Chemoenzymatic synthesis of optically active phosphinic analogues of S-substituted sulfur-containing amino acids

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The interaction of racemic 1-amino-3-(methylthio)propylphosphinic acid with benzylthiol catalysed by pyridoxal-5′-phosphate-dependent L-methionine-γ-lyase affords (*R*)-1-amino-3-(benzylthio)propylphosphinic acid, which was converted into the (*R*)-isomers of phosphinic analogues of homocysteine and methionine.

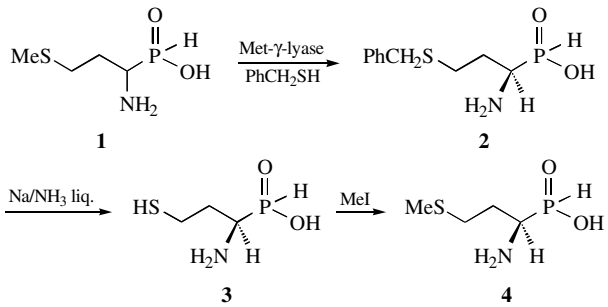
1-Aminoalkylphosphinic acids, analogues of natural α-amino acids, display pronounced biological activity and are extensively applied in enzymological studies.[1–3] However, only a few optically active compounds of this kind have been obtained until now. Thus, (*R*)-isomers of phosphinic analogues of alanine and phenylalanine have previously been prepared by the stereoselective alkylation of chiral Schiff bases derived from (1*R*,2*R*,5*R*)-2-hydroxypinan-3-one and the ethyl esters of aminomethyl(diethoxymethyl)phosphinic acid by methyl iodide and benzyl chloride, respectively, while the stereoselectivity of the reaction strongly depended on the structure of the alkyl halide.[4] The (*S*)- and (*R*)-isomers of 1-aminoalkylphosphinic acids, including (*S*)- and (*R*)-1-amino-3-(methylthio)propylphosphinic acids (analogues of methionine), have been obtained by separating diastereomeric salts of corresponding N-benzyloxycarbonyl derivatives with (*R*)- or (*S*)-methylbenzylamines.[5] The prospects of using enzymatic methods for the separation and/or synthesis of chiral phosphinic analogues of amino acids were first demonstrated by the example of interaction of the enantiomers of a tyrosine phosphinic analogue with PLP-dependent L-tyrosine-phenol-lyase.[3] Thus, only (*R*)-isomer of the phosphinic acid, whose configuration corresponds to natural (*S*)-tyrosine, proved to be the substrate in the reaction of α,β-elimination. Accordingly, (*R*)-isomer of the tyrosine phosphinic analogue was the product of the reverse enzymatic reaction.

In this work, a new method is proposed for the synthesis of optically active phosphinic analogues of *S*-substituted sulfur-containing amino acids. It is based on the use of L-methionine-γ-lyase for the transformation of racemic 1-amino-3-methylthiopropylphosphinic acid **1**† into optically active 1-amino-3-(benzylthio)propylphosphinic acid **2** and the subsequent preparation of chiral 1-amino-3-mercaptopropylphosphinic acid **3** followed by its S-methylation to known (*R*)-1-amino-3-methylthiopropylphosphinic acid **4**.

PLP-dependent L-methionine-γ-lyase [L-methionine-methanethiol lyase (deaminating) E.C. 4.4.1.11] from *Citrobacter intermedius* catalyses decomposition of L-methionine to methanethiol, α-ketobutyrate and the ammonium ion and displays specificity with respect to substrate structures and types of chemical reactions.[7]

An important feature of the enzyme is its ability to catalyse the enantioselective formation of S-alkylhomocysteins from L-methionine and thiols due to γ-substitution reaction. This reaction was also possible for analogue **1**, whose interaction with benzylthiol under the action of *Citrobacter intermedius* cells containing L-methionine-γ-lyase afforded optically active acid **2**, although with a low yield.[8]

We examined various conditions of the enzymatic synthesis of compound **2** from acid **1** and benzylthiol and found that the use of L-methionine-γ-lyase‡ instead of intact cells as a biocatalyst allowed us to increase the yield of **2** up to 45%.§ The (*R*)-configuration was ascribed to optically active acid **2** based on that the stereochemistry of the enzymatic reaction remains the same on going from the natural substrate to its phosphinic analogue, as it was observed for the reaction of L-tyrosine-phenol-lyase with the enantiomers of tyrosine phosphinic analogues. This assumption was supported experimentally by obtaining previously described phosphinic analogue **4** as a result of chemical transformation of amino acid **2** not affecting the chiral centre of the molecule. The debenzylation of aminophosphinate **2** by treatment with Na in liquid ammonia affords optically active amino acid **3**, which was not described before and may be used as a starting material for the synthesis of phosphinic analogues of various chiral S-substituted sulfur-containing amino acids.¶ By the methylation of analogue **3**, optically active acid **4** was prepared, for which the sign and value of specific rotation corresponded to (*R*)-isomer of the methionine phosphinic analogue[5] (Scheme 1).††



Scheme 1

Thus, the (*R*)-configuration corresponds to amino acids **2**–**4**, which agrees with our assumption that the enantioselectivity of enzymatic γ-substitution reactions is retained and shows that phosphinic analogues of amino acids are stable with respect to racemisation under the action of Na in liquid ammonia.

† Racemic 1-amino-3-methylthiopropylphosphinic acid **1** was synthesised according to a published procedure.[6]

‡ L-Methionine-γ-lyase was obtained from *C. intermedius* cells according to a published procedure.[8] The activity of the preparation was assayed by measuring the rate of α-ketobutyrate formation from L-methionine according to Friedemann.[9] One unit of enzymic activity was determined as the enzyme amount catalysing the transformation of 1 μmol of L-methionine per minute at 30 °C and a 40 mM concentration of L-methionine.

§ Benzylthiol (0.5 ml) was added to a solution of **1** (169 mg, 1 mmol) in 20 ml of a 0.1 M potassium phosphate buffer containing 0.1 mM PLP and 12.8 U of L-methionine-γ-lyase. The reaction mixture was stirred on a shaker for three days at 30 °C. The protein was denatured by adding 30% trichloroacetic acid (2 ml) and removed by centrifugation. The solvent was evaporated *in vacuo*; the residue was dissolved in water (1 ml) and applied to a 40 ml column with Dowex 50×8 resin (H^+ form). The column was washed with water (100 ml), and product **2** was eluted with a 5% ammonia solution. The fractions containing **2** were evaporated *in vacuo* to dryness. The residue was dried *in vacuo* over P_2O_5 to give phosphinic analogue **2** (110 mg, 45%), mp 221 °C, $[\alpha]_D^{20}$ –16.3° (*c* 0.5, 1M HCl). R_f 0.61 (Pr^iOH–25% NH_4OH–H_2O, 7:1:2), R_f 0.46 (BuOH–AcOH–H_2O, 12:3:5). ^1H NMR (400 MHz, 0.25 M NaOD in D_2O) δ: 1.63–2.05 (m, 2H, CH_2CH), 2.53–2.79 (m, 3H, SCH_2CH_2 and CH), 3.80 (s, 2H, CH_2Ph), 6.72 (dd, 1H, PH, *J* 486 Hz, *J* 1.8 Hz), 7.40 (s, 5H, Ph).

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¶ Sodium metal (20 mg, 0.86 mmol) was added to a solution of compound **2** (100 mg, 0.4 mmol) in 15 ml of boiling liquid ammonia. The reaction mixture was stirred for 30 min, and then solid NH_4Cl was added until the disappearance of a blue colour. The ammonia was allowed to evaporate, and the residue was concentrated with H_2O *in vacuo*. Thiol **3** was isolated on a 10 ml column with Dowex 50×8 resin (H^+ form) by elution with a 15% aqueous isopropanol solution. The fractions containing **3** were evaporated *in vacuo* to dryness. The residue was dried *in vacuo* over P_2O_5 to give phosphinic analogue **3** (27 mg, 43%). $[\alpha]_D^{20}$ –22° (*c* 1, H_2O). R_f 0.39 (Pr^iOH–25% NH_4OH–H_2O, 7:1:2), R_f 0.23 (BuOH–AcOH–H_2O, 12:3:5). ^1H NMR (400 MHz, D_2O) δ: 1.80–2.06 (m, 2H, CH_2CH), 2.48–2.78 (m, 3H, SCH_2), 3.21–3.25 (m, 1H, CH), 6.87 (d, 1H, PH, *J* 535 Hz).

†† MeOH (1.4 ml) and MeI (0.01 ml) were added to a solution of compound **3** (25 mg, 0.15 mmol) in 0.15 ml of 2 M NaOH. The mixture was allowed to stand for 16 h and concentrated *in vacuo* to dryness. Compound **4** (15 mg, 59%) was isolated as described above. Mp 230 °C, $[\alpha]_D^{20}$ –31° (*c* 1, H_2O) {lit.,[5] $[\alpha]_D^{22}$ –30° (*c* 1, H_2O)}. R_f 0.53 (Pr^iOH–25% NH_4OH–H_2O, 7:1:2), R_f 0.36 (BuOH–AcOH–H_2O, 12:3:5). ^1H NMR (400 MHz, D_2O) δ: 1.8–2.28 (m, 2H, CH_2CH), 1.97 (s, 3H, MeS), 2.51–2.65 (m, 2H, CH_2S), 3.14–3.19 (m, 1H, CH), 6.87 (d, 1H, PH, *J* 535 Hz).

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