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The Rational Design and Application of New Chiral Phosphonates for the Enantiomeric Excess Determination of Unprotected Amino Acids. Remarkable pH Dependency of the Diastereomeric Shift Differences.

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Abstract: Diastereomeric amide derivatives of chiral phosphorinane **8** and unprotected amino acids are easily prepared in aqueous solutions, showing well separated signals in the ^{31}P NMR spectra allowing accurate enantiomeric excess determination. Moreover, the obtained diastereomeric shift dispersion appears to be highly pH dependent, indicating the influence of intramolecular ion pair formation on the diastereomeric shift dispersion.

The development of new chiral derivatizing agents for the enantiomeric excess (*e.e.*) determination is a major endeavour[1]. It is obvious that with the design of such agents factors that govern the relationship between the intrinsic structural variations and the chemical shift differences of the diastereomeric products have to be taken into account[2]. It is well established that diastereomeric shift differences respond to steric effects, non-bonded interactions and can often be tuned by the distance between stereogenic centers in auxiliary and chiral substrate[3]. One of the most important factors in this respect is the restriction of conformational freedom resulting in two diastereomers with distinct conformations.

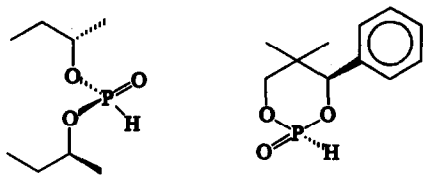
Our approach is the formation of distinct conformations by means of intramolecular *locking*, showing a larger diastereomeric shift dispersion when compared to the diastereomers that are not conformationally locked (Scheme 1).

unlocked locked

Scheme 1

Substrates that are highly suitable for such an approach are unprotected amino acids, possessing an α-carboxyl group with a pK_a that is typically close to 2 and an α-amine group having a pK_a between 9 and 10. Moreover, at physiological pH (pH 7) free amino acids exist largely as zwitterions, having both a

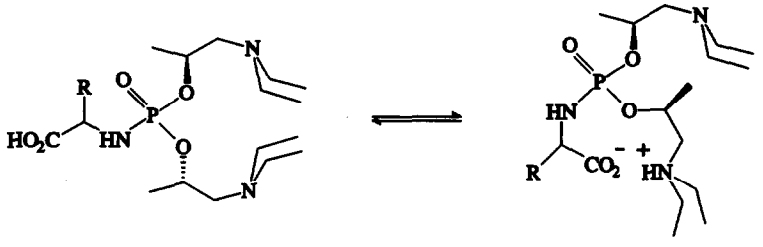
quaternary ammonium and a carboxylate ionic group. The conformational and chemical shift behaviour of the derivatized amino acids could thus be studied as a function of the pH.



1 **2**

Scheme 2

We recently developed two reagents, O,O-di-sec-(*S*)-butylphosphonate **1**[4] and (*S*)-2H-oxo-5,5-dimethyl-4(*R*)-phenyl-1,3,2-dioxaphosphorinane **2**[5] for the enantiomeric excess determination of unprotected amino acids in aqueous solutions. Although a number of other derivatizing agents are known for the enantiomeric excess determination of unprotected amino acids[6,7], these reagents do not easily allow the required structural modifications. In contrast, reagents **1** and **2** can easily be modified, being ideal structures for the purpose described above.



3 **3**

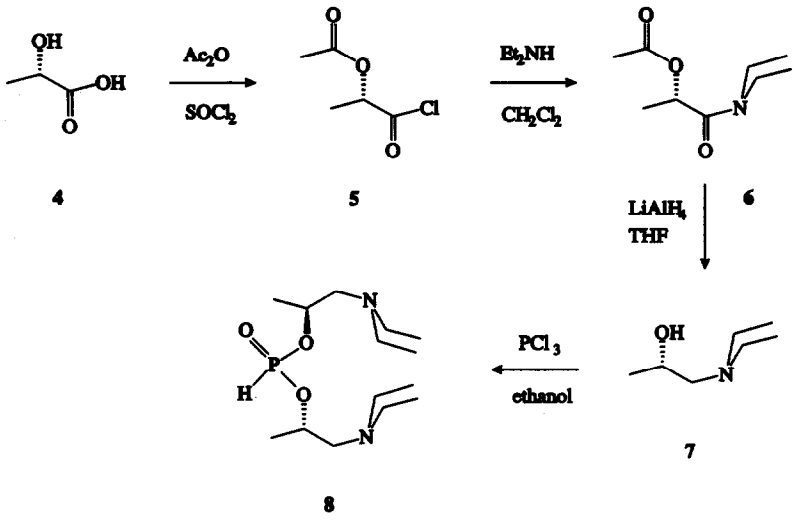
unlocked **locked**

Scheme 3

Adduct **3** for instance meets the requirements (Scheme 3). The protonated amine moiety in **3** could possibly *lock* the deprotonated acid part of the molecule by means of an intramolecular ion pair formation. The conformational behaviour is expected to be a function of the pH.

Therefore, we envisaged to introduce a tertiary amine functionality in reagent **1**, as is shown in structure **8** (Scheme 4). A synthetic route to target phosphonate **8** is based on enantiomerically pure lactic acid **4**. When *S*-lactic acid **4** (Scheme 4) was treated with acetic anhydride followed by thionyl chloride, the acetyl protected acid chloride **5** was obtained in 60% yield after distillation[8].

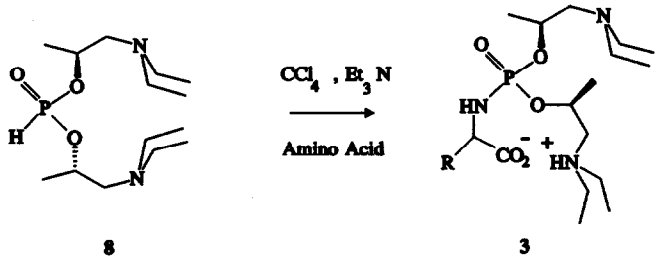
The enantiomeric composition of this material was checked by ^{1}H NMR after conversion with *l*-α-phenylethylamine. For racemic **5**, two distinct diastereomers of the corrseponding amides were obtained. A single diastereomer was present when **5** was prepared from (*S*)-**4**, and it was concluded that the conversion to the acid chloride did not influence the enantiomeric composition. The protected acid chloride **5** was subsequently converted into amide **6** in 91% yield by reaction with diethylamine. Subsequent reduction



Scheme 4

($LiAlH_4$ in THF) afforded amino alcohol **7** in 69% yield after Soxhlet extraction from the lithium and aluminium salts. The enantiomeric composition of amino alcohol **7** was checked with a chiral phospholidine based method[9] and was over 98%. The reaction of **7** (as HCl salt) with PCl_3 yielded several products, including the desired phosphonate **8** (37% yield). By ^{31}P NMR, using the method of Feringa et al[10], it was shown that **8** consists of one enantiomer only. Although several amines were used in the amide formation with **5**, like benzylamine, aniline and *S*-α-phenylethylamine, these are not presented here because they turned out to be less easy to handle. During subsequent reduction into the corresponding amino alcohols partial racemization took place and the subsequent functionalization with PCl_3 gave several side products, in particular elimination products.

Reagent **8** can be coupled with unprotected amino acids using the Atherton-Openshaw-Todd reaction conditions[11], employing Et_3N and CCl_4 as reagents in aqueous solutions (Scheme **5**).



Scheme 5

Subsequent workup by means of extraction using ethyl acetate and recording of a decoupled ^{31}P NMR spectrum in $CDCl_3$ afforded the enantiomeric compositions. The analysis of the amino acid products took

place at pH 7-8 ($CDCl_3$) and the observed diastereomeric shift differences Δδ are, as expected, pH dependent (*vide infra*). The use of reagent **8** in the enantiomeric excess determination of amines and unprotected amino acids is less facile than that of reagents **1** and **2**, primarily due to the rather low stability of **8** yielding several elimination products. Furthermore, it became clear that reagent **8** could not be properly coupled to alcohols, because of severe decomposition.

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

Table 1 ^{31}P NMR data of derivatives **3** using racemic amines and amino acids, recorded in $CDCl_3$, [L]= 0.01 M (PG= Phenylglycine)

In Table 1 several results are collected using derivatizing agent **8**, amines and unprotected amino acids. The structure of reagent **8** shows closest resemblance to that of reagent **1**[4], and for this reason a comparison is made between these two reagents in their ability to discriminate between enantiomers. When the product of *d,l*-2-butylamine and **8** (Δδ 0.151 ppm) is compared to the product of reagent **1** (Δδ 0.102 ppm) the difference in Δδ is not large, although somewhat larger for reagent **8**. Also when *d,l*-α-phenyl-ethylamine is used, the difference in Δδ is larger using reagent **8** (Δδ 0.213 ppm) compared to **1** (Δδ 0.185 ppm). Probably, the bulk of reagent **8** has a positive influence upon the diastereomeric shift dispersion of products **3** compared to products formed using reagent **1**. When *d,l*-alanine is coupled to reagent **8** a diastereomeric shift difference Δδ of 0.273 ppm is observed in comparison with 0.099 ppm for the product using reagent **1**. For *d,l*-phenylalanine (Δδ 0.243 ppm) the product **8** also showed a significantly larger diastereomeric shift dispersion than the values for corresponding product using reagent **1** being only Δδ 0.025 ppm. The amino acids *d,l*-valine, *d,l*-phenylglycine and *d,l*-tryptophan, with a Δδ of 0.252, 0.347 and 0.189 ppm, respectively, when coupled to reagent **8**, also show larger diastereomeric shift differences when compared to the shifts obtained with reagent **1**, being 0.069, 0.098 and 0.038 ppm. All data are recorded with half an equivalent of water complexed to the products; it was not possible to remove this water from the products **3** without inducing decomposition processes.
Furthermore, the enantiomeric compositions as determined using reagent **8** were compared to the ratios as obtained using reagent **1**[4], and were in good agreement.

If the larger diastereomeric shift differences using reagent **8** indeed arise from the restricted conformational freedom due to intramolecular ion pair formation (in **3**) rather than from small, structural differences in the vicinity of the phosphorus nucleus, compared to the corresponding adducts of reagent **1**, the observed shift differences should show a large pH dependency.

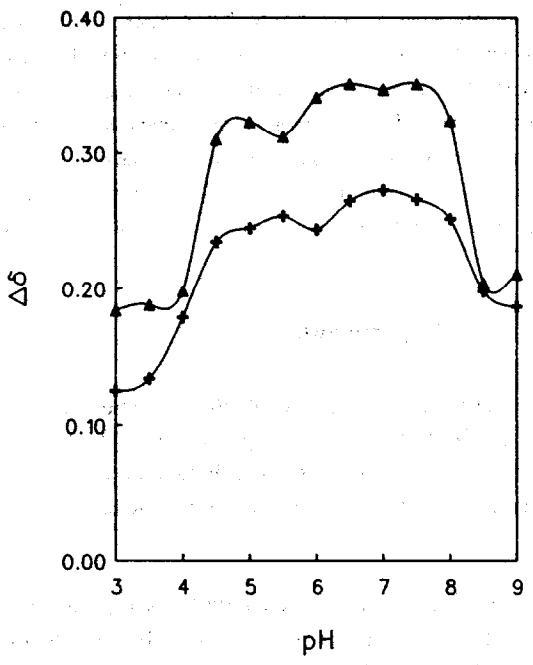


Figure 1 ^{31}P NMR Δδ values of derivatives **3** of d,l-Ala (+) and d,l-PG (▲) vs pH, recorded in D_2O [L]= 0.01 M

Using *d,l*-alanine as substrate coupled to reagent **8**, a dependence of the diastereomeric shift differences upon the pH was observed (Figure 1). The shift differences appear to be significantly higher in the pH range from 4.5 to 8.0, reaching a maximum Δδ value at pH 7. Furthermore, decomposition of the products above pH 10.0 was observed, as expected. In the pH domain of increased shift differences itself, the differences are relatively small. At lower pH, the protonation of the carboxylic acid group probably gives rise to a situation in which the intramolecular tight ion pair no longer exists. The product presumably resembles more strongly the structure of the analogous adduct using reagent **1**, which indeed shows a smaller diastereomeric shift dispersion (Δδ 0.12 vs 0.099 for **3** and **1**, respectively). At high pH, the total deprotonation of the product **3** also results in a situation in which the tight ion pair contribution to the restricted conformation is of little importance. As a result, the diastereomeric shift dispersion is smaller in the higher pH domain, when compared to the pH 4.5 to 8.0 domain. Using *d,l*-phenylglycine, analogous behaviour is found, as can be seen in Figure 1. Again, the diastereomeric shift dispersion is largest in the domain of pH 4-8. Although not extensively studied, the other amino acid products of reagent **8** showed the same type of behaviour. The products **3** with *d,l*-2-butylamine and *d,l*-α-phenylethylamine do not show this pH dependency. It should be emphasized that in control experiments where reagent **1** is coupled to amino acids, the shifts and ΔΔδ values also appear to be pH dependent, although the differences are very small (about 0.02 ppm maximum) and probably arise from the normal dependency of the ^{31}P nucleus to solvent and polarity effects.

The observed phenomena strongly suggest (but are not the ultimate proof) that a mechanism of

conformational locking must be in operation in the pH 4.5 to 8.0 domain when using amino acids as substrates. Therefore, the use of products **3** result in larger diastereomeric shift differences than observed outside this domain. Although reagent **8** can be used for the enantiomeric excess determination of unprotected amino acids in aqueous solutions, the low stability of reagent **8** and the adducts **3** *clearly limits the scope*. We showed, however, that the chiral auxiliaries and derivatizing agents can be tuned by the pH of the solution and this remarkable behaviour can be rationalized by a conformational locking model. This can serve as a working model for further developments towards a more rational design of chiral derivatizing agents.

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.

2-(S)-O-Acetylpropionylchloride **5**

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

2-(S)-O-Acetylpropionyl-N,N-diethyl amide **6**

A solution of 48.6 g (0.65 mol) of diethylamine and 67.4 g (0.65 mol) Et_3N in CH_2Cl_2 (500 mL) was cooled to 0 °C. A solution of 100.0 g of **5** in CH_2Cl_2 (125 mL) was added slowly, while the temperature was maintained at 0 °C. After the addition the temperature was allowed to reach room temperature again, and the mixture was stirred for 12 h. Subsequently, the mixture was washed three times with a saturated NH_4Cl solution (150 mL) and once with water (150 mL). The CH_2Cl_2 layer was dried over Na_2SO_4 and concentrated. The resulting slightly yellow oil was distilled at 126-127 °C (13 mm Hg) to afford **6** as a colorless oil. Yield 110.33 g (0.59 mol, 91%). $[\alpha]_D^{20}$= -26.44° (c 0.55, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.95 (dd, $^{3}J_1$=$^{3}J_2$= 7.08 Hz, 3H), 1.09 (dd, $^{3}J_1$=$^{3}J_2$= 7.32 Hz, 3H), 1.27 (d, ^{3}J= 6.83 Hz, 3H), 1.94 (s, 3H), 3.17 (m, 2H), 3.31 (m, 2H), 5.13 (q, ^{3}J= 6.83 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 12.34 (CH_3), 13.74 (CH_3), 16.76 (CH_3), 20.33 (CH_3), 40.08 (CH_2), 41.17 (CH_2), 66.54 (CH), 169.11 (C), 170.08 (C); Analysis calcd for $C_9H_{17}O_3N$, C: 57.73, H: 9.15, N: 7.48. Found C: 57.22, H: 8.89, N: 7.29; HRMS calcd 187.121, found 187.121.

2-(S)-N,N-Diethyl-1-amino-2-hydroxypropane **7**

A suspension of 25.1 g (0.64 mol) of $LiAlH_4$ in dry THF (500 mL) under nitrogen was cooled to 0 °C. A solution of 75.0 g (0.43 mol) amide **6** in 50 mL of THF was added slowly, while the temperature was not allowed to exceed 5 °C. After the addition was completed (1 h), the mixture was brought to reflux for 12 h. Subsequently, water was added (25 mL) followed by 25 mL of a 1 N KOH solution. The solution was stirred with 15.0 g of Celite, followed by a Soxhlet extraction of the Celite-salt mixture with THF. The THF layers were dried over Na_2SO_4 and concentrated. The yellow residue was purified by means of column chromatography over silica gel, using ethyl acetate-hexane as eluent. Yield 39.30 g (0.30 mol, 69%) of **7** as a colorless oil. $[\alpha]_D^{20}$= -34.21° (c 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.00 (dd, $^3J_1$=$^3J_2$= 4.80 Hz, 6H), 1.11 (d, ^{3}= 6.00 Hz, 3H), 2.19 (dd, $^2J_{AB}$= 11.40 Hz, ^3J= 10.80 Hz, 1H), 2.38 (dd, $^2J_{AB}$= 11.40 Hz, ^3J= 1.20 Hz, 1H), 2.44 (m, 2H), 2.63 (m, 2H), 3.52 (s, br, 1H), 3.71 (ddq, ^3J= 10.80 Hz, ^3J= 6.00 Hz, ^3J= 1.20 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 11.57 (CH_3), 15.52 (CH_3), 28.59 (CH_2), 41.54 (CH_2), 48.91 (CH_2), 70.12 (CH_3), 84.01 (CH); Analysis calcd for $C_7H_{17}ON$, C: 64.07, H: 13.06, N: 10.67. Found C: 63.88, H: 12.96, N: 10.39; HRMS calcd 131.131, found 131.131.

2-(S)-1-Amino-N,N-diethyl-2-hydroxypropane.HCl, HCl salt of **7**

Free **7** was dissolved in 250 mL dry ether and upon treatment with HCl gas the product crystallized spontaneously. The solid material was collected, washed with hexane and dried in vacuum at 40 °C. Mp 138-140 °C. ^1H NMR ($CDCl_3$): δ 1.24 (d, ^3J= 4.76 Hz, 3H), 1.42 (dd, $^3J_1$=$^3J_2$= 5.31 Hz, 6H), 2.98 (m, 2H), 3.01 (s, br, 1H), 3.22 (m, 2H), 4.38 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 8.63 (CH_3), 20.47 (CH_3), 48.65 (CH_2), 61.27 (CH_2), 61.94 (CH_3), 98.36 (CH).

O,O-Di-(2-(S)-(N,N-diethyl-2-hydroxypropyl)-phosphonate **8**

A solution of 1.00 g (5.98 mmol) of **7**.HCl in 75 mL of CH_2Cl_2 under nitrogen was cooled to 0 °C. To this solution was added dropwise a solution of 0.41 g (3.00 mmol) of PCl_3 in CH_2Cl_2 (10 mL) while the mixture was degassed regularly. After 1 h of stirring at 0 °C, 0.14 g (3.00 mmol) of ethanol was added with vigorous stirring. The reaction mixture was allowed to reach room temperature and the mixture was stirred for 1 h at this temperature. The reaction mixture was concentrated to dryness yielding a white solid material, which was purified by means of chromatography over silica gel (under a nitrogen atmosphere) using $CHCl_3$ as eluent. Yield 0.34 g (1.11 mmol, 37%) of white solid material, being **8**.2HCl. It was not possible to obtain a proper, reproducable rotation for this product. ^1H NMR ($CDCl_3$): δ 1.40 (dd, $^3J_1$=$^3J_2$= 9.01 Hz, 12H), 1.54 (dd, $^4J_{PH}$= 15.60 Hz, ^3J= 5.48 Hz, 6H), 2.98 (m, 4H), 3.22 (m, 4H), 4.31 (m, 2H), 7.41 (d, $^1J_{PH}$= 723 Hz, 1H), 10.82 (d, br, J= 52.94 Hz, 1H), 11.38 (d, br, J= 42.36 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 9.32 (CH_3), 22.23 (CH_3), 50.45 (d, $^3J_{PC}$= 5.40 Hz, CH_2), 64.78 (d, $^5J_{PC}$= 5.21 Hz, CH_2), 67.54 (d, $^3J_{PC}$= 6.98 Hz, CH_3), 101.34 (d, $^2J_{PC}$= 6.89 Hz, CH); ^{31}P NMR ($CDCl_3$): δ 5.81; No proper HRMS or elemental analysis could be obtained, due to decomposition reactions. The enantiomeric composition of this product was checked by the method as described by Feringa et al[10].

Typical procedure for the enantiomeric excess determination of amines and unprotected amino acids using phosphonate **8**

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 **8**.HCl (1.1 mmol) 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 analysis by ^{31}P NMR (by taking the residue in $CDCl_3$, C_6D_6 or D_2O).

Acknowledgement

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References

1. For a review, see: Parker, D., *Chem. Rev.* **1991**, *91*, 1441.
2. For an approach towards the prediction of diastereomeric shift dispersion on the basis of structural analysis see: Zijlstra, R.W.J., Hulst, R., de Vries, N.K., Feringa, B.L., manuscript submitted to *J. Org. Chem.*
3. See for examples:
   a) Fukushima, E., Roeder, S.B.W., in *Experimental Pulse NMR*, Addison-Wesley Publishing Company, London, **1981**.
   b) Oki, M., in *Applications of Dynamic NMR Spectroscopy to Organic Chemistry*, VCH Publishers, Deerfield Beach, **1985**.
   c) Gorenstein, D.G., *J. Am. Chem. Soc.* **1975**, *97*, 898.
4. Hulst, R., de Vries, N.K., Feringa, B.L., *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1092.
5. a) Hulst, R., Zijlstra, R.W.J., Feringa, B.L., de Vries, N.K., ten Hoeve, W., Wijnberg, H., *Tetrahedron Lett.* **1993**, *34*, 1339.
   b) Hulst, R., Zijlstra, R.W.J., de Vries, N.K., Feringa, B.L., *Tetrahedron: Asymmetry*, accepted for publication.
6. a) Brewer, W., Ugi, I., *J. Chem. Res.* **1982**, 271; **1982**, 2901.
   b) Hull, W.E., Seeholzer, K., Baumeister, M., Ugi, I., *Tetrahedron* **1986**, *42*, 547.
   c) Kolasa, T., Chimiak, A., *Rocz. Chem.* **1976**, *50*, 367; CA *82*, 86589b **1976**.
   d) Kolasa, T. Miller, M.J., *J. Org. Chem.* **1986**, *51*, 3055.
7. a) Kruizinga, W.H., Bolster, J., Kellogg, R.M., Kamphuis, J., Boesten, W.H.J., Meyer, E.M., Schoemaker, H.E., *J. Org. Chem.* **1988**, *53*, 1826.
   b) Moorlag, H., Kruizinga, W.H., Kellogg, R.M., *Recl. Trav. Chim., Pays-Bas* **1990**, *109*, 479.
8. a) Anschütz, R., Böcher, R., *Annalen* **1909**, *368*, 53.
   b) Slessor, K.N., King, G.G.S., Miller, D.R., Winston, M.L., Cutforth, H., *J. Chem. Ecol.* **1985**, *11*, 1659.
9. Hulst, R., de Vries, N.K., Feringa, B.L., *Tetrahedron: Asymmetry* **1994**, *4*, 699.
10. a) Wijnberg, H., Feringa, B.L., *Tetrahedron* **1976**, *32*, 2831.
    b) Feringa, B.L., Smaardijk, A., Wijnberg, H., *J. Am. Chem. Soc.* **1985**, *107*, 4798.
    c) Feringa, B.L., Strijtveen, B., Kellogg, R.M., *J. Org. Chem.* **1986**, *51*, 5484.
11. Atherton, F.R., Openshaw, H.T., Todd, A.R., *J. Chem. Soc.* **1945**, 660.

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