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Recueil Reviews

New methodologies for enantiomeric excess (ee) determination based on phosphorus NMR

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I Introduction

Although more than 50% of commercial drugs are chiral, less than half of these are marketed in an enantiomerically pure form. Dramatic examples of the differences in pharmacological responses of enantiomers are known: (S)-propranolol 1 (Scheme 1) is an antihypertensive and antiarhythmic used in the treatment of heart disease, whereas the R enantiomer acts as a contraceptive. The R enantiomer of asparagine 2 tastes sweet whereas the S enantiomer tastes bitter. Thalidomide 3, commercially known as Softenon, was originally used as a racemate. Only the R enantiomer is responsible for the desired (sedative) therapeutic effect whereas the S enantiomer causes teratogenic effects.

Such examples are a strong incentive for the industry to market chiral compounds as single enantiomers, in response to the requirements being imposed by the regulatory authorities in Europe and the United States. This resulted in a dramatic increase in research efforts towards the development of (new) chiral synths, catalysts and procedures leading to the synthesis of enantioselectively pure products. In particular the enormous improvement in enantioselective synthesis by means of stoichiometric or catalytic asymmetric transformations as well as kinetic resolution and biomimetic synthesis makes the availability of reliable analytical techniques for the correct assessment of the enantiomeric composition increasingly important.

An inherent difficulty in analyzing enantiomeric compositions is the fact that enantiomers have, apart from their chiroptical characteristics, identical physical and chemical properties (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. This involved measuring the optical rotation of the sample under accurately defined conditions and comparing the obtained value with the known rotation of the enantiomerically pure compound, measured under the same conditions. The optical purity determined this manner is often equated with the enantiomeric purity, although several examples are known in which uncritical use of absolute rotations quoted in the literature led to incorrect conclusions concerning the enantiomeric excess (ee) of an enantioselective reaction.

Any other method of distinguishing enantiomers must rely on the use of a chiral environment expressed by means of 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 distinguished four general approaches to determine the enantiomeric 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' or SR' to facilitate the determination. Thus it follows that the determination may be carried out on enantiomers with or without separation or on diastereomers with or without separation. A further distinction can be made depending on whether the determination of the enantiomeric composition can be performed without an auxiliary probe or only in the presence of a nonracemic chiral probe. Some examples are known in which an achiral auxiliary probe is used that reacts twice with the enantiomers to give diastereomers RR' or SR' to facilitate the determination. This has in large measure been a result of improvements in column lifetime and performance. In 1959 Karagunis and Lippold were able to separate the racemic mixtures arbitrary
butan-2-ol and of 2-bromobutane on optically active stationary phases making use of gas chromatographic (GC) methods. It took, however, seven more years before the first example of a fully reproducible separation of enantiomers by means of GC was reported. These methods have proved to be very sensitive and widely applicable for the determination of the ee. The enormous number of chiral selector-selectand systems developed since that time, e.g. by Pirkle and co-workers, made these methods probably the most powerful in the field of enantiomer separation. A breakthrough in this respect was made by Pirkle and co-workers by the introduction of N-(3,5-di-nitrobenzoyl)amino acids as immobilized, chiral charge-transfer-acceptor compounds used for high pressure liquid chromatography (HPLC). The introduction of (R)-2,2,2-trifluoro-1-(9-anthracenyl)-ethanol 4 as a chiral solvating agent, known as Pirkle’s alcohol, preceded this methodology. Chiral stationary phases based upon the ability to separate enantiomers via hydrogen bonding or via inclusion are now readily available. In general, the chromatographic methods tend to be very fast, sensitive (often very high resolutions are obtained), precise and reproducible, making them very attractive. Moreover, chromatographic methods can be used for ee determination of a broad range of substrates, including e.g. amino alcohols, thiols and amino acids. A drawback is the necessity of (often expensive) chiral stationary phases and selection of the proper chiral column might be rather time consuming.

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

Three types of chiral auxiliaries are commonly used. Chiral solvating agents and chiral lanthanide shift reagents form diastereomeric complexes in situ that allow a direct determination of the enantiomeric composition. The use of chiral (or achiral) derivatizing agents requires the formation of diastereomers prior to the NMR analysis. The sample can in principle serve as its own chiral probe, sometimes allowing quantification under strictly defined conditions.

Scheme 2.

Scheme 3.

Determination of the ee using (chiral) derivatizing reagents now is the most widely used NMR technique, as the discrete diastereomers show chemical shift nonequivalences $\Delta \delta$ that are typically five times higher than for related chiral solvating agents. The formation of the diastereomers must occur under strictly defined conditions that exclude racemization or kinetic resolution. When purification is necessary only methods that exclude selective enrichment of one of the diastereomers must be used. In spite of these restrictions, many chiral derivatizing reagents are known and commonly used. Some examples (10–14) are shown in Scheme 4.
The derivatizing agents contain a reactive group which can be coupled to the substrate. For example (S)-2-chloropropanoyl chloride 14 reacts with a large variety of nucleophiles, including alcohols, amines and unprotected amino acids. Excellent diastereomeric shift dispersions are observed in the $^1$H NMR spectra. The most widely used chiral derivatizing reagent is $\alpha$-methoxy-$\alpha$-(trifluoromethyl)benzeneacetic acid 10 (MTPA), introduced by Mosher in 1969. It offers the possibility to use not only $^1$H- but also $^{19}$F-NMR to determine the diastereomeric composition.

Chiral derivatizing reagents often contain more than one NMR active nucleus, e.g. $^{19}$F, $^{29}$Si, $^{77}$Se and $^{31}$P nuclei useful for enantiomeric excess analysis. It should be emphasized that chiral compounds often have a complex $^1$H NMR spectrum with multiplets due to H-H and/or H-X coupling patterns, making analysis difficult because of overlapping signal groups. With most other NMR active nuclei, in particular $^{19}$F and $^{31}$P, the chemical shift dispersion is large compared to $^1$H NMR and the nuclei are very sensitive to small structural changes in the diastereomeric adducts. When broad-band proton decoupling is used most of the spectra are simple, being a major advantage over $^1$H NMR and allowing easy quantification of the diastereomeric signals. Since both $^{19}$F and $^{31}$P nuclei have an abundance of 100%, the analysis can be performed very quickly provided the proper technical settings are used (vide infra).

In this review several new $^{31}$P NMR methods for the ee determination developed in our laboratories based on chiral (section II) and non-chiral (section III) phosphorus reagents, as well as related methodology, will be discussed.

II Chiral phosphorus derivatizing reagents

The attractiveness of the phosphorus-31 nucleus for NMR analysis has led to the introduction of various chiral pentavalent (thio)phosphoryl chlorides as derivatizing agents for chiral alcohols, amines, thiols and esters of amino acids. Derivatization proceeds by means of a displacement reaction on the phosphorus atom affording the diastereomeric products.

In order to determine the enantiomeric composition using (phosphorus) derivatizing reagents several criteria must be met:

(i) The reagents or precursors must be available in enantiomerically pure form.

(ii) In the process of adduct formation reactions at (the) chiral center(s) should not occur or must be stereo-specific.

(iii) The coupling reactions should proceed in high (quantitative) yield without enrichment of one diastereomer.

(iv) The adducts obtained must not be subjected to purification techniques, as these are a potential source for diastereomeric enrichment.

(v) The diastereomeric adducts must show a diastereomeric shift difference that is large enough to allow proper quantification of the selected NMR signals.

For derivatizing agents like 15 the phosphorus atom is stereochemically as well as a chirotopic center; displacement reactions can (in principle) proceed with inversion or retention of configuration, affording the diastereomeric products ($R$) and ($S$)-16 as shown in Scheme 5. Although under controlled conditions normally either quantitative inversion or retention can be achieved, this potential complication has led to the development of several reagents possessing $C_2$ symmetry, for example 17 so that inversion or retention of configuration at the phosphorus center upon treatment with an enantiomerically pure substrate yields the same product 18.

For reasons of synthetic availability, stability and desired control of stereochemistry during reaction at the phosphorus center, most of the derivatizing reagents are cyclic and based upon readily accessible chiral diols, amino alcohols or diamines that are functionalized by means of reaction with POCl$_3$ or PSCI. The reagents prepared in this way are very reactive towards a large variety of substrates. They are, however, sensitive to moisture, which restricts their use to non-aqueous solutions.

A way to circumvent the need for (thio)-phosphoryl chlorides is the use of chiral phosphonates, which upon treatment with CCl$_4$ and Et$_3$N, in situ afford the corresponding trichloromethyl phosphonates, which readily react with amines even in aqueous solutions.

Diastereomeric pentavalent phosphorus adducts generally show moderate diastereomeric shift differences in the decoupled $^{31}$P and $^1$H NMR, the shifts being sensitive to several factors like (im)purity of the sample, solvents (or combinations of solvents) used and the temperature. The largest diastereomeric shift differences are obtained using trivalent phosphorus reagents like e.g. phospholines. These reagents are readily coupled to alcohols, amines and thiols, often without the need of additional reagents and allow ee determination using $^1$H, $^1$C or $^{31}$P NMR. Phospholines are, however, not suitable for derivatization in aqueous solvent systems.

Besides the preparation of covalently bonded diastereomers, several phosphorus containing compounds can also be used for the formation of diastereomeric noncovalent associative complexes, like e.g. diastereomeric salts or hydrogen bonded complexes.
II.A Chiral pentavalent chloro(thio)phospholane oxides as derivatizing reagents

In 1983, Wynberg and Smaardijk \(^\text{37}\) suggested optically pure alcohol 19, containing a phosphonate moiety, as potential reagent for the determination of the enantiomeric composition of chiral acids. It was shown that the diastereomeric shift differences by \(^{31}\)P-NMR of adduct 20 are even larger than by \(^{19}\)F-NMR using Mosher's acid \(^\text{33}\) (Scheme 6).

Anderson and Shapiro \(^\text{38}\) introduced C\(_2\)-symmetrical chlorodioxaphospholane oxide 21 for the ee determination of chiral primary and secondary alcohols (Scheme 7). In the presence of base diastereomeric phosphonate esters 22 are obtained for which the diastereomeric shift dispersions were small but distinct; typically \(\delta\) values are between 0 and 0.13 ppm in the \(^{31}\)P-NMR. Representative examples are given in Table I.

Reagent 21 can also be used for the analysis of primary alcohols, as they are prone to elimination with other derivatizing agents. Also sterically hindered alcohols can be analyzed using this method e.g. when racemic alcohol 23 is used, a diastereomeric ratio of 48:52 is found whereas the use of Mosher's reagent yielded a 63:37 ratio \(^\text{39}\). It should be noted that anomalous reactions, like ring opening, give rise to the formation of several byproducts which, however, appear not to interfere with the actual derivatization and subsequent analysis.

Based upon earlier work of Hall and Inch \(^\text{40}\), Johnson and co-workers \(^\text{41}\) introduced chloro-1,3,2-oxazaphospholidine 22-sulfide 24 and the corresponding oxide 25, derived from D- or L-ephedrine as chiral derivatizing reagents for chiral amines and alcohols (Scheme 8). Derivatization of chiral primary amine proceeds smoothly when triethylamine is used as a base to facilitate nucleophilic attack, whereas chiral primary and secondary alcohols are only coupled after transformation to the alkoxides using n-butyllithium in ether. Substitution of the halide in 24 or 25 is known to proceed with complete retention of configuration at the phosphorus center \(^\text{42}\), although a warning for some stereocchemical scrambling has been reported more recently \(^\text{43}\).

Both the thio derivatives 26 and 27 and the oxygen analogues 28 and 29 are suitable for ee determination by means of \(^{31}\)P-NMR, although the former show superior diastereomeric shift dispersions. Typical \(\delta\) values are between the 0.175 ppm (D,L-\(\alpha\)-methylbenzenemethan-amine) and 0.843 ppm (D,L-4-methylpentan-2-amine) for adducts with chiral amines and 0.111 ppm and 0.301 ppm for the adducts of D,L-\(\alpha\)-ethylenbenzenemethanol and D,L-4-methyl-2-pentanol, respectively.

Diastereomeric adducts 26 and 27 can also be analyzed by means of HPLC, using a silica gel column and hexane/ethyl acetate as eluent. Using HPLC, adduct recovery is over 99.5\%, indicating that the diastereomeric adducts are very robust.

Clearly, adducts 27 lead to larger diastereomeric shift differences in the \(^{31}\)P-NMR compared with phosphate esters 22, which do not have a stereogenic phosphorus atom.

C\(_2\)-symmetrical (S)-1,1'-binaphthalene-2,2'-diyl phosphoril chloride 30, initially described as unstable by Johnson and co-workers \(^\text{41}\), was reintroduced by Kato \(^\text{44}\) for the ee determination of chiral secondary alcohols (Scheme 9). On treatment of 30 with 1-methylimidazole and a racemic chiral alcohol, diastereomeric phosphate esters 31 are formed, which can be analyzed by means of \(^1\)H NMR, although some diastereoselectivity is observed during coupling. Surprisingly, no \(^{31}\)P NMR data have been given for the diastereomeric products 31. The diastereomeric shift dispersions in the \(^1\)H NMR are small but distinct; typically \(\Delta\delta\) values between 0.01 and 0.20 ppm are found. For racemic 32, \(\Delta\delta\) values are obtained for several signals including the OAc (0.12 ppm), 10-Me (0.06 ppm) and 13-Me (0.20 ppm) moieties. Severe doubts have been expressed, however, concerning the products obtained from 30 and their subsequent analysis \(^\text{41,45}\).

Alexakis and co-workers \(^\text{46}\) introduced C\(_2\)-symmetrical chiral diamine based (thio)phosphoramides 33–37 (Scheme 10) as chiral derivatizing reagent for primary and sec-

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Table I  \(^{31}\)P-NMR non-equivalences of diastereomeric products 22 obtained from reagent 21 and racemic alcohols (C\(_4\)H\(_6\) and C\(_4\)D\(_8\)).

<table>
<thead>
<tr>
<th>D,L-Alcohols</th>
<th>(\Delta\delta) (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2O-benzyl-3-O-octadeoxylglycerol</td>
<td>2.40</td>
</tr>
<tr>
<td>exo-norborneol</td>
<td>6.11</td>
</tr>
<tr>
<td>endo-norborneol</td>
<td>0</td>
</tr>
<tr>
<td>butan-2-ol</td>
<td>0.5</td>
</tr>
<tr>
<td>meNthol</td>
<td>12.2</td>
</tr>
<tr>
<td>pent-1-yne-3-ol</td>
<td>10.13</td>
</tr>
</tbody>
</table>

Scheme 8.
condary chiral alcohols. The reagents are readily prepared by reaction of suitable diamines with POCl₃ or PSCI₃. Like 17 these reagents possess a chirotopic non-stereo- genetic phosphorus atom, so that either inversion or retention of configuration at phosphorus during derivatization with an enantiomerically pure alcohol yields the same single diastereomer, as shown for 38 (Scheme 11). Due to the reduced electrophilicity of the phosphorus atom in these reagents, associated with the presence of two P–N bonds, more forcing conditions are required in the coupling reactions with alcohols. For example, butan-2-ol does not react with 34 in THF in the presence of 2 equivalents of triethylamine, but gives several byproducts probably arising from opening of the diazaphospholane ring. The use of other bases (DMAP, DBU) or other solvents (CH₃Cl, DMF) does not lead to improvements. However, with the sodium alcoholates quantitative and clean conversions into the diastereomeric phosphoramide derivatives take place. All the phosphoramides give significant diastereomeric shift dispersion in the ³¹P NMR (Table II), the shifts and shift differences Δδ being highly solvent dependent.

<table>
<thead>
<tr>
<th>D,L-Alcohol</th>
<th>Δδ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>butan-2-ol</td>
<td>0.269</td>
</tr>
<tr>
<td>mesitol</td>
<td>0.606</td>
</tr>
<tr>
<td>isomenthol</td>
<td>0.404</td>
</tr>
<tr>
<td>ethyl lactate</td>
<td>0.337</td>
</tr>
<tr>
<td>β-citronellol</td>
<td>0.032</td>
</tr>
</tbody>
</table>

The shift dispersion for diastereomeric derivatives 38 ranges from Δδ 0.004 ppm to 1.335 ppm. With racemic butan-2-ol, the diastereomeric shift difference is 0.269 ppm, whereas for 22 and 29 Δδ values are much smaller (0.006 and 0.20 ppm, respectively). So far, reagent 37 seems to be the most promising for ³¹P NMR analysis, although unfortunately no useful diastereomeric shift differences have been obtained by ¹⁹F NMR. The corresponding diamine, however, was described as an effective reagent for the analysis of chiral aldehydes. In contrast to the results obtained by Johnson and Feringa, this analogue 36 induces smaller shift differences than 35. Furthermore, because of the lower degree of polarization of the P=S bond in comparison to the P=O bond, the amide 36 is much less reactive compared to the oxygen analogue 35; the more forcing conditions needed for reaction clearly limits the scope by preventing the analysis of sensitive substrates.

On examination of currently available methods for the ee determination of alcohols reagents 33–37 compare favourably, as large structural variations in substrate are allowed and sufficient diastereomeric shift dispersions are obtained. The diamines used are readily available, and can be structurally modified if desired (vide supra). Dehmlow and Sauerbier introduced phosphorylhydrazone 39 based upon l-ephedrine as a derivatizing reagent for carbonyl derivatives (Scheme 12). Chiral ketones can form two pairs of syn/anti diastereomeric hydrazones 40 which can be analyzed by means of ¹H or ³¹P NMR. Alternatively, HPLC analysis gives (only occasionally) separation of the four diastereomers and furthermore the results obtained by means of ³¹P NMR and HPLC analysis sometimes contradict each other. Moreover, since the method is restricted to some chiral, monosubstituted cyclohexanones, its use appears to be limited.

The introduction of cyclic phosphoric acids 41 as excellent resolving agents for amines, amino alcohols and amino acids by ten Hoeve and Wynberg, initiated the development in our laboratory of the corresponding chlorophosphorinanes 42 as a derivatizing reagents for chiral primary and secondary alcohols and amines (Scheme 13). Phosphoric acids 41 are readily obtained upon treatment of isobutyraldehyde and two equivalents of benzaldehyde with base (NaOH), followed by reaction with POCl₃ and basic hydrolysis affording the racemic phosphoric acids. Subsequent facile resolution by means of e.g. l-ephedrine yields the phosphoric acids as single enantiomers, which can be transferred diastereoselectively into the air and moisture stable chlorophosphorinanes 42 by
means of reaction with PCl_5. This synthetic protocol allows large structural variation in the substituents on the phenyl moiety and, moreover, guarantees the availability of both enantiomers of the derivatizing reagent. Chiral primary amines and esters of amino acids react with 42 using Et_3N as base in THF at reflux temperature to afford diastereomeric amides 44. Chiral secondary amines and alcohols require more forcing conditions, although with n-butyllithium in THF at room temperature the corresponding amides 44 and esters 45 are obtained in quantitative yields (Scheme 13). The same holds for the thio analogue 43. Diastereomeric amides 44 and esters 45 can be analyzed by means of ^1H and ^31P NMR, although the latter is preferred. As long as the substrates are stable towards the basic conditions needed to achieve adequate coupling, large structural variations in substrate are tolerated. The use of n-butyllithium or NaH makes this method of limited use with respect to multifunctional substrates. Some representative examples are given in Table III.

As can be seen from Scheme 14, amide formation proceeds with inversion of configuration at the phosphorus center, whereas the esters are formed with complete retention of configuration as proven by extensive 2D NMR (NOESY) and X-ray studies. The reactions normally proceed without the formation of side products, although sometimes small amounts of pyrophosphate 46 are formed (Scheme 13), which is recognized by a ^31P NMR signal at δ = -20.56 ppm. The formation of 46, however, does not influence the actual ee determination.

Insight in the structural requirements needed for efficient derivatizing reagents that show large shift dispersion for the diastereomeric adducts could lead to a more rational design of this type of reagents. On comparison of the derivatizing reagents described so far a number of important conclusions can be drawn:

(i) The largest diastereomeric chemical shift dispersion is found when diastereomeric phosphorothioic amides or esters instead of the corresponding oxygen analogues are used, although the diazaphospholidines show an inverse behaviour.

(ii) On comparison of chiral amines, thiols and alcohols the following order of diastereomeric shift dispersion is found for the adducts: amine > thiol > alcohol.

(iii) The chemical shift behaviour of the diastereomeric products is very sensitive to temperature and solvent polarity effects.

(iv) The lower reactivity of the chlorophospholane sulfides 43 limits their use to substrates that are not sensitive to treatment with strong base. Because of the large structural differences of the derivatizing agents described so far, it would be desirable if a reagent was available that is easily modified systematically without changing its most important stereochemical features. Fortunately, we were able to develop new chiral chlorophosphorinane oxides and sulfides 47–50, based upon enantiomerically pure amino alcohols, which are structurally related to chlorophosphorinane 42 and the thio analogue 43 (Scheme 15).

The new chlorophosphorinanes provide upon reaction with d,l-alanine methyl ester the corresponding diastereomeric phosphorus amides 51, 53 and 55, using Et_3N as base and CH_2Cl_2 as solvent at reflux temperature. Due to the lower reactivity of the amides 43, 48 and 50, the addition of a catalytic amount of 4-(dimethylamino)pyridine was required to obtain the amides 52, 54 and 56, respectively (Scheme 15).

For 51, the observed shift difference (∆δ 0.066 ppm) is comparable with the diastereomeric shift dispersion of thio-analogue 52 (∆δ 0.053 ppm) (Table IV). However, when the diastereomeric adducts 53 and 54 are compared the latter gives a large shift dispersion of ∆δ 2.01 ppm

<table>
<thead>
<tr>
<th>D,L-Compound</th>
<th>∆δ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhPhNH2</td>
<td>0.63</td>
</tr>
<tr>
<td>PhPhNH2</td>
<td>0.48</td>
</tr>
<tr>
<td>PhPhNH2</td>
<td>0.51</td>
</tr>
<tr>
<td>PhPhNH2</td>
<td>0.07</td>
</tr>
<tr>
<td>PhPhNH2</td>
<td>0.17</td>
</tr>
<tr>
<td>PhPhNH2</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Table III: ^31P-NMR diastereomeric shift differences of products 44 and 45 obtained from 42 and racemic alcohols and amines, recorded in CDCl_3; 0.01 M.
(illustrated in Figure 1) compared to $\Delta \delta$ 0.487 ppm for the former. The same tendency is observed for the adducts 55 and 56.

Reactions with the thio derivatives, however, proved to be more troublesome than with the oxygen analogues. The use of stronger basic conditions results in unfavourable side reactions like i.e. ring opening and polymerization.

In conclusion, pentavalent phosphorus reagents have shown to be efficient and successful derivatizing reagents for alcohols, amines and thiols, showing moderate to excellent diastereomeric shift dispersion. Moreover, by using the readily available phosphorus chlorides, pentavalent chlorophospholane oxides and sulfides are not suitable for use in aqueous solutions. Fortunately, by employing the Atherton-Openshaw-Todd reaction phosphonic amides can be obtained from dibenzylphosphonate and amines using CCl$_4$ and strong bases as reagents. Moreover, Zhao and co-workers reported that on using weakly basic conditions, $\alpha$-amino acids are easily transformed into $N$-(diisopropylphosphoryl)-$\alpha$-amino acids in aqueous media using diisopropyl phosphate, CCl$_4$ and Et$_3$N as reagents.

This led us to develop C$_2$-symmetrical phosphonate 57, which is readily accessible from commercially available $(S)$-butan-2-ol and PCl$_3$. Chiral amino acids are quantitatively transferred into the diastereomeric amides 58 upon treatment with phosphonate 57, CCl$_4$ and Et$_3$N in ethanol water mixtures (Scheme 16).

After extraction of the crude reaction mixture with ethyl acetate, the obtained diastereomeric phosphonate amides 58 and esters 59 are analyzed by means of $^{31}$P NMR. Alternatively, $^1$H NMR can be used, although the spectra do not always show proper quantification due to excess H-H and P-H coupling. The method allows large structural variations since also amines, alcohols, amino acid esters, amino acid amides and sterically hindered $\alpha$-alkylated amino acids can be analyzed. When amines and alcohols are to be analyzed, water can be omitted as (co-)solvent. Moreover, when alcohols are analyzed, more forcing coupling conditions (KO'Bu) are required. Typically, the diastereomeric shift dispersions for 58 and 59 in the decoupled $^{31}$P NMR spectra ranges from $\Delta \delta$ 0.079 ppm (D,L-serine) to $\Delta \delta$ 0.487 ppm (D,L-phenylglycine) for amines and amino acids and from $\Delta \delta$ 0.103 ppm (D,L-methylbenzenemethanol) to $\Delta \delta$ 0.127 ppm (D,L-menthol) for alcohols. Typical $^{31}$P NMR data are collected in Table V. The diastereomeric shift dispersions compare favourably with those obtained by using other chiral derivatizing agents, which illustrates the advantage of $^{31}$P NMR analysis; only two signals are obtained in the case racemic amino acids are used whereas other methods are based upon using the (multiple) $^1$H NMR signals (Figure 2).

The method not only allows broad structural variation in substrate but also in solvents; besides ethanol water solvent systems, CH$_2$Cl$_2$, CHCl$_3$, THF, benzene and combinations of these can be used. The most important feature of this new method is the easy derivatization of unprotected amino acids in aqueous solutions and the subsequent

![Scheme 15.](image1)

![Scheme 16.](image2)
$ee$ determination by means of $^{31}$P NMR. Moreover, reagent 57 is easily formed from commercially available starting materials and is very stable. Although phosphonate 57 is readily coupled with amino acids, an increase in diastereomeric shift differences is sometimes warranted. Therefore, phosphorinane 60 which strongly resembles chlorophosphorinane 42 was developed as an alternative derivatizing reagent for aqueous media$^{51}$ (Scheme 17). Phosphorinane 60 reacts with a variety of nucleophiles including chiral alcohols, amines, amino acid esters and unprotected amino acids using $\text{CCl}_4$ and $\text{Et}_3\text{N}$ as reagents and ethanol containing solvent mixtures. Furthermore, water is acceptable as (co)-solvent if desired when unprotected amino acids are allowed to react with reagent 60. After extraction of the crude reaction mixture with ethyl acetate or chloroform, the diastereomeric phosphonate amides 44, amide acids 61 and esters 45 are analyzed by means of $^{31}$P NMR.

It appears that large substituents, such as a phenyl group present in the substrate, usually have a positive influence upon the diastereomeric shift dispersion of products 44, 45 and 61. Using amino acids, the diastereomeric shift differences are the largest for $\text{D,L-phenylglycine} (\Delta \delta 1.218 \text{ ppm})$ and relatively small for $\text{D,L-alanine} (\Delta \delta 0.256 \text{ ppm})^{62}$. Also $\alpha$-alkylated amino acids and amino acid amides can be analyzed, which give diastereomeric shift differences between $\Delta \delta 0.786 \text{ ppm}$ for $\text{D,L-}\alpha$-methylphenylalanine amide and $\Delta \delta 1.653 \text{ ppm}$ (D,L-\alpha-methylphenylglycine). These products, however, are less easily formed compared to the $\alpha$-amino acids probably due to steric hindrance by the $\alpha$-alkyl group. The ester protected amino acids show only little differentiation in the diastereomeric chemical shift dispersion, except for $\text{D,L-}\alpha$-methylserine, which appears to be coupled through the alcohol group rather than the amine functionality ($\delta - 7.45 \text{ ppm}, \Delta \delta 0.201 \text{ ppm}$).

It is important to note that, although the reaction conditions and the derivatizing reagents 42 and 60 are different, the products as well as the stereochemistry at the phosphorus center are the same upon the use of chiral alcohols, amines and amino acid esters as substrates.

Figure 2. (a) Selected part of the $^1H$ spectrum of 58 using $\text{D-Phe}$ and (b) $\text{D,L-Phe}$. (c) $^{31}P$-NMR spectrum of adduct 58 using $\text{D,L-Phe}$ recorded in CDC$_3$, 0.1 M.
<table>
<thead>
<tr>
<th>D,L-Compound</th>
<th>( \Delta \delta ) (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{NH}_2 ) ( \text{CO}_2 \text{H} )</td>
<td>0.116</td>
</tr>
<tr>
<td>( \text{Ph} ) ( \text{NH}_2 ) ( \text{CO}_2 \text{H} )</td>
<td>0.106</td>
</tr>
<tr>
<td>( \text{CO}_2 \text{H} )</td>
<td>0.091</td>
</tr>
<tr>
<td>( \text{NH}_2 ) ( \text{Me} ) ( \text{CO}_2 \text{H} )</td>
<td>0.487</td>
</tr>
<tr>
<td>( \text{NH}_2 ) ( \text{CONH}_2 ) ( \text{Me} )</td>
<td>0.185</td>
</tr>
<tr>
<td>( \text{NH}_2 ) ( \text{CO}_2 \text{H} )</td>
<td>0.185</td>
</tr>
<tr>
<td>( \text{OH} )</td>
<td>0.103</td>
</tr>
</tbody>
</table>

Based upon the X-ray structures of 42 and the corresponding amides 44 and esters 45, extensive 2D NMR NOE experiments and the fact that reagent 42 does not tolerate water as (co)-solvent, it is concluded that the reactive intermediates are probably not the same, and we suggest that the derivatization reaction proceeds via trichloromethyl ester 62 (Scheme 18).

Since it is known that large substituents at phosphorus preferentially assume the axial position, leaving the double bonded oxygen in the equatorial position, the most likely route involves two subsequent retentions of configuration on the phosphorus center when alcohols are allowed to react and one retention followed by an inversion of configuration on reaction of amines or amine containing substrates.

In conclusion, reagents 57 and 60 show excellent shift differences for the diastereomeric amide 44, 58 and 61 and ester 45 and 59 derivatives using \( ^{31} \text{P} \) NMR. Both of the enantiomers of derivatizing reagents 57 and 60 are available and structural variations are easily introduced. Moreover, large variations in the derivatizing conditions, for example the use of aqueous media, and substrate structure including unprotected amino acids and \( \alpha \)-alkylated amino acids, are tolerated.

It is well established that diastereomeric shift differences respond to steric effects, non-bonded interactions and conformational mobility in the diastereomeric derivatives, affording larger shift differences for diastereomers that are conformationally more restricted. Adducts 64 were designed to meet such requirements. The protonated amine moiety in 64 could possibly conformationally lock the deprotonated acid part of the molecule by means of intramolecular ion pair formation (Scheme 19), and the conformational locked-unlocked equilibrium should be strongly pH dependent.

Diastereomeric amides 64 are obtained upon treatment of derivatizing reagent 63 with \( \text{CCl}_4 \), \( \text{Et}_3 \text{N} \) and racemic amino acids (\textit{vide supra}), and can be analyzed by means of \( ^1 \text{H} \) and \( ^{31} \text{P} \) NMR.

The observed diastereomeric shift dispersion (\( ^{31} \text{P} \) NMR) shows, as expected, a large pH dependency. Using e.g. D,L-alanine as substrate, shift differences are significantly higher in the pH range from 4.5 to 8.0, reaching a maximum value at pH 7 (Figure 3).

However, within this pH domain 4.5 to 8.0, the deviations are relatively small. At low pH, the protonation of the carboxylic group probably gives rise to a situation in which the intramolecular tight ion pair no longer exists. At high pH, total deprotonation takes place and also results in a situation where tight ion pair contributions vanish. With D,L-phenylglycine as substrate, analogous behaviour is found. This phenomenon is not observed when amines are used as substrates, strongly suggesting that some kind of conformational locking must be in operation in the pH 4.5 to 8.0 domain with amino acids. Unfortunately, decomposition above pH 10 was observed.
Table VI  $^{31}$P-NMR diastereomeric shift differences of 70 obtained from reagent 67 and racemic alcohols, recorded in $C_6D_6; 0.1 \text{ M}$.

<table>
<thead>
<tr>
<th>d,l-Alcohol</th>
<th>$\Delta \delta$ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Methylbutan-1-ol</td>
<td>0.202</td>
</tr>
<tr>
<td>$\beta$-citronellol</td>
<td>0.538</td>
</tr>
<tr>
<td>butan-2-ol</td>
<td>3.702</td>
</tr>
<tr>
<td>menthol</td>
<td>6.259</td>
</tr>
<tr>
<td>Ephedrine</td>
<td>11.442</td>
</tr>
<tr>
<td>$\alpha$-ethyl-$\alpha$-methylbenzene methanol</td>
<td>1.726</td>
</tr>
</tbody>
</table>

Although reagent 63 can be used for the ee determination of unprotected amino acids in aqueous solutions, the low stability of 63 and the adducts 64 limit the scope. It was, however, shown that tuning of the derivatizing reagents and the products by changing the pH of the solution is possible. This remarkable behaviour can be rationalized by a conformational locking model and can be used as a model for further developments towards a more rational design of chiral derivatizing reagents.

II. C Trivalent phosphorus derivatizing reagents

As shown by Burgada and Mukaiyama in 196666, exo-cyclic P–N bonds of amino-phosphines are very easily cleaved by alcohols67, thiols and amines. Alexakis and co-workers68 recently used this principle for the development of chiral derivatizing reagents based on trivalent phospholidines (Scheme 20).

Derivatizing reagents 65, 66 and 67 are obtained by amine exchange of the appropriate C$_2$-symmetrical diamines with HMPT. These trivalent phosphorus reagents are stable for months under an inert atmosphere although they are very sensitive to moisture. Reagents 65, 66 and 67 are very reactive and diastereomeric derivatives 68, 69 and 70 are readily formed upon reaction with a large variety of chiral primary, secondary and tertiary alcohols without the necessity of additional reagents by simple stirring in $C_6D_6$ at 20°C. Diastereomeric adducts 68–70 can be analyzed directly without the need of isolation and/or purification by means of $^1$H or $^{31}$P NMR, although the latter is preferred. The largest diastereomeric shift dispersions are obtained for derivatives 70 prepared from reagent 67 (See Table VI for some selected examples).

For example with d,l-butan-2-ol, the diastereomeric shift dispersion ($\Delta \delta$ 3.702 ppm) compares favourably with the values previously obtained by Shapiro38 ($\Delta \delta$ 0.0056 ppm), by Johnson41 ($\Delta \delta$ 0.200 ppm) and the closely related pentavalent adduct 3846 ($\Delta \delta$ 0.269 ppm). In contrast to allylic alcohols, which are readily analyzed, prop-2-yn-1-ols undergo rapid [2,3]-sigmatropic rearrangement69. The initially formed chiral phosphoallene reacts with the dimethylamine produced upon cleavage of the P–N bond to afford ultimately an enamine with loss of the stereocenter in the former alcohol70.

The use of diols as substrate gives rise to the formation of dioxaphospholanes with cleavage of the diazaphospholane ring. The phosphorus atom becomes a new stereogenic center, resulting in many signals in the $^{31}$P NMR spectrum that cannot be quantified properly. Also chiral thiols can be analyzed with reagent 67. Diastereomeric thiaphospholanes 71 are formed, for which excellent diastereomeric shift dispersions are obtained. With d,l-2-butanethiol as substrate, $\Delta \delta$ is 1.82 ppm. For most of the examples shown, $^1$H and $^{13}$C NMR also allow ee determination, although $^{31}$P NMR is preferred for its superior diastereomeric shift dispersion and simple spectra. It should be noted that the $^1$H spectra of these derivatives are often very complex, due to extensive P–H and H–H coupling.

Diastereomeric derivatives 69–71 are not stable to TLC or GC analysis. However, virtually instantaneous reaction with sulfur (S$_8$) powder provides air stable derivatives 72 quantitatively as shown for 70 (Scheme 21). Many of these derivatives can be analyzed by means of GC or alternatively by means of HPLC to afford baseline separation that allow easy and accurate quantification. Moreover, these pentavalent thiio derivatives can be analyzed by means of $^{31}$P NMR, although the shift differences are much smaller compared to the trivalent derivatives71.
We introduced phospholides based upon the very cheap (R) or (S) α-methylbenzenemethanamine. Reagents 73 and 74 react with a variety of chiral alcohols, but also with amines, esters of amino acids, thiols, α-sulfanyl acids and the corresponding mercapto esters affording diastereomeric adducts 75–78 (Scheme 22).

We were also able to functionalize several free amino acids by using reagent 73 under phase transfer conditions (solid-liquid phase).

The diastereomeric shift differences of derivatives 75–78 are comparable with the results as obtained by Alexakis and co-workers (Table VII). An example is shown in Figure 4. Furthermore, the same side reactions, as described by Alexakis and co-workers (vide supra), were observed including a [2,3]-sigmatropic rearrangement with propynols and an intramolecular cyclization reaction when using diols.

To overcome these problems encountered with propynols and diols, which are probably due to the reactivity of reagents 65–67, recently a new derivatizing protocol was developed by Alexakis and co-workers in which the reactive exocyclic P–NMe₂ moiety was replaced by a better but less basic leaving group. Thus, an appropriate C₂-symmetrical chiral diamine, for example 79, is allowed to react with one equivalent of PCl₃ in the presence of excess base (Et₃N, pyridine etc.) leading to the in situ formation of the derivatizing reagent 80. This reagent is subsequently allowed to react with chiral alcohols, thiols or, due to its higher reactivity also with chiral amines (Scheme 23).

The entire derivatization reaction is performed in an NMR tube using CDCl₃ or CH₂Cl₂. It should be noted that the products using this new procedure are the same as those obtained from using reagents 65–67. In addition, owing to the higher reactivity also propynols and/or hindered alcohols will enter into reaction (See Table VIII for some selected examples).

As expected, it is possible to analyze propynols without any problem using this new protocol. Also 1,2 and 1,3 diols can be analyzed since the intermolecular reaction is faster than the intramolecular cyclization reaction due to the higher reactivity of the in situ prepared 80. Furthermore, it is possible to transfer the adducts quantitatively

### Table VII

<table>
<thead>
<tr>
<th>d,l-Compound</th>
<th>Δδ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-methylbenzenemethanol</td>
<td>1.38</td>
</tr>
<tr>
<td>butan-2-ol</td>
<td>2.73</td>
</tr>
<tr>
<td>menthol</td>
<td>1.69</td>
</tr>
<tr>
<td>[1R-(1a,2β,5α)],5-methyl-2-(1-methylethyl)cyclohexanol</td>
<td>1.31</td>
</tr>
<tr>
<td>α-methylbenzenemethanamine</td>
<td>2.46</td>
</tr>
<tr>
<td>[2S-(2α,5β)].2,5-dimethylpyrrolidine</td>
<td>1.31</td>
</tr>
<tr>
<td>2-sulfanylpropanoic acid</td>
<td>2.30</td>
</tr>
<tr>
<td>β-(prop-2-etyl)-β-sulfanylbenzeneethanol, acetate</td>
<td>0.53</td>
</tr>
</tbody>
</table>

Scheme 23.
Table VIII. $^{31}$P-NMR diastereomeric shift differences of $\delta$ obtained from racemic alcohols and 79 with $\text{PCI}_3$, recorded in CDCl$_3$; 0.2 M.

<table>
<thead>
<tr>
<th>d,l-alcohol</th>
<th>$\Delta\delta$ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,2-dimethyl-1,3-dioxolane-4-methanol</td>
<td>0.748</td>
</tr>
<tr>
<td>3-ethynylxirane-2-methanol</td>
<td>0.905</td>
</tr>
<tr>
<td>2-[4-(tert-butyl)cyclohexylidene]ethanol</td>
<td>0</td>
</tr>
<tr>
<td>menthol</td>
<td>2.759</td>
</tr>
<tr>
<td>1-(trimethylsilyl)oct-1-yn-3-ol</td>
<td>4.913</td>
</tr>
<tr>
<td>3-methylpent-1-yn-3-ol</td>
<td>0.479</td>
</tr>
<tr>
<td>propane-1,2-diol</td>
<td>0.202</td>
</tr>
<tr>
<td>[1,1'-binaphthalene]-2,2'-dilol</td>
<td>0.202</td>
</tr>
</tbody>
</table>

By using this new protocol very good reactivity is obtained allowing the analysis of a large variety of sensitive alcohols, thiols and amines. The adducts show excellent diastereomeric shift dispersion in the $^{31}$P NMR spectra and the derivatizing reagents can easily be modified. It was shown by Buono and co-workers$^{76}$, however, that trivalent dioxaphospholanes can be used as derivatizing reagents for the ee determination of chiral alcohols. Using diesters of ($R,R$)-tartaric acid$^{82}$ as $C_2$-symmetrical building block, they were able to functionalize these reagents in situ by reaction with $\text{PCI}_3$ into the trivalent phosphorus reagent$^{83}$, which reacts readily with chiral alcohols upon the addition of Et$_3$N as base (Scheme 24).

A variety of alcohols was derivatized quantitatively, and the diastereomeric products$^{84}$ were shown to give small to moderate $\Delta\delta$ values, i.e. for d,l-α-methylbenzene-methanol and d,l-menthol $\Delta\delta$ values of 1.4 and 0.4 ppm were found, respectively.

The method allows easy structural modification in the ester part and is based upon the use of the cheap $C_2$-symmetrical tartaric acid. A major drawback, connected with the use of all the trivalent phosphorus derivatizing reagents treated here, is the high sensitivity to moisture and problems related herewith.
II.D Phosphorus reagents based on non-covalent diastereomeric interactions

It is also possible to form diastereomers by means of noncovalent interactions, and several phosphorus methods have been developed based on this principle. The utility of (R) or (S)-1,1'-binaphthalene-2,2'-dyl hydrogen phosphate (85, BNHP) as chiral complexing agent for chiral amines was shown by Shapiro and Jarema (Scheme 25).

The derivatization procedure consists of salt formation by simply mixing one equivalent of amine and of BNHP in CDCl₃ or C₆D₆. The ee is subsequently determined by means of ¹H-NMR using the diastereotopic shifts of substrate protons. Often more than one signal allows quantification. For the cyclic amines 86 and 87, respectively, two (ΔΔδ 0.161 and 0.246 ppm) and three (ΔΔδ 0.147, 0.016 and 0.067 ppm) different proton signals can be used for analysis (Scheme 25). By using the BNHP-pyridined-d₅ salt it was possible to determine the ee of two non-amine materials (88 and 89, Scheme 25), although the diastereomeric shift dispersion (ΔΔδ 0.005 and 0.006 ppm) is only of importance when high fields are applied. The method, however, is very easy to perform and appears to be very accurate; enantiomeric impurities as low as 0.5% were detected without any problems.

Parker and Taylor used organometallic C₂-symmetric biphosphine ethene complexes 90 and 91 based on zerovalent platinum and palladium for the in-situ ³¹P-NMR assay of the enantiomeric purity of several chiral π² donors (Scheme 26).

The displacement of ethene with alkenes, allenes and electron-poor or strained alkenes proceeds readily in THF or C₆D₆, yielding diastereomeric complexes 92 that show good chemical shift dispersion (Table IX). The spectral analysis may be complicated since binding to the Si or Re face of a non-C₂-symmetric two electron donor gives rise to constitutionally isomeric species. Furthermore, the de-coupled ³¹P-NMR spectra show also two different platinum couplings for each diastereomer. Although the troublesome spectral interpretation can be seen as a drawback, the method readily affords the ee of a class of substrates that is difficult to analyze by other NMR methods.

Mikołajczyk and co-workers reported the use of (S)-(tert-buty1)phenylphosphinothioc acid (93) as a chiral solvating agent for the ee determination of chiral sulfoxides (Scheme 27).

The procedure for the enantiomeric analysis consists of the mixing of one equivalent of the chiral sulfoxide and one or two equivalents of the phosphoryl compound in C₆D₆. The diastereomeric solvatation complexes are analyzed by means of ¹H-NMR; small diastereomeric shift dispersions are obtained for signals in the (partly) racemic substrates. Some typical values are collected in Table X. The observed spectral non-equivalences for the diastereomeric complexes are probably due to the formation of hydrogen bonded complexes or ion pairs.

III Acchiral phosphorus reagents

Although Ladenburg as early as 1895 showed that mixing of (R)- and (S)-conine was accompanied by a change in temperature and Uskoković and co-workers were able to demonstrate the nonequivalence of the NMR

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Table IX ³¹P-NMR diastereomeric non-equivalences of 90 using 91 and racemic alkenes, recorded in C₆D₆; 0.02 M. Δδₐ arbitrarily assigned as resonating at higher frequency.

<table>
<thead>
<tr>
<th>D,L-Alkene</th>
<th>Δδₐ (ppm)</th>
<th>Δδₐ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table X ¹H-NMR non-equivalences of diastereomeric phosphinothioc sulfoxide complexes obtained from 93 and racemic 94, recorded in C₆D₆.

<table>
<thead>
<tr>
<th>D,L-Sulfoxide</th>
<th>Δδ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

Scheme 26.

Scheme 27.
The consequences of the differences in chemical reactivity and product composition between a single enantiomer (R or S isomer) or the (partly) racemic compound (R and S isomers), due to enantiomer recognition and interactions, have been discussed by Wynberg and Feringa. Chiral self-recognition leading to non-linear effects in asymmetric synthesis has now several precedents. Horeau was the first to recognize the potential of using the intrinsic differences in chirality of an enantiomerically pure and (partly) racemic substrate for ee determination. When two enantiomers dimerize (Eqn. 1), either via covalent bond formation or via noncovalent associative interactions, diastereomers are formed in the (partly) racemic case.

$$R \rightarrow RR$$
$$R + S \rightarrow RR + SS + RS \ (SR) \quad (1)$$

If the enantiomers are coupled via an achiral agent A, a single isomer $R-A-R$ is obtained with an enantiomerically pure substrate whereas two diastereomers $R-A-R$ ($S-A-S$), a d,l-pair, and $R-A-S$ ($S-A-R$), a meso compound, are formed starting from a racemic substrate (Eqn. 2).

$$R + A \rightarrow R-A-R$$
$$R + S + A \rightarrow R-A-R + R-A-S \ (S-A-R) \quad (2)$$

We found that the differences in properties between the diastereomers can be used for ee determination provided that the coupling reagent A fulfills in number of requirements:

(i) The coupling reaction proceeds in high yield, preferably with quantitative conversions.

(ii) No deviation from the statistical ratios of coupled products occurs (no antipodal effects).

(iii) Chemical shift differences of the diastereomeric products are large enough to ensure accurate integration.

(iv) It is highly desirable that agent A contains a unique atom that makes analysis of each diastereomer via a single NMR absorption possible, e.g. that A contains a spectator atom.

The phosphorus nucleus is highly suitable for this purpose in view of the great advantage of $^1$H-decoupled $^{31}$P-NMR.
for ee determination using chiral derivatizing agents (vide supra).

Several ee determination methods based on PCl₃, RP=OCl₂ (95) or RP=SCl₂ (96) as achiral phosphorus derivatizing agents have been developed by us (Scheme 28).

### III.A PCl₃ method

Using PCl₃ in deuteriochloroform various chiral alcohols are converted in diastereomeric phosphonates in the absence or presence of an equivalent of base in a fast and quantitative reaction via initial phosphate formation followed by an Arbuzov rearrangement. Application of this reaction for the ee determination is illustrated for butan-2-ol 97. Racemic 97 yields a mixture of phosphonates 98 (R,R,S,S), 99 (R,S, meso-1) and 100 (R,S, meso-2) in a 2:1:1 ratio (Scheme 29).

The formation of two meso-phosphonates 99 and 100 is due to the presence of a stereogenic, achiraltopic phosphorus center. The ¹H decoupled ³¹P NMR spectrum of the reaction mixture of racemic 97 shows three well separated singlet signals in the expected ratio for the diastereomers 98, 99 and 100 (Figure 59). Enantiomerically pure (S,)-97 yields exclusively (S,S)-98 resulting in the absence of the meso-absorptions (Figure 59).

For (partially) enriched alcohols the absorptions of the meso isomers decrease relative to the absorptions of the D,L-pair. The enantiomeric excess (p X 100; p = enantiomeric purity) is calculated from the integrated signal areas Q and Q' of the D,L and meso isomers, respectively, with D,L/meso ratio K = Q/Q' using Horeau's formula p² = (K - 1)/(K + 1).

It was shown by independent ee determinations that no racemization during phosphate formation occurs and that loss of part of the alcohol as the halide does not affect the accuracy. Typical examples of chiral alcohols analyzed via this technique are given in Table XI. The method tolerates large variations in the alcohol structure, e.g. primary, secondary, benzylic and aliphatic alcohols and α-hydroxy-esters and amides. Baseline separations were found except for borneol. Chiral recognition during phosphate formation is negligible. Even for the case of hindered alcohols, such as menthol, only small deviations (< 3%) from the expected ratios are observed.

Important advantages of the PCl₃ method are the cheap achiral reagent employed, the very fast and easy (in situ) ee determination without the necessity of product isolation and purification. Major drawbacks are the loss of one equivalent of chiral alcohol and the difficulty of handling small quantities of PCl₃ due to volatility and moisture sensitivity. Furthermore, the method is restricted to alcohols (vide infra).

Under an inert atmosphere (N₂) phosphonation formation appears to be much slower and Welch has shown that the enantiomeric excess of a number of chiral, secondary alcohols can also be determined from the ³¹P NMR absorptions of the trialklyphosphites 102 initially obtained.

<table>
<thead>
<tr>
<th>d,L-Alcohols</th>
<th>Δδ (meso, meso, d,l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>butan-2-ol</td>
<td>454, 387, 425</td>
</tr>
<tr>
<td>3-hydroxytetrahydrofuran</td>
<td>481, 447, 464</td>
</tr>
<tr>
<td>menthol</td>
<td>459, 309, 413</td>
</tr>
<tr>
<td>α-methylbenzenemethanol</td>
<td>416, 344, 370</td>
</tr>
<tr>
<td>4-[2-furyl]-butan-2-ol</td>
<td>449, 395, 433</td>
</tr>
<tr>
<td>β-ethylbenzeneethanol</td>
<td>652, 590, 623</td>
</tr>
<tr>
<td>buten-3-en-2-ol</td>
<td>393, 349, 369</td>
</tr>
<tr>
<td>cyclohex-2-en-1-ol</td>
<td>450, 416, 432</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table XI Some of the alcohols analyzed using the PCl₃ method (Δδ [meso, meso, d,l] in Hz in parenthesis).</th>
</tr>
</thead>
</table>

**Scheme 30.**
Table XII 31P-NMR data of the diastereomeric shift differences of 103–105 as a function of the substituent R, using α-methylbenzenemethanethiol.

<table>
<thead>
<tr>
<th>R substituent</th>
<th>Δδ (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₃</td>
<td>109.30</td>
</tr>
<tr>
<td>C₆H₅CH₂</td>
<td>24.19</td>
</tr>
<tr>
<td>C₆H₅</td>
<td>7.38</td>
</tr>
<tr>
<td>C₆H₅CH₂S</td>
<td>12.33</td>
</tr>
</tbody>
</table>

* Absolute values between the d,L pair and respective meso diastereomers.

Table XIII 31P-NMR data for diastereomeric phosphonates 103, 104 and 105 (X = S, R = CH₃), recorded in CDCl₃; 0.1 M.

<table>
<thead>
<tr>
<th>d,L-Thiol</th>
<th>Δδ (meso)</th>
<th>Δδ (meso)</th>
<th>Δδ (d,L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sulfanylecetaceticester</td>
<td>4660</td>
<td>4987</td>
<td>4725</td>
</tr>
<tr>
<td>α-methylbenzenemethanethiol</td>
<td>4523</td>
<td>4935</td>
<td>4632</td>
</tr>
<tr>
<td>thionemethanol</td>
<td>4640</td>
<td>4707</td>
<td>4694</td>
</tr>
<tr>
<td>tetrahydrofurane-3-methanethiol</td>
<td>5129</td>
<td>5088</td>
<td>5050</td>
</tr>
<tr>
<td>α-ethylbenzenemethanethiol</td>
<td>4940</td>
<td>4977</td>
<td>4954</td>
</tr>
<tr>
<td>N,N-dimethylsulfanylacetamide</td>
<td>4883</td>
<td>5088</td>
<td>5010</td>
</tr>
</tbody>
</table>

from PCI₃ and three equivalents of the chiral alcohol. A typical example is α-ethylbenzenemethanol 101 (Scheme 30).

Quartets are observed at δ 140.7 and δ 142.7 ppm in a statistical ratio of 3:1 for the RRR (SSS) isomers and the RRS (SRR) isomers of the phosphites, respectively. In the case of enantiomerically pure R (or S) alcohol only the signal belonging to the RRR (or SSS) isomer was observed at δ 142.7 ppm.

The enantioselective excess is calculated from % ee = (2a - 1)·100, were a is the molar fraction of one isomer which is related to the signal integral ratio Q by Q = 3·a·(1 - a).

In cases where the subsequent Arbuzov rearrangement can be sufficiently retarded by using 3 equivalents of base, this method is complementary to the phosphonate procedure although so far it seems to be of rather limited use.

III.B CH₃P(=O)Cl₂ method

Extension of the PCI₃ method to the ee determination of chiral thiols was not possible because thio-trialkylphosphites are obtained from PCI₃ and thiols instead of phosphonodithioates and the former do not give well resolved 31P NMR signals for the diastereomers.

As there was no general method available for the ee determination of chiral thiols we were pleased to find that alkylphosphonic dichlorides 95 are highly suitable for this purpose (Scheme 31).

Alkylphosphonic dichlorides 95 are very reactive towards thiols leading to quantitative formation of one d,L-pair, (S,S) and (R,R)-103, and two meso adducts 104 and 105 (X = S) in a few minutes. Reagent 95 can also be used as an alternative to PCI₃ for the ee determination of alcohols (Scheme 31, X = O), in particular acid sensitive ones, as excess base can be used to remove efficiently the liberated HCl.

The largest chemical shift differences for the diastereomeric products of 95 are obtained with methylphosphonic dichloride (95, R = CH₃). Increase of the size of the alkyl substituent R leads to a decrease in chemical shift dispersion Δδ, as is shown for α-methylbenzenemethanethiol derivatives (Table XII).

Diastereomeric chemical shift dispersion for adducts of 95 (R = CH₃, X = S) compare favourably with adducts of other chiral derivatizing agents in the case of d,L-α-methylbenzenemethanethiol. Diastereomeric shift differences for the meso isomers and d,L pair of Δδ 1.35 and Δδ 3.74 ppm were observed whereas Mosher’s reagent showed a difference of only Δδ 0.06 ppm (19F NMR, Figure 6) and Pirkle’s reagent resulted in a maximum separation of Δδ 0.05 ppm in the 1H NMR spectrum.

The scope and limitations of CH₃P(=O)Cl₂ as a reagent for chiral thiols are illustrated in Table XIII. It can be seen that the method is broadly applicable including aliphatic and secondary benzylic thiols, α-sulfanyl carboxylic esters and α-sulfanyl amides. In the few examples that no baseline separation is observed using CDCl₃ as solvent, recording of the 31P NMR spectrum in the more polar CD₃OD results in (nearly) baseline separated signals. In addition a large increase of the diastereomeric shift dispersion is observed at lower temperatures. This temperature effect is particular advantageous in case the absorptions of meso 104 and 105 and d,L-103 are not completely separated at room temperature; the effect widens the applicability of the method. Again, only small deviations from the statistical ratio of 50:50 for meso and d,L isomers are found.

Figure 6. Comparison of peak separations for derivatives of α-methylbenzenemethanethiol using Mosher’s reagent (a. 19F-NMR) and 95 (b. 31P-NMR).
Although extensive investigations revealed that CH$_3$P(=O)Cl$_2$ was not a satisfactory reagent for the derivatization of chiral amines, recent research by Fedin and co-workers showed that the diastereomeric shift differences, the self-induced diastereomeric anisochronity, may be enhanced provided the measurements are performed in toluene at low temperature (−20°C). Probably, hydrogen bonding between the phosphoryl and amide moiety lies at the basis of these observations.

### III.C CH$_3$P(=S)Cl$_2$ method

It became clear that neither PCl$_3$ nor CH$_3$P(=O)Cl$_2$ is a satisfactory reagent for the derivatization of chiral amines (vide supra). Fortunately, alkylphosphonothioic dichlorides [RP(=S)Cl$_2$] are relatively stable, resistant to hydrolysis and provide good yields of the alkylphosphonothioic diamides upon treatment with amines. Reagent 96 (R = CH$_3$) reacts in the presence of triethylamine quantitatively in a few minutes at −20°C with two equivalents of α-allylglycine methyl ester (R = allyl) to afford diastereomeric methylphosphonic diamides 106, 107 and 108 (Scheme 32).

The decoupled $^{31}$P NMR spectrum shows three nicely separated singlets for the two meso compounds 106 and 108 and the racemic (RR and SS)-106 adducts, with a meso/D,L ratio (49/51) virtually identical with theory (Figure 7).

The scope of this method encompasses a variety of primary amines and amino acid esters including sterically hindered ones. Representative examples are given in Table XIV.

### Table XIV  $^{31}$P-NMR data of diastereomeric methylphosphonic diamides 106–108 recorded in CDCl$_3$; 0.1 M.

<table>
<thead>
<tr>
<th>D,L-Amine</th>
<th>δ(meso) (Hz)</th>
<th>δ(meso) (Hz)</th>
<th>δ(D,L) (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>butan-2-amine</td>
<td>4995</td>
<td>5035</td>
<td>5015</td>
</tr>
<tr>
<td>phenylalanine</td>
<td>5507</td>
<td>5404</td>
<td>5395</td>
</tr>
<tr>
<td>α-methylmethionine</td>
<td>5504</td>
<td>5575</td>
<td>5485</td>
</tr>
<tr>
<td>α-allylglycine</td>
<td>5355</td>
<td>5472</td>
<td>5393</td>
</tr>
<tr>
<td>α-methylbenzenemethanamine</td>
<td>5086</td>
<td>5208</td>
<td>5168</td>
</tr>
</tbody>
</table>

It should be noted that alkylphosphonothioic dichlorides are not satisfactory for the analysis of chiral secondary or tertiary amines. However, chiral diamine 109, which is a typical example of a bifunctional compound containing both a primary and a tertiary amine center, readily forms the diastereomeric phosphonothioic diamide 110, which shows well separated absorptions in the decoupled $^{31}$P NMR spectra, allowing accurate ee determination (Scheme 33).

The commercially available C$_6$H$_5$P(=S)Cl$_2$ can be used although CH$_3$P(=S)Cl$_2$, with a small alkyl substituent, is preferred because it gives superior diastereomeric chemical shift dispersions (see also III.B). Again, as with the use of thiols, a strong temperature dependency is observed, resulting in an approximately 1.5 fold increase of the diastereometric shift dispersion at −20°C. Examination of the currently available methods for the ee determination of chiral amines leads to the conclusion that the CH$_3$(P=S)Cl$_2$ method compares favourably in view of the large shift differences obtained for the diastereomers, the simple experimental procedure (no workup is required) and the ready availability of this achiral reagent.

A disadvantage of this method is that hydroxy amines or α-hydroxyamino acid derivatives (unless hydroxy protected) cannot be analyzed by this technique, due to severe side product formation. Information on the structural requirements for efficient derivatizing reagents was obtained by comparing a number of reagents 111 with a variety of X, Y and Z around phosphorus. Typical data are summarized in Tables XII, XIII and XIV.

A number of important observations can be made:

(i) It appears that the largest chemical shift dispersion is found when X = H or a small alkyl group. This arrangement is also synthetically easier to achieve.
(ii) Thioderivatives (Y = S) give in general superior shift differences, except for these cases were Z = S.

(iii) Smaller δ values are found in the order X = OR < X = SR for diastereomeric (thio)phosphonates.

(iv) On comparison of chiral amines, thiols and alcohols the following order of diastereomeric shift dispersion is found: Z = NH > Z = S > Z = O.

(v) The chemical shift behavior of the diastereomeric products is very sensitive to solvent polarity effects and temperature of the measurements. These observations are in accordance with the results using chiral phosphorus derivatizing agents (vide supra).

IV Related methodology

IV.A Phosphorus based methods

The 31P NMR nonequivalence of diastereomeric dialkyl phosphorodithioates 1113 (X = S) has also been used for the enantiomeric excess determination of chiral alcohols104. When Horeau’s principle (vide supra) is applied to (thio)phosphonates 114 (X = O, S; R1 = H, alkyl) or (thio)phosphates 112 (X = O) or 113 (X = S), there is a distinct stereochemical difference (Scheme 34).

In the case of phosphonates 114, derived from racemic alcohols, a d.L-pair and two meso isomers are formed whereas in (thio)phosphates 112 and 113 the phosphorus atom is stereogenic but achiral result87ing in a d,L-pair and one meso isomer only. As an achiral reagent (C6H5O)2P(=S)SH 115 (Scheme 34) was used. Reaction of 115 with d,L-butan-2-ol gives diastereomeric di-sec-butyl phosphorodithioates 116 and 117 (Scheme 35). In the 31P NMR spectrum a singlet is observed for 116 obtained from enantiomerically pure l-butan-2-ol, whereas two singlets in a 50:50 ratio, for d,L-116 and meso-117, are found using d,L-butan-2-ol (Figure 8). Again, 31P NMR nonequivalence of the diastereomeric (d.L. and meso) phosphorodithioates allow ee determination of chiral alcohols. It should be emphasized that chiral phosphates (112, X = O) generally show only one (broad) absorption. It was also shown that it was possible to discriminate between meso- and d.L-phosphorodithioic acids using chiral tertiary amines (see Figure 8).

It is remarkable that despite the high symmetry at phosphorus in 113 when racemic alcohols are used diastereomeric nonequivalence is found in the decoupled 31P NMR spectrum. The fact that only two singlets are observed is an advantage compared to derivatives 114, although the diastereomeric shift differences are generally smaller, making them less suitable for practical application. The conversion of chiral alcohols into diastereomeric phosphonates can also be used for assessment of the enantiomeric composition by means of achiral HPLC105.

IV.B Non-phosphorus based methods: A comparison

Horeau demonstrated the intrinsic differences in chirality of an enantiomerically pure and racemic compound using dichlorides as achiral coupling agents for alcohols and showed the possibility to examine the enantiomeric composition by means of 1H NMR and chromatographic techniques (see eqns. 1 and 2). Recently an efficient method for ee determination of chiral alcohols by means of GC of dialkoxysilanes 118 was developed based on this principle using dialkylsilyl dichloride as a coupling reagent106 (Scheme 36). Pertinent examples are furthermore N,N'-ethylene-bridged amino acid dimers 119107 and the N,N'-bis(y-alkoxybutyl)acetone-substituted diamines 120108. In all cases the Horeau principle applies, although complicated spectra are often seen and these systems in general have not resulted in an effective methodology for ee determination.

CoIII-porphyrines 121 proved to be useful as NMR shift reagents due to their complexing power for instance with amines109, as well as the fact that the cobalt-porphyrin systems, unlike the naturally occurring porphyrins, do not possess planar chirality. These CoIII-systems can form complexes with two nitrogen ligands in the axial positions, and these features make it possible, owing to the slow
ligand exchange on the NMR time scale in the six-coordinate complex, to observe both diastereomeric complexes (RR, SS and RS) in the $^1H$ NMR spectrum provided that a racemic amine is used (Scheme 37).

Complex 122 with R (or S) amines is optically active and exhibits planar chirality through the porphyrin ring plane, whereas the meso R,S-adduct 123 (not shown) is optically inactive due to the pseudo-chirality or pseudo-asymmetry about the porphyrin ring plane.$^{110}$ Abraham and coworkers$^{111}$ were able to determine the enantiomeric composition of $\alpha$-methylbenzenemethanamine and levamisole in CHCl$_3$. Although the chemical shifts are usually very large (in the order of several ppm's), the diastereomeric shift differences (ΔΔδ values of about 0.005 ppm are observed) are only significant when high field strengths are applied. This method can also be applied to chiral amino alcohols.

Recently, a water-soluble Co$^{III}$-shift reagent was developed based upon meso-tetrakis-(4-sulfonatophenyl)porphyrin 124. Following Horeau’s principle complexation of two amino acids (or derivatives) to Co$^{III}$-124 allows ee determination of these compounds even in polar solvents like water or water/alcohol mixtures.$^{112}$ Typical examples are summarized in Table XV.

Sufficient resolution is seen in particular with $\alpha$-alkylated amino acids. Shifts are, however, strongly variable depending on the pH and solubility of the Co$^{III}$-substrate complexes D,L-125 and meso-126 (not shown). Only those substrates that give simple resonances (at least for some protons) in the $^1H$ NMR spectrum provide enough resolution to allow accurate analysis. The attempted use of $^{59}$Co NMR as an alternative technique was not successful.

### Table XV

<table>
<thead>
<tr>
<th>D,L-Amino Acid</th>
<th>ΔΔδ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>alanine</td>
<td>0.06 (β)</td>
</tr>
<tr>
<td>phenylglycine</td>
<td>0.02 (β)</td>
</tr>
<tr>
<td>phenylalanine</td>
<td>0.08 (β)</td>
</tr>
<tr>
<td>a-vinylalanine</td>
<td>0.12 (β)</td>
</tr>
<tr>
<td>a-phenylalanine</td>
<td>0.11 (β)</td>
</tr>
</tbody>
</table>

V Enantiomeric excess (ee) determination of phosphorus compounds

When phosphorus containing reagents are used for the ee determination it is of great importance to have access to ee determining methods for this type of materials. The methods described (vide supra) can in principle$^{113}$ also be used to determine the ee of the phosphorus derivatizing reagents by using enantiomerically pure substrates and the partially racemic phosphorus materials. There are, however, few methods known especially developed to determine the enantiomeric composition of chiral phosphorus compounds. The enantiomeric composition of chiral, chelating diphenosphines can be determined by means of in situ derivatization with (−)-bis(μ-chloro)bis[(R)-dimethyl(α-methylbenzy)aminato-C$^2$]-N$_2$ dipalladium(II) 127$^{114}$ (Scheme 38).

Upon dissolving 1 equivalent 127 and 2 equivalents chiral diphenosphine in CDCl$_3$, diastereomeric adducts 128 are formed, which are analyzed by means of $^1H$ or $^{31}$P NMR. Although there is evidence for decooordination of one of the chelating phosphines to form a tricoordinate species, the method has proven to be applicable for several diphenosphines, including (R,R)-2,2-dimethyl-4,5-bis[(diphenylphosphinomethyl)dialkeoxy] DIOP. It has to be noted that, after the determination is done, the phosphines can be recovered essentially quantitatively, if desired.

Duñach and Kagan$^{115}$ were able to determine the enantiomeric composition of a variety of chiral phosphate oxides 130 by using (R or S)-N-(3,5-dinitrobenzoyl)-$\alpha$-methylbenzenemethanamine 129 as chiral shift reagent (Scheme 39).

The procedure consists of mixing of equimolar amounts of shift reagent and phosphate oxide in CDCl$_3$ followed by analysis by means of $^1H$ NMR. The diastereomeric shift dispersion is relatively small, i.e. ΔΔδ 7 Hz (when spin decoupling is used) for D,L-DIOP, which is transformed to the oxide in situ by treatment with tBuOOH just prior to determination. The enantiomeric composition of chiral (1-hydroxyalkyl)phosphonic acids 131 can be determined by means of derivatization into diastereomeric phosphonic acid diesters 132, which can be analyzed using $^{31}$P NMR$^{116}$ (Scheme 40).

The derivatization method consists of a coupling of the 1-hydroxyalkylphosphonic acids 131 with N-protected 1-
amino acids using DCC to facilitate the coupling. The obtained phosphonodidespeptides 132 are quantitatively distinguishable in the decoupled \(^{31}\)P NMR. The values of the diastereomeric shift dispersions differ with the change of the L-amino acids and the protecting groups, and range from \(\Delta \delta \) 0.06 to 0.60 ppm. The method is performed readily, since e.g. Boc-L-Val and Boc-L-Phe are commercially available.

Another method for the \(ee\) determination of (1-hydroxalkyl)phosphonic acids 131 is based upon the formation of diastereomeric salts using \(\alpha\)-methylnaphthalene-1-methanamine 5 (\(R = H\)) as chiral auxiliary \(^{11}\) in nonpolar solvents like \(\text{CDCl}_3\). Although the magnitude of the diastereomeric shift dispersion is sensitive to concentration, temperature and (enantiomeric) purity of the phosphonates and the amine, the magnetic nonequivalence is large enough to allow proper quantification. The \(ee\) of chiral phosphorus thioacids 133 (Scheme 40) can be determined by means of diastereomeric salt formation using optically active amines such as 5 (\(R = H\)).

The salts show diastereomeric nonequivalences in the \(^1\)H, \(^{13}\)C and \(^{31}\)P NMR spectra, provided that these are taken in nonpolar solvents (vide supra). Diastereomeric shift dispersions are sensitive to several factors, and are generally small but distinct, i.e. between the \(\Delta \Delta \delta\) 0.6 and 18.6 Hz (\(^1\)H NMR) for phosphorus thio acids 133 (\(R^1 = Me\), \(R^2 = \text{OMe}\)) and 133 (\(R^1 = Me\), \(R^2 = \text{BuO}\)), respectively, using (\(S\)-\(\alpha\)-methylbenzenemethanamine. Since the \(^1\)H NMR spectra tend to be complex due to P–H and H–H coupling, \(31\)P NMR proved to be an alternative since these provide only two signals for (partially) racemic phosphorus thio acids. Moreover, the two techniques appeared to be complementary as is shown for phosphorus thio acid 133 (\(R^1 = Ph\), \(R^2 = Me\)), which shows a \(\Delta \Delta \delta\) value of 14.4 Hz in the \(^1\)H-NMR and no separation in the \(^{31}\)P-NMR using (\(S\)-\(\alpha\)-methylbenzenemethanamine whereas for 133 (\(R^1 = Ph\), \(R^2 = t-Bu\)) no separation was found in the \(^1\)H NMR but \(\Delta \Delta \delta\) 24.4 Hz in the \(^{31}\)P NMR using (\(S\)-\(\alpha\)-methyl naphthalene-1-methanamine. It is interesting to note that nonequivalences were also observed in the case the chirality on phosphorus was introduced by isotopic exchange (\(D\) for \(H\)) as is shown for 133 (\(R^1 = \text{CH}_3\), \(R^2 = \text{CD}_3\)). Despite the slight differences between these groups, a remarkable \(\Delta \Delta \delta\) value of 4.1 Hz was observed in the \(^1\)H NMR! At this point it should be noted that the diastereomeric shift dispersion is also visible in the \(^{13}\)C-NMR, although the prolonged time to record a \(^{13}\)C spectrum that also contains excessive P–C coupling is clearly less attractive. Furthermore, this particular article provides a very detailed study concerning the chirality aspects of diastereomeric salt formation. Diastereomeric salt formation can also serve as a tool to follow the resolution on a continuous manner as has been shown by \textit{Kuchen} and \textit{Kutter}. They were able to resolve (4-methoxyphenyl)methylphosphinothioic acid by means of crystallization of the diastereomers using optically active quinine and use the same combination to determine the enantiomeric composition of the mixture at any moment by means of \(^{31}\)P-NMR, for which a \(\Delta \Delta \delta\) value of 0.3 ppm were observed.

We were able to use diastereomeric salt formation as method for the \(ee\) determination of phosphoric acids 41 (Scheme 13), employing enantiomerically pure amines or amino alcohols like e.g. \(L\)-ephedrine (Figure 9).

Surprisingly, the method is not applicable for the determination of the \(ee\) of the amines or amino alcohols (the reciprocal situation), by using enantiomerically pure phosphoric acid. This clearly shows that great care has to be taken when dynamic systems are used for \(ee\) determining processes.

Self-association of enantiomers, i.e. of chiral phosphonates or phosphonic amides in solution, would result in diastereomeric aggregates which in principle allow \(ee\) determination by \(^{31}\)P NMR, following the principle introduced by \textit{Horeau} (vide supra).

![Figure 9](image-url)

**Figure 9.** \(^{31}\)P-NMR spectra of \(L\)-ephedrine with: (a) racemic 41 (\(R = 2-\text{Cl}\)), (b) \((-\text{-})\)-41 (\(R = 2-\text{Cl}\)) 45% \(ee\) and (c) \((-\text{-})\)-41 (\(R = 2-\text{Cl}\)) 98% \(ee\).
Harger\textsuperscript{123} has demonstrated the $^1$H NMR nonequivalence of amide 134 (and several analogues), which exhibits two distinct doublets (due to a phosphorus coupling) for the methyl groups whereas enantiomerically pure 134 and racemic 134 show a single doublet signal (Scheme 41). The observed NMR nonequivalences can be rationalized via molecular association involving H-bonding to yield diastereomeric complexes 135 and 136. This allows ee determination of this type of chiral phosphorus compounds based on self-recognition, although the applicability seems to be limited to special cases so far. Also combinations of amides and phosphonates can be analyzed.

Pasquier and Marty were able to determine the enantiomeric composition of 1-(diphenylphosphino)propane-2-thiol (137) by the addition of less than one equivalent of Ni(NO$_3$)$_2$·6H$_2$O in CH$_2$Cl$_2$/MeOH (1°/1)\textsuperscript{124}. By complexation of two molecules of 137 to Ni\textsuperscript{11} the meso and D,L diastereomers of trans-Ni\textsuperscript{11} 138 are formed in situ (Scheme 42), which can be analyzed by means of $^{31}$P NMR, showing two nicely separated singlets with a diastereomeric shift dispersion of $\Delta \delta$ 0.80 ppm.

A further example using this methodology is the chelation of two molecules of chiral (1-aminoalkyl)phosphonic acid 139 with Pd\textsuperscript{11} in alkaline D$_2$O solutions\textsuperscript{125}. The obtained diastereomers, the optically active R, R (or S,S) and meso (R,S) forms 140 (Scheme 43), give diastereomeric shift differences up to $\Delta \delta$ 0.18 ppm, depending on the structure of the phosphonic acid used.

Another remarkable example, where the substrate serves as its own reference, is by making use of the different crystallization behaviour of enantiomers and (partially) racemates which can be detected by using $^{31}$P solid-state magic-angle spinning NMR ($^{31}$P MAS NMR)\textsuperscript{126}. The enantiomers and racemates generally crystallize in different point groups, and crystallization of a mixture of two enantiomers gives some racemic crystallite the amount of which is governed by the quantity of the enantiomer representing the minor constituent.

VI Conclusions

Due to the increasing number of phosphorus derivatizing reagents and the ready availability of NMR spectrometers able to record phosphorus spectra on a routine basis, phosphorus NMR rapidly gained importance in the field of ee determination. The high sensitivity and abundance of the phosphorus nucleus and the relatively simple spectra of the diastereomeric products if broad-band $^1$H-de-coupling is used, clearly indicate the advantages. Moreover, phosphorus derivatizing reagents are very reactive towards a large number of substrates, including alcohols, amines, thiols, amino alcohols and amino acids. Also chiral alkenes and sulfoxides can be analyzed using phosphorus-based methods. The high reactivity is sometimes, however, a disadvantage since the application of these reagents require drastic exclusion of e.g. water and/or air.

Alternatively, chiral phosphorus reagents designed to be used in aqueous solutions have been successfully applied. Although in the past many phosphorus derivatizing reagents were based on expensive chiral starting materials, several reagents are now available based on much cheaper auxiliaries and even very cheap achiral reagents, like PCl$_3$.

Our knowledge of steric and electronic factors of the phosphorus reagents influencing the diastereomeric shift dispersions is rapidly growing and several new or improved reagents can be foreseen to be developed in the near future.

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1. **V. Schurig, Kontakte (Darmstadt)** 1, 54 (1985); 
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13. **V. Schurig, Kontakte (Darmstadt)** 1, 3 (1986); 

See for some selected examples;
27. **S.G. Allenmark,** J. Biochim. Biophys. Methods 9, 1 (1984); 
35. See for some selected examples;
43. **Mislow and Raban** introduced the concept of stereotopicity. See also;
46. At this point it should be noted that stereogenicity and chirality are conceptually distinct. For a discussion see also Ref. 21; 
48. The accuracy of NMR spectra can be discussed in a forthcoming manuscript;
50. The self-association between homochiral and heterochiral molecules will produce diastereomeric interactions for the pure enantiomers (R,R or S,S) and the racemate (S,R or R,S) making a direct determination possible;
It should be noted that the reactions are best performed when high concentrations are applied, which normally means that a slurry is used at the start of the reaction. These observations are in full agreement with the observations made by Johnson and co-workers.  

Using D,L-serine at a ratio differing from the expected 50:50 ratio so that the reaction goes to completion, we have shown that a competitive attack of the alcohol moiety also takes place, leading to some diastereoselectivity.

This is surprising, since it is possible to synthesize chlorophosphorinane 42 from reagent 40, using the same reagents CCl₄ and Et₂O, without additional sulfur to give a quantitative yield. The liberated chloride attacks the initially formed trichloroacetyl ester 42, yielding chloroform and chlorophosphorinane 42.

For an outstanding example of double salt formation between a phosphorinane and dimethyl sulfoxide see:

- R. Huse, H. Wynberg, Tetrahedron Lett. 22, 322 (1981);

And for the isolation of a chlorine atom in the phosphorinane derivative 42, see for example:


Since diastereomeric interactions of this kind are dynamic processes, only the protons of the (partially) racemic material will show a non-equivalence; J.P. Guedel, L. Lacombe, A. Horeau, C. R. Acad. Sc. Paris 267, 166 (1968).

Due to the insolubility of BNHP in organic solvents the pyridine salt was prepared to overcome this problem.

In fact only one other NMR method based on chiral silver shift reagents is known for this purpose while also HPLC or GC techniques are scarce.

- W. Offermann, A. Mannschreck, Tetrahedron Lett. 22, 322 (1981);

Four for an outstanding example of double salt formation between a phosphorinane and dimethyl sulfoxide see:


As for the isolation of a chlorine atom in the phosphorinane derivative 42, see for example:


The use of equimolar or excess quantities of base is possible as no HCl is needed to promote the conversion of trialkylphosphite into dialkyl phosphate.

On the basis of these observations, HPO₃Cl₂ should be the best derivatizing reagent for chiral thiols. This is, however, a very unstable compound and alternative single step preparations of S,S-dialkylphosphonothioates HPO₃OK⁺ are lacking. See for example:


- The products as obtained upon reaction with sulfur powder are the same as the products obtained by reaction using 36 as derivatizing reagent.
- Adducts 75 and 76 are obtained upon reaction of (R) or (S)-α-methylbenzenemethanamine with dibromomethane or dibromopropane. Subsequent reaction with HMPT affords the desired reagents.
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97 In various cases the ratio of the meso isomers deviate from the statistical 1/1 ratio, but this has no detectable influence on the accuracy of the ee determination.

98a B.L. Feringa, B. Strijveen, R.M. Kellogg, unpublished results.


102 CH₃P(=S)C₁₂ is obtained in 92% yield from CH₃PCI₂ by a simple procedure involving treatment with sulfur (S₈) powder in the presence of AlCl₃.


113 This is, however, not the case when noncovalent associative complexes are used, since their dynamic behaviour is not reciprocal.


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