

Determination of absolute configuration of the phosphonic acid moiety of fosfazinomycins†

Katharina Schiessl,^a Alexander Roller^b and Friedrich Hammerschmidt^{*a}Cite this: *Org. Biomol. Chem.*, 2013, **11**, 7420Received 1st August 2013,
Accepted 9th September 2013

DOI: 10.1039/c3ob41574k

www.rsc.org/obc

Fosfazinomycins A and B produced by *Streptomyces lavendofoliae* share the same phosphonate moiety with one chiral centre of unknown configuration which was determined by synthesising both enantiomers of 2-hydroxy-2-phosphonoacetic acid methyl ester. A chiral cyclic phosphite was reacted with methyl glyoxylate in a Pudovik reaction to give a pair of diastereomeric α -hydroxyphosphonates, which were separated by HPLC. The configurations at C-2 were assigned on the basis of single crystal X-ray structure analysis. Deprotection of these diastereomers furnished the enantiomeric α -hydroxyphosphonic acids, of which the (S)-configured had the same sign of optical rotation as the phosphonic acid moiety of the two fosfazinomycins.

Introduction

Phosphonates and phosphinates are organic compounds characterised by either one or two phosphorus–carbon bonds. Over the past few decades they have been studied extensively and are nowadays used in medicine and agriculture.¹ They have interesting biological properties,² which can be attributed to their structural similarity to phosphoric acid esters and carboxylic acids, as well as to the high stability of the incorporated P–C bonds.^{3,4}

They can be found both free and bound to structural components such as lipids or proteins. As conjugates with macromolecules they either enhance the structural rigidity of the latter or protect them against enzymatic degradation.⁵ There is a steadily growing, fascinating group of about a dozen small molecules of natural origin containing a P–C bond, some of which are bioactive.^{1,6} Their properties range from antibacterial, antiviral or antibiotic to pesticidal and enzyme inhibitory.

Fosfomycin (**1**) is a clinically used antibiotic,^{1,7} phosphinothricin (**2**) a commercially important and very potent herbicide,⁸ fosmidomycin (**3**) an antimalarial agent⁹ and 2-amino-1-hydroxyethylphosphonic acid (**4**)^{10,11} is a component of the

lipophosphonoglycan of the plasma membrane of *Acanthamoeba castellanii* (Fig. 1).

The current work deals with two other members of the group of small bioactive phosphonates, namely fosfazinomycins A (**5a**) and B (**5b**) (Scheme 1). They were first isolated in

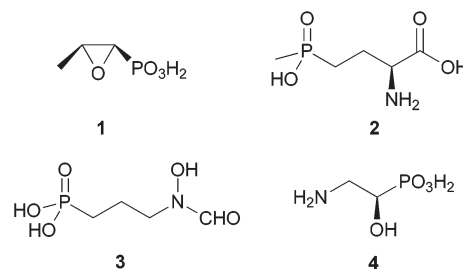
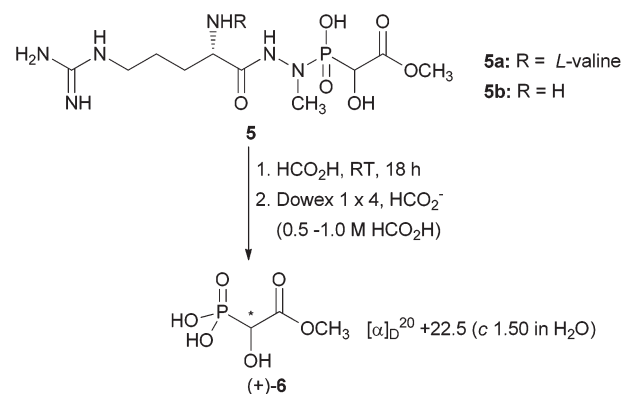


Fig. 1 Naturally occurring P–C compounds.



Scheme 1 Partial hydrolysis of fosfazinomycins A and B to get phosphonic acid (+)-6 of unknown configuration.

^aUniversity of Vienna, Institute of Organic Chemistry, Währingerstraße 38, 1090, Vienna, Austria. E-mail: friedrich.hammerschmidt@univie.ac.at; Fax: +43 (0)1 4277 9521; Tel: +43 (0)1 4277 52105

^bUniversity of Vienna, Institute of Inorganic Chemistry, Währingerstraße 42, 1090, Vienna, Austria

†Electronic supplementary information (ESI) available: Copies of ¹H and ¹³C NMR spectra of the compounds prepared, X-ray data. CCDC 952037 for **16a** and 952038 for **16b**. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3ob41574k



1983 from the fermentation broth of *Streptomyces lavendofoliae* and are active against some filamentous fungi.¹²

Structure elucidation revealed fosfazinomycin to be a mixture of two components, A and B. They both contain L-arginine as well as a unique phosphonohydrazine moiety.^{13,14} The latter somehow relates them to FR-900137, an antibacterial antibiotic particularly active against *Escherichia coli*.¹⁵ Fosfazinomycin A differs from B by containing L-valine attached to the α -amino group of L-arginine. Furthermore, they share the same α -hydroxyphosphonate moiety with one stereogenic centre. The corresponding free phosphonic acid has been isolated by acid hydrolysis of fosfazinomycins and purification by ion exchange chromatography. Its optical rotation was determined $\{[\alpha]_D^{20} +22.5$ (c 1.50 in H_2O) $\}$, but the absolute configuration remained elusive.¹⁶ The purpose of this work was to synthesise both enantiomers of 2-hydroxy-2-phosphonoacetic acid methyl ester (**6**) of known absolute configuration. Their specific optical rotation will allow assigning the configuration to the natural product. This information might be helpful in unravelling the biosynthesis of fosfazinomycins.

Results and discussion

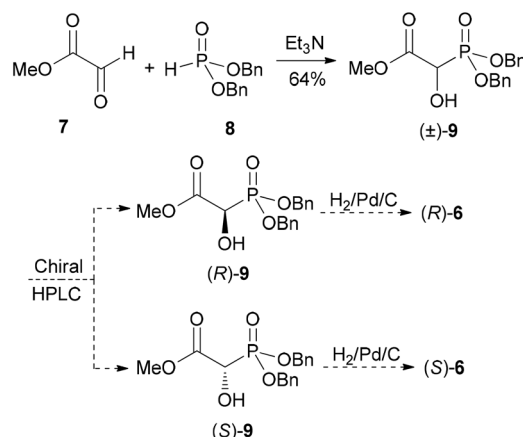
Synthetic challenges

P–C bonds in phosphonates are generally chemically very stable towards cleavage by bases and acids. However, α -hydroxyphosphonates are chemically labile.¹⁷ Their formation from aldehyde and phosphite and cleavage to the same compounds are catalysed by a base. Although chiral α -hydroxyphosphonate **6** looks very simple, we anticipated some obstacles during its synthesis. First, racemisation can interfere if the chiral, non-racemic hydroxyphosphonate is treated with a base. Second, the stereogenic centre here is base-labile as the α -hydrogen is acidified by the ester and the phosphonate group, irrespective of whether it is protected or not. Third, the base can induce an α -hydroxyphosphonate–phosphate rearrangement¹⁸ assisted by the ester group, which itself can be hydrolysed. To avoid these problems, very mild reaction conditions and catalytic removal of protecting group(s) from phosphorus at the end were mandatory.

Original synthetic strategy

Initially, we envisaged to generate racemic dibenzyl α -hydroxyphosphonate (\pm)-**9**, possibly separable by HPLC on a chiral stationary phase. Catalytic removal of the protecting groups would give the enantiomeric 2-hydroxy-2-phosphonoacetic acid methyl esters (*R*)- and (*S*)-**6** in the final step (Scheme 2).

Therefore, methyl glyoxylate (**7**) was prepared from glyoxylic acid monohydrate and methyl dimethoxyacetate by a literature procedure.¹⁹ It was reacted immediately in a Pudovik reaction²⁰ with dibenzyl phosphite (**8**) at -78°C in the presence of Et_3N as a base catalyst to give the desired racemic α -hydroxyphosphonate (\pm)-**9** in moderate yield (64%). Unfortunately, the two enantiomers could not be separated by HPLC on a



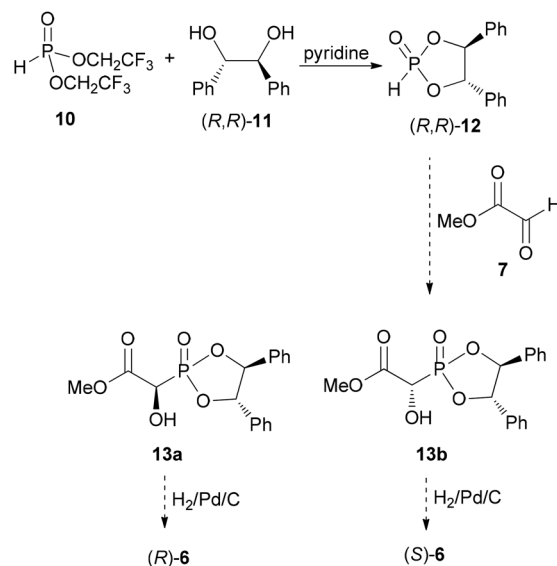
Scheme 2 Initial route to prepare α -hydroxyphosphonic acids (*R*)- and (*S*)-**6**.

Chiralcel OD-H column using various mixtures of iso-propanol–hexanes.

Revised strategy and synthesis

Therefore the synthetic strategy had to be changed. We decided to prepare a cyclic phosphite of known absolute configuration, which will yield a pair of diastereomeric cyclic α -hydroxyphosphonates upon reaction with methyl glyoxylate. Flash column chromatography and deprotection would give the free phosphonic acids (*R*)- and (*S*)-**6**.

Thus, C_2 -symmetric (*R,R*)-1,2-diphenylethane-1,2-diol (**11**)²¹ was heated at 80°C with commercially available bis(2,2,2-trifluoroethyl) phosphite (**10**)²² in dry pyridine hoping to get cyclic phosphite (*R,R*)-**12** (Scheme 3). As the phosphorus atom in this case is a chirotopic, non-stereogenic center,²³ only two diastereomers would be formed with methyl glyoxylate compared to four, when not using a C_2 -symmetric diol. However, only polymeric material resulted upon transesterification for



Scheme 3 Revised strategy for the synthesis of (*R*)- and (*S*)-**6**.

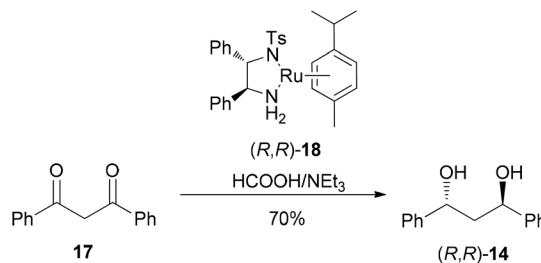


unknown reasons. Increasing the reaction temperature to 120 °C or replacing phosphite **10** by diethyl phosphite did not yield cyclic five-membered phosphite (*R,R*)-**12** either.

Assuming that a cyclic six-membered phosphite would be more favourable, (*R,R*)-1,2-diphenylethane-1,2-diol was replaced by (*R*,R**)-1,3-diphenylpropane-1,3-diol [(*R*,R**)-**14**]. First trials were performed using the racemic diol (\pm)-**14**, which was readily available.²⁴ Transesterification proceeded quantitatively within 1.5 h at room temperature in pyridine as judged by NMR spectroscopy (Scheme 4).

As it proved to be useful, we tried to prepare diol (*R,R*)-**14** by a literature procedure from (*S*)-2-chloro-1-phenylethanol *via* the dilithiated species, which was added to benzaldehyde. Contrary to the literature, which reported only the (*R,R*)-product to be formed in 79% yield, our (*R,R*)-**14** : *meso*-**14** ratio was 1.0 : 0.8 and the yield was low (35%).²⁵ As it could not be improved, enantioselective reduction of dibenzoylmethane (**17**) with Ru(*p*-cymene)[(*R,R*)-Ts-DPEN] (**18**) seemed to be an attractive alternative to get this chiral diol (Scheme 5).²⁶ This asymmetric transfer hydrogenation worked best with a mixture of 4.4 equiv. of HCO₂H and 2.6 equiv. of Et₃N as a hydrogen source and the yield of the crystallised product was 71% (ee >99% by chiral HPLC).²⁷

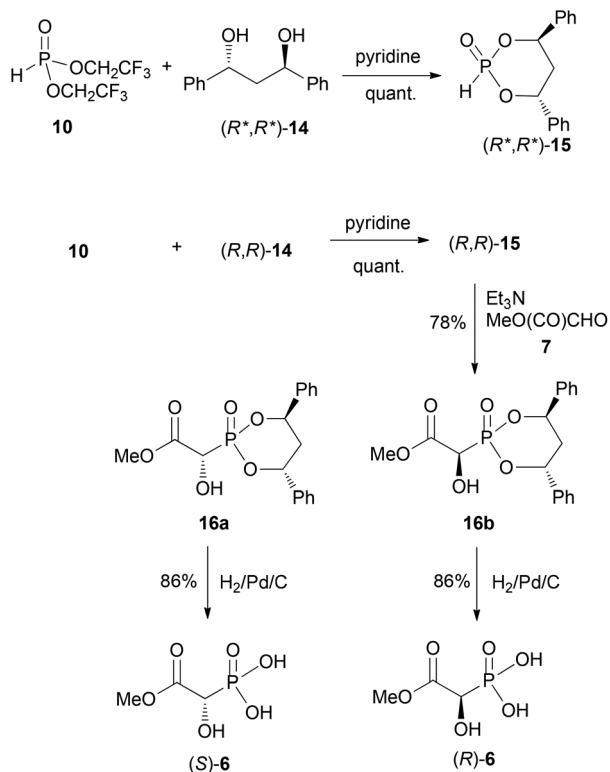
Having the enantiomerically pure diol (*R,R*)-**14** in hand, it was used to generate the cyclic phosphite (*R,R*)-**15** which was added to methyl glyoxylate (**7**) at −78 °C in a Pudovik reaction²⁰ catalysed by Et₃N (Scheme 4). When the reaction mixture was allowed to warm to −50 °C in the cooling bath,



Scheme 5 Highly enantioselective preparation of (*R,R*)-**14**.

the two diastereomeric α -hydroxyphosphonates **16a** and **16b** were formed. Addition of an equimolar amount of CF₃CO₂H relative to Et₃N, extractive workup and flash chromatography gave an inseparable mixture of the two diastereomers (in a 1 : 1 ratio) in low (50–55%) yield. Replacing CF₃CO₂H by CH₃SO₃H to neutralise the amine caused a lower yield. However, when the transformation was finished (as can be easily determined by NMR spectroscopy) and the reaction mixture was directly applied to the silica gel column for flash chromatography without the addition of an acid, the diastereomeric mixture of **16a** and **16b** was obtained in good (78%) yield. Unfortunately, the two diastereomers could not be separated by flash column chromatography, but only by preparative HPLC (CH₂Cl₂–EtOAc = 1 : 1; *t_R* (**16a**) 7.8 min, *t_R* (**16b**) 9.9 min). Crystallisation of both diastereomers from CH₂Cl₂ produced crystals suitable for single crystal X-ray structure analysis (Fig. 2 and 3). Diastereomers **16a** and **16b** were found to have (*S*)- and (*R*)-configuration, respectively, at the carbon atom bearing the hydroxyl group.

These α -hydroxyphosphonates were used for the next and final step in the reaction sequence, removal of the diol protecting group from phosphorus by hydrogenolysis in MeOH. The 1,3-diphenylpropane formed was removed by extraction with hexanes. Concentration of the methanolic solutions furnished the α -hydroxyphosphonates (*R*)- and (*S*)-**6** in sufficient purity for collecting the analytical data. Their specific optical rotations



Scheme 4 Revised strategy for the synthesis of (*R*)- and (*S*)-**6**.

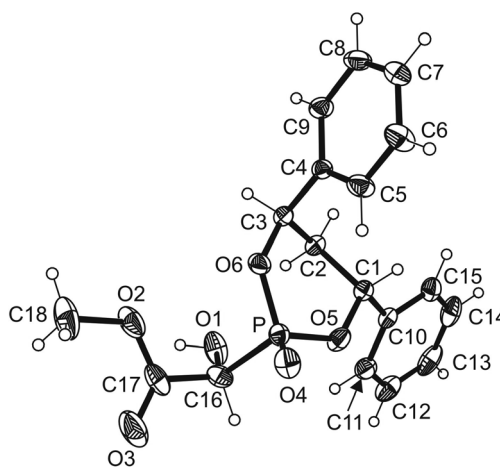


Fig. 2 ORTEP view of the molecule **16a** with thermal ellipsoids drawn at the 50% probability level.



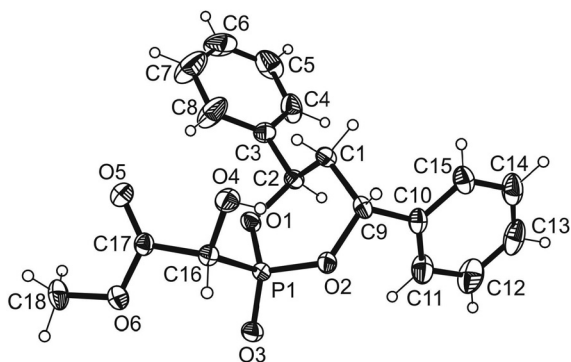


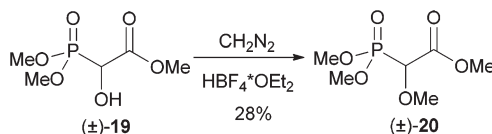
Fig. 3 ORTEP view of one crystallographically independent molecule of **16b** with thermal ellipsoids drawn at the 50% probability level.

were $[\alpha]_D^{20} -28.8$ (c 1.63 in H_2O) and $[\alpha]_D^{20} +29.6$ (c 1.40 in H_2O), respectively. Surprisingly, the enantiomers **6** are configurationally stable. The optical rotation of an aqueous solution of (*R*)-**6** did not change when left at room temperature for 8 days. However, this product racemised completely after 16 h at pH 7–8. The optical rotation measured after 1 h $\{[\alpha]_D^{20} -19.7$ (c 0.95 in H_2O) $\}$ decreased within the next 15 h to $[\alpha]_D^{20} -0.3$ (c 0.95 in H_2O).

Acidic hydrolysis of fosfazinomycins, followed by ion exchange chromatography, furnished the free phosphonic acid moiety with $[\alpha]_D^{20} +22.5$ (c 1.50 in H_2O).¹⁶ By comparison with the synthetic samples, it was concluded that it has (*S*)-configuration and has evidently partly racemised during the cleavage process. The finding that the only other known α -hydroxyphosphonate of biological origin, which is 2-amino-1-hydroxyethylphosphonic acid (**4**), has (*R*)-configuration will have biosynthetic implications.¹¹

To underpin the consistency between the structure of the α -hydroxyphosphonic acid **6** isolated from fosfazinomycins and the synthetic samples, and to determine the ee of our samples, (*R*)-**6** was esterified with diazomethane in methanol (Scheme 6).

The crude product obtained by concentration of the reaction mixture under reduced pressure was dimethyl phosphonate (*R*)-**19** (ee 99%, by NMR spectroscopy using (*R*)-(+)-*t*-butyl-(phenyl)monothiophosphinic acid as a chiral shift reagent)^{28,29} in admixture with a small amount of a compound tentatively assigned the structure of methyl ether (*R*)-**20** (molar ratio of (*R*)-**19** : (*R*)-**20** = 88 : 12). They were separated by flash chromatography. The NMR spectrum of homogeneous (*R*)-**19** was identical to that of an authentic sample of (\pm)-**19**,³⁰ prepared by base-catalysed addition of dimethyl phosphite to methyl



Scheme 7 Synthesis of (\pm)-**20** from (\pm)-**19**.

glyoxylate in 72% yield, but its ee was only 31%. Partial racemisation evidently occurred during flash chromatography on silica gel by removal of the fairly acidic proton α to phosphorus. To prove the formation of methyl ether (*R*)-**20** during esterification, its racemate was synthesised by etherification of racemic methyl 2-(dimethoxyphosphinyl)-2-hydroxyacetate $[(\pm)\text{-}19]$ with CH_2N_2 in the presence of $HBF_4 \cdot OEt_2$ as a catalyst³¹ (Scheme 7). The product yield was poor (29%), but sufficient for collecting the necessary analytical data and proving that (*R*)-**20** was formed as a side product during the esterification of (*R*)-**6**.

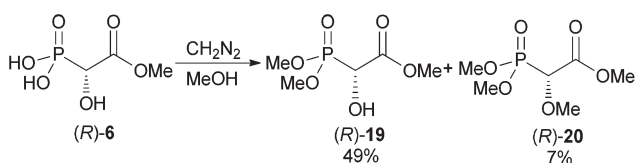
Experimental

General experimental

1H , ^{13}C and ^{31}P NMR spectra were recorded in $CDCl_3$, d_4 -methanol and d_8 -toluene using a Bruker Avance DRX 400 (1H : 400.13 MHz, ^{13}C : 100.61 MHz, ^{31}P : 161.98 MHz), AV 400 (1H : 400.27 MHz, ^{13}C : 100.65 MHz, ^{31}P : 162.03 MHz) or DRX 600 (1H : 600.13 MHz) spectrometer. Chemical shifts were referenced to residual $CHCl_3$ (δ_H 7.24), CHD_2OD (δ_H 3.31) or $C_6D_5CD_2H$ (δ_H 2.09) and $CDCl_3$ (δ_C 77.23), CD_3OD (δ_C 49.15) and external H_3PO_4 (85%). Chemical shifts (δ) are given in ppm and coupling constants (J) in Hz. IR spectra were run using a Bruker VERTEX 70 IR spectrometer as ATR spectra. Optical rotations were measured at 20 °C using a Perkin-Elmer 341 polarimeter in a 1 dm cell. $[\alpha]_D$ values are given in $10^{-1} \text{ deg cm}^2 \text{ g}^{-1}$. Analytical HPLC for the determination of the ee of (*R,R*)-**14** was performed on a Jasco system (PU-980 pump, UV 975 and RI 930) using a Chiralcel OD-H column, \varnothing 0.46 cm \times 25 cm. Preparative HPLC for the separation of **16a** and **16b** was performed using a Dynamix Model SD-1 equipped with a Model UV-1 absorbance detector using a Nucleosil 50–5 column, \varnothing 3.2 cm \times 25 cm. Melting points were determined using a Leica Galen III Reichert Thermovar instrument and were uncorrected.

TLC was carried out on 0.25 mm thick Merck plates with silica gel 60 F₂₅₄. Spots were visualised by UV and/or dipping the plate into a solution of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (25.0 g) and $Ce(SO_4)_2 \cdot 4H_2O$ (1.0 g) in 10% aqueous H_2SO_4 (500 mL), followed by heating with a heat gun. Flash (column) chromatography was performed with Merck silica gel 60 (230–400 mesh).

Pyridine was dried by refluxing over powdered CaH_2 , followed by distillation and storage over molecular sieves (4 Å). Dichloromethane was dried by passing through aluminium oxide 90 active neutral (0.063–0.200 mm, activity I) and stored



Scheme 6 Esterification of (*R*)-**6** with ethereal diazomethane.



over molecular sieves (3 Å). Et₂O was refluxed over LiAlH₄, THF over potassium and distilled prior to use. Bis(2,2,2-trifluoroethyl) phosphite was distilled under reduced pressure (b.p. 48–50 °C/9 mm; lit.,²² 43–44 °C/2 mm). All other chemicals were used as purchased from Sigma-Aldrich, Acros, Fluka or Merck.

Methyl glyoxylate (7).¹⁹ Glyoxylic acid monohydrate (2.348 g, 25.5 mmol), methyl dimethoxyacetate (2.521 g, 2.3 mL, 19.0 mmol) and *p*-TsOH·H₂O (72 mg, 0.4 mmol) were combined and refluxed for 16 h under argon. The resulting yellowish liquid was allowed to cool to room temperature and treated with P₂O₅ (2.0 g). The suspension was stirred at 80 °C for 4.5 h and then bulb-to-bulb distilled (110–138 °C/14 mm). A second bulb-to-bulb distillation (115–130 °C/12 mm) was necessary to completely depolymerise the product and to get methyl glyoxylate (2.777 g, 72%) as a colourless liquid, which was sufficiently pure for the following reactions. It should be used immediately, as it polymerises easily. If storage is necessary, methyl glyoxylate can be recovered by distillation prior to use to depolymerise polymeric material; δ_{H} (400.27 MHz, CDCl₃) 3.91 (3H, s, OCH₃), 9.39 (1H, s, CHO).

(±)-Methyl 2-(dibenzoxyposphinyl)-2-hydroxyacetate [(±)-9]. Dibenzyl phosphite (524 mg, 0.44 mL, 2.0 mmol) was dissolved in dry CH₂Cl₂ (4 mL) under argon at –78 °C. Methyl glyoxylate (7, 176 mg, 0.16 mL, 2.0 mmol) and Et₃N (40 mg, 0.19 mL, 0.4 mmol) were added dropwise and the reaction mixture was stirred at –78 °C for 1 h. Completion of the reaction was monitored by NMR spectroscopy and the cold reaction mixture was directly applied to a silica gel column for flash chromatography (CH₂Cl₂–EtOAc 1 : 1, *R_f* 0.57). α -Hydroxyphosphonate (±)-9 (445 mg, 64%) was obtained as colourless crystals; mp 43–45 °C (CH₂Cl₂/hexanes); found: C, 58.28; H, 5.71; O, 27.55. Calc. for C₁₇H₂₀O₆P: C, 58.29; H, 5.47; O, 27.40%; $\nu_{\text{max}}/\text{cm}^{-1}$ 3311, 2930, 2855, 1753, 1513, 1461, 1379, 1249, 1091, 1037; δ_{H} (400.27 MHz, CDCl₃) 3.32–3.47 (1H, br m, OH), 3.76 (3H, s, OCH₃), 4.58 (1H, dd, *J*_{HP} 15.9, *J*_{HH} 7.0, P–OCH₃), 5.04–5.17 (4H, m, 2 × CH₂), 7.27–7.38 (10H, m, Ph); δ_{C} (100.65 MHz, CDCl₃) 53.58 (OCH₃), 69.08 (d, *J*_{CP} 6.9, CH₂), 69.30 (d, *J*_{CP} 155.8, CH–P), 69.31 (d, *J*_{CP} 6.9, CH₂), 128.21 (d, *J*_{CP} 2.9, Ph), 128.77 (Ph), 128.80 (Ph), 135.96 (d, *J*_{CP} 5.8, Ph), 136.02 (d, *J*_{CP} 5.9, Ph), 169.96 (d, *J*_{CP} 1.2, C=O); δ_{P} (162.03 MHz, CDCl₃) 16.15; HRMS: (ESI) observed [*M* + *H*]⁺ 351.0990, calculated for C₁₇H₂₀O₆P⁺ 351.0992.

Activation of hydrogenation catalyst.³² Commercially available RuCl(*p*-cymene)[(*R,R*)-Ts-DPEN] (18) (100 mg, 0.16 mmol) was dissolved in CH₂Cl₂ (2 mL) and treated with KOH (9 mg, 0.16 mmol) dissolved in water (2.5 mL) at room temperature whereupon the solution turned from brownish red to purple. The aqueous phase was separated and the organic phase was dried (CaH₂). The solution was filtered and concentrated under reduced pressure to give Ru(*p*-cymene)[(*R,R*)-Ts-DPEN] (78 mg, 81%) as a purple solid, which could be stored at room temperature under an inert atmosphere for about two weeks.

(*R,R*)-(+)-1,3-Diphenylpropane-1,3-diol [(*R,R*)-14].²⁷ Formic acid (1.013 g, 0.83 mL, 22 mmol) was added dropwise to Et₃N

(1.315 g, 1.80 mL, 13 mmol) at 0 °C under argon. The mixture was allowed to warm to room temperature before the addition of dibenzoylmethane (1.121 g, 5 mmol) and Ru(*p*-cymene)-[(*R,R*)-Ts-DPEN] (18, 45 mg, 0.075 mmol) dissolved in dry CH₂Cl₂ (0.5 mL). The reaction mixture was stirred at 40 °C. After 1 h an additional portion of the catalyst (20 mg, 0.03 mmol) dissolved in CH₂Cl₂ (0.2 mL) was added and heating was continued for 16 h. Water (2 mL) was added. The brownish precipitate was dried *in vacuo* and purified by flash chromatography (EtOAc–hexanes 1 : 2 (*R_f* 0.40) to EtOAc), followed by crystallisation from *i*PrOH to give diol (*R,R*)-14 (799 mg, 70%) as slightly reddish crystals; ee >99% before and after crystallisation (by analytical HPLC on chiral stationary phase; 10% *i*PrOH–hexanes, *t_R* (*S,S*) 10.52 min, *t_R* (*R,R*) 12.16 min). The product was directly used for the next step. A small sample was further purified by HPLC for characterisation to give colourless needles; mp 150–152 °C (from *i*PrOH); [α]_D²⁰ +72.15 (*c* 0.79 in methanol) {lit.,³³ for (*S,S*)-enantiomer: [α]_D²⁰ –72 (methanol)}; $\nu_{\text{max}}/\text{cm}^{-1}$ 3382, 3295, 1454, 1400, 1201, 1061, 1023; δ_{H} (400.27 MHz, CDCl₃) 2.16 (1H, d, *J* 6.1, CH₂), 2.17 (1H, d, *J* 6.1, CH₂), 2.74 (2H, br s, 2 × OH), 4.97 (2H, t, *J* 6.1, 2 × CH–O), 7.22–7.37 (10H, m, Ph); δ_{C} (100.65 MHz, CDCl₃) 46.75 (CH₂), 71.99 (2C, 2 × CH–O), 125.84 (4C, Ph), 127.72 (2C, Ph), 128.73 (4C, Ph), 144.40 (2C, Ph).

(4*R*,6*R*)-2-Hydro-2-oxo-4,6-diphenyl-1,3,2-dioxaphosphorinane (15). (*R,R*)-1,3-Diphenylpropane-1,3-diol [(*R,R*)-14] (457 mg, 2.0 mmol) was dissolved in dry pyridine (10 mL) at room temperature under argon. Bis(2,2,2-trifluoroethyl) phosphite (541 mg, 0.35 mL, 2.2 mmol) was added and the reaction mixture was stirred for 1.5 h. The solvent was removed *in vacuo* and traces of the remaining pyridine were removed by azeotropic distillation with toluene (2 times). The crude cyclic phosphite (*R,R*)-15 (549 mg, quant.) was isolated as a colourless oil of sufficient purity for the next step. An analytical sample was purified by flash chromatography (EtOAc, *R_f* 0.70); $\nu_{\text{max}}/\text{cm}^{-1}$ 3200, 1746, 1453, 1208, 1057, 973; δ_{H} (400.27 MHz, CDCl₃) 2.62 (ABXX'P-system, A-part: 1H, dddd, *J*_{AB} 15.1, *J*_{AX} 5.7, *J*_{AX'} 3.9, *J*_{AP} 1.5; B-part: 1H, dddd, *J*_{AB} 15.1, *J*_{BX} 4.6, *J*_{BX'} 7.5, *J*_{BP} 1.1; CH₂), 5.54 (1H, td, *J*_{X'B/X'P} 7.5, *J*_{X'A} 3.9, O–CH), 5.65 (1H, ddd, *J*_{XA} 5.7, *J*_{XB} 4.6, *J*_{XP} 8.5, OCH), 7.34 (1H, d, *J*_{HP} 710.3, H–P), 7.29–7.47 (10H, m, Ph); δ_{C} (100.65 MHz, CDCl₃) 39.67 (d, *J*_{CP} 9.6, CH₂), 77.02 (d, *J*_{CP} 6.3, CH–O), 77.46 (d, *J*_{CP} 6.9, CH–O), 125.56, 125.60, 128.83, 129.06, 129.21 (10C, 6 × s, Ph), 138.57 (d, *J*_{CP} 10.0, Ph), 138.62 (d, *J*_{CP} 10.9, Ph); δ_{P} (162.03 MHz, CDCl₃) –1.56; HRMS: (ESI) observed [*M* + *Na*]⁺ 297.0654, calculated for C₁₅H₁₅O₃PNa⁺ 297.06565.

Cyclic α -hydroxyphosphonates 16a and 16b. The crude phosphite (*R,R*)-15 (1.840 g, 6.7 mmol) was dissolved in dry CH₂Cl₂ (12 mL) under argon at –78 °C. Methyl glyoxylate (7, 0.885 g, 0.79 mL, 10.1 mmol) and Et₃N (0.136 g, 0.19 mL, 1.3 mmol) were added dropwise and the reaction mixture was stirred at –78 °C for 30 min. Completion of the reaction was monitored by ¹H and ³¹P NMR spectroscopy and the cold reaction mixture was directly applied to the silica gel column for flash chromatography (hexanes–acetone 3 : 2, *R_f* 0.22) to obtain a mixture of the two diastereomeric α -hydroxyphosphonates



16a and **16b** (1.888 g, 78%) as an oil. The ratio of **16a** to **16b** was 1.16 : 1.00 (by ^{31}P NMR).

The diastereomers were separated by preparative HPLC (CH_2Cl_2 -EtOAc 1 : 1, t_{R} (**16a**) 7.8 min, t_{R} (**16b**) 9.9 min) and crystallised. A single crystal of each diastereomer was picked directly from the solution for X-ray structure analysis.

α -Hydroxyphosphonate 16a. 42%; mp 156–158 °C (from CH_2Cl_2); $[\alpha]_{\text{D}}^{20}$ –63.5 (c 0.96 in CH_2Cl_2); found: C, 59.5; H, 5.0. Calc. for $\text{C}_{18}\text{H}_{19}\text{O}_6\text{P}$: C, 59.7; H, 5.3%; $\nu_{\text{max}}/\text{cm}^{-1}$ 3254, 1735, 1235, 1208, 1095, 1057, 1017, 976; δ_{H} (600.13 MHz, CDCl_3) 2.62 (ABXX'P-system, A-part: 1H, dddd, J_{AB} 14.9, $J_{\text{AX'}}$ 9.8, J_{AX} 5.2, J_{AP} 1.1, CH_2 ; B-part: 1H, dddd, J_{AB} 14.9, $J_{\text{BX'}}$ 2.9, J_{BX} 3.3, J_{BP} 2.1, CH_2), 3.08 (1H, br s, OH), 3.92 (3H, d, J 0.4, OCH_3), 4.78 (1H, d, J_{HP} 15.5, CH-P), 5.46 (1H, ddd, J_{XP} 2.1, J_{XA} 9.8, J_{XB} 2.9, O-CH), 5.76 (1H, ddd, J_{XP} 8.8, J_{XA} 5.2, J_{XB} 3.3, O-CH), 7.29–7.48 (10H, m, Ph); δ_{C} (100.61 MHz, CDCl_3) 39.56 (d, J_{CP} 7.4, CH_2), 54.18 (OCH_3), 68.88 (d, J_{CP} 158.4, CH-P), 76.83 (d, overlap with CDCl_3 signal, J_{CP} 8.3, O-CH), 79.92 (d, J_{CP} 8.6, O-CH), 125.53, 126.06, 128.58, 128.93, 128.97, 129.13 (10C, Ph), 138.85 (d, J_{CP} 2.4, Ph), 139.03 (d, J_{CP} 6.9, Ph), 170.61 (C=O); δ_{P} (161.98 MHz, CDCl_3) 9.48; HRMS: (ESI) observed $[\text{M} + \text{Na}]^+$ 385.0820, calculated for $\text{C}_{18}\text{H}_{19}\text{O}_6\text{PNa}^+$ 385.08169.

α -Hydroxyphosphonate 16b. 36%; mp 117–119 °C (from CH_2Cl_2); $[\alpha]_{\text{D}}^{20}$ –23.85 (c 0.96 in CH_2Cl_2); found: C, 59.1; H, 5.55. Calc. for $\text{C}_{18}\text{H}_{19}\text{O}_6\text{P}$: C, 59.7; H, 5.3%; $\nu_{\text{max}}/\text{cm}^{-1}$ 3302, 2951, 1752, 1519, 1452, 1262, 1112, 1056; δ_{H} (600.13 MHz, CDCl_3) 2.60 (ABXX'P-system, A-part: 1H, dddd, J_{AB} 14.9, $J_{\text{AX'}}$ 3.6, J_{AX} 4.5, J_{AP} 1.9, CH_2 ; B-part: 1H, dddd, J_{AB} 14.9, $J_{\text{BX'}}$ 8.9, J_{BX} 5.2, J_{BP} 1.2, CH_2), 2.63 (1H, br s, OH), 3.90 (3H, s, OCH_3), 4.78 (1H, d, J_{HP} 15.7, CH-P), 5.51 (1H, ddd, J_{XP} 3.0, J_{XA} 3.6, J_{XB} 8.9, O-CH), 5.83 (1H, td, $J_{\text{XA}} = J_{\text{XB}}$ 4.9, J_{XP} 8.0, O-CH), 7.25–7.49 (10H, m, Ph); δ_{C} (100.61 MHz, CDCl_3) 40.05 (d, J_{CP} 7.2, CH_2), 54.17 (OCH_3), 69.23 (d, J_{CP} 157.7, CH-P), 76.46 (d, J_{CP} 7.5, O-CH), 79.62 (d, J_{CP} 8.5, O-CH), 125.57, 125.60, 128.63, 128.89, 129.04, 129.11 (10C, Ph), 139.03 (d, J_{CP} 6.9, Ph), 139.24 (d, J_{CP} 4.2, Ph), 170.57 (C=O); δ_{P} (161.98 MHz, CDCl_3) 9.95; HRMS: (ESI) observed $[\text{M} + \text{Na}]^+$ 385.0817, calculated for $\text{C}_{18}\text{H}_{19}\text{O}_6\text{PNa}^+$ 385.08169.

(S)-(+)- and (R)-(–)-Methyl 2-phosphono-2-hydroxyacetate [(S)- and (R)-6]. α -Hydroxyphosphonate **16a** (100 mg, 0.28 mmol) was dissolved in methanol (10 mL) and Pd/C (70 mg, 10% Pd) was added. The reaction mixture was hydrogenated in a Parr apparatus for 2 h at room temperature (50 psi), filtered over celite and cooled to 0 °C. The filtrate was extracted with hexanes (3 \times 10 mL), precooled to –25 °C, and concentrated *in vacuo* to yield the desired (S)-**6** (41 mg, 86%); $[\alpha]_{\text{D}}^{20}$ +29.6 (c 1.40 in H_2O); δ_{H} (400.13 MHz, d_4 -methanol) 3.79 (3H, s, OCH_3), 4.48 (1H, d, J_{HP} 17.6, CH-P), 4.83 (3H + residual water, s, 3 \times OH); δ_{C} (100.61 MHz, d_4 -methanol) 52.48 (OCH_3), 71.01 (d, J_{CP} 175.1, C-P), 171.47 (C=O); δ_{P} (161.98 MHz, d_4 -methanol) 15.31.

Similarly, diastereomer **16b** (100 mg) was converted to α -hydroxyphosphonic acid (R)-**6** of opposite configuration (43 mg, 90%); $[\alpha]_{\text{D}}^{20}$ –28.8 (c 1.63 in H_2O); the spectroscopic data were identical to that of the (S)-enantiomer.

Configurational stability of (R)-(–)-methyl 2-phosphono-2-hydroxyacetate. The optical rotation of the free phosphonic

acid (R)-**6** did not change significantly in aqueous medium over a period of 8 days {after 8 days: $[\alpha]_{\text{D}}^{20}$ –28.4 (c 1.63 in H_2O)}. After the pH had been adjusted to 7 by the addition of NaOH (2 M) the optical rotations were measured after 1 h { $[\alpha]_{\text{D}}^{20}$ –19.7 (c 0.95 in H_2O)} and 16 h { $[\alpha]_{\text{D}}^{20}$ –0.3 (c 0.95 in H_2O)}.

(\pm)-Methyl 2-(dimethoxyphosphinyl)-2-hydroxyacetate [(\pm)-19]. Methyl glyoxylate (440 mg, 0.40 mL, 5.0 mmol) and dimethyl phosphite (550 mg, 0.46 mL, 5.0 mmol) were stirred at –78 °C under argon in CH_2Cl_2 (10 mL). Et_3N (100 mg, 0.14 mL, 1.0 mmol) was added slowly and the resulting turbid solution was stirred at –78 °C for 30 min. The cooling bath was removed and the reaction mixture was allowed to warm to room temperature. The solvents were removed *in vacuo* and the hydroxyphosphonate was purified by flash chromatography (hexanes–acetone 2 : 3, R_{f} 0.26) and crystallisation (EtOAc– Et_2O) to yield colourless needles (712 mg, 72%); mp 60–63 °C (lit.,³⁰ 59–61 °C); $\nu_{\text{max}}/\text{cm}^{-1}$ 3269, 2961, 1754, 1440, 1247, 1185, 1108, 1028; δ_{H} (400.27 MHz, CDCl_3) 3.29 (1H, br s, OH), 3.84 (3H, d, J_{HP} 10.8, P– OCH_3), 3.85 (3H, d, J_{HP} 10.7, P– OCH_3), 3.88 (3H, d, J_{HP} 0.5, CO_2CH_3), 4.57 (1H, d, J_{HP} 16.0, CH-P); δ_{C} (100.65 MHz, CDCl_3) 53.81 (CO_2CH_3), 54.26 (d, J_{CP} 6.8, P– OCH_3), 54.60 (d, J_{CP} 6.8, P– OCH_3), 68.76 (d, J_{CP} 156.1, CH-P), 170.06 (C=O); δ_{P} (162.02 MHz, CDCl_3) 17.70.

(R)-(–)-Methyl 2-(dimethoxyphosphinyl)-2-hydroxyacetate [(R)-19]. α -Hydroxyphosphonic acid (R)-**6** (51 mg, 0.30 mmol) was esterified in dry MeOH (3 mL) with a distilled ethereal solution of CH_2N_2 (prepared from *N*-nitroso-*N*-methylurea; added until faint yellow colour persisted) at room temperature. The reaction mixture was immediately concentrated *in vacuo* at room temperature. The crude product was a mixture of (R)-**19** and (R)-**20**; ee >99% (by $^{31}\text{P}/^1\text{H}$ NMR using (R)-(+)-*tert*-butyl-phenyl-monothiothiophosphinic acid as a chiral solvating agent).^{28,29} Homogeneous (R)-**19** was obtained by flash chromatography (hexanes–acetone 2 : 3, R_{f} 0.38) as a colourless solid (29 mg, 49%); ee 34% (by $^{31}\text{P}/^1\text{H}$ NMR using the chiral shift reagent); $[\alpha]_{\text{D}}^{20}$ –10.64 (c 1.10 in methanol). The spectroscopic data agreed with that for the racemic compound (\pm)-**19**.

(\pm)-Methyl 2-(dimethoxyphosphinyl)-2-methoxyacetate [(\pm)-20].³¹ Hydroxyphosphonate (\pm)-19** (29 mg, 0.15 mmol) was dissolved in dry CH_2Cl_2 (3 mL) at 0 °C. $\text{HBF}_4 \cdot \text{OEt}_2$ (8 drops, 0.16 M solution in CH_2Cl_2) was added, followed by freshly distilled ethereal CH_2N_2 , until its yellow colour persisted in the reaction mixture. Then the solution was concentrated to about 2 mL and subjected to flash chromatography (hexanes–acetone 2 : 3, R_{f} 0.49) to obtain (\pm)-**20** (9 mg, 28%) as a colourless solid; $\nu_{\text{max}}/\text{cm}^{-1}$ 1744, 1443, 1259, 1190, 1119, 1027; δ_{H} (400.27 MHz, CDCl_3) 3.50 (3H, d, J 0.4, CH– OCH_3), 3.82 (3H, s, CO_2CH_3), 3.82 (3H, d, J_{HP} 10.9, P– OCH_3), 3.84 (3H, d, J_{HP} 10.9, P– OCH_3), 4.24 (1H, d, J_{HP} 18.5, CH-P); δ_{C} (100.65 MHz, CDCl_3) 53.04 (CH– OCH_3), 54.32 (d, J_{CP} 7.1, P– OCH_3), 54.40 (d, J_{CP} 7.3, P– OCH_3), 60.75 (d, J_{CP} 13.0, CO_2CH_3), 78.20 (d, J_{CP} 158.2, CH-P), 167.74 (d, J_{CP} 1.6, C=O); δ_{P} (162.02 MHz, CDCl_3) 16.35; HRMS: (ESI) observed $[\text{M} + \text{H}]^+$ 213.05217, calculated for $\text{C}_6\text{H}_{14}\text{O}_6\text{P}^+$ 213.05280.**



Conclusions

The first chemical synthesis of both enantiomers of α -hydroxyphosphonic acid **6** was accomplished. A synthetic route taking advantage of a chirotopic, non-stereogenic phosphorus centre in a cyclic phosphite was chosen to generate two diastereomeric α -hydroxyphosphonates in a Pudovik reaction. The diastereomer with (*S*)-configuration at the stereogenic centre bearing the hydroxyl group was converted to the dextrorotary α -hydroxyphosphonic acid, which was also obtained by partial hydrolysis of the fosfazinomycins A and B. Therefore, the natural α -hydroxyphosphonic acid also has (*S*)-configuration.

Acknowledgements

We greatly acknowledge the financial support by the Austrian Science Fund (FWF) (P19869-N19). We thank S. Felsinger for recording NMR spectra and J. Theiner for performing combustion analyses.

Notes and references

- W. W. Metcalf and W. A. van der Donk, *Annu. Rev. Biochem.*, 2009, **78**, 65.
- A. K. White and W. W. Metcalf, *Annu. Rev. Microbiol.*, 2007, **61**, 379.
- S. C. Fields, *Tetrahedron*, 1999, **55**, 12237.
- A. Mucha, P. Kafarski and L. Berlicki, *J. Med. Chem.*, 2011, **54**, 5955.
- F. R. McSorley, P. B. Wyatt, A. Martinez, E. F. DeLong, B. Hove-Jensen and D. L. Zechel, *J. Am. Chem. Soc.*, 2012, **134**, 8364.
- H. Seto and T. Kuzuyama, *Nat. Prod. Rep.*, 1999, **16**, 589.
- F. M. Kahan, J. S. Kahan, P. J. Cassidy and H. Kropp, *Ann. N. Y. Acad. Sci.*, 1974, **235**, 364.
- F. Hammerschmidt and H. Kählig, *J. Org. Chem.*, 1991, **56**, 2364.
- J. Wiesner, S. Borrmann and H. Jomaa, *Parasitol. Res.*, 2003, **90**(Suppl. 2), 71.
- E. D. Korn, D. G. Dearborn, H. M. Fales and E. A. Sokoloski, *J. Biol. Chem.*, 1973, **248**, 2257.
- F. Hammerschmidt and H. Völlenkle, *Liebigs Ann. Chem.*, 1989, 577.
- S. Gunji, K. Arima and T. Beppu, *Agric. Biol. Chem.*, 1983, **47**, 2061.
- T. Ogita, S. Gunji, Y. Fukazawa, A. Terahara, T. Kinoshita, H. Nagaki and T. Beppu, *Tetrahedron Lett.*, 1983, **24**, 2283.
- I. J. Kang and Y. J. Kim, *Bull. Korean Chem. Soc.*, 1994, **15**, 595.
- (a) Y. Kuroda, T. Goto, M. Okamoto, M. Yamashita, E. Iguchi, M. Kohsaka, H. Aoki and H. Imanaka, *J. Antibiot.*, 1980, **33**, 272; (b) Y. Kuroda, H. Tanaka, M. Okamoto, T. Goto, M. Kohsaka, H. Aoki and H. Imanaka, *J. Antibiot.*, 1980, **33**, 280.
- A. Terahara, personal communication.
- B. L. Wanner, *Biodegradation*, 1994, **5**, 175.
- F. Hammerschmidt, *Monatsh. Chem.*, 1993, **124**, 1063.
- (a) M. S. Foster, C. D. Oldham and S. W. May, *Tetrahedron: Asymmetry*, 2011, **22**, 283; (b) J. M. Hook, *Synth. Commun.*, 1984, **14**, 83; (c) T. R. Kelly, T. E. Schmidt and J. G. Haggerty, *Synthesis*, 1972, 544.
- A. N. Pudovik and I. V. Konovalova, *Synthesis*, 1979, 81.
- Z.-M. Wang and K. B. Sharpless, *J. Org. Chem.*, 1994, **59**, 8302.
- D. E. Gibbs and C. Larsen, *Synthesis*, 1984, 410.
- For an explanation of chirotopic non-stereogenic centers see: K. Mislow and J. Siegel, *J. Am. Chem. Soc.*, 1984, **106**, 3319.
- J.-P. Deprés and C. Morat, *J. Chem. Educ.*, 1992, **69**, A232.
- J. Barluenga, J. Flórez and M. Yus, *J. Chem. Soc., Perkin Trans. 1*, 1983, 3019.
- J. Cossy, F. Eustache and P. I. Dalko, *Tetrahedron Lett.*, 2001, **42**, 5005.
- T. Ikariya, S. Hashiguchi, K. Murata and R. Noyori, *Org. Synth.*, 2005, **82**, 10.
- M. Drescher, S. Felsinger, F. Hammerschmidt, H. Kählig, S. Schmidt and F. Wuggenig, *Phosphorus, Sulfur Silicon Relat. Elem.*, 1998, **140**, 79.
- E. Zymanczyk-Duda, M. Skwarczynski, B. Lejczak and P. Kafarski, *Tetrahedron: Asymmetry*, 1996, **7**, 1277.
- D. Horne, J. Gaudino and W. J. Thompson, *Tetrahedron Lett.*, 1984, **25**, 3529.
- (a) M. Neeman, M. C. Caserio, J. D. Roberts and W. S. Johnson, *Tetrahedron*, 1959, **6**, 36; (b) A. B. Smith III, K. J. Hale, L. M. Laakso, K. Chen and A. Riéra, *Tetrahedron Lett.*, 1989, **30**, 6963.
- K. Matsumura, S. Hashiguchi, T. Ikariya and R. Noyori, *J. Am. Chem. Soc.*, 1997, **119**, 8738.
- K. Yamamoto, H. Ando and H. Chikamatsu, *J. Chem. Soc., Chem. Commun.*, 1987, 334.

