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Enzymes in organic chemistry. Part 10:[1] Chemo-enzymatic synthesis of L-phosphaserine and L-phosphaisoserine and enantioseparation of amino-hydroxyethylphosphonic acids by non-aqueous capillary electrophoresis with quinine carbamate as chiral ion pair agent

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Abstract

Diisopropyl 2-azido-1-acetoxyethylphosphonate (±)-**7** was hydrolysed with high enantioselectivity by lipase SP 524 to give α-hydroxyphosphonate (*S*)-(–)-**6** and ester (*R*)-(–)-**7**, which was saponified to give (*R*)-(+)-**6**. The two α-hydroxyphosphonates (*R*)- and (*S*)-**6** were transformed into l-phosphaisoserine and L-phosphaserine, respectively. Their enantiomeric excesses were determined to be 97% by HPLC on an chiral stationary phase. A mixture of all four stereoisomeric amino-hydroxyethylphosphonic acids can be separated by non-aqueous capillary electrophoresis with quinine carbamate as the chiral ion pair agent applying the partial filling technique.

1. Introduction

Recently, we reported the chemo-enzymatic synthesis of phosphonic acid analogues of L-valine, L-leucine, L-isoleucine, L-methionine and L-α-aminobutyric acid.[1] These α-aminophosphonic acids having apolar side chains were prepared by chemical manipulation of the corresponding (*S*)-α-hydroxyphosphonates which were obtained by enzymatic resolutions. The enantiomeric excesses of the α-aminophosphonic acids were determined by HPLC of *N*-protected derivatives, employing quinine-derived chiral anion exchangers.[2] Additionally, these stationary phases allow the assignment of absolute configurations of a broad variety of both aliphatic and aromatic α- and β-aminophosphonic acids.

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2. Results and discussion

In continuation of this work, we wanted to demonstrate that the chemo-enzymatic approach can be extended to aminophosphonic acids containing an additional functional group. We selected L-phosphaserine (*R*)-(–)-**1**, the phosphonic acid analogue of the proteinogenic amino acid L-serine, and L-phosphaisoserine (*R*)-(–)-**2** (Fig. 1), a naturally occurring β-amino-α-hydroxyphosphonic acid. It was isolated from the plasma membrane of the protozoon *Acanthamoeba castellanii*.[3,4] Furthermore, we wanted to study the enantioseparation of all four stereoisomeric amino-hydroxyethylphosphonic acids by non-aqueous capillary electrophoresis with quinine carbamate as the chiral ion pair agent. Both phosphonic acids have been prepared in enantiomerically pure form by multistep syntheses using chemical resolution.[4–6] L-Phosphaserine was also obtained by diastereoselective addition of dialkyl phosphite to a *N*-glycosyl nitrone.[7] A highly enantioselective synthesis of L-phosphaisoserine based on a hydrolytic kinetic resolution of racemic diethyl epoxyethylphosphonate was published last year.[8]

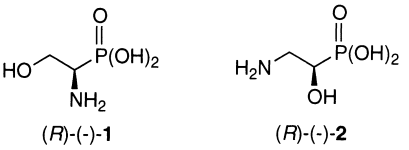
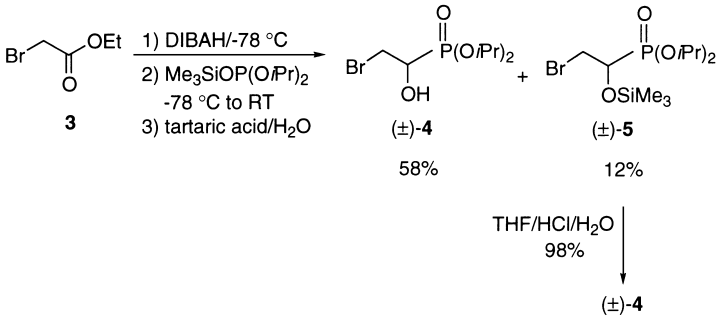


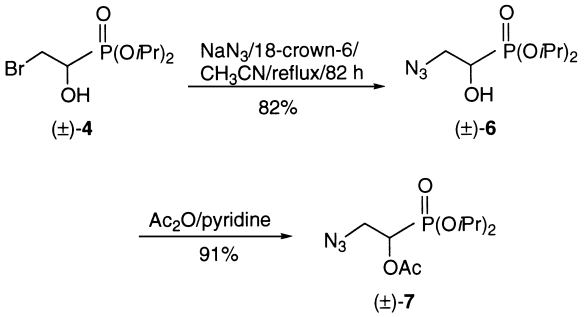
Figure 1.

Our synthesis is short and uses a lipase-catalysed, kinetic resolution which furnished at the same time the starting materials for L-phosphaserine and L-phosphaisoserine. It started with the reduction of ethyl bromoacetate **3** with DIBAH at –78°C, followed by the addition of diisopropyl trimethylsilyl phosphite and warming to room temperature (Scheme 1). Workup gave α-hydroxyphosphonate (±)-**4** in 58% yield and its silylated derivative (±)-**5** in 12% yield. The latter was deblocked easily in THF with hydrochloric acid. This synthesis of hydroxyphosphonate (±)-**4** is very simple, circumvents the manipulation of bromoacetaldehyde and can also be used to make the corresponding chloro derivative. On warming up the reaction mixture of the reduction of ester with DIBAH, the intermediate acetal decomposes to form ethoxydiisobutylaluminum and bromoacetaldehyde which is intercepted by the silylated phosphite. A similar approach was used by Hägele and Haas to generate, in situ, α-fluorinated aldehydes which were reacted with P- and N-nucleophiles.[9]



Scheme 1.

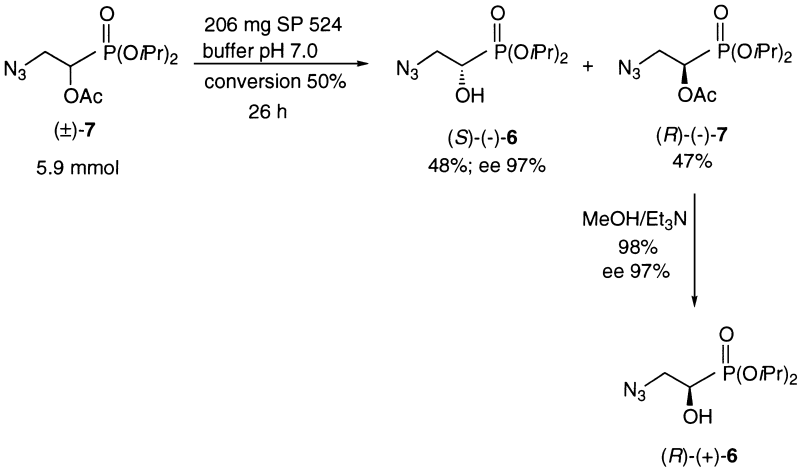
The bromide was transformed into azide (±)-**6** using excess sodium azide (4 equiv.), 0.2 equiv. of 18-crown-**6** in refluxing acetonitrile (82 h) (Scheme 2). When only 2 equiv. of sodium azide were used and the reaction time was 40 h, a 1.8:1 mixture of bromide (±)-**4** and azide (±)-**6** resulted. Esterification of hydroxy azide (±)-**6** with acetic anhydride/pyridine furnished acetate (±)-**7** in 91% yield.[10]



Scheme 2.

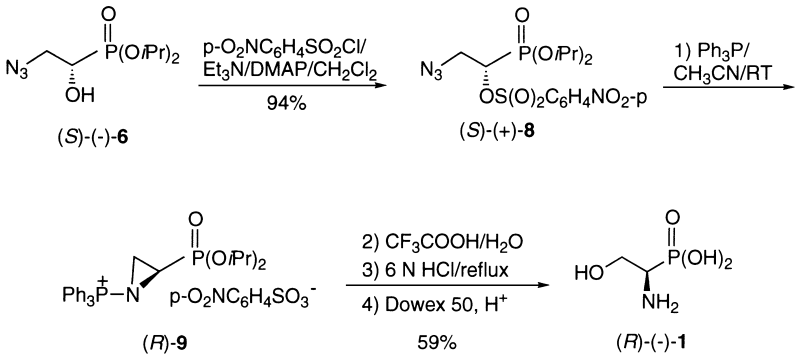
Lipase SP 524 (the genetic coding for the lipase has been transfered from a selected fungus, *Mucor*, to the host organism, *Aspergillus oryzae*) was found to hydrolyse diisopropyl 1-acetoxyethyl-[11] and diisopropyl 1-acetoxypropylphosphonate[1] and α-acetoxy phosphonates with a bromomethyl, chloromethyl or cyanomethyl side chain with high enantioselectivity. Hoping that this hydrolase would also accept (±)-**7** as substrate, a preliminary experiment was carried out on a 0.5 mmol scale and 56 mg of enzyme as reported earlier.[12] The reaction was stopped after 10.5 h at a conversion of 50%. The enantiomeric excess of the α-hydroxyphosphonate was determined by NMR spectroscopy to be 99%. A ^{31}P NMR spectrum (161.97 MHz) was recorded of a solution of the crude mixture of ester and α-hydroxyphosphonate in deuterated benzene, containing 2 equiv. of the chiral solvating agent (*S*p)-(–)-*t*-butyl-phenylmonothiophosphinic acid.[13] Only one signal at $\delta = 20.80$ was visible. A reference sample showed two signals at 20.80 and 20.99.

Then the resolution was carried out on a preparative scale using 5.9 mmol of (±)-**7**, 206 mg of lipase SP 524, 50 ml of phosphate buffer pH 7.0 and 15 ml of hexane and 5 ml *t*-butyl methyl ether (Scheme 3).[12]



Scheme 3.

At a conversion of 50% after 26 h the consumption of base was minimal and the reaction mixture was worked up. The ester (*R*)-(–)-**7** and the α-hydroxyphosphonate (*S*)-(–)-**6** were separated by flash chromatography {(*S*)-(–)-**6**: yield: 48%; $[\alpha]_D^{20} = -7.9$ (*c* 1.2, acetone); ee 97% by use of chiral solvating agent and ^{31}P NMR spectroscopy; (*R*)-(–)-**7**: yield: 47%; $[\alpha]_D^{20} = -14.3$ (*c* 1.0, acetone)}. The (*S*) configuration was assigned on the basis of precedence with 1-acetoxyethyl- and 1-acetoxypropylphosphonate and the relative intensity of the ^{31}P NMR signals of diastereomeric complexes. We showed that the diastereomeric complexes between the (*S*)-configured solvating agent and (*R*)-α-hydroxyphosphonates resonate at lower field than that between solvating agent and (*S*)-α-hydroxyphosphonates.[13] Finally, the assignment was proved by the transformation of the α-hydroxyphosphonates into aminophosphonic acids (*R*)-(–)-**1** and (*R*)-(–)-**2** of known configuration. The ester (*R*)-(–)-**7** was hydrolysed chemically[10] ($MeOH/H_2O/Et_3N$) to give α-hydroxyphosphonate (*R*)-(+)-**6** {yield: 98%; $[\alpha]_D^{20} = +8.0$ (*c* 1.0, acetone); ee 97%}. Hydroxyphosphonate (*S*)-(–)-**6** was transformed into L-phosphaserine via *p*-nitrobenzenesulfonate (*S*)-(+)-**8** (Scheme 4). Treatment with triphenylphosphine induced a Staudinger reaction and the intermediate iminophosphorane cyclised smoothly at room temperature. The corresponding mesylate did not give the aziridine, although the dimethyl 2-azido-1-mesyloxyethylphosphonate did.[4] Regioselective opening of the aziridine, followed by removal of protecting groups with refluxing hydrochloric acid and ion exchange chromatography furnished L-phosphaserine in 59% yield.[4]

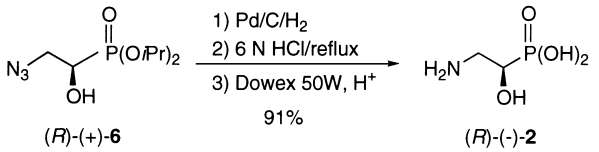


(*S*)-(-)-**6** (*S*)-(+)-**8** (*R*)-**9** (*R*)-(-)-**1**

Scheme 4.

2-Azido-1-hydroxyethylphosphonate (*R*)-(+)-**6** furnished L-phosphaisoserine in high yield (Scheme 5). Catalytic reduction of the azide, deprotection and purification gave the crystalline natural product.

The ee of both aminophosphonic acids was 97% as determined by HPLC using *t*-butyl carbamoyl quinine as chiral stationary phase (CSP)[14] and the respective *N*-DNP (2,4-dinitrophenyl) and



(*R*)-(+)-**6** (*R*)-(-)-**2**

Scheme 5.

N-DNZ (3,5-dinitrobenzyloxycarbonyl) derivatives of the analyte. The absolute configuration of both compounds (*R*)-**1** and (*R*)-**2** was derived, based on observed elution order, employing structurally similar aminocarboxylic acids as reference compounds of known stereochemistry.[2] Thus, threonine and allo-threonine were used as a reference for α-amino-β-hydroxyphosphonic acids, while β-phenylalanine served as a reference for β-amino-α-hydroxyphosphonic acids. As a general finding, a reversal of elution order was observed for the *N*-DNZ and *N*-DNP derivatives of α- and β-amino acids, of the carboxylic and phosphonic acid types. Clearly, the amino function is the dominating factor in the overall chiral recognition mechanism on this type of CSP. For DNP derivatives of α-amino-β-hydroxyphosphonic acids, the (1*R*,2*R*) diastereomer is eluted before the corresponding (1*S*,2*S*) isomer, and the (1*R*,2*S*) before the (1*S*,2*R*) diastereomer. The β-amino congeners, *N*-DNP modified β-amino-α-hydroxyphosphonic acids, show an elution behaviour characterized by (1*S*,2*S*) < (1*R*,2*R*) and (1*R*,2*S*) < (1*S*,2*R*). It should be noted that this change in the elution order is not due to a different stereochemically driven chiral recognition mechanism, it is only a result of changes in priorities according to the Cahn–Ingold–Prelog rule. Among the studied amino phosphonic acids, compound **2** is of particular interest as the amino function is not located at the stereogenic centre. Unfortunately, enantioselective HPLC with quinine based CSPs failed to some extent to separate all four stereoisomers of compounds **1** and **2** in one chromatogram, respectively. However, application of the partial filling technique in non-aqueous capillary electrophoresis (NACE) using *t*-butylcarbamoyl quinine as the chiral selector (SO)[15] allowed convenient separation of all four stereoisomers within one electropherogram (run) [see Fig. 2; **A** racemic mixture of DNP derivatives of **1** and **2**; **B** racemic mixture of DNP derivatives of **1** and **2** with DNP derivative of (*R*)-**2**]. Once again, the elution order for compound **2** was (*S*) before (*R*) in agreement with previous investigations.[2]

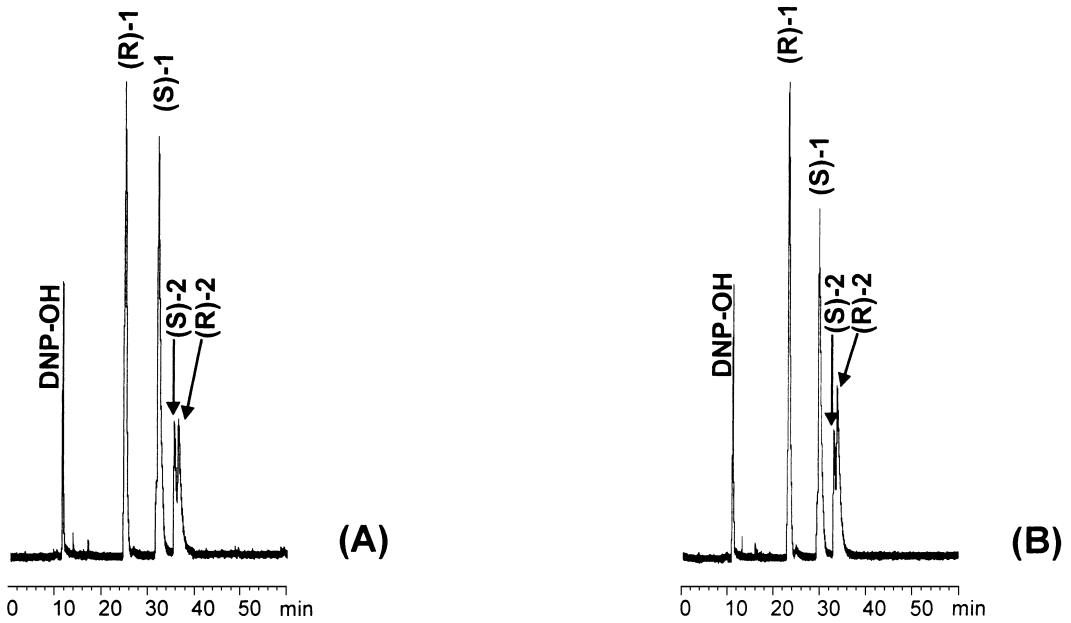


Figure 2.

3. Conclusions

We have demonstrated that L-phosphaserine and L-phosphaisoserine can be prepared by a chemoenzymatic approach with a high enantiomeric excess. The two starting 1-hydroxy-2-azidoethylphosphonates were obtained by a single lipase-catalysed resolution and were transformed into the amino-hydroxyphosphonic acids (*R*)-**1** and (*R*)-**2**. The ee (97%) and absolute configurations of both aminophosphonic acids can be determined by HPLC using a chiral stationary phase (CSP). Non-aqueous capillary electrophoresis (NACE) using a chiral selector allowed covenient separation of all four stereoisomers of amino-hydroxyethylphosphonic acids within one electropherogram.

4. Experimental

4.1. General

All starting materials and enzymes were obtained from commercial suppliers and were generally used without further purification. ^{1}H and ^{13}C NMR (*J* modulated) spectra were recorded in $CDCl_3$, unless otherwise stated, using tetramethylsilane as internal standard on a Bruker AM 400 WB at 400.13 and 100.61 MHz, respectively. ^{31}P NMR spectra were recorded on the same spectrometer at 161.97 MHz using H_3PO_4 (85%) as external standard. In order to get undistorted ^{31}P signal intensities for an accurate integration, adequate relaxation times were used without irradiation during this period to avoid NOE enhancements. Chemical shifts (δ) are given in ppm and coupling constants (*J*) in hertz. IR spectra were run on a Perkin–Elmer 1600 FT-IR spectrometer; liquid samples were measured as films between NaCl plates or on a silicon disc,[16] solids as Nujol mulls between NaCl plates. Optical rotations were measured at 20°C on a Perkin–Elmer 341 polarimeter in a 1 dm cell. TLC was carried out on 0.25 mm thick Merck plates, silica gel 60 F_{254}. Flash chromatography was performed with Merck silica gel 60 (230–400 mesh). Spots were visualized by dipping the plate into a solution of 24 g of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ and 1 g of $Ce(SO_4)_2\cdot 4H_2O$ in 500 ml of 10% H_2SO_4 in water, followed by heating with a hot gun. A Metrohm 702 SM Titrino instrument was used as an autotitrator. Lipase SP 524 was stored at +4°C and used as supplied. Abbreviations used: MC = methylene chloride; EA = ethyl acetate, PE = petroleum ether, AC = acetone, ee = enantiomeric excess.

Liquid chromatography and data system: The HPLC system employed consisted of the following components: L-7100 intelligent pump, L-7400 UV–visible spectrophotometric detector, L-7200 autosampler, D-7000 interface, HSM 7000 chromatography data station software all from Merck, Darmstadt, Germany and a column thermostat from W.O. Electronics (Langenzersdorf, Austria). The column dimensions were 150×4.6 mm i.d. packed with the chiral stationary phase described elsewhere.[15]

The standard operating conditions were as follows: the mobile phase consisted of 80% methanol and 20% of a 50 mM aqueous phosphate buffer (Na_2HPO_4). The pH of the mixture was adjusted with phosphoric acid to the apparent $pH_a = 5.6$. It was measured with a pH 540 GLP meter from Aigner-Unilab (Vienna). The flow rate was 1 ml min^{-1}, and the column temperature was 40°C. The derivatives of the enantiomerically enriched and the racemic

derivatives of the analytes were detected at 360 nm (2,4-dinitrophenyl derivatives, DNP) and 250 nm (3,5-dinitrobenzyloxycarbonyl derivatives, DNZ).

For CE experiments an HP^{3D} capillary electrophoresis system (Hewlett Packard, Waldbronn, Gemany) equipped with a diode array detector was used. The capillary was thermostated (air ventilation) at 15°C.

4.2. (±)-Diisopropyl 2-bromo-1-hydroxyethylphosphonate (±)-4

A solution of diisobutylaluminum hydride in heptane (1 M, 24 ml, 24 mmol) was added slowly at –78°C (bath temperature) to a stirred solution of 3.34 g (20 mmol) of ethyl bromoacetate in 30 ml of dry toluene. After stirring for 2 h at –78°C, 4.77 g (20 mmol) of diisopropyl trimethylsilyl phosphite were added. The reaction mixture was allowed to warm up gradually in the cooling bath to 15°C overnight and was then poured into 150 ml of a vigorously stirred 2 M aqueous solution of tartaric acid. After 2 h the organic phase was separated and the aqueous phase was extracted three times with EA. The combined organic layers were washed with brine, dried with $MgSO_4$ and concentrated in vacuo. Purification of the residue by flash chromatography (EA; $R_f = 0.60$, EA) furnished 3.36 g (58%) of (±)-**4**, mp 42°C (PE) and 0.89 g (12%) of (±)-**5** as an oil, $R_f = 0.80$ (EA).

Compound (±)-**5**: ^{1}H NMR: δ 4.79 and 4.73 (dsept, $J = 6.3$, 7.5, 1H, OCH), 4.09 (ddd, $J = 2.0$, 9.5, 10.5, 1H, CHP), 3.76 (ddd, $J = 2.0$, 3.3, 10.5, 1H, CH_2Br), 3.43 (dt, $J = 5.5$, 10.5, 1H, CH_2Br), 1.36, 1.355, 1.35 and 1.34 (d, $J = 6.3$, 3H, Me), 0.25 (s, 9H, Me_3Si); ^{13}C NMR: δ 71.94 (d, $J = 6.1$), 71.76 (d, $J = 7.7$), 71.10 (d, $J = 164.5$), 34.44 (d, $J = 13.0$), 24.27 (d, $J = 3.8$), 24.10 (d, $J = 4.6$), 23.96 (d, $J = 3.8$), 23.83 (d, $J = 5.4$), 0.25.

Compound (±)-**4**: IR (NaCl) ν_{max} cm^{-1}: 3282, 2980, 2935, 1467, 1387, 1376, 1233, 1179, 1143, 1104, 1085, 991; ^{1}H NMR: δ 4.72 (m, 2H, OCH), 3.98 (ddt, $J = 2.5$, 5.5, 10.0, 1H, CHP), 3.72 (dd, $J = 6.0$, 10.0, 1H, OH), 3.68 (ddd, $J = 2.5$, 5.0, 10.8, 1H, CH_2), 3.47 (dt, $J = 5.5$, 10.8, 1H, CH_2), 1.29 and 1.277 (d, $J = 6.0$, 3H, Me), 1.280 (d, $J = 6.0$, 6H, Me); ^{13}C NMR: δ 72.19 and 71.98 (d, $J = 7.6$), 69.05 (d, $J = 159.1$), 33.66 (d, $J = 11.7$), 24.08 and 23.91 (d, $J = 3.8$), 24.04 (d, $J = 4.6$), 23.90 (d, $J = 5.4$). Anal. calcd for $C_8H_{18}BrO_4P$: C, 33.24; H, 6.28. Found: C, 33.48; H, 6.06.

4.3. (±)-Diisopropyl 2-azido-1-hydroxyethylphosphonate (±)-6

Sodium azide (1.33 g, 20.43 mmol) and 18-crown-**6** (1.08 g, 4.09 mmol) were added under argon to a solution of (±)-**4** (2.36 g, 8.17 mmol) in 50 ml of dry acetonitrile. The mixture was refluxed for 82 h. The solvent was removed under reduced pressure and the residue was taken up in water. The aqueous solution was extracted three times with EA. The combined organic layers were dried with $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash chromatography with EA as eluent to yield 1.69 g (82%) of (±)-**6** as a viscous oil containing a trace of diisopropyl phosphite as impurity; $R_f = 0.66$ (EA).

IR (Si) ν_{max} cm^{-1}: 3277, 2982, 2936, 2875, 2101, 1468, 1454, 1387, 1376, 1355, 1315, 1222, 1179, 1143, 1104, 990; ^{1}H NMR: δ 4.71 (m, 2H, OCH) overlapping with 4.68 (m, 1H, OH), 3.96 (m, 1H, CHP), 3.42 (AB system, $J_{AB} = 13.1$, $J = 3.3$, 6.5, 6.8, 9.2, 2H, N_3CH_2), 1.29 (d, $J = 7.0$, 3H, Me), 1.27 (d, $J = 7.0$, 6H, Me), 1.26 (d, $J = 6.0$, 3H, Me); ^{13}C NMR: δ 72.07 and 71.76 (d, $J = 7.6$), 68.15 (d, $J = 161.4$), 52.26 (d, $J = 7.6$), 24.08 (d, $J = 3.1$), 24.00 (d, $J = 3.8$), 23.89 and 23.87 (d, $J = 4.6$). Anal. calcd for $C_8H_{18}N_3O_4$: C, 38.25; H, 7.22; N, 16.73. Found: C, 38.38; H, 6.92; N, 16.75.

4.4. (±)-Diisopropyl 2-azido-1-acetoxyethylphosphonate (±)-7

Compound (±)-**6** (1.984 g, 7.90 mmol) was acetylated by a known procedure and purified by flash chromatography (PE:EA = 1:4) to yield 2.11 g (91%) of (±)-**7** as an oil; $R_f = 0.55$ (PE:EA = 1:4).

IR (NaCl) ν_{max} cm^{-1}: 2983, 2937, 2100, 1757, 1453, 1375, 1218, 1179, 1143, 1105, 1063, 990; ^{1}H NMR: δ 5.32 (m, 1H, CHP), 4.70 (m, 2H, OCH), 3.54 (t, $J = 6.3$, 2H, N_3CH_2), 2.10 (s, 3H, MeCO), 1.28 (d, $J = 7.0$, 6H, Me), 1.27 (d, $J = 5.7$, 3H, Me), 1.26 (d, $J = 6.5$, 3H, Me); ^{13}C NMR: δ 169.36 (d, $J = 5.3$), 72.25 and 72.20 (d, $J = 6.9$), 67.47 (d, $J = 167.5$), 50.36 (d, $J = 6.1$), 24.05 and 23.97 (d, $J = 3.8$), 23.87 and 23.76 (d, $J = 4.6$), 20.57. Anal. calcd for $C_{10}H_{20}N_3O_5P$: C, 40.96, H, 6.87, N, 14.33. Found: C, 41.10, H, 6.67, N, 13.63.

4.5. (S)-(+)-Diisopropyl 2-azido-1-(4-nitrobenzenesulfonyloxy)ethylphosphonate (S)-(+)-8

A solution of *p*-nitrobenzenesulfonylchoride (397 mg, 1.79 mmol) in 5 ml of dry MC, followed by 0.50 ml (0.36 g, 3.58 mmol) of dry triethylamine and 15 mg of DMAP, was added to a stirred solution of (*S*)-(–)-**6** (375 mg, 1.49 mmol) in 8 ml of dry MC under argon at 0°C. After stirring for 30 min at 0°C and 2 h at room temperature (TLC-control: PE:EA = 1:4), 10 ml of water and 1 ml of concentrated HCl were added. The organic phase was separated and the aqueous phase was extracted twice with MC. The combined organic layers were washed with a saturated aqueous solution of $NaHCO_3$, dried with $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash chromatography (PE:EA = 1:1) to yield 615 mg (94%) of (*S*)-(+)-**8** as a viscous oil; $R_f = 0.43$ (PE:EA, 1:1); $[\alpha]_D^{20} = +13.4$ (*c* 1.0, AC).

IR (Si) ν_{max} cm^{-1}: 2984, 2108, 1608, 1535, 1453, 1378, 1351, 1314, 1264, 1188, 1144, 1104, 994; ^{1}H NMR: δ 8.23 (AA′BB′ system, $J = 9.0$, 4H, C_6H_4), 4.88 (ddd, J = 3.0, 8.5, 11.5, 1H, CHP), 4.69 (m, 2H, OCH), 3.61 (AB system, $J_{AB} = 14.0$, $J = 3.0$, 5.0, 8.0, 8.5, 2H, N_3CH_2), 1.28 (d, $J = 6.0$, 6H, Me), 1.26 and 1.25 (d, $J = 6.0$, 3H, Me); ^{13}C NMR: δ 150.83, 142.28, 129.41 (2×C), 124.23 (2×C), 75.87 (d, 167.5), 73.22 and 73.18 (d, $J = 6.9$), 50.94 (d, $J = 6.1$), 24.04 and 24.00 (d, $J = 3.8$), 23.88 (d, $J = 5.3$), 23.72 (d, $J = 4.6$). Anal. calcd for $C_{14}H_{21}N_4O_8PS$: C, 38.53; H, 4.85; N, 12.84. Found: C, 38.83; H, 4.74; N, 12.85.

4.6. (R)-(–)-1-Amino-2-hydroxyethylphosphonic acid (R)-(–)-1

A solution of (*S*)-(+)-**8** (585 mg, 1.34 mmol) (ee 97%) and triphenylphosphine (422 mg, 1.61 mmol) in 15 ml of dry acetonitrile was stirred under argon at room temperature for 18 h. After evaporation of the solvent, the residue was treated with 1.8 ml of trifluoroacetic acid and 17 ml of water for 1 h at room temperature and for 3 h at reflux.[4] Concentrated HCl (28 ml) was added and the reaction mixture was refluxed for 31 h. Then water (50 ml) was added and the cold solution was extracted three times with diethyl ether. The aqueous solution was concentrated in vacuo. The residue was dried over KOH in a vacuum desiccator and purified by ion exchange chromatography on Dowex 50 WX4, H^+ with water as eluent.[4] Ninhydrin positive fractions were pooled, concentrated in vacuo and finally freeze dried to give 112 mg (59%) of (*R*)-(–)-**1** as a crystalline solid; melting range 80–120°C (lit.:[4] 95–110°C); $[\alpha]_D^{20} = -26.0$ (*c* 1.0, H_2O) {lit.:[4] $[\alpha]_D^{20} = -30.2$ (*c* 1.27, H_2O), ee ≥98%}.

IR (Nujol mull) ν_{max} cm^{-1}: 3570, 1614, 1538, 1169, 1036, 916; ^1H NMR (D_2O): δ 3.96 (ddd, $J=3.8$, 6.7, 12.5, 1H, CH_2), 3.71 (ddd, $J=$ 4.4, 9.8, 12.5, 1H, CH_2), 3.36 (ddd, $J=3.8$, 9.8, 14.0, 1H, CHP); ^{13}C NMR (D_2O): δ 59.02 (d, $J=3.1$), 51.39 (d, $J=139.2$); ^{31}P NMR (D_2O): δ 11.25.

4.7. (R)-(–)-2-Amino-1-hydroxyethylphosphonic acid (R)-(–)-2

Compound (*R*)-(+)-**6** (326 mg, 1.30 mmol) (ee 97%) was reduced to the amine, deblocked by refluxing with 6N HCl for 7 h and then purified by ion exchange chromatography as reported in the literature;[4] yield: 167 mg (91%) of (*R*)-(–)-**2** as a crystalline solid; mp 257–260°C (decomp.) [lit.:[4] 242–245°C (H_2O/ethanol)]; $[\alpha]_D^{20}=-31.8$ (0.9, H_2O) {lit.:[4] $[\alpha]_D^{20}=-30.5$ (0.525, H_2O)}.

The ^1H NMR spectrum is identical with the one reported; ^{13}C NMR (D_2O): δ 65.60 (d, $J=156.0$), 41.77 (d, $J=9.2$); ^{31}P NMR (D_2O): δ 16.27.

4.8. Protocol of derivatization of aminophosphonic acids

About 0.5–1 mg of the respective α- or β-aminophosphonic acid was dissolved in 200 μl of carbonate buffer (mixture of 0.1 M $NaHCO_3$ and 0.1 M Na_2CO_3 to obtain pH 9.5) to which 100 μl of the reagent solution of 2,4-dinitrofluorobenzene (DNFB) or 3,5-dinitrobenzyloxycarbonyl chloride (DNZ-Cl) (2.5% in acetonitrile, w/v) were added. The mixtures were heated for 30 min at 50°C. Then 100 μl of the reaction mixture were diluted with 500 μl of LC mobile phase and 20 μl of this sample were injected onto the column.

4.9. Standard procedure for CE experiments with NACE in the partial filling technique modus (see also Ref. 15)

In brief, before each run, the capillary was pre-conditioned by flushing with the plain non-aqueous electrolyte solution containing no chiral selector (SO), e.g. an ethanol:methanol mixture (60:40) containing 100 mM glacial acetic acid and 12.5 mM triethylamine. This solution furnished as a background electrolyte (BGE). Then the capillary was filled with non-aqueous electrolyte solution containing 10 mM of the SO applying a pressure of 50 mbar to the injection end of the capillary over a period of 50 min. Under these conditions a plug of ca. 30 cm in length from the injection end of the 50 μm i.d. FS capillary ($L_{tot}=45.5$ cm, $L_{eff}=37$ cm) is filled (calculated from the breakthrough curve). Then the sample dissolved in BGE without SO was injected hydrodynamically by application of a pressure of 50 mbar for 5 s. Finally, the analytical run was performed applying a constant voltage of –25 kV using plain non-aqueous electrolyte solution without chiral SO as running buffer at both inlet and outlet home vials.

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