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Penicillin G acylase-mediated kinetic resolution of racemic 1-(*N*-acylamino)alkylphosphonic and 1-(*N*-acylamino)alkylphosphinic acids and their esters

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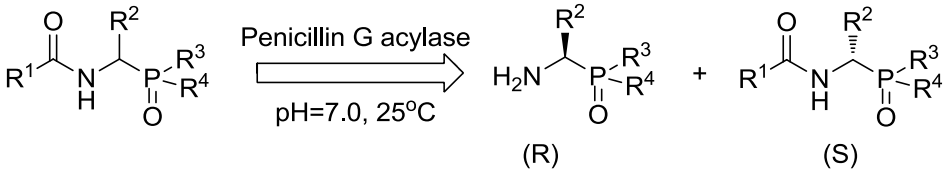
Graphical abstract



R^1= Me, $PhCH_2$, $PhCH_2O$
R^2= Me, $PhCH_2$, *i*-Pr, *i*-$PrCH_2$, CH_2CONH_2, CH_2OtBu
R^3= H, OH, OMe, OEt
R^4= OH, OMe, Ph

22 examples

Highlights

- The penicillin G acylase-mediated hydrolysis of 1-(*N*-acylamino)alkylphosphonic acids and 1-(*N*-acylamino)alkylphosphinic acids, as well as their dimethyl esters proved to be a highly effective method of their kinetic resolution for 13 of 16 tested substrates.
- The initial hydrolysis rates of 1-(*N*-acylamino)alkylphosphonic acids and their esters rapidly decrease with the increasing steric effect of the substituent at the α-position.
- Bulky substituents at phosphorus hinder the enzymatic hydrolysis to a much lesser degree.
- Penicillin G acylase exhibited stereochemical preference for the (*R*)-substrate.

Abstract

Extensive studies on the penicillin G acylase-mediated kinetic resolution of *N*-acylated 1-aminoalkylphosphonic and 1-aminoalkylphosphinic acids as well as their esters were carried out to recognise the relationships between the substrate structure, reaction conditions, and the enzymatic hydrolytic deacylation efficiency and stereoselectivity. Reactivity of 1-(*N*-acylamino)alkylphosphonic and 1-(*N*-acylamino)alkylphosphinic acids and their esters in the penicillin G acylase-mediated hydrolytic deacylation reaction depends strongly on the kind of their *N*-acyl group, with high preference to the hydrolytic splitting of the *N*-phenylacetyl moiety. The initial hydrolysis rates of 1-(*N*-phenylacetylamino)alkylphosphonic acid dimethyl esters **2** are mostly distinctly lower in comparison with the corresponding free acids **3** and rapidly decrease with the increasing steric effect of the substituent at the α-position. In contrary to the substituents at the α-carbon, bulky substituents at the phosphorus hinder the enzymatic hydrolysis to a much lesser degree. The penicillin G acylase-mediated stereospecific hydrolysis of *N*-acyl group of both racemic 1-(*N*-acylamino)alkylphosphonic acids **3** and their dimethyl esters **2** proved to be, in most cases, a highly effective method for

the kinetic resolution of these compounds. High enzyme enantioselectivity *E*-values exceeding 100, or synthetically useful *E*-values exceeding 20 (in two cases) were obtained for the *N*-acylated phosphonic acid analogues of alanine, phenylalanine, valine, leucine, and asparagine, as well as for their dimethyl esters, with the exception of the dimethyl ester of phosphonic analogue of valine **2e**, that *E*-value was low (*E* = 1.2). Also for the *N*-acylated *H*-phosphinic acid analogues of alanine, as well as phenylphosphinic acid analogue of alanine, high enzyme enantioselectivity values exceeding 100 were obtained. In contrary, *E*-values for both diastereomers of ethyl ester of phenylphosphinic analogue of alanine **2k** were low (*E* = 7 and 13). For the all accomplished assignments penicillin G acylase exhibited stereochemical preference for the (*R*)-substrate.

Keywords:
Penicillin G acylase
Enantioselective kinetic resolution
Hydrolytic deacylation
1-(*N*-acylamino)alkylphosphonic acids
1-(*N*-acylamino)alkylphosphinic acids

1. Introduction

Chiral, non-racemic α-aminoalkylphosphonic and α-aminoalkylphosphinic acids, as structural analogues and mimetics of natural α-amino acids, exhibit inhibitory activity against a plethora of different enzymes, resulting in their diversified biological activity, with a wide range of application, mainly as pharmaceuticals and agrochemicals [1-6]. Due to that, they attract significant attention of chemists and biochemist for many years. The biological activities of these compounds are strongly dependent on their configuration at the stereogenic α-carbon [3,4,6]. Several groups of methods for the asymmetric synthesis of 1-aminoalkylphosphonic and 1-aminoalkylphosphinic acids derivatives have been developed [2,7-10]; however, due to disadvantages most of them (complicated and laborious procedures, expensive chiral catalysts) the enzymatic kinetic resolution of racemic mixtures of these compounds has become an interesting alternative [2].

Hydrolase-mediated kinetic resolution of racemic α-amino acid derivatives is a well established method for producing optically pure products, with a large number of practical applications [11-13]. In contrast to α-amino acids, literature data on hydrolases-mediated

kinetic resolution of 1-aminoalkylphosphonic and 1-aminoalkylphosphinic acids derivatives are limited to a few papers. The resolution of racemic 1-(*N*-acylamino)alkylphosphonic acids using porcine kidney acylase described by Telegdi et al. [14,15] was not confirmed by other authors in later experiments [16]. Solodenko et al. [4, 17-19] applied penicillin acylase from *E. coli* for stereoselective hydrolytic kinetic resolution of a few relatively simple *N*-acylated 1-aminoalkylphosphonic acids, 1-(*N*-phenylacetylamino)ethylphosphonous acid, as well as dimethyl and diisopropyl esters of 1-(*N*-phenylacetylamino)ethylphosphonic acid. Zimmermann et al. described in the US Patent [20] and other analogical patents the kinetic resolution of phosphonic analogues of a few *N*-acylated 1-amino acids using lyophilised, carrier–bound penicillin G acylase (Boehringer Mannheim GmBH).

Recently, we have developed a new, effective two-stage method for the transformation of *N*-acyl-α-amino acids into their phosphonic or phosphinic analogues via the electrochemical decarboxylative α-methoxylation of *N*-acyl-α-amino acids to *N*-(1-methoxyalkyl)amides and the Michaelis-Arbuzov-type amidoalkylation of the proper phosphorus nucleophile with the latter compounds [21-24]. The method enables easy access to a variety of *N*-acylated 1-aminoalkylphosphonic and 1-aminoalkylphosphinic acid esters of diversified structure, including the most interesting group of these compounds with the substituents at the α-position identical with those ones characteristic for natural, α-amino acids, both proteinogenic and unproteinogenic. The main drawback of this method is the racemization of *N*-acyl-α-amino acid already on the step of decarboxylative α-methoxylation of *N*-acyl-1-amino acid. With a large library of structurally diversified racemic 1-aminoalkylphosphonic and 1-aminoalkylphosphinic acid derivatives in hand, we performed an extensive study on the penicillin G acylase-mediated kinetic resolution of *N*-acylated 1-aminoalkylphosphonic and 1-aminoalkylphosphinic acids as well as their esters to recognise the relationships between the substrate structure, reaction conditions, and the enzymatic hydrolytic deacylation efficiency and stereoselectivity.

2. Experimental

2.1. General

Melting points were determined using capillary tubes in Stiriling SMP 3 apparatus and were uncorrected. IR-spectra were measured on a Nicolett 6700 FT-IR spectrophotometer (ATR method). ^{1}H and ^{13}C NMR spectra were recorded on Varian instruments at operating

frequencies of 400 or 600 and 100 or 150 MHz, respectively, using TMS as resonance shift standard. ^{31}P NMR spectra were recorded on a Varian instrument at operating frequency of 161.8 MHz with 80% orthophosphoric acid as an external resonance shift standard. All chemical shifts (δ) are reported in ppm and coupling constants (*J*) are in Hz. HPLC analyses were carried out using a LaChrom ULTRA HPLC system (Merck Hitachi, Germany), including a binary pump (Model L-2 2160U), diode array detector (Model L-2455U), autosampler (Model L-2200), a temperature–controled column compartment (Model L-2350U), and a degasser module. The data were evaluated by EZ Chrom Elite System Manager. High-resolution mass spectrometry analyses were performed on a Waters Xevo G2 Q-TOF mass spectrometer (Waters Corporation) equipped with an ESI source operating in positive ion modes. Full-scan MS data were collected from 100 to 1000 Da in a positive ion mode with a scan time of 0.1 s. Column chromatography was carried out using Merck 60 silica gel (70-230 mesh). Routine monitoring of reactions was done with the use of thin layer chromatography, using TLC plates coated with silica gel (Merck 60 F_{254}). Ion-exchange chromatography was carried out using DOWEX 50W X8 in the H^+ form. Optical rotations were measured on a JASCO V-650 polarimeter.

2.2. Chemicals and enzyme

All used α-amino acids, *N*-Cbz-DL-alanine, *N*-Cbz-*O*-*t*-Bu-DL-serine, acetyl chloride, phenylacetyl chloride, *N*,*N*-diisopropylethylamine, ammonium phosphinate, hexamethyldisilazane and 3-(1-piperidino)propyl functionalized silica gel (SiO_2-Pip, 200-400 mesh, 1.1 mmol/g) were purchased from Sigma-Aldrich. Penicillin G acylase from *Escherichia coli* (20.34 U/mg) was purchased from CBC Biotech S.r.l. (Italy).

The specific activity of the penicillin G acylase was determined with penicillin G as a substrate according to the PDAB method [25].

2.3. Substrate synthesis

2.3.1. N-Phenylacetylation of α-amino acids

To a solution of amino acid (20 mmol) in 2M NaOH (20 mL), phenylacetyl chloride (2.9 mL, 3.4 g, 22 mmol) was added. The reaction mixture was stirred at room temperature for 2 hours and then acidified by the addition of 2M HCl. The crude product was filtered off, washed with distilled water and recrystallized from ethanol.

2.3.2. Bis-(trimethylsilyl) phosphonite

The synthesis was performed following the procedure described by Olszewski et al. [26] and Boyd et al. [27]. Ammonium phosphinate (3.32g, 40 mmol) and hexamethyldisilazane (8.8 mL, 6.78 g, 42 mmol) were heated at 110°C for 2 hours under argon in a three-necked flask with a septum and a condenser. The reaction mixture was cooled to room temperature, dichloromethane (12.5 mL) was added, and the mixture was stirred for another hour. The solution of the crude product in CH_2Cl_2 was used for further reactions without purification.

*2.3.3. Racemic dimethyl 1-(N-acylamino)alkylphosponates **2** (Procedure A)*

To an undivided cylindrical glass electrolyser (85 mL) with a thermostatic jacket, a magnetic stirrer, a cylindrical Pt mesh anode (47 cm^2), and a similar cathode (44 cm^2), arranged concentrically each to other at a distance of 2.5 ± 0.5 mm, methanol (30 mL), *N*-acyl-α-amino acid **1** (3.0 mmol), and SiO_2-Pip (200 mg, 0.22 mmol) were added. Electrolysis was carried out with stirring at a current density of 0.3 A/dm^2 at 10ºC until 3-3.75 F/mol charge was passed. SiO_2-Pip was filtered off and methanol was evaporated under reduced pressure to obtain the corresponding *N*-(1-methoxyalkyl)amide. The crude *N*-(1-methoxyalkyl)amide and triphenylphosphonium tetrafuluoroborate (2.0 mmol) were dissolved in CH_2Cl_2 (2 mL) and the solution was left at room temperature for 0.5 h. After evaporation of the solvent, the corresponding crude 1-(*N*-acylamino)alkyltriphenylphosphonium tetrafluoroborate was obtained. A solution of the phosphonium salt (2 mmol) in CH_2Cl_2 (6 mL), triphenylmethylphosphonium iodide (0.5 mmol), diisoprophylethylamine (0.03 mL, 0.2 mmol) and trialkylphosphite (3 mmol) or diethoxy phenyl-phosphonite (3 mmol) were placed in a glass vial sealed with a screw-cap. The mixture was kept at 60 ºC for the time given in Table 1. On the reaction completion, the solvent was evaporated under reduced pressure and the residue was extracted with toluene (4 x 2 mL). After evaporation of toluene, the crude product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 20:1 v/v).

2.3.3.1 1-(N-Acetylamino)ethylphosphonic acid dimethyl ester **2a** [28]

^{31}P NMR ($CDCl_3$): δ = 30.9;

^{1}H NMR ($CDCl_3$): δ= 1.35 (dd, J_1 = 7.6, J_2 = 16.8 Hz, 3H, CH_3), 2.02 (s, 3H, CH_3CO), 3.76 (d, J = 10.4 Hz, 3H, OMe), 3.78 (d, J = 10.4 Hz, 3H, OMe), 4.54 (ddq, J_1 = 7.2 Hz, J_2 = 9.6 Hz, J_3 = 16.4 Hz, 1H, CH), 6.22 (d, J = 8.0 Hz, 1H, NH).

2.3.3.2 1-(N-Benzyloxycarbonylamino)ethylphosphonic acid dimethyl ester **2b** [29,30]

^{31}P NMR ($CDCl_3$): δ = 28.5;

^{1}H NMR ($CDCl_3$): δ= 1.40 (dd, J_1 = 7.2, J_2 = 16.8 Hz, 3H, CH_3), 3.74 (d, J = 11.2 Hz, 3H, OMe), 3.75 (d, J = 10.4 Hz, 3H, OMe), 4.18 (m, 1H, CH), 4.95 (d, J = 8.0 Hz, 1H, NH), 5.12 (s, 2H, O*CH*$_2$Ph), 7.25-7.36 (m, 5H, Ph).

2.3.3.3 1-(N-Phenylacetylamino)ethylphosphonic acid dimethyl ester **2c** [31]

^{31}P NMR ($CDCl_3$): δ = 28.21;

^{1}H NMR ($CDCl_3$): δ = 1.30 (dd, J_1 = 7.6 Hz, J_2 = 17.2 Hz, 3H, CH_3), 3.55 (s, 2H, *CH*$_2$Ph), 3.68 (d, J = 10.4 Hz, 3H, OMe), 3.60 (d, J = 10.4 Hz, 3H, OMe), 4.53 (ddq, J_1 = 7.6 Hz, J_2 = 10.0 Hz, J_3 = 15.2 Hz, 1H, CH), 6.18 (d, J = 9.6 Hz, NH), 7.24-7.32 (m, 5H, Ph).

2.3.3.4 1-(N-Phenylacetylamino)-2-phenylethylphosphonic acid dimethyl ester **2d**

^{31}P NMR ($CDCl_3$): δ = 26.2;

^{1}H NMR ($CDCl_3$): δ = 2.78 (ddd, J_1 = J_2 = 10.0 Hz, J_3 = 14.8 Hz, 1H, CH*CH*$_2$Ph), 3.17 (ddd, J_1 = 4.8 Hz, J_2 = 8.4 Hz, J_3 = 14.4 Hz, 1H, CH*CH*$_2$Ph), 3.45 (s, 2H, *CH*$_2$Ph), 3.64 (d, J = 10.6 Hz, 3H, OMe), 3.74 (d, J = 10.6 Hz, 3H, OMe), 4.78 (dddd, J_1 = 4.0 Hz, J_2 = 9.6 Hz, J_3 = 10.4 Hz, J_4 = 15.2 Hz, 1H, CH), 5.81 (d, J = 9.6 Hz, NH), 6.94-7.82 (m, 10H, Ph);

^{13}C NMR ($CDCl_3$): δ = 35.3 (d, J = 3.2 Hz) 43.4, 45.7 (d, J = 154.8 Hz), 53.0 (d, J = 6.6 Hz), 53.2 (d, J = 7.1 Hz), 126.8, 127.2, 128.4, 128.9, 129.2, 132.1, 134.3, 136.2, 170.3 (d, J = 4.4 Hz);

IR (ATR) ν (cm^{-1}): 3252, 2956, 1672, 1541, 1228, 1180, 1018, 838, 734, 697;

HRMS(ESI): calc. for $C_{18}H_{23}NO_4P$ $[M + H]^+$ m/z 348.1365, found m/z 348.1366.

2.3.3.5 1-(N-Phenylacetylamino)-2-methylpropylphosphonic acid dimethyl ester **2e**

^{31}P NMR ($CDCl_3$): δ = 26.6;

^{1}H NMR ($CDCl_3$): δ = 0.85 (d, J= 6.9 Hz, 3H, CH(*CH*$_3$)$_2$), 0.93 (dd, J_1 = 1.3, J_2 = 6.8 Hz, 3H, CH(*CH*$_3$)$_2$), 2.06-2.22 (m, 1H, *CH*(CH$_3$)$_2$), 3.62 (d, J = 10.6 Hz, 3H, OMe), 3.63 (s, 2H, *CH*$_2$Ph), 3.73 (d, J = 10.7 Hz, 3H, OMe), 4.38 (ddd, J_1 = 4.4 Hz, J_2 = 10.4 Hz, J_3 = 17.9 Hz, 1H, CH), 5.66 (d, J = 9.7 Hz, NH), 7.25-7.40 (m, 10H, Ph);

^{13}C NMR ($CDCl_3$): δ = 17.7 (d, J = 4.6 Hz), 20.3 (d, J = 12.5 Hz), 28.8 (d, J = 3.5 Hz), 43.7, 49.6 (d, J = 152.2 Hz), 52.7 (d, J = 7.5 Hz), 52.8 (d, J = 6.7 Hz), 127.4, 129.0, 129.2, 134.6, 170.7 (d, J = 5.1 Hz);

IR (ATR) ν (cm^{-1}): 3261, 1665, 1541, 1244, 1041, 1021, 828, 746, 711, 697;

HRMS(ESI): calc. for $C_{14}H_{23}NO_4P$ $[M + H]^+$ m/z 300.1365, found m/z 300.1354.

2.3.3.6 1-(N-Phenylacetylamino)-3-methylbutylphosphonic acid dimethyl ester **2f**

^{31}P NMR ($CDCl_3$): δ = 27.6;

^{1}H NMR ($CDCl_3$): δ = 0.88 (d, *J* = 6.0 Hz, 6H, CH(*CH*$_3$)$_2$), 1.45-1.60 (m, 3H, C*H*$_2$*CH*(CH$_3$)$_2$), 3.59 (s, 2H, *CH*$_2$Ph), 3.63 (d, *J* = 10.8 Hz, 3H, OMe), 3.73 (d, *J* = 10.4 Hz, 3H, OMe), 4.47-4.59 (m, 1H, CH), 5.56 (d, *J* = 10.0 Hz, 1H, NH), 7.23-7.53 (m, 5H, Ph);

^{13}C NMR ($CDCl_3$): δ = 21.2, 23.2, 24.5 (d, *J* = 13.3 Hz), 38.1 (d, *J* = 1.7 Hz), 43.2 (d, *J* = 154.6 Hz), 43.7, 52.9 (d, *J* = 6.4 Hz), 53.0 (d, *J* = 7.1 Hz), 127.4, 128.9, 129.2, 134.5, 170.4 (d, *J* = 4.2 Hz);

IR (ATR) *v* (cm^{-1}): 3255, 2956, 1655, 1541, 1229, 1031, 831, 725;

HRMS(ESI): calc. for $C_{15}H_{25}NO_4P$ $[M + H]^+$ m/z 314.1521, found m/z 314.1523.

2.3.3.7 1-(N-Phenylacetylamino)-2-carbamoylethylphosphonic acid dimethyl ester **2g**

^{31}P NMR (DMSO-d6): δ = 27.1;

^{1}H NMR (DMSO-d6): δ = 2.33-2.42 (m, 1H, C*H*$_2$CH), 2.45-2.55 (m, 1H, C*H*$_2$CH), 3.42 (d, *J* = 14.0 Hz, 1H, *CH*$_2$Ph), 3.46 (d, *J* = 14.0 Hz, 1H, *CH*$_2$Ph), 3.56 (d, *J* = 10.0 Hz, 3H, OMe), 3.60 (d, *J* = 10.4 Hz, 3H, OMe), 4.65-4.75 (m, 1H, CH), 6.91 (s, 2H, NH_2), 7.19-7.29 (m, 5H, Ph), 8.36 (d, *J* = 9.2 Hz, 1H, NH);

^{13}C NMR (DMSO-d6): δ = 35.5 (d, *J* = 4 Hz), 42.2 (d, *J* = 157.2 Hz), 42.3, 53.0 (d, *J* = 6.4 Hz), 53.3 (d, *J* = 6.8 Hz), 126.7, 128.5, 129.3, 136.6, 169.8 (d, *J* = 2 Hz), 170.6 (d, *J* = 15.1 Hz);

IR (ATR) *v* (cm^{-1}): 3404, 1671, 1548, 1224, 1030;

HRMS(ESI): calc. for $C_{13}H_{20}N_2O_5P$ $[M + H]^+$ m/z 315.1110, found m/z 315.1101.

2.3.3.8 1-(N-Benzyloxycarbonylamino)-2-tert-butoxyethylphosphonic acid dimethyl ester **2h**

^{31}P NMR ($CDCl_3$): δ = 26.1;

^{1}H NMR ($CDCl_3$): δ =1.18 (s, 9H, *t*-Bu), 3.54 (dd, J_1 = 4.0 J_2 = 9.6 Hz, 1H, CH_2), 3.61 (dd, J_1 = 3.6 Hz, J_2 = 9.2 Hz, 1H, CH_2), 3.75 (d, *J* = 10.8 Hz, 3H, OMe), 3.76 (d, *J* = 10.8 Hz, 3H, OMe), 4.26 (m, 1H, CH), 5.13 (s, 2H, O*CH*$_2$Ph), 5.28 (d, *J* = 10.4 Hz, 1H, NH), 7.31-7.37 (m, 5H, Ph);

^{13}C NMR ($CDCl_3$): δ = 27.4, 48.3 (d, *J* = 155.6 Hz), 52.7 (d, *J* = 6.1 Hz), 53.3 (d, *J* = 6.1 Hz), 60.6, 67.2, 73.7, 128.1, 128.2, 128.5, 136.2, 153.5 (d, *J* = 5.1 Hz),

IR (ATR) *v* (cm^{-1}): 3225, 2971, 1705, 1552, 1276, 1223, 1033, 904, 776, 696;

HRMS(ESI): calc. for $C_{16}H_{27}NO_6P$ $[M + H]^+$ m/z 360.1576, found m/z 360.1578.

2.3.3.9 N-Phenylacetylpyrrolidine-2-phosphonic acid dimethyl ester **2j**

^{31}P NMR ($CDCl_3$): δ = 22.5;

^{1}H NMR ($CDCl_3$): δ = 1.84-2.34 (m, 4H, $(CH_2)_2$), 3.50-3.61 (m, 2H, NCH_2), 3.69 (d, J = 10.8 Hz, 3H, OMe), 3.79 (d, *J* = 10.4 Hz, 3H, OMe), 3.70 (s, 2H, *CH*$_2$Ph), 5.60-5.66 (m, 1H, CH), 7.21-7.35 (m, 5H, Ph);

^{13}C NMR ($CDCl_3$): δ = 24.7, 26.3, 42.1, 47.3, 52.1 (d, *J* = 158.2 Hz), 52.8 (d, *J* = 6.4 Hz), 52.9 (d, *J* = 7.7 Hz), 126.8, 128.6, 128.9, 134.5, 169.9 (d, *J* = 2 Hz);

IR (ATR) *ν* (cm^{-1}): 3471, 2955, 1640, 1405, 1231, 1023, 828, 722;

HRMS(ESI): calc. for $C_{14}H_{21}NO_4P$ $[M + H]^+$ m/z 298.1208, found m/z 298.1209.

2.3.3.10 1-(N-Phenylacetylamino)ethyl(phenyl)phosphinic acid ethyl ester **2k** *(a mixture of diastereomers in a ratio of 0.55:0.45)*

^{31}P NMR ($CDCl_3$): δ = 36.8 (major), 36.3 (minor);

^{1}H NMR ($CDCl_3$): δ = 1.18 (dd, J_1 = 7.8 Hz, J_2 = 15.8 Hz, 3H, α-CH_3, minor), 1.19 (t, *J* = 7.1 Hz, 3H, OCH_2*CH*$_3$, minor), 1.27 (t, *J* = 7.1 Hz, 3H, OCH_2*CH*$_3$, major), 1.39 (dd, J_1 = 7.3 Hz, J_2 = 14.6 Hz, 3H, α-CH_3, major), 3.37 (s, 2H, *CH*$_2$Ph, major), 3.59 (s, 2H, *CH*$_2$Ph, minor), 3.77-4.22 (m, 2H, O*CH*$_2$$CH_3$), 4.48-4.62 (m, 1H, CH), 4.62-4.81 (m, 1H, CH), 6.16 (d, *J* = 9.7 Hz, 1H, NH, major), 6.24 (d, *J* = 8.1 Hz, NH, minor), 6.90-7.87 (m, 5H, Ph);

^{13}C NMR ($CDCl_3$): δ = 14.4, 15.0 (d, *J* = 3.2 Hz), 16.4 (d, *J* = 6.2 Hz), 42.9 (d, *J* = 113.8 Hz), 43.4, 43.8, 43.9 (d, *J* = 108.5 Hz), 61.4 (d, *J* = 6.8 Hz), 61.41 (d, *J* = 6.7 Hz), 127.2, 127.4, 128.4, 128.5, 128.6, 128.76, 128.8, 128.9, 129.1, 129.3, 132.0 (d, *J* = 15.2 Hz), 132.1 (d, *J* = 15.9 Hz), 132.6 (d, *J* = 3.0 Hz), 132.8 (d, *J* = 3.2 Hz), 134.4, 134.7, 170.0 (d, *J* = 5.4 Hz), 170.2 (d, *J* = 5.3 Hz);

IR (ATR) *ν* (cm^{-1}): 3244, 1668, 1545, 1209, 1122, 1024, 953, 733, 702, 690;

HRMS(ESI): calc. for $C_{18}H_{23}NO_3P$ $[M + H]^+$ m/z 332.1415, found m/z 332.1416.

2.3.4. Racemic 1-(N-acylamino)alkylphosponic acids ***3*** *and 1-(phenylacetylamino)ethyl(phenyl)phosphinic acid* ***4d*** *(Procedure B)*

A mixture of dimethyl 1-(*N*-acylamino)alkylphosponate **2** or ethyl 1-(*N*-phenylacetyl)ethyl(phenyl)phosphinate **2k** (0.2 mmol), aqueous HCl (1M, 2 mL), and acetone (1 mL; only in the case of compounds **2d**-**f**) was heated under reflux for 2 hours (for compounds **2a**-**c**, **2e** and **2g**-**j**) or for 4 hours (for compounds **2d** and **2f**). After evaporation of the solvent under reduced pressure, the residue was recrystallized by dissolution in ethanol (96%) and precipitation with diethyl ether.

2.3.4.1 1-(N-Acetylamino)ethylphosphonic acid **3a** [32]

^{31}P NMR (D_2O): δ = 21.7;

^{1}H NMR (D_2O): δ = 1.31 (dd, J_1 = 6.8 Hz, J_2 = 16.0 Hz, 3H, CH_3), 2.01 (d, *J* = 1.2 Hz, 3H, *CH*$_3$CO), 4.21 (dq, J_1 = 7.2 Hz, J_2 = 14.8 Hz, 1H, CH).

2.3.4.2 1-(N-Phenylacetylamino)ethylphosphonic acid **3b** [4]

^{31}P NMR (D_2O): δ = 23.9;

^{1}H NMR (D_2O): δ = 1.33 (dd, J_1 = 7.6 Hz, J_2 = 16.4 Hz, 3H, CH_3), 3.64 (s, 2H, CH_2Ph), 4.22 (dq, J_1 = 7.6 Hz, J_2 = 14.8 Hz, 1H, CH), 7.32-7.44 (m, 5H, Ph).

2.3.4.3 1-(N-Phenylacetylamino)-2-phenylethylphosphonic acid **3c** [4]

^{31}P NMR (D_2O): δ = 19.9;

^{1}H NMR (D_2O): δ = 2.78 (ddd, J_1 = 7.6 Hz, J_2 = 12.4 Hz, J_3 = 14.0 Hz, 1H, CHCH_2Ph), 3.23-3.29 (m, 1H, CHCH_2Ph), 3.41 (d, J = 14.8 Hz, 1H, CH_2Ph), 3.48 (d, J = 14.8 Hz, 1H, CH_2Ph), 4.43 (ddd, J_1 = 2.8 Hz, J_2 = 12.4 Hz, J_3 = 16.4 Hz, 1H, CH), 7.21-7.32 (m, 10H, Ph).

2.3.4.4 1-(N-Phenylacetylamino)-2-methylpropylphosphonic acid **3d** [20]

^{31}P NMR (D_2O): δ = 22.3;

^{1}H NMR (D_2O): δ = 0.92 (d, J = 2.9 Hz, 3H, CH(CH_3)$_2$), 0.94 (d, J = 3.2 Hz, 3H, CH(CH_3)$_2$), 2.07-2.25 (m, 3H, CH(CH_3)$_2$,), 3.65 (d, J = 14.8 Hz, 1H, CH_2Ph), 3.73 (d, J = 14.8 Hz, 1H, CH_2Ph), 4.06 (dd, J_1 = 5.6 Hz, J_2 = 17.6 Hz, 1H, CH), 7.30-7.76 (m, 5H, Ph);

^{13}C NMR (D_2O): δ = 18.4 (d, J = 6.1 Hz) 20.9 (d, J = 11.0 Hz), 29.6 (d, J = 3.1 Hz), 43.3, 53.5 (d, J = 150.2 Hz), 128.2, 129.8, 130.1, 136.0, 175.4 (d, J = 5.7 Hz);

IR (ATR) ν (cm^{-1}): 3415, 2969, 2285, 1530, 1181, 981, 935, 709;

HRMS(ESI): calc. for $C_{12}H_{19}NO_4P$ $[M + H]^+$ m/z 272.1052, found m/z 272.1052.

2.3.4.5 1-(N-Phenylacetylamino)-3-methylbutylphosphonic acid **3e** [4]

^{31}P NMR (D_2O): δ = 22.9;

^{1}H NMR (D_2O): δ = 0.78 (d, J = 6.0 Hz, 3H, CH(CH_3)$_2$), 0.87 (d, J = 6.0 Hz, 3H, CH(CH_3)$_2$), 1.47-1.67 (m, 3H, $CH_2CH(CH_3)_2$ + $CH_2CH(CH_3)_2$), 3.59 (d, J = 14.8 Hz, 1H, CH_2Ph), 3.67 (d, J = 14.4 Hz, 1H, CH_2Ph), 4.23 (ddd, J_1 = 2.8 Hz, J_2= 12.4 Hz, J_3 = 15.6 Hz, 1H, CH), 7.29-7.41 (m, 5H, Ph).

2.3.4.6 1-(N-Phenylacetylamino)-2-carbamoylethylphosphonic acid **3f**

^{31}P NMR (D_2O): δ = 18.1;

^{1}H NMR (D_2O): δ = 2.55-2.63 (m, 1H, CH_2), 2.84-2.91 (m, 1H, CH_2), 3.60 (d, J = 14.8 Hz), 3.65 (d, J = 14.8 Hz), 4.45-4.56 (m, 1H, CH), 7.29-7.42 (m, 5H, Ph);

^{13}C NMR (D_2O): δ = 35.4 (d, J = 3.8 Hz), 42.2, 44.9 (d, J = 153.3 Hz), 127.2, 128.8, 129.0 (d, J = 3.0 Hz), 134.8, 173.9 (d, J = 4.6 Hz), 174.9;

IR (ATR) ν (cm^{-1}): 3263, 2954, 1732, 1656, 1542, 1226, 1165, 1000, 695;

HRMS(ESI): calc. for $C_{11}H_{16}N_2O_5P$ $[M + H]^+$ m/z 287.0797, found m/z 287.0799.

2.3.4.7 N-Phenylacetylpyrrolidine-2-phosphonic acid **3h**

^{31}P NMR (D_2O): δ = 23.2;

^{1}H NMR (D_2O): δ = 1.89-2.24 (m, 4H, $(CH_2)_2$), 3.59-3.78 (m, 2H, N*CH*$_2$), 3.81-3.85 (m, 2H, *CH*$_2$Ph), 4.24-4.33 (m, 1H, CH), 7.24-7.42 (m, 5H, Ph);

^{13}C NMR (DMSO-d6): δ = 27.2, 29.3, 31.1, 32.5, 45.6, 46.2, 51.0, 52.6, 59.6 (d, *J* = 148.6 Hz), 60.0 (d, *J* = 153.9 Hz), 131.3, 131.6, 133.2, 133.4, 134.5, 134.6, 140.4, 141.9, 174.4, 175.6;

IR (ATR) *v* (cm^{-1}): 1608, 1451, 1178, 998, 772;

HRMS(ESI): calc. for $C_{12}H_{17}NO_4P$ $[M + H]^+$ m/z 270.0895, found m/z 270.0892.

2.3.4.8 1-(N-Phenylacetylamino)ethyl(phenyl)phosphinic acid **4d**

^{31}P NMR (D_2O): δ = 37.7;

^{1}H NMR (D_2O): δ = 1.37 (dd, J_1 = 7.4 Hz, J_2 = 14.9 Hz, 3H, CH_3), 3.40 (s, 1H, *CH*$_2$Ph), 4.38 (dq, J_1 = 7.2 Hz, J_2 = 9.6 Hz, 1H, CH), 6.98-7.87 (m, 10H, Ph);

^{13}C NMR (D_2O): δ= 12.7, 42.0, 44.4 (d, *J* = 111.4 Hz), 127.1, 128.5, 128.7, 128.9, 131.4, 131.5, 132.7, 134.5, 172.8 (d, *J* = 4.6 Hz);

IR (ATR) *v* (cm^{-1}): 3233, 3057, 1634, 1544, 1167, 950, 690;

HRMS(ESI): calc. for $C_{16}H_{19}NO_3P$ $[M+H]^+$ m/z 304.1103, found m/z 304.1102.

2.3.4.9 1-(N-Benzyloxycarbonylamino)-2-hydroxyethylphosphonic acid dimethyl ester **2i**

^{31}P NMR (D_2O): δ = 26.4;

^{1}H NMR (D_2O): δ = 3.72-3.80 (m, 1H, C*H*$_2$OH), 3.75 (d, *J* = 12.0 Hz, 6H, OMe), 3.85-3.90 (m, 1H, C*H*$_2$OH), 4.22-4.29 (m, 1H, CH), 5.12 (d, *J* = 12.0 Hz, 1H, PhC*H*$_2$O), 5.19 (d, *J* = 12.0 Hz, 1H, PhC*H*$_2$O), 7.37-7.68 (m, 5H, Ph);

^{13}C NMR (D_2O): δ = 26.4, 49.7 (d, *J* = 152.5 Hz), 53.8 (d, *J* = 7.6 Hz), 53.8 (d, *J* = 7.1 Hz), 67.3, 127.6, 128.4, 128.7, 136.2, 157.8;

IR (ATR) *v* (cm^{-1}): 3283, 2956, 1698, 1533, 1215, 1024, 696;

HRMS(ESI): calc. for $C_{12}H_{19}NO_6P$ $[M + H]^+$ m/z 304.0949, found m/z 304.0950.

2.3.5. Racemic 1-(N-acylamino)alkyl-H-phosphinic acids ***4a-c*** *(Procedure C)*

Syntheses of crude 1-(*N*-acylamino)alkyltriphenylphosphonium tetrafluoroborates from the corresponding *N*-acylaminoacids **1** were carried out as described in procedure A. A solution of the phosphonium salt (2 mmol) in CH_2Cl_2 (6 mL), triphenylmethylphosphonium iodide (0.020 g, 0.5 mmol), diisoprophylethylamine (0.03 mL, 0.2 mmol) and bis(trimethylsilyl)phosphonite (3 mmol) was placed in a glass vial sealed with a screw-cap. The mixture was kept at room temperature for a given period of time (Table 1). Then methanol (2 mL) was added and the mixture was left to stay overnight at room temperature. After filtration, the solvent was evaporated under reduced pressure and the remaining was

extracted with toluene (4 x 2 mL). The residue from extraction was subjected to ion-exchange chromatography using DOWEX 50W X8 in the H^+ form, and water as elution agent to isolate the product.

2.3.5.1 1-(N-Benzyloxycarbonylamino)ethylphosphinic acid **4a** [33]

^{31}P NMR (D_2O): δ =28.1;

^{1}H NMR (D_2O): δ = 1.28 (dd, J_1 = 7.6 Hz, J_2 = 16.4 Hz, 3H, CH_3), 3.78 (dq, J_1 = 7.2 Hz, J_2 = 15.2 Hz, 1H, CH), 5.13 (s, 2H, C*H*$_2$Ph), 6.87 (d, J = 543.6 Hz, 1H, P*H*), 7.37-7.46 (m, 5H, Ph).

2.3.5.2 1-(N-Phenylacetylamino)ethylphosphinic acid **4b** [31]

^{31}P NMR (D_2O): δ = 26.9;

^{1}H NMR (D_2O): δ = 1.25 (dd, J_1 = 7.6 Hz, J_2 = 15.6 Hz, 3H, CH_3), 3.64 (s, 2H, C*H*$_2$Ph), 3.96 (dq, J_1 = 7.2 Hz, J_2 = 14.8 Hz, 1H, CH), 6.81 (d, J = 530 Hz, 1H, P*H*), 7.31-7.42 (m, 5H, Ph).

2.3.5.3 1-(N-Benzyloxycarbonylamino)-2-tert-butoxyethylphosphinic acid **4c**

^{31}P NMR (D_2O): δ = 23.3;

^{1}H NMR (D_2O): δ = 1.16 (s, 9H, *t*-Bu), 3.52-3.65 (m, 1H, CH), 3.62-3.78 (m, 2H, *CH*$_2$-O-*t*-Bu), 5.18-5.87 (m, 2H, O*CH*$_2$Ph), 7.35-7.64 (m, 5H, Ph);

^{13}C NMR (D_2O): δ = 27.2, 51.2 (d, J = 103.5 Hz), 59.9, 67.4, 73.9, 128.5, 132.0, 132.1, 136.0, 156.5;

IR (ATR) ν (cm^{-1}): 2380, 1697, 1525, 1365, 1123, 962, 794, 696;

HRMS(ESI): calc. for $C_{14}H_{23}NO_5P$ $[M + H]^+$ m/z 316.1313, found m/z 316.1308.

2.4. Enzymatic reactions

Racemic substrates **2a-k**, **4a-d** or **3a-i** (1 mmol) were dissolved in 0.2 M pH 7.0 phosphate buffer (20 mL) and the temperature of the mixture was adjusted to 25ºC. The pH was adjusted to 7.0 by the addition of 1 M aqueous NaOH as necessary. A solution of the proper amount of penicillin G acylase (see Table 1) in phosphate buffer (0.45 mL) was then added and the resulting mixture was stirred at 25ºC for the time given in Table 1.

2.4.1. Kinetic experiments

In the case of kinetic experiments, the progress of the substrate conversion was determined by extracting a 2 mL of aliquot of the reaction mixture, acidification to pH 3.5 with 0.1M to aqueous HCl (in the case of free acids **3** or **4**) or with 0.2 M pH 3.5 acetate buffer in the case of esters **2**. After evaporation of water under reduced pressure, a precisely measured amount of a solution of pivalic acid in D_2O was added as the internal standard (about 0.5 mg of the pure compound), the residue was dissolved in D_2O, and the amount of substrate and hydrolysis product was determined by ^{1}H NMR.

2.4.2. Preparative experiments

*2.4.2.1.Separation of deacylation products **5a-h** and remaining N-acylated esters **2a-k***

The reaction mixture was acidified with 0.2 M pH 3.5 acetate buffer to pH = 4.0 and water was evaporated under reduced pressure. The residue was extracted with CH_2Cl_2 (4 x 3 mL), the extract was dried with $MgSO_4$, the solvent was evaporated under reduced pressure, and the residue was separated by column chromatography (silica gel, CH_2Cl_2/MeOH 20:1).

2.4.2.1.1 (R)-1-Aminoethylphosphonic acid dimethyl ester **5a** [34]

^{31}P NMR ($CDCl_3$): δ = 31.1;

^{1}H NMR ($CDCl_3$): δ = 1.45 (dd, J_1 = 7.2 Hz, J_2 = 18.0 Hz, 3H, CH_3), 3.21 (dq, J_1 = 7.2 Hz, J_2 = 9.6 Hz, 1H, CH), 3.87 (d, J = 10.8 Hz, 3H, OMe), 3.89 (d, J = 10.4 Hz, 3H, OMe).

2.4.2.1.2 (R)-1-Amino-2-phenylethylphosphonic acid dimethyl ester **5b** [35]

^{31}P NMR ($CDCl_3$): δ = 30.1;

^{1}H NMR ($CDCl_3$): δ = 3.19 (ddd, J_1 = 4.0 Hz, J_2 = 7.6 Hz, J_3 = 14.0 Hz, 1H, CHCH_2), 3.34 (ddd, J_1 = 3.6 Hz, J_2 = 11.2 Hz, J_3 = 14.8 Hz, 1H, CHCH_2), 3.74 (ddd, J_1 = 3.6 Hz, J_2 = 8.4 Hz, J_3 = 14.0 Hz, 1H, CH), 3.78 (d, J = 10.4 Hz, 3H, OMe), 3.80 (d, J = 10.4 Hz, 3H, OMe), 7.33-7.19 (m, 5H, Ph);

HRMS(ESI): calc. for $C_{10}H_{17}NO_3P$ $[M + H]^+$ m/z 230.0946, found m/z 230.0947.

2.4.2.1.3 (R)-1-Amino-2-methylpropylphosphonic acid dimethyl ester **5c**

^{31}P NMR ($CDCl_3$): δ = 31.0;

^{1}H NMR ($CDCl_3$): δ = 1.02 (d, J = 6.4 Hz, 3H, CH(CH_3)$_2$), 1.05 (d, J = 7.2 Hz, 3H, CH(CH_3)$_2$), 2.08-2.15 (m, 1H, CH(CH$_3$)$_2$), 2.94 (dd, J_1 = 4.0 Hz, J_2 = 14.4 Hz, 1H, CH), 3.79 (d, J = 10.4 Hz, 3H, OMe), 3.78 (d, J = 10.4 Hz, 3H, OMe);

^{13}C NMR ($CDCl_3$): δ = 17.3 (d, J = 4.6 Hz), 20.5 (d, J = 12.9 Hz) 29.2 (d, J = 3.5 Hz), 52.7 (d, J = 6.8 Hz), 52.8 (d, J = 7.6 Hz), 53.8 (146.4 Hz);

IR (ATR) ν (cm^{-1}): 3392, 2958, 1563, 1465, 1373, 1221, 1027, 827, 699;

HRMS(ESI): calc. for $C_6H_{17}NO_3P$ $[M + H]^+$ m/z 182.0946, found m/z 182.0945.

2.4.2.1.4 (R)-1-Amino-3-methylbutylphosphonic acid dimethyl ester **5d** [34]

^{31}P NMR ($CDCl_3$): δ = 29.2;

^{1}H NMR ($CDCl_3$): δ = 0.94 (d, J = 6.4 Hz, 3H, CH(CH_3)$_2$), 0.98 (d, J = 6.4 Hz, 3H, CH(CH_3)$_2$), 1.64-1.82 (m, 3H, CH_2CH(CH$_3$)$_2$ + CH(CH$_3$)$_2$), 3.64 (ddd, J_1 = 4.0 Hz, J_2 = 10.0 Hz, J_3 = 14.4 Hz, 1H, CH), 3.87 (d, J = 10.8 Hz, 3H, OMe), 3.89 (d, J = 10.8 Hz, 3H, OMe).

2.4.2.1.5 (R)-1-Amino-2-carbamoylethylphosphonic acid dimethyl ester **5e**

^{31}P NMR (CD_3CN): δ = 29.9;

^{1}H NMR (CD_3CN): δ = 2.28 (ddd, J_1 = 8.2 Hz, J_2 = 10.9 Hz, J_3 = 18.7 Hz, 1H, CHCH_2), 2.51 (ddd, J_1 = 3.2 Hz, J_2 = 8.6 Hz, J_3 = 15.2 Hz, 1H, CHCH_2), 3.44 (ddd, J_1 = 3.6 Hz, J_2 = 10.9 Hz, J_3 = 14.1 Hz, 1H, CH), 3.71 (d, J = 10.4 Hz, 3H, OMe), 3.74 (d, J = 10.4 Hz, 3H, OMe);

^{13}C NMR (CD_3CN): δ = 38.5, 46.6 (d, J = 153.7 Hz), 54.1 (d, J = 6.9 Hz), 54.3 (d, J = 7.0 Hz), 174.0 (d, J = 3.7 Hz);

IR (ATR) ν (cm^{-1}): 3342, 1668, 1555, 1408, 1203, 1042, 780;

HRMS(ESI): calc. for $C_5H_{14}N_2O_4P$ $[M + H]^+$ m/z 197.0691, found m/z 197.0690.

2.4.2.1.6 (R)-1-Aminoethyl(phenyl)phosphinic acid ethyl ester **5i** *(a mixture of diastereomers in a ratio of 0.63 : 0.37)* [36]

^{31}P NMR ($CDCl_3$): δ = 48.0 (minor), 48.9 (major);

^{1}H NMR ($CDCl_3$): δ = 1.16 (dd, J_1 = 7.2 Hz, J_2 = 17.2 Hz, 3H, α-CH_3, major), 1.307 (dd, J_1 = 7.2 Hz, J_2 = 16.4 Hz, 3H, α-CH_3, minor), 1.309 (t, J = 6.8 Hz, 3H, OCH_2CH_3, major), 1.312 (t, J = 7.2 Hz, 3H, OCH_2CH_3, minor), 3.18-3.26 (m, 1H, CH), 3.87-3.97 and 4.07-4.19 (m, 2H, OCH_2CH_3), 7.18-7.82 (m, 6H, NH, Ph).

2.4.2.2.Separation of deacylation products ***6a-f*** *or* ***7a-c*** *and remaining N-acylated acids* ***3a-h*** *or* ***4a-d***

The reaction mixture was acidified with 0.1 M aqueous HCl to pH 3.5 and water was evaporated under reduced pressure. The residue was dissolved in water (2 mL) and passed through a DOWEX 50W X8 (H^+ form) column (50 mL), which was rinsed with water. The first fractions contained remaining free acids **3a**-**h** or **4a**-**d**, whereas further, ninhydrin-positive fractions contained deacylation products **5a**-**i**.

2.4.2.2.1 (R)-1-Aminoethylphosphonic acid **6a** [37,38]

^{31}P NMR (D_2O): δ = 14.9;

^{1}H NMR (D_2O): δ = 1.45 (dd, J_1 = 6.8 Hz, J_2 = 15.6 Hz, 3H, CH_3), 3.44 (m, 1H, CH).

2.4.2.2.2 (R)-1-Amino-2-phenylethylphosphonic acid **6b** [4,6,37]

^{31}P NMR (D_2O): δ = 11.7;

^{1}H NMR (D_2O): δ = 2.63-2.88 (m, 1H, CH_2Ph), 3.21-3.29 (m, 1H, CH_2Ph), 3.57-3.64 (m, 1H, CH) 7.22-7.42 (m, 5H, Ph).

2.4.2.2.3 (R)-1-Amino-2-methylpropylphosphonic acid **6c** [6,37,38]

^{31}P NMR (D_2O): δ = 13.9;

^{1}H NMR (D_2O): δ = 1.11 (d, J = 6.8 Hz, 3H, CH(CH_3)$_2$), 1.14 (d, J = 7.2 Hz, 3H, CH(CH_3)$_2$), 2.23-2.32 (m, 1H, CH(CH_3)$_2$), 3.30 (dd, J_1 = 6.0 Hz, J_2 = 14.4 Hz, 1H, CH).

2.4.2.2.4 (R)-1-Amino-3-methylbutylphosphonic acid **6d** [4,37]

^{31}P NMR (D_2O): δ = 14.4;

^{1}H NMR (D_2O): δ = 0.94 (d, *J* = 6.0 Hz, 3H, CH(C*H*$_3$)$_2$), 0.98 (d, *J* = 5.6 Hz, 3H, CH(C*H*$_3$)$_2$), 1.67-1.70 (m, 3H, C*H*$_2$C*H*(CH$_3$)$_2$ + CH$_2$C*H*(CH$_3$)$_2$), 3.13-3.18 (m, 1 H, CH).

2.4.2.2.5 (R)-1-Amino-2-carbamoylethylphosphonic acid **6e** [39]

^{31}P NMR (D_2O): δ =11.5;

^{1}H NMR (D_2O): δ = 2.69-2.73 (m, 1H, CHC*H*$_2$), 2.91-2.96 (m, 1H, CHC*H*$_2$), 3.63-3.67 (m, 1H, CH).

2.4.2.2.6 (R)-1-Aminoethyl-H-phosphinic acid **7a** [40]

^{31}P NMR (D_2O): δ = 21.4;

^{1}H NMR (D_2O): δ = 1.41 (dd, J_1 = 7.2 Hz, J_2 = 15.6 Hz, 3H, CH$_3$), 3.24 (dq, J_1 = 7.2 Hz, J_2 = 9.6 Hz 1H, CH), 6.97 (d, *J* = 531.6 Hz, 1H, P*H*).

2.4.2.2.7 (R)-1-Aminoethyl(phenyl)phosphinic acid **7c** [41]

^{31}P NMR (D_2O): δ = 27.3;

^{1}H NMR (D_2O): δ = 1.38 (dd, J_1 = 7.2 Hz, J_2 = 14.0Hz, 3H, CH$_3$), 3.54 (dq, J_1 = 7.2 Hz, J_2 = 9.6 Hz, 1H, CH), 7.41-7.80 (m, 5H, Ph).

2.5. Enantiomeric excess determination

2.5.1. NMR methods

The enantiomeric excesses of unreacted *N*-acylated acids **3a**-**h** and **4a**-**c** (ee$_s$) were determined by using ^{31}P NMR spectroscopy in the presence of quinine as the chiral discriminating agent. The analysed sample (5 mg) and quinine (10-25 mg) were dissolved in a mixture of $CDCl_3$ (0.55 mL) and CD_3OD (0.1 mL) and ^{31}P NMR spectrum was measured. The enantiomeric excess was determined based on the integration of phosphorus signals of both enantiomers.

The enantiomeric excesses of unreacted *N*-acylated esters **2b**-**g**, **2k**, as well as ethyl(phenyl)phosphinic acid **4d,** was determined by ^{1}H NMR spectroscopy in the presence of quinine. The analysed sample (5 mg) and quinine (10-25 mg) ware dissolved in $CDCl_3$ (0.65 mL) and ^{1}H NMR spectrum was measured. The enantiomeric excesses of esters **2a**-**g** were determined based on the integration of OMe group signals of both enantiomers. In the case of ethyl(phenyl)phosphinic acid derivatives **2k** and **4d**, signals of CH_2 protons of benzyl group and signals of α-Me group were integrated, respectively

In the all cases, the satisfactory resolution of enantiomers signals was verified by adding 1-2 mg of the corresponding racemate to the analysed sample and repeating the measurement.

2.5.1. Chiral HPLC

For most *N*-acylated acids **3a-f** and **4a** and **4d** as well as esters **2c, 2e** and **2f**, enantiomeric excesses were also successfully determined by means of chiral HPLC. The chromatographic separation of enantiomers of acids **3a-f** and **4a** and **4d** was performed on a CHIRALPAK® QN-AN (150x4.6 mm, 5 μm) column (Daicel/Chiral Technologies Illkirch, France) maintained at 25°C. The mobile phase was composed of MeOH/CH_3CO_2H/ammonium acetate (98:2:0.5; v/v/w) using an isocratic elution with a flow rate set at 0.8 mL/min. The detector wavelength was set at 232 nm and injection volume was 20 μL. Chromatographic separation of enantiomers of esters **2c, 2e** and **2f** was achieved on a CHIRALCEL® OD-RH (150x4.6 mm, 5 μm) column (Daicel/Chiral Technologies Illkirch, France). Mobile phase consisted of a mixture of hexane/isopropanol (92:8; v/v for ester **2c** and **2e**, and 97:3; v/v for ester **2f**) at 1.0 mL/min flow rate. The column temperature was maintained at 25°C, the injection volume was 20 μL, the detection was performed at 222 nm.

3. Results and discussion

The *N*-acylated 1-aminoalkylphosphonic and 1-aminoalkylphosphinic acids esters used in this work were prepared as racemic mixtures using the method described in our previous papers [21-24] (Table 1). Their hydrolysis in 2 M hydrochloric acid to *N*-acylated acids was satisfactorily performed according to a well-known method [42] (Scheme 1, Table 1). Only in the case of ester **2h**, the product of deprotection of hydroxymethyl group with untouched dimethoxyphosphonyl group **2i** was obtained instead of the expected product **3g**. The obtained *N*-acylated acids were isolated by cation-exchange chromatography and purified by recrystallization. The structures of the obtained compounds were confirmed by their spectroscopic (^{1}H-, ^{13}C-, ^{31}P NMR, IR) and HRMS data (see Experimental).

Enzymatic deacylation of *N*-acylated 1-aminoalkylphosphonic and 1-aminoalkylphosphinic acids as well as their esters was carried out in a water solution of phosphate buffer (pH 7.0) at 25°C, using various reaction time and amount of enzyme depending on the specific reactivity of substrate. The optimal reaction conditions for every specific substrate, assuring a racemic substrate conversion close to 50%, were determined in a series of preliminary experiments, in which initial hydrolysis rates were measured (Table 2). The concentrations of the reaction product and unconverted substrate in reaction mixtures were determined by means of ^{1}H NMR spectroscopy using pivalic acid as the internal standard.

Analysis of the initial reaction rates obtained (Table 2) showed, that the reactivity of a substrate depends strongly on the kind of its *N*-acyl group. The ratio of the initial hydrolysis rate of 1-(*N*-acetylamino)- and 1-(*N*-phenylacetylamino)ethylphosphonic acids was found to be about 1 : 38000, whereas the same ratio for methyl esters of 1-(*N*-acetylamino)-, 1-(*N*-benzyloxycarbonyamino)-, and 1-(*N*-phenylacetylamino)ethylphosphonic acids was about 1 : 95: 5100000, respectively. Therefore, our results confirmed that penicillin G acylase showed a high preference in catalysing the hydrolysis of *N*-phenylacetyl moiety [4,18,19].

The initial hydrolysis rates of 1-(*N*-phenylacetylamino)akylphosphonic acid dimethyl esters **2** in most cases are distinctly lower compared to the rates obtained for the corresponding free acids **3**. Also the initial hydrolysis rate of ethyl ester of ethyl(phenyl)phosphinic acid **2k** is about two times lower compared to the corresponding acid **4d**. Only in the case of the 1-(*N*-phenylacetylamino)ethylaminophosphonic acid dimethyl ester **2c**, reactivity of ester is about three times higher than the reactivity of the parent acid **3b**.

For a series of 1-(*N*-phenylacetylamino)alkylphosphonic acids **3b-f** the initial hydrolysis rate rapidly decreased with the increasing steric effect of the substituent at the α-position. Reactivity of the phosphonic acid analogue of alanine (**3b**, R^2 = Me) was found to be 2-3 orders of magnitude higher compared to the reactivities of phosphonic analogues of phenylalanine, valine, leucine and asparagine (**3c-f**; R^2 = $PhCH_2$, *i*-Pr, *i*-$PrCH_2$, and H_2NCOCH_2, respectively). The analogous, but even more distinct effect of the reduction of reactivity by bulky substituents at the α-position was seen in a series of dimethyl esters of *N*-acylated 1-aminophosphonic acids **2c-g**; the reactivity of the phosphonic analogue of alanine (**2c**, R^2 = Me) was, in this case, 4-6 orders of magnitude higher than the reactivity of the corresponding phenylalanine, valine, leucine and asparagine, *t*-Bu-serine and serine (**2d-i**; R^2 = $PhCH_2$, *i*-Pr, *i*-$PrCH_2$, H_2NCOCH_2, t-$BuOCH_2$, and $HOCH_2$, respectively).

Our efforts to hydrolyse phosphonic analogues of *N*-phenylacetylproline, both as the free acid (**3h**) and as its methyl ester (**2j**), failed, even with high concentration of penicillin G acylase and a prolonged reaction time. It seems, that the presence of proton at the amide nitrogen of the acylamine moiety of substrate is crucial to ensure the catalytic activity of penicillin G acylase in hydrolysis of *N*-acylated α-aminophosphonic acids and their esters.

The presence of bulky phenyl group at the phosphorus atom in phenylphosphinic acid analogue of 1-(*N*-phenylacetylamino)alanine **4d** exerts unexpectedly slight reduction of its reactivity in comparison with its *H*-phosphinic acid analogue **4b**; moreover, only a slightly lower reactivity was found for its diethyl ester **2k**. It seems that in contrary to the substituents

at the α-carbon, the bulky substituents at the phosphorus hinder the enzymatic hydrolysis to a much lesser degree.

Enantiomeric excesses of unreacted *N*-acylated acids **3a-f, 4a-b,** and **4d** were determined based on the integration of ^{31}P NMR signals of both enantiomers of a substrate, using quinine as the chiral discriminating agent (Fig.1a). In the case of unconverted *N*-acylated esters **2b-g**, and **2k**, as well as ethyl(phenyl)phosphinic acid **4d**, more satisfactory resolutions were obtained for ^{1}H NMR signals of OMe groups, CH_2 protons of benzyl group or α-Me group, respectively (Fig. 1b). In the case of most unconverted *N*-acylated acids **3** and **4**, as well as esters **2c**, **2e** and **2f** enantiomeric excesses were also successfully determined by chiral reversed-phase HPLC, using a CHIRALPAK® QN-AX column (for acids), or by chiral normal-phase chromatography, using a CHIRALPAK® OD-RH column (for esters). The obtained results were in a reasonable agreement with the data obtained using the ^{31}P or ^{1}H NMR method (Table 2).

The enzyme enantioselectivity *E* was calculated as a function of extent of conversion *c* and enantiomeric excess of the remaining substrate ee_s, measured at conversions close to 50% (Table 2). *E* was defined as:

$$E = \frac{\ln[(1-c)(1-ee_S)]}{\ln[(1-c)(1+ee_S)]} \qquad [12]$$

In the case of 1-(*N*-acetylamino)alkylphosphonic acids **3a-h** highly effective kinetic resolutions with the *E*-values exceeding 100 were obtained for phosphonic acid analogues of alanine **3a** and **3b**, phenylalanine **3c**, leucine **3e**, and asparagine **3f**; synthetically useful *E*-value exceeding 20 was obtained also for the analogue of valine **3d** (*E* = 22). Enzyme enantioselectivities obtained for 1-(*N*-acylamino)alkylphosphonic acid methyl diesters **2** also exceeded the value 100 for phosphonic analogues of alanine **2b** and **2c**, phenylalanine **2d**, and asparagine **2g**; a satisfactory result was obtained also for the leucine analogue **2f** (E = 66). Only in the case of dimethyl ester of phosphonic analogue of valine **2e**, the *E*-value was low (*E* = 1.2), and lower that this one for the corresponding free acid **3d**.

Also for the 1-*N*-acylated *H*-phosphinic acid analogues of alanine (**4a-b**), as well as phenylphosphinic acid analogue of alanine **4d**, high enzyme enantioselectivity values exceeding 100 were determined. In contrary, the *E*-value was low for both diastereomers of the ethyl ester of the phenylphosphinic analogue of alanine **2k** (*E* = 7 and 13).

In the case of a few investigated compounds of extremely low reactivity (**2a**, **2h**, **2i** and **4c**) attempts of kinetic resolution were not undertaken.

The absolute configuration of hydrolysis products and unconverted substrates was assigned by measurements of their optical rotation and the comparison with literature data. For all the accomplished assignments, penicillin G acylase exhibited stereochemical preference for the (*R*)-substrate. The same stereochemical preference of penicillin G acylase was found by Zimmermann et al. [20] and Solodenko et al. [4,18] for phosphonic analogues of 1-*N*-phenylacetylated alanine, phenylalanine, and leucine, as well as *H*-phosphinic analogue of 1-*N*-phenylacetylalanine.

Conclusions

Reactivity of 1-(*N*-acylamino)alkylphosphonic and 1-(*N*-acylamino)alkylphosphinic acids and their esters in the penicillin G acylase-mediated hydrolytic deacylation reaction depends strongly on the kind of its *N*-acyl group; the obtained results confirm high preference of penicillin G acylase to catalyse the hydrolysis of the *N*-phenylacetyl moiety.

The initial hydrolysis rates of 1-(*N*-phenylacetylamino)alkylphosphonic acid dimethyl esters **2** are, in most cases, distinctly lower compared to the corresponding free acids **3**. Only in the case of 1-(*N*-phenylacetylamino)ethylaminophosphonic acid dimethyl ester **2c**, the reactivity of ester is about three times higher than the reactivity of the parent acid **3b**.

The initial hydrolysis rates of 1-(*N*-acylamino)alkylphosphonic acids and their esters rapidly decrease with the increasing steric effect of the substituent at the α-position. Reactivities of the phosphonic analogues of alanine, both free acid **3b** and their dimethyl esters **2c** (R^2 = Me), are 2-6 orders of magnitude higher compared to that of the corresponding phosphonic analogues of phenylalanine, valine, leucine, asparagine, *t*-Bu-serine, and serine (**3c-f** and **2d-i)**. It seems that in contrary to the substituents at the α-carbon, bulky substituents at phosphorus hinder the enzymatic hydrolysis to a much lesser degree. The efforts to hydrolyse phosphonic analogues of *N*-phenylacetylproline, both as the free acid (**3h**) and as its dimethyl ester (**2j**), failed.

The penicillin G acylase-mediated stereospecific hydrolysis of *N*-acyl group of both,racemic 1-(*N*-acylamino)alkylphosphonic acids **3** and their dimethyl esters **2** proved to be, in most cases, the highly effective method for the kinetic resolution of these compounds. The enzyme enantioselectivity *E*-values exceeding 100 were obtained for the *N*-acylated phosphonic acid analogues of alanine, phenylalanine, leucine, and asparagine, as well as for their dimethyl esters, with the exception of the leucine diester. A synthetically useful *E*-value exceeding 20 was obtained also for the phosphonic acid analogue of valine **3d** and for the

dimethyl ester of phosphonic analogue of leucine **2f**; only in the case of dimethyl ester of phosphonic analogue of valine **2e** the *E*-value was low (*E* = 1.2).

Also for the 1-*N*-acylated *H*-phosphinic acid analogues of alanine (**4a-c**), as well as phenylphosphinic acid analogue of alanine **4d**, high enzyme enantioselectivity values exceeding 100 were determined. In contrary, the *E*-value was low for both diastereomers of the ethyl ester of the phenylphosphinic analogue of alanine **2k** (*E* = 7 and 13).

For all the accomplished assignments, penicillin G acylase exhibited stereochemical preference for the (*R*)-substrate.

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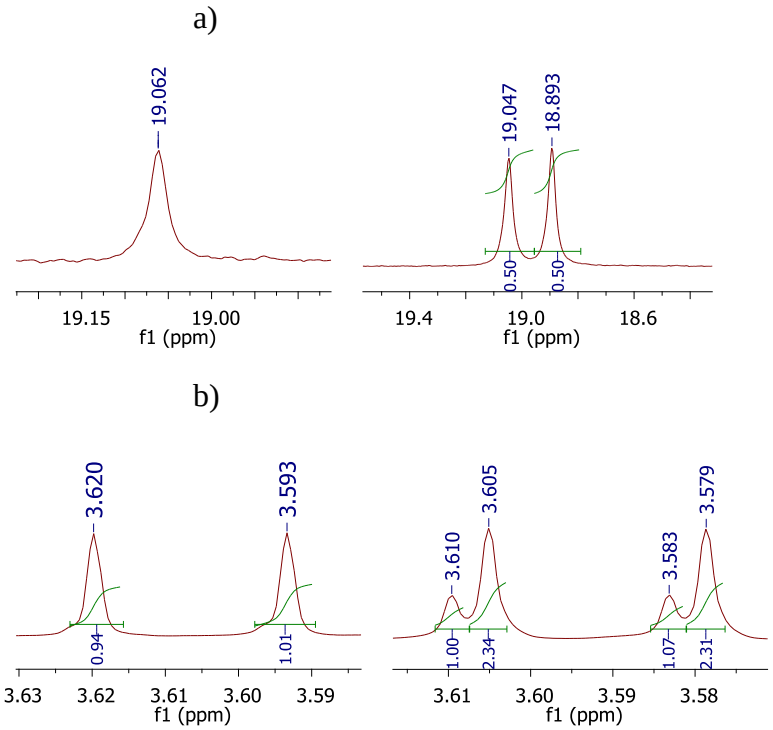
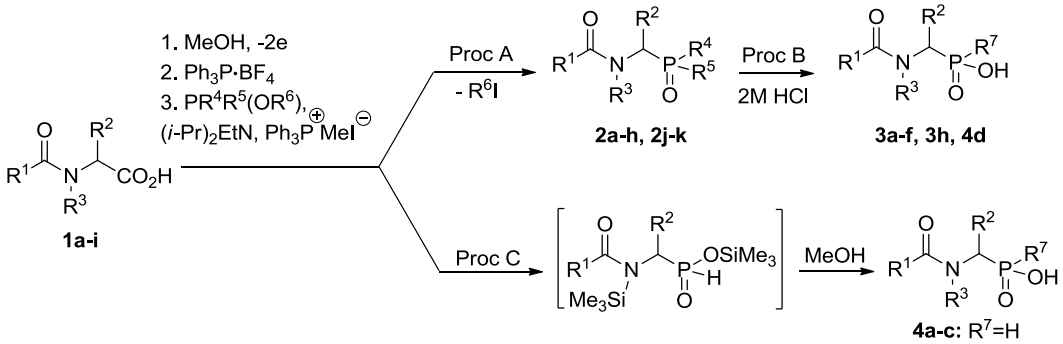
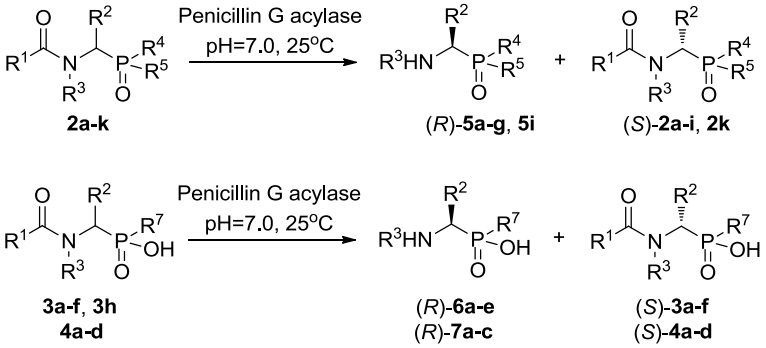


Figure 1. Enatiomeric excess determination using NMR methods: a). ^{31}P NMR; left - (*R*)-1-(*N*-phenylacetylamino)ethylphosphonic acid [(*R*)-**3b**] obtained by kinetic resolution (8 mg) with quinine (22 mg) in $CDCl_3$/CD_3OD (0.6 mL/ 0.1 mL); right – racemic (*R*,*S*)-**3b** – the same conditions. b). ^{1}H NMR; left – (*R*)-1-(*N*-phenylacetylamino)ethylphosphonic acid dimethyl ester [(*R*)-**2c**] (5 mg) with quinine (17 mg) in $CDCl_3$; left – sample of (*R*)-**2c** enriched with racemic (*R*,*S*)-**2c** (2 mg) – the same conditions.



Scheme 1. Syntheses of racemic 1-(*N*-acylamino)alkylphosphonic and 1-(*N*-acylamino)alkylphosphinic acids and their esters.



Scheme 2. Kinetic resolution of 1-(*N*-acylamino)alkylphosphonic and 1-(*N*-acylamino)alkylphosphinic acids and their esters.

Table 1. Transformation of *N*-acyl-α-amino acids **1** into *N*-acylated 1-aminoalkylphosphonic and 1-aminoalkylphosphinic acids esters **2** and free acids **3** or **4**

N-acyl-α-amino acid				Phosphorus nucleophile $PR^4R^5(OR^6)$			Michaelis-Arbuzov reaction conditions and results					Free acids **3** or **4**				
No.	R^1	R^2	R^3	R^4	R^5	R^6	Proc.	Time [h]	No.	Yield [%]	m.p. [°C]	No.	R^7	Proc.	Yield [%]	m.p.[°C]
1a	Me	Me	H	OMe	OMe	Me	A	2	**2a**	83	65-67	**3a**	OH	B	97	181-182
1b	$PhCH_2O$	Me	H	OMe	OMe	Me	A	2	**2b**	78	oil					
1c	$PhCH_2$	Me	H	OMe	OMe	Me	A	2	**2c**	84	85-86	**3b**	OH	B	97	140-141
1d	$PhCH_2$	$PhCH_2$	H	OMe	OMe	Me	A	4	**2d**	61	93-94	**3c**	OH	B	98	145-146
1e	$PhCH_2$	*i*-Pr	H	OMe	OMe	Me	A	2	**2e**	92	62-63	**3d**	OH	B	94	142-143
1f	$PhCH_2$	*i*-$PrCH_2$	H	OMe	OMe	Me	A	4	**2f**	83	102-104	**3e**	OH	B	76	139-142
1g	$PhCH_2$	H_2NCOCH_2	H	OMe	OMe	Me	A	4	**2g**	66	156-157	**3f**	OH	B	99	oil
1h	$PhCH_2O$	*t*-$BuOCH_2$	H	OMe	OMe	Me	A	2	**2h**	86	68-69	**3g**	OH	B	-[a]	-
1i	$PhCH_2$	$-(CH_2)_3-$		OMe	OMe	Me	A	4	**2j**	50	oil	**3h**	OH	B	75	oil
1b	$PhCH_2O$	Me	H	H	OTMS	TMS	C	24	not	isolated		**4a**	H	-	85	109-110
1c	$PhCH_2$	Me	H	H	OTMS	TMS	C	24	not	isolated		**4b**	H	-	91	139-140
1h	$PhCH_2O$	*t*-$BuOCH_2$	H	H	OTMS	TMS	C	24	not	isolated		**4c**	H	-	94	oil
1c	$PhCH_2$	Me	H	Ph	OEt	Et	A	2	**2k**	90	81-83	**4d**	Ph	B	97	91-92

[a]The product of deprotection of CH_2OH group **2i** (R^1 = $PhCH_2O$, R^2= CH_2OH, R^3= H, R^4= R^5= OMe) was obtained in a yield of 91% (oil) instead of the expected product **3g**.

Table 2. Enzymatic hydrolysis of *N*-acylated 1-aminoalkylphosphonic and 1-aminoalkylphosphinic esters **2** and free *N*-acylated acids **3** or **4**

Substrate **2**, **3** or **4**									Kinetic resolution results									
No.	R^1	R^2	R^3	R^4	R^5	R^7	Enzyme U/mmol	Initial hydrolysis (μmol/Umin)	Product No.	Time [h]	Conversion	Yield [%][a]		$[\alpha]_D^{20}$ [°]		Product config.	ee_s[b] x100 [%]	E[c]
												Substrate	Product	Substrate	Product			
2a	Me	Me	H	OMe	OMe	-	2000	2.86 ±0.13 x10^{-6}	**5a**	504	0.15	-	-	-	-		-	
3a	Me	Me	H	-	-	OH	2000	1.32 ±0.21 x10^{-4}	**6a**	216	0.43	48	35	+15.6[d]	-16.3[e]	R	67(77)	>100
2b	$PhCH_2O$	Me	H	OMe	OMe	-	500	2.71 ±0.24 x10^{-4}	**5a**	144	0.46	46	41	+14.2[f]	-6.2[g]	R	87	>100
2c	$PhCH_2$	Me	H	OMe	OMe	-	4	1.46 ±0.1 x10^{1}	**5a**	0.5	0.49	42	45	+42.4[h]	-6.8[i]	R	99(99)	>100
3b	$PhCH_2$	Me	H	-	-	OH	4	5.01 ±0.70	**6a**	1	0.50	37	41	+37.9[j]	-5.1[k]	R	99	>100
2d	$PhCH_2$	$PhCH_2$	H	OMe	OMe	-	500	5.21 ±0.40 x10^{-4}	**5b**	72	0.46	49	42	+27.5[l]	-22.5[m]	R	84	380
3c	$PhCH_2$	$PhCH_2$	H	-	-	OH	500	4.31 ±0.67 x10^{-3}	**6b**	47	0.52	39	48	+40.4[n]	-50.6[p]	R	99(99)	>100
2e	$PhCH_2$	*i*-Pr	H	OMe	OMe	-	2000	5.69 ±0.04 x10^{-5}	**5c**	92	0.37	48	33	+3.33[r]	-1.38[s]		14(8)	1.2
3d	$PhCH_2$	*i*-Pr	H	-	-	OH	500	7.16 ±0.82 x10^{-4}	**6c**	48	0.47	45	41	-0.62[t]	+0.58[u]		75(73)	22
2f	$PhCH_2$	*i*-$PrCH_2$	H	OMe	OMe	-	500	6.89 ±0.03x10^{-4}	**5d**	72	0.48	31	34	+25.4[v]	-16.6[w]		82(85)	66
3e	$PhCH_2$	*i*-$PrCH_2$	H	-	-	OH	400	7.44 ±0.55 x10^{-3}	**6d**	23	0.49	45	40	+31.4[z]	-24.8[aa]	R	99(99)	>100
2g	$PhCH_2$	H_2NCOCH_2	H	OMe	OMe	-	500	6.83 ±0.07 x10^{-4}	**5e**	48	0.48	49	41	+6.6[ab]	-2.2[ac]		99	>100
3f	$PhCH_2$	H_2NCOCH_2	H	-	-	OH	500	4.19 ±0.72 x10^{-3}	**6e**	24	0.49	42	45	+7.9[ad]	-32.5[ae]	R	99(95)	660
2h	$PhCH_2O$	*t*-$BuOCH_2$	H	OMe	OMe	-	2000	5.12±1.44 x10^{-6}	**5f**	336	0.17	-	-	-	-		-	-
2i	$PhCH_2O$	$HOCH_2$	H	OMe	OMe	-	2000	~5.30x10^{-6}	**5g**	288	0.16	-	-	-	-		-	-
2j	$PhCH_2$	$-(CH_2)_3-$		OMe	OMe	-	2000	no reaction	**5h**	216	no reaction	-	-	-	-		-	-
3h	$PhCH_2$	$-(CH_2)_3-$		-	-	OH	2000	no reaction	**6f**	190	no reaction	-	-	-	-		-	-
4a	$PhCH_2O$	Me	H	OH	-	H	4	1.70 ±0.09 x10^{-1}	**7a**	24	0.46	40	41	+47.5[af]	-5.9[ag]	R	85(87)	>100
4b	$PhCH_2$	Me	H	OH	-	H	4	6.20 ±0.37	**7a**	1	0.49	29	34	+71[ah]	-6.7[ai]	R	99	>100
4c	$PhCH_2O$	*t*-$BuOCH_2$	H	OH	-	H	2000	3.89±0.38 x10^{-5}	**7b**	336	0.25	-	-	-	-	-	-	-
2k[aj]	$PhCH_2$	Me	H	OEt	Ph	-	10	2.00 ±0.16[ak]	**5i**	2	0.45[al]/048[am]	49[an]	47[ap]	+72.0[ar]	-14.7[as]		52[al]/68[am]	7[al]/13[am]
4d	$PhCH_2$	Me	H	OH	-	Ph	8	4.28 ±0.37	**7c**	2	0.47	19	48	+76.5[at]	-35.8[aw]		79(85)	>100

[a]Isolated yield after chromatography. [b]Based on ^{31}P- or ^{1}H NMR in the presence of quinine; results based on chiral HPLC are in parenthesis. [c]If ee_s value was determined independently by HPLC and NMR method, the E value was calculated based on the data obtained by a chiral HPLC method. [d]c 0.3, 1M NaOH, [e]c 0.2, 1M NaOH, Lit.[37] $[\alpha]^{20}_D$ -16.9° (R) c 1, 1M NaOH, [f]c 0.5, CH_2Cl_2, Lit. [29] $[\alpha]^{20}_D$ +14.4 ° (S) c 1 CH_2Cl_2, [g]c 0.4, MeOH, [h]c 0.4 MeOH, Lit. [4] $[\alpha]^{20}_D$ +44.5° (S) c 1, MeOH, [i]c 0.5, MeOH, [j]c 0.5, H_2O, Lit.[18] $[\alpha]^{20}_D$ +38° (*S*), c 0.5, H_2O, [k]c 0.5, H_2O, Lit.[4] $[\alpha]^{20}_D$ -5.5° (*R*) c 1, H_2O, Lit. [37] $[\alpha]^{20}_D$ -16.9° (*R*) c 1, 1M NaOH, [l]c 0.9, $CHCl_3$, [m]c 1.1, MeOH, Lit. [35] $[\alpha]^{20}_D$ +25.9 (S) c 0.9 $CHCl_3$, [n]c 0.2, 1M NaOH, [p]c 0.4, 1M NaOH, Lit.[37] $[\alpha]^{20}_D$ -49.0° (R) c 0.25 1M NaOH, [r]c 0.2, $CHCl_3$, [s]c 0.2, MeOH, [t]c 0.2, 1M NaOH, [u]c 0.2 1M NaOH, [v]c 0.6, $CHCl_3$, [w]c 0.3, $CHCl_3$, [z]c 0.5, 1M NaOH, [aa]c 0.5 1M NaOH, Lit. [37] $[\alpha]^{20}_D$ -25.0° (R) c 1 1M NaOH, [ab]c 0.5 CH_3CN, [ac]c 0.4 CH_3CN, [ad]c 0.4 1M NaOH, [ae]c 0.3 H_2O, Lit. [39 $[\alpha]^{20}_D$ -33.0° (R) c 1 H_2O, [af]c 0.1 1M NaOH, Lit. [33] $[\alpha]^{20}_D$+48.3° (R) c 1 Acetic Acid, [ag]c 0.5 H_2O, Lit.[18] $[\alpha]^{20}_D$ -6.8° (R) c 0.5 H_2O, [ah]c 0.1 H_2O, Lit.[18] $[\alpha]^{20}_D$ +74.0° (S) c 0.5 H_2O, [ai]c 0.8 H_2O, Lit.[18] $[\alpha]^{20}_D$ -6.8° (R) c 0.5 H_2O, [aj]A mixture of diastereomers in a ratio of 0.55:0.45. [ak]For a mixture of diastereomers. [al]Major diastereomer. [am]Minor diastereomer. [an]A mixture of diastereomers in a ratio of 0.56:0.44. [ap]A mixture of diastereomers in a ratio of 0.63:0.37.[ar]c 2.2 $CHCl_3$, [as]c 1.5 $CHCl_3$, [at]c 0.6, 1M NaOH, [aw]c 0.2 1M NaOH.