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(*S*)-2H-2-oxo-5,5-dimethyl-4(*R*)-phenyl-1,3,2-dioxaphosphorinane, a New Reagent for the Enantiomeric Excess Determination of Unprotected Amino Acids using ^{31}P NMR

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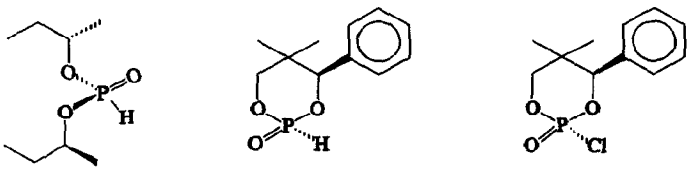
Abstract: Diastereomeric amide derivatives of title phosphorinane **2** and unprotected amino acids are easily prepared in aqueous solutions, showing well separated signals in the ^{31}P NMR spectra allowing accurate *e.e.* determination.

The tremendous effort in asymmetric synthesis and the rapidly increasing use of enantiomerically pure compounds as chiral building blocks, auxiliaries, ligands or catalysts requires the development of fast and accurate methodologies for the determination of the enantiomeric composition[1]. Especially the rapid assessment of the stereochemical results of the enzymatic resolution of *synthetic amino acids* and the increasing use of natural and unnatural amino acids and their derivatives in protein modifications and the applications as chiral ligands in numerous asymmetric syntheses is a strong incentive for the development of new methods for the enantiomeric excess determination of this type of compound[2].

The enantiomeric purity of amino acids can routinely be analyzed by means of several gas or liquid chromatographic techniques[3]. Moreover, a number of *NMR* methods for the analysis of *amino acid derivatives* exist[4,5,6]. Methods for the analysis of *free amino acids*, however, are scarce, mainly due to their low solubility in organic solvents. Besides the use of aqueous chiral shift reagents[7], new methodologies developed in our laboratory include the derivatization using α-chloropropionyl chloride (for ^{1}H NMR)[8] and chiral phosphorinane **1** (for ^{31}P NMR)[9]. Although phosphorinane **1** is readily coupled with amino acids in aqueous solutions using the Atherton, Openshaw and Todd reaction conditions[10], the diastereomeric shift differences obtained are relatively small and sometimes resolution is not sufficient for quantification purposes[9].

We now wish to report a simple and efficient new chiral derivatizing agent, (*S*)-2H-2-oxo-5,5-dimethyl-4(*R*)-1,3,2-dioxaphosphorinane **2**, for the derivatization of amino acids in aqueous solutions using the

conditions mentioned before (Scheme 1).

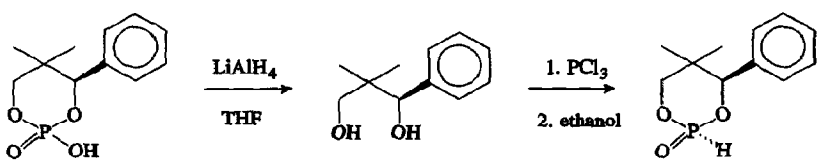


1 2 3

Scheme 1

Phosphorinane 2 strongly resembles phosphoric acid chloride 3, that we recently reported as a chiral derivatizing agent for the enantiomeric excess determination of alcohols, amines and esters of amino acids, showing excellent diastereomeric shift differences using ^{31}P NMR[11]. It appeared, however, not to be possible to use reagent 2 for enantiomeric excess determination of substrates in aqueous solutions, due to substantial hydrolysis and pyrophosphate formation (*vide infra*).

Enantiomerically pure 2 is easily obtained in 65% yield from (*R*)-phencyphos 4[12] by reduction to the free diol 5 using $LiAlH_4$ in THF (or ether). Subsequent reaction with PCl_3, followed by an Arbuzov rearrangement[13] using ethanol (or water[14]), yields (*S*)-2H-2-oxo-5,5-dimethyl-4(*R*)-phenyl-1,3,2-dioxaphosphorinane 2 as a single diastereomer in 83% overall yield (Scheme 2).

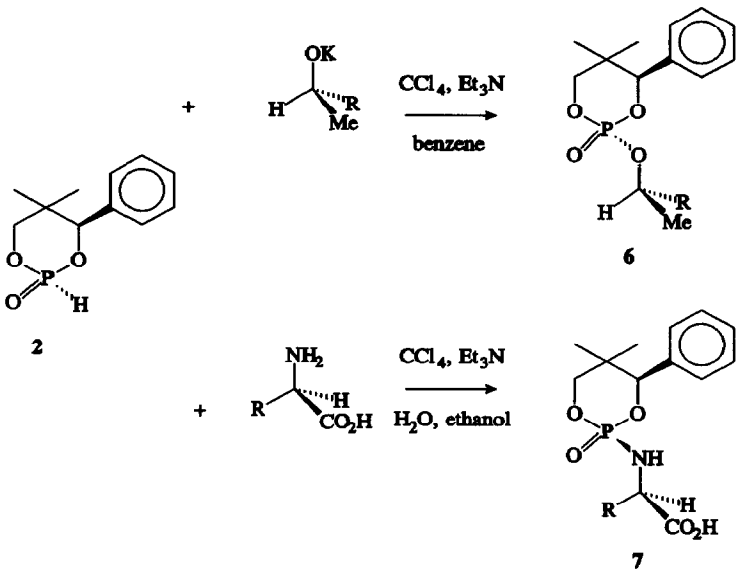


4 5 2

Scheme 2

Phosphorinane 2 reacts with a variety of nucleophiles including alcohols, amines, amino acid esters and unprotected amino acids using the Atherton, Openshaw and Todd coupling with CCl_4 and Et_3N as reagents and ethanol as (co)-solvent[10] (Scheme 3). Furthermore, water is acceptable as (co)-solvent if desired when unprotected amino acids are reacted with reagent 2.

A typical derivatizing procedure for *unprotected amino acids* involves the mixing of the substrate of interest (0.1 mmole) and phosphonate 2 (0.1 mmole) with Et_3N (1 mL), ethanol and water (both 1 mL) and cooling the resulting suspension to 0 °C with stirring. CCl_4 (1 mL) is added dropwise to the suspension, and the mixture is stirred for 8 h at room temperature. The mixture is subsequently acidified



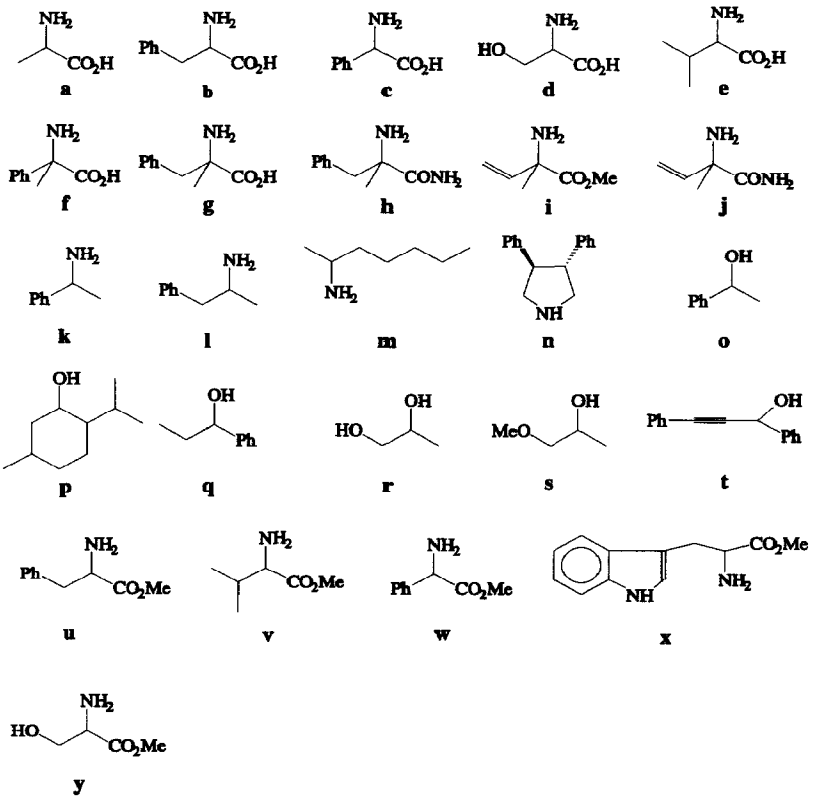
Scheme 3 *The use of reagent 2 as derivatizing agent for alcohols and amino acids.*

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 ratios. In several cases, the products can be purified by crystallization from ethyl acetate-petroleum ether mixtures or by column chromatography on silica gel, providing the products **6** or **7** 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. Alcohols that are reacted with reagent **2** can best be transferred into the potassium salts prior to the actual reaction to enhance the nucleophilicity.

Some of the results are collected in Table **1**, the indices refer to Scheme **4**. As can be seen from the data collected in Table **1**, 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 $\Delta\delta$ were the largest for *d,l*- phenylglycine (**c**, $\Delta\delta$ 1.218 ppm) and relatively small for *d,l*-alanine (**a**, $\Delta\delta$ 0.256 ppm). The other amino acids gave comparable $\Delta\delta$ values, all situated in this chemical



Scheme 4 *Representative chiral alcohols, amines and amino acids used in the ^{31}P NMR analysis with reagent 2.*

shift range. For *d,l*-serine (**d**) a ratio different from the expected 50:50 value was found (47:53). We assume that a competitive attack of the alcohol moiety also takes place.

Also, *α-alkylated amino acids* can be analyzed with reagent **2**, which gave diastereomeric shift differences between Δδ 0.786 (**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.

For amines, the obtained diastereomeric differences typically are between the Δδ 0.112 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 stereogenic 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

Substrate	δ (ppm)	Δδ (ppm)	ratio
a	1.01	0.26	49.5:50.5
b	0.56	1.02	49.5:50.5
c	0.92	1.22	49.5:50.5
d	0.78	0.93	47:53
e	0.89	0.87	49.5:50.5
f	-2.09	1.65	49.5:50.5
g	-1.20	0.93	49.5:50.5
h	0.32	0.79	49.5:50.5
i	0.56	1.35	49:51
j	0.12	0.60	50:50
k	5.05	0.63	49.5:50.5
l	1.86	0.54	49.5:50.5
m	2.25	0.11	50:50
n	3.35	0.48	49.5:50.5
o	-7.34	0.07	49:51
p	-1.25	0.05	49.5:50.5
q	-7.91	0.26	50:50
r	-7.41	0.91	49.5:50.5
s	-5.23	0.07	49.5:50.5
t	-11.65	0.17	49:51
u	4.73	0.51	49.5:50.5
v	2.28	0.21	49.5:50.5
w	0.82	0.30	49:51
x	11.51	0.44	49.5:50.5
y	-7.45	0.20	49.5:50.5

Table 1 *^{31}P NMR data of products **6** and **7**, complexed with 1 mole H_2O, recorded in $CDCl_3$ L= 0.1 M.*

rather than the amine functionality (δ -7.45 ppm, Δδ 0.201 ppm). *d,l*-Methyltryptophan (**x**) probably is coupled through the ring nitrogen, and gives a Δδ value of 0.442 ppm. For the alcohol substrates, no relation between the observed diastereomeric shift differences and structure was found, the shift differences being between the Δδ 0.048 (**p**) and Δδ 0.912 ppm (**r**). A typical example is illustrated in Figure **1**.

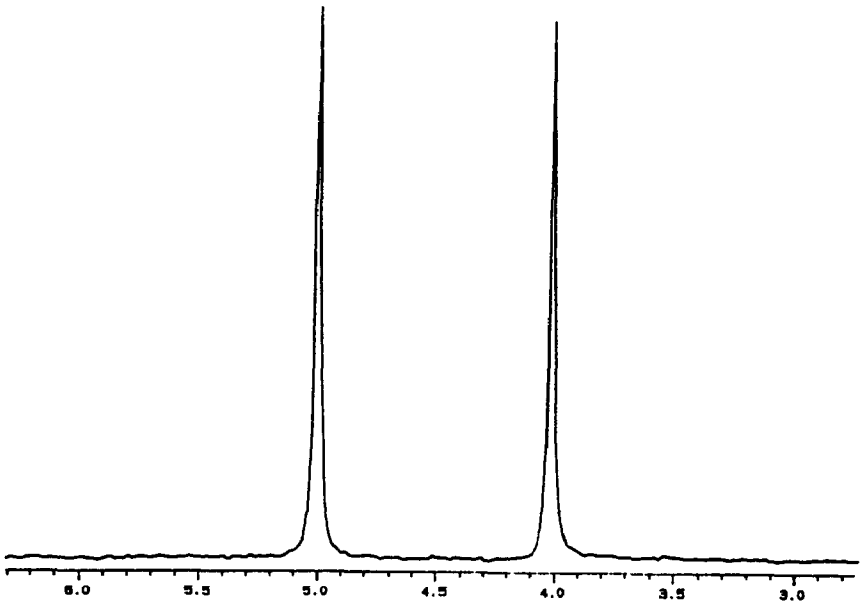


Figure 1 *Decoupled ^{31}P NMR spectrum of derivative* ***6****, R= Ph, recorded in C_6D_6, L= 0.1 M.*

The reactions normally proceed without the formation of side products, although sometimes small amounts of the *pyrophosphate* of phencyphos are formed (^{31}P NMR signal at δ -20.56 ppm)[15]. The formation of this pyrophosphate, however, does not influence the actual *e.e.* determination.

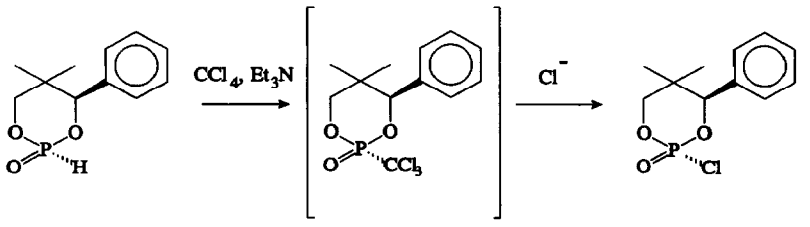
The enantiomeric composition of some enriched samples as determined with reagent **2** was checked by comparison with the enantiomeric purity determined by the α-chloropropionyl chloride[8] and the *sec*-butylphosphonate[9] **1** method. The results appeared to be within the experimental error (2%).

Substrate	**polarimetry**	**α-chloropropionyl method**	**reagent 2**
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-phenylethylamine	70.8:29.2	70.1:29.9	70.9:29.1.

Table 2 *Comparison of the enantiomeric composition as determined using reagent 2, optical rotation and the α-chloropropionyl chloride method.*

It is important to note that, although the reaction conditions and the derivatizing reagents are different when comparing reagents **2** and **3**, the products as well as the stereochemistry at phosphorus are the same.

Using (*S*)-phencyphos, the absolute stereochemistry of the phosphorus atom in reagent **3** is *S*, based upon the X-ray structure. For **2**, 2D NMR NOE experiments (NOE interactions were observed for the 2H and the benzylic protons) clearly indicated that the phosphorus atom in reagent **2** has the *S* configuration when using (*R*)-phencyphos[16].



2 **8** **3**

Scheme 5 *Proposed reaction sequence using Et_3N, CCl_4 and reagent (4R)-2.*

Based upon the fact that reagent **3** does not tolerate water as (co)-solvent during derivatization, contrary to reagent **2**, it is concluded that the reactive intermediates are probably not the same. This is surprising, since it is possible to synthesize chlorodioxaphosphorinane **3** from reagent **2**, using the same reagents CCl_4 and Et_3N, but without additional substrate in nearly quantitative yield. The liberated chloride probably attacks the initially formed trichloromethylester **8**, yielding chloroform and the chlorodioxaphosphorinane **3** with overall retention of configuration on phosphorus. It is known that *large* substituents at phosphorus preferentially assume the axial position, leaving the double bonded oxygen in the equatorial position[17]. Although there is no evidence, except for the absolute configuration of **2** and **3**, the most likely route to **3** would involve two subsequent retentions of configuration on the phosphorus center (Scheme 5).

The phosphorus atom in reagent **2** is chiral, and derivatization reactions in principle can proceed with retention or inversion of configuration at the phosphorus center. However, ^1H NMR, NOESY and X-ray analyses (not shown) clearly indicated that reactions with amines proceed with inversion and with alcohols with retention of configuration at the phosphorus center (Scheme 3). These phenomena as well as the relationship between intrinsic structure and diastereomeric shift differences of adducts **6** and **7**, will be treated in detail in a forthcoming manuscript[16].

In conclusion, the new chiral derivatizing agent **2** gives excellent results in the *e.e.* determinations by ^{31}P NMR, showing larger diastereomeric shift differences compared to the use of reagent **1**, allowing broad structural variation in substrates, including unprotected amino acids. Moreover, reagent **2** can be used for derivatization purposes in aqueous solutions.

Acknowledgement

We wish to thank Prof. Dr. R.M. Kellogg and Dr. B. Kaptein (DSM Research) for the gift of α-alkylated amino acids and amino acid amides and DSM Research for financial support.

Experimental

^{31}P, ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR 300 instrument at 30 °C. The chemical shifts are expressed relative to $CDCl_3$ for ^{1}H NMR (at δ 7.26 ppm) or ^{13}C NMR (at δ 76.91 ppm) and to $(NPCl_2)_3$ (at δ 19.91 ppm) for ^{31}P NMR spectra. All solvents were dried according to literature procedures. Deuterated solvents were dried over an Al_2O_3 (activity I) column just prior to use.

(R)-1-Phenyl-2,2-dimethyl-1,3-propanediol 5

This material was prepared according to a modified literature procedure[12].

A solution of 7.00 g (180 mmole) of $LiAlH_4$ in dry ether (150 mL) was brought to reflux. (R)-Phencyphos[12] (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 **5**. Yield 9.36 g (52.10 mmole, 65%). Mp 65-66 °C; $[\alpha]_D^{20}$= -49.9 (c 1.0, $CHCl_3$); ^{1}H 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, $^{2}J_{AB}$= 10.75 Hz, 1H), 3.53 (d, $^{2}J_{AB}$=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 2

A solution of 5.00 g (27.80 mmole) diol **5** 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 **2** as a white solid. Yield 5.21 g (23.07 mmole, 83%). Mp 151-153 °C; $[\alpha]_D^{20}$= -73.02 (c 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.81 (s, 3H), 1.08 (s, 3H), 4.01 (dd, $^{2}J_{AB}$= 11.53 Hz, $^{3}J_{PH}$= 25.85 Hz, 1H), 4.20 (dd, $^{2}J_{AB}$= 11.53 Hz, $^{3}J_{PH}$= 3.85 Hz, 1H), 5.16 (d, $^{3}J_{PH}$= 3.20 Hz, 1H), 7.03 (d, $^{1}J_{PH}$= 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, $^{3}J_{PC}$= 4.78 Hz, C), 86.82 (d, $^{2}J_{PC}$= 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.

Typical procedure for the enantiomeric excess determination of alcohols, amines and amino acids using phosphonate 2:

For alcohols and amines; 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 **2** (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 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. Alcohols are best coupled by using the corresponding potassium salts.

For amino acids;

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 phosphonate **2** 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.

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