the 2D ^{31}P COSY spectrum, concerning the intermediates of a still developing reaction, appeared to be rather troublesome, because of the larger time interval needed to record a 2D spectrum. The intermediates were allowed to build up at 47.5 °C. When they had developed adequately (based upon the 1D ^{31}P NMR spectral data), the temperature was quickly (within 30 sec) brought to −50 °C. This temperature allowed us to record a 2D phosphorus COSY spectrum, without observable progression of the reaction (only a small amount of $CDCl_3$ had to be added to the reaction mixture to prevent freezing). The experiments were performed using unlabelled as well as with labelled materials to obtain maximum information from the 2D phosphorus COSY experiments. The results as obtained are collected in Table **7.4**.

The doublets at δ −11.43 and δ −13.73 ppm are connected, giving a $^2J_{PP}$ coupling of 18.22 Hz, and probably arise from intermediate **7.37** (Scheme **7.18**). Although there is no *direct* evidence for this assigment, the observed chemical shift together with the fact that both signals at δ −11.43 and δ −13.73 ppm double when partially oxygen labelled **7.1**, formed in situ from labelled **7.2a**, is used in this reaction, forms a strong indication that this is a

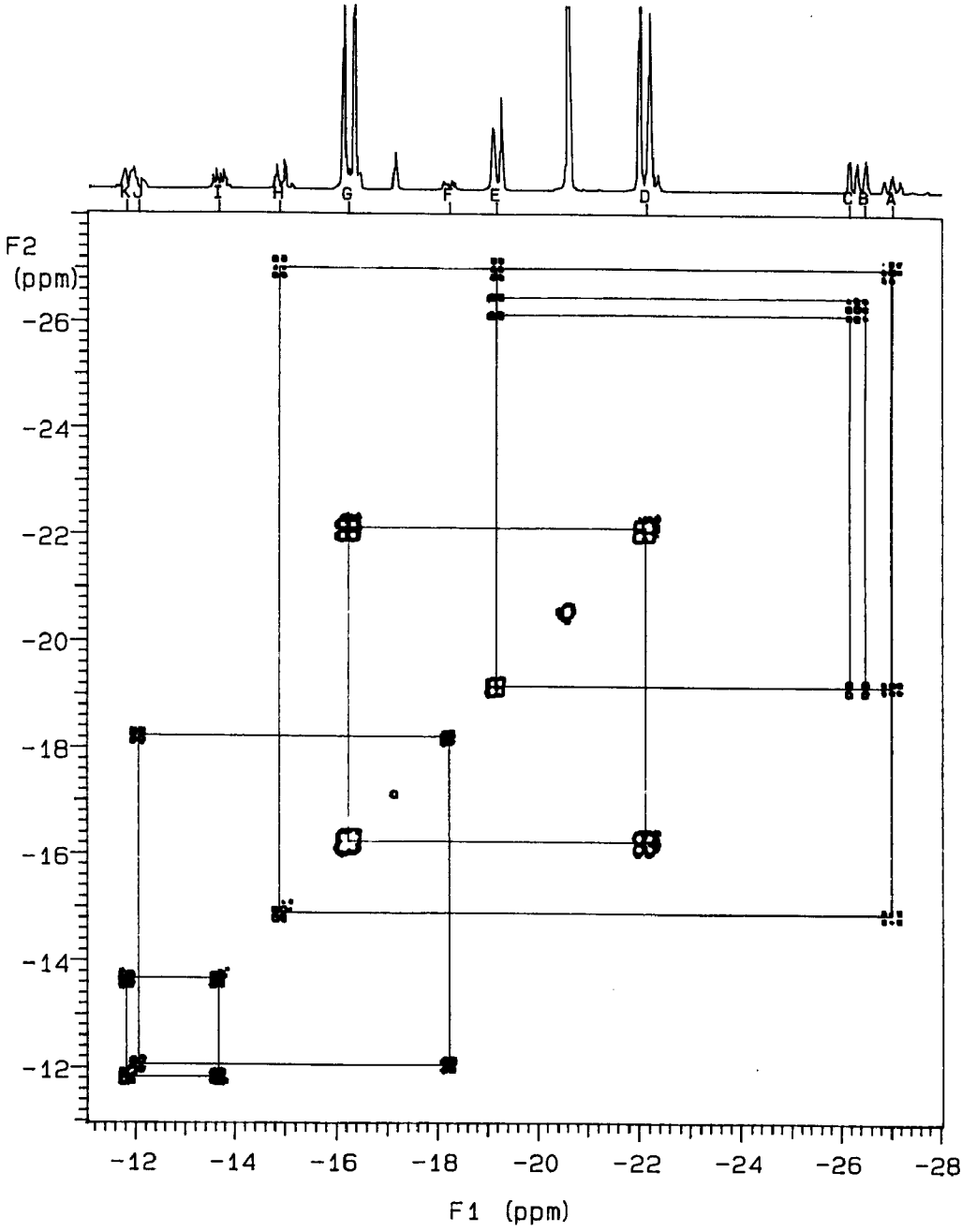
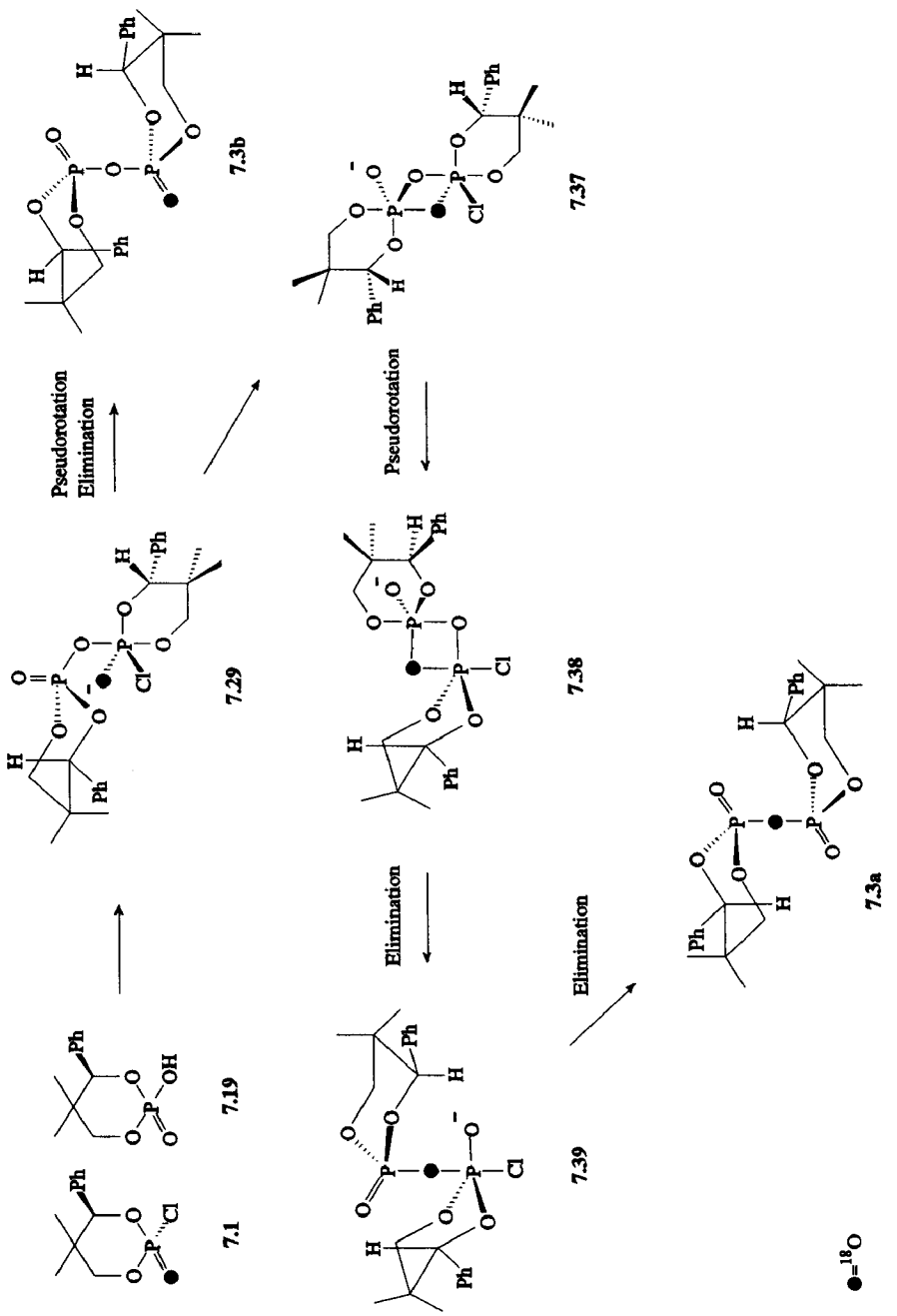


Figure 7.10 ^{31}P *NMR COSY spectrum during the formation of (R)-7.3 recorded at -50° C, solvent* $CDCL_3$ *[L]= 0.01 M*



Scheme 7.18 The formation of 7.3 via dioxaphosphetanes

phosphetane intermediate. The labelled oxygen is shared by the two phosphorus atoms. The same holds for the group of signals at δ −11.68 and −18.26 ppm ($^2J_{PP}$= 21.02 Hz). These also belong to two *O*-connected phosphorus atoms. The labelling experiment, as described above, shows that both signals double upon the use of partially ^{18}O labelled starting material **7.1**. This indicates that the labelled oxygen is bridged between the two phosphorus atoms. Based upon these observations and the chemical shifts, the signals could belong to intermediate **7.38**. The breakdown of **7.38** should give bridge ^{18}O labelled intermediate **7.39**, although it was not observed. Intermediate **7.39** contains a chlorine in the axial position. It is likely that the elimination of this chlorine is too fast to allow detection by NMR techniques.

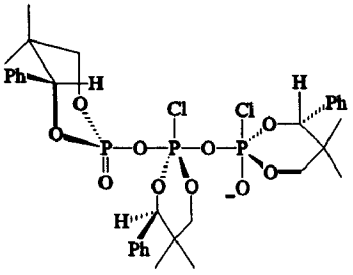
compound	δ ^{31}P NMR (ppm)	δ ^{31}P NMR (ppm)	δ ^{31}P NMR (ppm)	J_{PP} (Hz)
7.37	−11.43	−13.73	–	18.22
7.38	−11.68	−18.22	–	21.02
7.39	–	–	–	–
7.40	−14.92	−26.33	−19.22	22.41 and 22.39
7.41	−19.42	−25.90	−25.90	22.44 and 22.44

Table 7.4 ^{31}P NMR chemical shifts and coupling constants of intermediates and products of the reaction of 7.1 and 7.19, temperature 47.5 °C, recorded at −50 °C

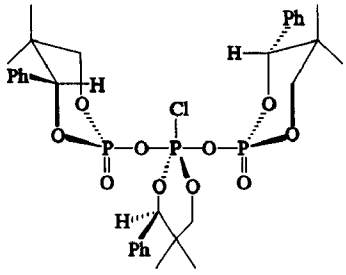
So far, the Cullis model provides an acceptable and unambiguous explanation for this part of the observations made. That the situation is more complex, however, is indicated by the appearance of two more *sets* of signals.

The responsible intermediates are built up of three connected phosphorus units, based upon the 2D COSY spectrum, as well as the observed coupling constants and integration data. The doublet at δ −14.92 ppm is connected with the double doublet at δ −26.33 ppm ($^2J_{PP}$= 22.41 Hz). The latter also shows a COSY cross interaction with the doublet at δ −19.22 ppm, leading to a $^2J_{PP}$ coupling of 22.39 Hz. It is clear that the phosphorus atom that is leading to the signal at δ −26.33 ppm is connected with two other, nonequivalent, phophorus atoms that are not connected themselves. The only possible explanation for this peculiar phenomenon is the coupling of three phosphorus-containing units into an intermediate non-cyclic trimeric structure. The use of partially oxygen-labelled **7.1** (the effects are unfortunately only observable in the early stages of the reaction) shows that the signals at δ −26.33 ppm double, and the signals at δ −14.92 ppm show a complex behaviour. The other signal, at δ −19.22 ppm, is not influenced by the oxygen labelling. The chemical shift data together with the integration data, coupling constants and the data obtained from the oxygen labelling experiments, indicate that the trimer intermediate might have structure **7.40** (Scheme **7.19**). This compound contains three different phosphorus atoms and is formed of phosphorus acid **7.19**, which has attacked phosphorus

acid chloride **7.1**. Subsequently, this unit attacks another acid chloride **7.1**, yielding the trimer structure as suggested.



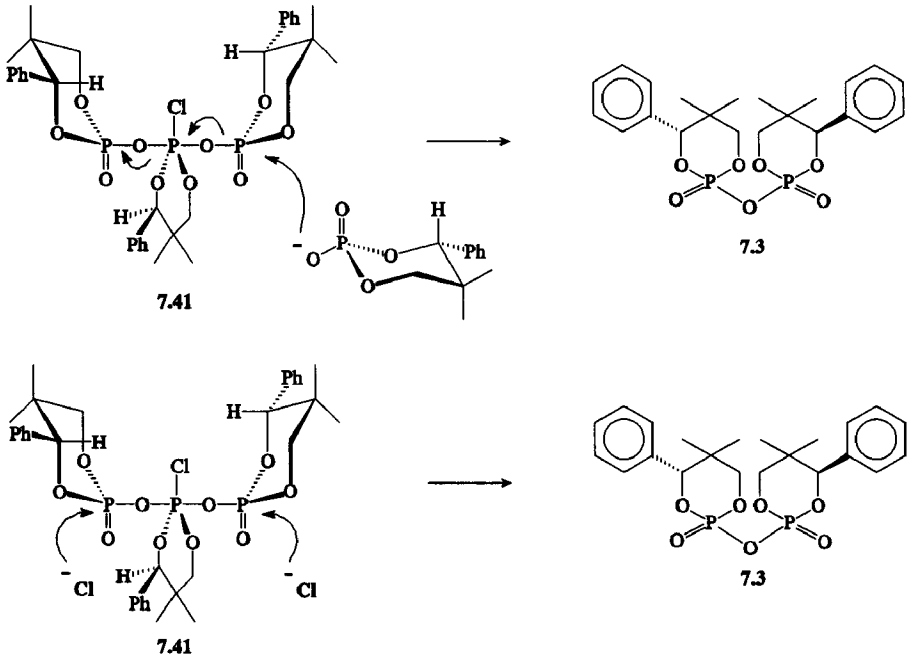
7.40



7.41

Scheme 7.19 *The proposed structures of intermediates **7.40** and **7.41***

The other trimer also contains three phosphorus atoms, although two of them appear to be equal, resulting in a doublet signal at δ −19.24 ppm ($^2J_{PP}$= 22.44 Hz) and a double doublet signal at δ −25.90 ppm. These signals show two equal $^2J_{PP}$ couplings of 22.44 Hz and a measured intensity half the size of the signal at δ −19.24 ppm.



7.41

7.3

7.41

7.3

Scheme 7.20 *Alternative mechanism for the formation of pyrophosphate **7.3***

The use of partially ^{18}O labelled **7.2a** leads to the doubling of the signals at δ −25.90 ppm and to a partial doubling of the signal at δ −19.24 ppm, while a part of this signal remains unaffected (again, only in the early stages of this reaction). Obviously, phosphoric acid **7.19** reacted with one equivalent of acid chloride **7.1**, that on its turn reacted with another molecule of acid chloride **7.1**, giving intermediate **7.41**. This is the elimination product of intermediate **7.40**. Intermediate **7.41** can only be transferred into **7.3** after another attack of a molecule of deprotonated phosphorus acid **7.19** has taken place. This would afford one molecule of acid **7.19** and one molecule of acid chloride **7.1**, together with one molecule of pyrophosphate **7.3** (Scheme **7.20**). Alternatively, chloride attack on **7.41** could result in one molecule of **7.1** and one molecule of pyrophosphate **7.3**, together with one chloride ion (Scheme **7.20**). Furthermore, intermediate **7.40** can either be converted into **7.41** by chloride elimination or pyrophosphate **7.3** by elimination of **7.1** and a chloride ion.
Unfortunately, it appeared not possible to generate the same type of intermediate structures by using one of the other phosphorus acids or acid chlorides with substituted phenyl groups, or combinations of acids and acid chlorides, regardless the reaction conditions employed (see Section 7.3.3). It is clear that besides the *direct* route as described in Section 7.3.1 and the chloride attack, followed by the direct route as described in Section 7.3.3 another reaction mechanism, involving trimeric structures, can also give rise to the formation of pyrophosphate **7.3**.

7.4 *Conclusions*

In this chapter an unusual *minor* side reaction is described that occurs when reagents **7.1** or **7.2** are used for enantiomeric excess determination of amines, alcohols or amino acids. Although this reaction does not influence the actual analyses, the appearance of side products gave rise to a more detailed investigation.
The products formed were characterized as pyrophosphate **7.3** and intermediate products leading to its formation. Due to the conformational *locking* of the phenyl group in the equatorial position of the phencyphos building–units, the pseudorotation products are blocked, and in this way allow detection of intermediates using ^{1}H and, in particular, ^{31}P NMR techniques. Based upon the data from several NMR experiments, the use of ^{18}O–labelled phosphoric acid chloride **7.1** and the stereochemical information from racemic and homochiral phosphoric acid chloride **7.1** and acid **7.19**, it was concluded that the nucleophilic displacement reaction proceeds with complete stereocontrol at the phosphorus atom. An explanation can be given on basis of a mechanism involving phosphetane intermediates (*mechanism 1*), as is depicted in Scheme **7.18**. These results are in agreement with the results using related *achiral* compounds, as reported by Zwierzak[1,2] and Cullis[5].
More important, however, is the fact that the results as obtained by Cullis and Zwierzak are only based upon the products obtained. Using the phencyphos derivatives, several intermediate structures can actually be observed using ^{31}P NMR techniques.
Labelling experiments with ^{18}O labelled **7.2a**, and hence **7.1** together with the use of racemic phosphoric acids **7.19** and **7.31** or phosphoric acid chlorides **7.30** and **7.1**, made the assignment of the observed intermediates possible. Furthermore, the scrambling of the

oxygen over all possible exocyclic oxygens in this system showed that not only a direct nucleophilic displacement reaction of phosphoric acid **7.19** and phosphoric acid chloride **7.1** occurred, but also that a *second* mechanism must operate (Scheme **7.17** and **7.18**).
The use of *mixed* phencyphos acids (**7.19** and **7.31**) and acid chlorides (**7.1** and **7.31**) showed that the eliminated chloride is able to act as a nucleophile, attacking the mixed pyrophosphate **7.32**. This yields combinations of phosphoric acid chlorides and reaction intermediates, which were not apparent at the start of the reaction following mechanism 1, or could not be formed during the reaction otherwise. These observations *could* be another explanation for the scrambling of the ^{18}O label.
Using 2D COSY NMR techniques, together with ^{18}O labelling experiments, a *third* mechanism was found (Scheme **7.20** an **7.21**). In this mechanism, acyclic trimer phosphorus containing intermediates are formed, that lead to the direct formation of pyrophosphate **7.3**, or to the formation of the pyrophosphate after the attack of a deprotonated phosphoric acid. This results in complete scrambling of ^{18}O labelled oxygens over all the exocyclic oxygens. Alternatively, chloride attack on the trimer intermediates would also give the same products. In this way, phosphoric acids and phosphoric acid chlorides bearing a ^{18}O label are formed, that were not present at the beginning of the reaction. These compounds can react further, and give rise to double or triple ^{18}O labelled pyrophosphate **7.3**.
Although probably all three mechanisms, involving (*a*), the formation of phosphetanes as proposed by Zwierzak and Cullis and co-workers, (*b*) by means of a chloride attack at the pyrophosphate formed or trimer formation (*c*), followed by attack of deprotonated phosphoric acid or chloride, are not in disagreement with each other, the appearance of the least two routes has never been proposed or observed before.
It is remarkable that, although the reaction routes are rather complex and markedly different, the final product, pyrophosphate **7.3**, is always formed as the only enantiomerically pure, product. This means that the reactions proceed with complete stereocontrol with regard to the phosphorus atom, regardless the route followed.

7.5 *Experimental*

See for details concerning the NMR experiments Section 2.5. All NMR measurements were performed in well dried and sealed NMR tubes, using dried solvents.
The phosphoric acids and phosphoric acid chlorides were prepared as described by Ten Hoeve and Wynberg[17], and as described in Chapter 5. ^{18}O-labelled water (labelling degree around 85 %) was kindly provided by Dr. G. Visser (PET Center, University Hospital, Groningen). Phencyphos pyrophosphate **7.3** was prepared for comparison purposes according to the method described by Edmundson[4b].

2,2'-Oxy-bis-(5,5-dimethyl-4-(R)-phenyl-1,3,2-dioxaphosphorinan-2-one) (7.3)
White crystalline material. Mp 143-145 °C; ^1H NMR ($CDCl_3$): δ 1.81 (s, 6H), 1.05 (s, 6H), 4.01-4.20 (ddd, 2H), 4.62-4.79 (d, 2H), 5.43 (dd, 2H), 7.19-7.38 (m, 10H); ^{13}C NMR ($CDCl_3$): δ 16.99 (dd, CH_3), 20.49 (dd, CH_3), 45.43 (CH_2), 79.76 (dd, C), 89.29 (dd,

CH), 127.16 (CH), 127.76 (dd, CH), 128.54 (CH), 134.49 (C); ^{31}P NMR ($CDCl_3$): δ 20.56 ppm; HRMS calcd 466.131, found 466.131.

7.6 *References*

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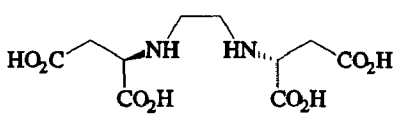
Summary

The synthesis of enantiomerically pure compounds is an important objective of the organic chemistry. Especially in the field of medicine and pesticides, mostly one enantiomer is responsible for the biological activity. Certain examples are known where the other enantiomer is more than *isomerical balast*, and its presence gives rise to side-effects. It is not surprising that the regulatory authorities in Europe and the United States usually impose that chiral compounds are marketed as single enantiomers. Growing interest and improvement in *enantioselective synthesis* leads to an increased demand for accurate, reliable and convenient methods of measuring the enantiomeric composition. A large number of methods for the determination of the enantiomeric excess (*e.e.*) of regardless which chiral substrate have been developed over the years. A popular, accurate, and moreover, fast method for the determination of the enantiomeric composition is to make use of NMR techniques (Nuclear Magnetic Resonance).

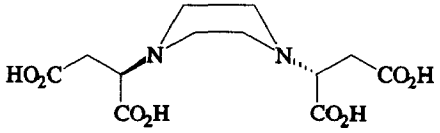
This thesis describes the research carried out towards the development of new methods for enantiomeric excess determination of amino acids and not naturally occuring α-alkylated amino acids by means of NMR techniques.

In Chapter 1 an introduction is given about the determination of the enantiomeric excess in general and amino acids in particular. Several definitions are treated together with a short review of the most important analyzing methods. The few NMR methods known for the enantiomeric excess determination of amino acids are discussed. Problems associated with the *e.e.* determination of amino acids are described, illustrating that the difficulties arise from the presence of two reactive groups and the restricted solubility in organic solvents, making water the solvent by necessity.

Chapter 2 focusses on the synthesis and the use of the chiral ligands **1**, **2** and **3** that, after complexation to europium, yield paramagnetic, water-soluble shift reagents (Scheme **1**).



1



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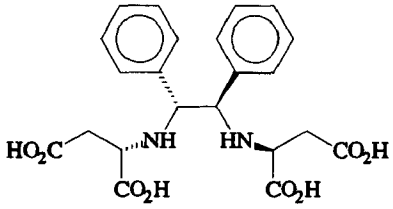
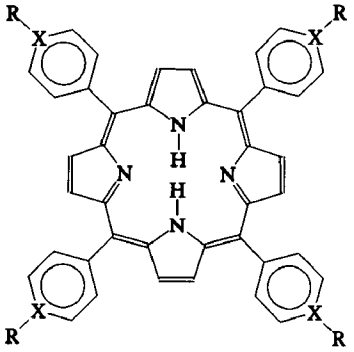


3

Scheme 1

The ligands are available from menthyloxyfuranone, a synthon developed in our research group. In contrast with known water-soluble shift reagents, our synthetic route offers the opportunity to modify the ligand systems structurally. The enantiomeric excess of a large number of α-amino acids and α-alkylated amino acids is determined by the use of these systems. Especially with ligand **3**, large diastereomeric shift dispersion was obtained. This is important because of the line broadening that takes place when paramagnetic shift reagents are used. Chapter 2 also deals with the synthesis and use of Co^{III}-porphyrines in the enantiomeric excess determination (Scheme **2**). These systems are capable of complexing two amines or two amino acids in axial positions, by which process diastereomers are formed. The obtained diastereomeric shift differences, however, were not sufficient to warrant adequate determination of the enantiomeric composition. Also ^{59}Co NMR techniques, appeared not to be a reasonable alternative.

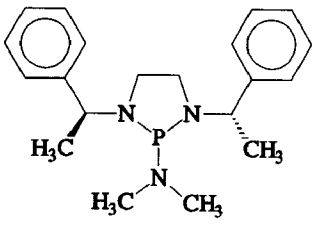


X= C; R= H, Me, COOH, OMe
X= C; R= SO_3H X= N

4

Scheme 2

The use of other than ^1H NMR techniques in the enantiomeric excess determination is described in Chapter 3. The synthesis and the use of trivalent phosphorus containing derivatizing agents **5** and **6** are treated (Scheme **3**). The derivatizing reagents are readily available from optically pure (*S*)-α-phenylethylamine and HMPT; their use in the



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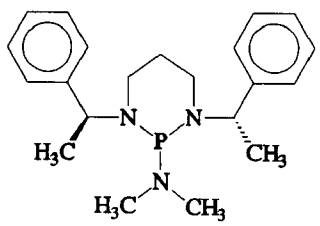
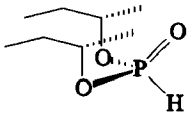


6

Scheme 3

enantiomeric excess determination of chiral alcohols, amines, amino alcohols, thiols and

esters of amino acids gives rise to large diastereomeric shift dispersions in the decoupled ^{31}P NMR spectra.
Although it appeared to be possible to determine the enantiomeric composition of several free amino acids, reagents **5** and **6** are not stable towards water as solvent. Therefore, several modified systems were developed, that, although not stable towards water as solvent, appeared to be remarkably stable.
Pentavalent phosphorus containing reagents are also successful in the enantiomeric excess determination of amino acids, as is presented in Chapter 4. The structurally simple, chiral, phosphorinane **7** (Scheme 4) is readily coupled with free amino acids, using water as



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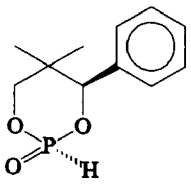

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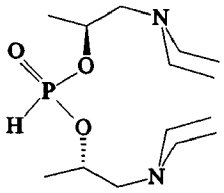
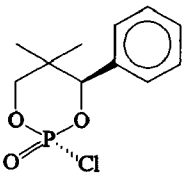


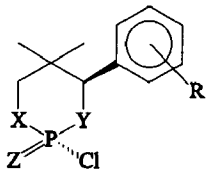
9

Scheme 4

solvent. Using ^{31}P NMR the enantiomeric composition of chiral amines, amino alcohols, alcohols, thiols and unprotected amino acids is easily revealed. Furthermore, the method shows a large tolerance towards the used solvents, including water. In Chapter 4 the synthesis and the application of chiral phosphorinanes **8** and **9** (Scheme 4) are described also. Reagent **8** is prepared from phencyphos developed by Ten Hoeve and Wynberg, which is available in both enantiomers. Reagent **8** is coupled to amino acids in water-containing solvents, yielding diastereomers, allowing analysis by ^{1}H or ^{31}P NMR. Phosphorinane **9** gives, after coupling with free amino acids, a pH-dependent diastereomeric shift dispersion. This behaviour is explained by a restriction of the conformational freedom, due to formation of internal tight ion pairs.
Phosphoric acid chlorides can also be used in the enantiomeric excess determination, as is shown in Chapter 5. Using reagent **10** (Scheme 5), based upon phencyphos, diastereomeric



10

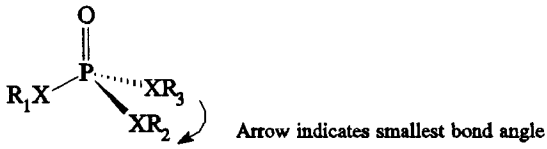


11

X= O, NH
Y= O, NH
Z= O, S

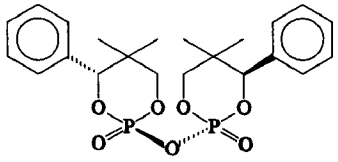
Scheme 5

products are obtained with chiral amines, alcohols, amino alcohols, thiols and esters of amino acids. Subsequent ^{31}P NMR analysis provides the enantiomeric excess. Reagent system **10** is easily structurally modified, as is proven by model system **11**. The structural modifications possess a large influence upon the diastereomeric shift dispersion after reaction with amino acids.
In Chapter 6 a conformational survey regarding the relation between the conformation of the compounds treated in Chapter 5 and the diastereomeric shift dispersion is described. A relation was found, showing that the smallest *O–P–O* bond angle of both diastereomers (Scheme **6**) correlates linear with the diastereomeric shift differences in the decoupled ^{31}P NMR spectra. Using this methodology, diastereomeric shift differences can be predicted based upon the structure of the derivatizing agents.



Scheme 6

The use of derivatizing reagents **8** or **10** in the enantiomeric excess determination also gives rise to the formation of a side product: pyrophosphate **12** (Scheme **7**). In Chapter 7 reaction mechanisms for the formation of this product are given, based upon 1D and 2D



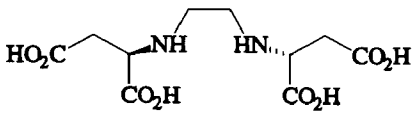
12

Scheme 7

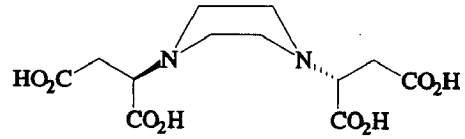
NMR techniques, like ^{31}P COSY. The formation of pyrophosphate **12** is shown to proceed stereoselectively with regard to phosphorus. Moreover, intermediates containing three phosphorus units were observed, and the role of nucleophilic chloride ions is discussed. This type of intermediates has not been observed before, and their appearance made the study of the reaction mechanisms possible. The elucidation of the mechanism of the pyrophosphate formation is of great importance. The phencyphos based pyrophosphate system appears to be a satisfactory model system.

Samenvatting

De synthese van enantiomeer zuivere verbindingen speelt een steeds belangrijker rol binnen de organische chemie. Met name bij geneesmiddelen en bestrijdingsmiddelen is het werkzame bestanddeel dikwijls een chirale verbinding, waarbij in vrijwel alle gevallen slechts één enantiomeer de gewenste effecten sorteert. In een aantal gevallen vormt het *verkeerde* enantiomeer zelfs meer dan enkel *isomere ballast*, en geeft de aanwezigheid hiervan aanleiding tot schadelijke bijwerkingen. Het is dan ook niet verbazingwekkend dat steeds strengere toelatingseisen worden gehanteerd voor het op de markt brengen van nieuwe synthetische geneesmiddelen; in vrijwel alle gevallen wordt geeist dat het product enantiomeer zuiver is. Om gehoor te kunnen geven aan deze ontwikkelingen is de beschikbaarheid van nauwkeurige, en liefst snelle, analysemethoden van primair belang. Er is dan ook een groot aantal methoden ontwikkeld om de enantiomere samenstelling van een breed scala aan chirale verbindingen te bepalen. Een populaire, accurate, en tevens snelle, methode om de enantiomere samenstelling, de *e.e.* (enantiomeric excess), te bepalen is door gebruik te maken van NMR-technieken (Nuclear Magnetic Resonance).
Dit proefschrift beschrijft het onderzoek naar de ontwikkeling van nieuwe methoden waarbij met behulp van NMR de enantiomere samenstelling van een bijzondere klasse van chirale verbindingen kan worden bepaald: van de α-aminozuren en de niet-natuurlijk voorkomende α-gealkyleerde aminozuren.
In Hoofdstuk 1 wordt nader ingegaan op de problematiek van de *e.e.* bepaling in het algemeen en van aminozuren in het bijzonder. Daartoe worden enige begrippen behandeld in samenhang met een kort overzicht van de belangrijkste analysemethoden. Tevens worden de weinige, reeds bekende, NMR-technieken voor de bepaling van de enantiomere samenstelling van aminozuren besproken, en wordt er ingegaan op de redenen waarom deze klasse van verbindingen kennelijk een probleem vormt in dit verband in vergelijking tot andere klassen van chirale verbindingen. Deze redenen zijn terug te voeren op de aanwezigheid van twee reactieve groepen in het aminozuur en de beperkte keuze van oplosmiddelen (water). Vrijwel alle bekende analysetechnieken zijn gebaseerd op het gebruik van organische oplosmiddelcombinaties.
In Hoofdstuk 2 zijn de synthese en het gebruik beschreven van de chirale liganden **1**, **2** en **3** die, na complexering aan europium, paramagnetische water-oplosbare shiftreagentia vormen (Schema **1**). Deze liganden zijn toegankelijk uitgaande van het in onze werkgroep ontwikkelde menthyloxyfuranon. In tegenstelling tot het gebruik van bestaande water-oplosbare shiftreagentia, biedt deze benadering de mogelijkheid de liganden structureel te modificeren. De enantiomere samenstelling van een groot aantal α-aminozuren en α-gealkyleerde aminozuren is met behulp van deze shiftreagentia bepaald. Met name bij het gebruik van ligandsysteem **3** worden grote diastereomere shiftverschillen verkregen, hetgeen van belang kan zijn om voldoende resolutie te verkrijgen in verband met de toenemende lijnverbreding bij het gebruik van paramagnetische shiftreagentia.
In Hoofdstuk 2 zijn verder de synthese en het gebruik van water-oplosbare, niet-chirale Co^{III}-porphyrines **4**, beschreven (Schema **2**). Deze systemen zijn in staat om met twee



1



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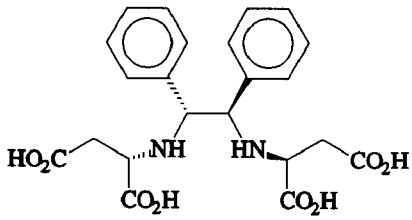
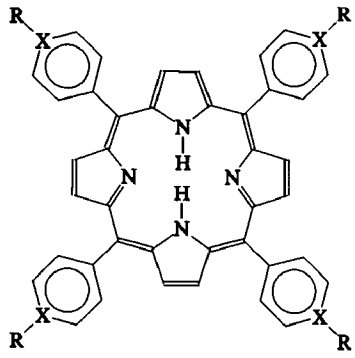


3

Schema 1

amines of twee aminozuren in de axiale posities te complexeren, waarbij diastereomeren ontstaan die vervolgens geanalyseerd kunnen worden. Helaas bleek de diastereomere shift-dispersie in een aantal gevallen niet voldoende te zijn om met behulp van ^{1}H NMR de enantiomere samenstelling te bepalen. Ook ^{59}Co NMR bleek geen redelijk alternatief te zijn, zoals is beschreven in Sectie 2.3.4.

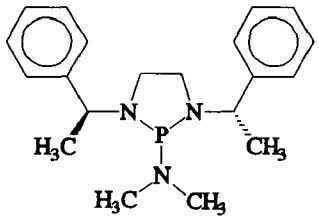


4

X= C; R= H, Me, COOH, OMe
X= C; R= SO_3H X= N

Schema 2

Dat andere dan ^{1}H NMR-technieken zeer attractief kunnen zijn, met name voor *e.e.* bepalingen, wordt in behandeld Hoofdstuk 3. Daartoe worden zowel de synthese en het gebruik van de op driewaardig fosfor gebaseerde derivatiseringsreagentia **5** en **6** beschreven (Schema 3). Deze reagentia zijn eenvoudig toegankelijk uitgaande van (*S*)-α-phenylethylamine en HMPT; ze geven in de bepaling van de *e.e.* door middel van de koppeling aan alcoholen, amines, amino alcoholen, thiolen en esters van aminozuren zeer hoge dia-



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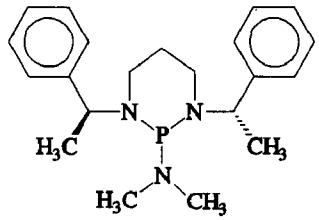
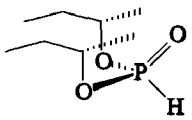


6

Schema 3

stereomere shiftdispersies in de ^{31}P NMR-spectra. Ondanks het feit dat, gebruik makend van deze methode, de analyse van een aantal vrije aminozuren eveneens mogelijk is, blijken reagentia **5** en **6** niet stabiel te zijn ten aanzien van water als oplosmiddel. Een aantal gemodificeerde systemen is dan ook ontwikkeld, die echter geen van alle kunnen worden gebruikt voor een effectieve *e.e.*-bepaling van vrije aminozuren. De opmerkelijke stabiliteit van sommige van de ontwikkelde derivaten geeft echter aanleiding tot vervolg-onderzoek.
Zeer succesvol is het gebruik van vijfwaardige fosforreagentia als derivatiseringsreagens, zoals wordt aangetoond in Hoofdstuk 4.



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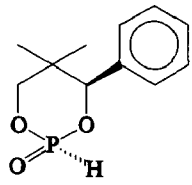

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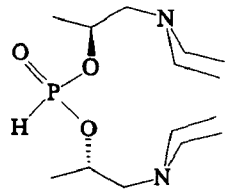
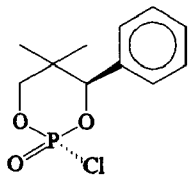


9

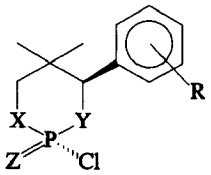
Schema 4

Met behulp van het synthetisch eenvoudig toegankelijke chirale fosfiet **7** (Schema **4**), kan in waterig milieu op eenvoudige wijze een koppelingsreactie met aminozuren worden uitgevoerd, waarna met behulp van ^{31}P NMR de enantiomere samenstelling kan worden bepaald. Behalve aminozuren kunnen tevens amines, (amino)-alcoholen en thiolen worden geanalyseerd, waarbij de gebruikte methodiek een grote oplosmiddeltolerantie blijkt te bezitten. De verkregen diastereomere shiftverschillen zijn over het algemeen ruim voldoende om een adequate *e.e.*-bepaling te garanderen. Tevens zijn in Hoofdstuk 4 de synthese en het gebruik van cyclische chirale fosfieten van het type **8** en **9** (Schema **4**) beschreven. Als bouwsteen voor fosfiet **8** wordt het door Ten Hoeve en Wynberg ontwikkelde phencyphos gebruikt, waarvan beide enantiomeren eenvoudig toegankelijk zijn. Nadat dit reagens, in waterige milieus, is gekoppeld aan aminozuren, kan met behulp van

zowel ^{1}H als ^{31}P NMR de enantiomere samenstelling worden bepaald. De diastereomere shiftdispersie is aanzienlijk groter dan die welke is bereikt met fosfiet **7**. Fosfiet **9** geeft, na koppeling aan vrije aminozuren, een pH-afhankelijke diastereomere shiftdispersie te zien, hetgeen wordt verklaard door een beperking van de conformationele vrijheid door de vorming van interne ion paren.
Hoofdstuk 5 behandelt de synthese van fosforzuurchlorides als derivatiseringsreagentia. Met behulp van het op phencyphos gebaseerde systeem **10** (Schema 5) zijn de diastereomere koppelingsproducten van amines, alcoholen, thiolen en esters van aminozuren met behulp van ^{31}P NMR-technieken te analyseren. Bovendien is het basisskelet zeer eenvoudig synthetisch te modificeren, hetgeen blijkt uit modelsysteem **11**. De structurele modificaties hebben een zeer grote, positieve, invloed op de verkregen diastereomere shiftdispersie na koppeling aan aminozuren.



10

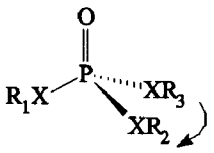


11

X= O, NH
Y= O, NH
Z= O, S

Schema 5

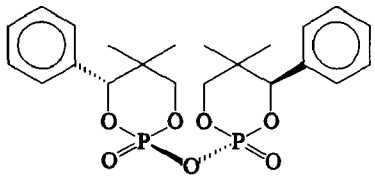
In Hoofdstuk 6 is een modellingonderzoek beschreven naar de relatie tussen de conformaties en de diastereomere shiftverschillen van alcohol- en amine-gekoppelde phencyphosderivaten, die zijn beschreven in Hoofdstuk 5. Er is een relatie gevonden, waarbij het verschil in de kleinste *O-P-O* bindingshoek voor beide diastereomeren (Schema **6**) lineair correleert met de gevonden diastereomere shiftverschillen. Deze ontwikkeling maakt het mogelijk de diastereomere shiftverschillen te *voorspellen*, en een gerichte synthese van de derivatiseringsreagentia uit te voeren.



Pijl geeft de kleinste bindingshoek aan

Schema 6

Gedurende de derivatiseringsreacties van fosfiet **8** of fosforzuurchloride **10** wordt dikwijls een nevenproduct gevonden. Dit product blijkt het pyrofosfaat **12** (Schema 7) te zijn. Hoofdstuk 7 behandelt het mogelijke mechanisme van de vorming van dit product. Met behulp van met name 2D NMR-technieken, zoals ^{31}P COSY, kon worden aangetoond dat de vormingsreactie stereoselectief is ten aanzien van de beide fosforcentra in **12**.



12

Schema 7

Tevens werd de aanwezigheid van intermediairen met drie verschillende fosforbevattende eenheden aangetoond. Ook bleek duidelijk dat chloride-ionen als nucleofiel kunnen dienen, waarbij waarschijnlijk het reeds ontstane pyrofosfaat **12** wordt aangevallen. Doordat voor het eerst intermediaren zijn waargenomen in dit type pyrofosfaat vormingsreacties, bleken we in staat te zijn het bestaan van meerdere, simultaan optredende, reactiemechanismen aan te tonen. De opheldering van het mechanisme van de pyrofosfaatvorming is van groot belang voor met name sommige biochemische processen. Het phencyphospyrofosfaat systeem is hiervoor een doeltreffend modelsysteem.