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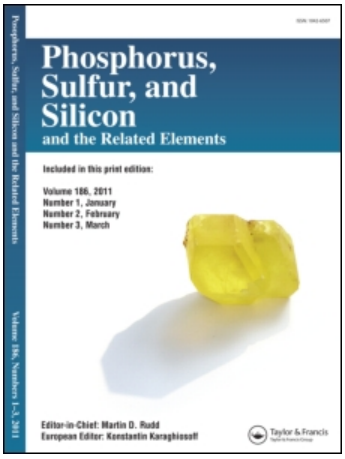

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RESOLUTION OF FLUORINATED AMINOMETHANPHOSPHONIC ACIDS CATALYSED BY PENICILLIN G ACYLASE - III

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RESOLUTION OF FLUORINATED AMINOMETHANPHOSPHONIC ACIDS CATALYSED BY PENICILLIN G ACYLASE - III

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In this work, we have used Penicillin G acylase (EC 3.5.1.11) from *E. coli* to resolve kinetically four fluorinated aminomethanphosphonic acids : **1**: 1-trifluoromethyl-, **2**:1-p-fluorophenyl-, **3**:1-m-fluorophenyl-, **4**: 1-p-trifluoromethylphenyl–1-aminomethanphosphonic acids. This enzyme catalyses enantioselectively the hydrolysis of the N-phenylacetylated derivatives of **1-4.** The enantiomeric excesses determined via ^{31}P NMR spectra of the diastereomeric Pd(II) complexes of **1-4** were high for each compound studied.

Dans ce travail, on a utilisé la Penicilline G acylase (EC 3.5.1.11) de *E. coli* pour effectuer la résolution cinétique de quatre acides aminométhanphosphoniques fluorés : acides **1:** 1-trifluorométhyl-, **2**: 1-p-fluorophényl-, **3:** 1-m-fluorophényl-, **4:** 1-p-trifluorométhylphenyl–1-aminométhanphosphoniques. Cette enzyme catalyse énantiosélectivement l'hydrolyse des dérivés N-phénylacétylés des composés **1-4.** Les excès énantiomériques calculés grâce aux spectres RMN du ^{31}P des complexes diastéréomères du Pd(II) des produits **1-4** se sont révélés excellents dans chacun des cas étudiés.

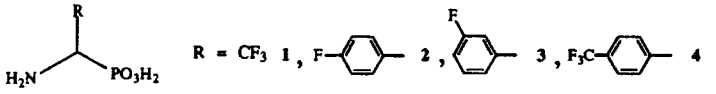
Keywords: Fluorinated Aminomethanphosphonic acids; Pencillin G acylase; kinetic resolution; ^{31}P NMR spectroscopy

INTRODUCTION

1-Aminoalkanphosphonic acids constitute a very interesting class of compounds due to their biological activity. The use of the concept of the mimicking of the tetrahedral transition states in the hydrolysis of the peptide bond led to the design of phosphonopeptides as a new class of protease inhibitors[1-4]. As a consequence, aminophosphonic acids have found many industrial applications as pharmaceuti-

cals and agrochemicals[5-8]. The bioactivity of these compounds is known to be strongly dependent on the configuration of the α-asymmetric carbon. Up to now only a few methods such as diastereomeric resolution[9,10] and asymmetric synthesis[11-19], are available for the synthesis of optically pure aminophosphonic acids.

Furthermore, chemoenzymatic methods have not been widely used for this purpose[20,21,22]. We have recently described an efficient method for the resolution of the N-protected isoserine-P diethyl ester [1] [N-Cbz-isoSER-P(O)(OEt)$_2$] using *Candida rugosa* lipase[23a] while under similar conditions a low selectivity was observed with isothreonin analogues[23b]. Penicillin G acylase was also used to resolve some 1-aminoalkanphosphonic acids[20]. The aim of this paper is to show the ability of this latter enzyme to hydrolyse enantioselectively some fluorinated N-phenylacetyl–1-aminomethanphosphonic acids (see Scheme 1). Such uncommon compounds are of interest because of the modified properties afforded by the presence of the fluorinated substituent (pK_a, lipoidal solubility, etc.)



SCHEME 1 Structure of the fluorinated 1-aminomethanphosphonic acids

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RESULTS AND DISCUSSION

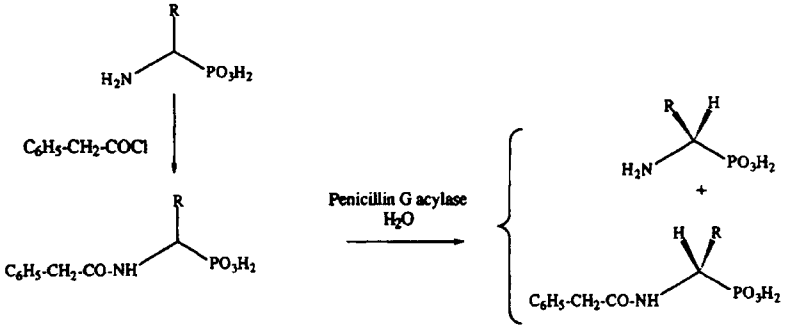
The four fluorinated 1-aminomethanphosphonic acids studied in this work are listed in Scheme 1. They were prepared according to already published methods[25-27].

Enzymatic resolution using penicillin G acylase from *E. Coli* (EC 3.5.1.11) as a chiral biocatalyst is now commonly used to synthesize optically pure α-aminoacids[28-30] and β-aminoacids[31,32]. This enzyme also accepts a broad range of substrates making possible not only the synthesis of the amide bond[33] and the hydrolysis of the esters[34], but also the hydrolysis of the N-phenylacylated 1-aminoalkylphosphonic acids[20]. The latter property is of great interest since not all the acylases accept these compounds as substrates. This is the case for instance for the overexpressed *Bacillus stearothermophilus* aminoacylase EC 3.5.1.14[35] which was shown to resolve efficiently the N-acetyl-α-amino acids[36] while no reaction was observed with the aminophosphonic analogues[37].

Penicillin G acylase selectively hydrolyzes or synthesizes the N-phenylacetyl bond. Several strategies are thus possible for the resolution of the compounds **1-4:**

a. Hydrolysis of the N-phenylacetyl derivatives
b. Hydrolysis of the dialkyl N-phenylacetylaminophosphonates
c. Synthesis of the N-phenylacetyl derivatives
d. Synthesis of the dialkyl N-phenylacetylaminophosphonates

Strategies b and d were not considered since in each case, the relatively unstable dialkyl α-aminophosphonates have to be handled. Furthermore, in the case b, the substrates are insoluble in water, making the hydrolysis difficult. Similarly, in case c, the insolubility in any convenient organic solvent of the starting free α-aminophosphonic acids prevents the use of this reaction. The latter considerations led to the selection of the strategy a (Scheme 2).



SCHEME 2 Strategy selected for the kinetic resolution of compounds **1-4**

The N-phenylacetylated derivatives were easily prepared according to standard procedures (see experimental section). Enzymatic hydrolysis was conducted at pH 7 and the course of the reaction was monitored by means of continuous adjustment of the pH with a 1M NaOH solution. The hydrolysis was quenched by acidification at pH 5 when the conversion reached 40-45%. Then, the product was separated from the remaining starting material by solvent extraction. The product of the reaction was purified chromatographically on a cation exchange resin (see experimental section). The enantiomeric purities were determined by means of ^{31}P NMR spectroscopy on the free 1-aminoalkanphosphonic acids via their diastereomeric Pd(II) complexes[38]. The accuracy of this method supposes that the rate of formation of the (RR) and/or (SS) species is the same as that of the meso (RS). This seems to be true since all the racemates **1–4** tested, showed ^{31}P NMR signals of equal areas for each of the two diastereomeric complexes. A simple relationship (see below) which stands between the ratio α = [R]/[S] (1-aminophosphonic acids) and the ratio r = [RS] / [RR + SS] of the Pd(II) complexes allows the calculation of the enantiomeric excesses[39].

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$$\alpha = \frac{[R]}{[S]} = \frac{1}{r} \times \left(1 + \sqrt{1 - r^2}\right)$$

The Figure 1 shows the ^{31}P NMR spectra of the complexes obtained in the case of **3** (racemate, product and remaining substrate of the enzymatic resolution). It should be pointed out that this method does not give any information on the structure of the enantiomers as can be provided for instance by Mosher's derivatives. It has been shown that the latter derivatives for free 1-aminoalkanphosphonic acids are impossible to prepare via standard procedures. On the basis of the optical activity of known 1-aminoalkanphosphonic acids, the L configuration was shown to be preferred by *E. coli* Penicillin G acylase[20]. Similarly, we have assumed the same behaviour of this enzyme with the fluorinated 1-aminomethanphosphonic acids **1-4**.

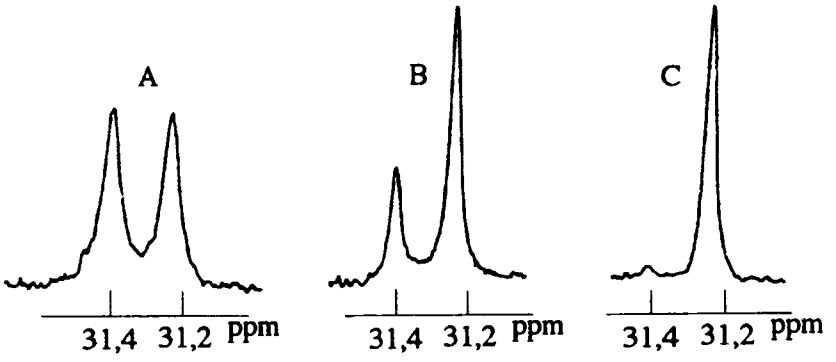

FIGURE 1 ^{31}P NMR spectra of the diastereomeric Pd(II) complexes of 1-m-fluorophenyl-1-aminomethanphosphonic acid 3 : A : racemate, B : from the unreacted N-phenylacetylated derivative and C : produced via the enzymatic hydrolysis

The results of the kinetic resolution are given in the Table I. In each case, high enantiomeric excesses (ee) are obtained with good yields.

TABLE I Enzymatic hydrolysis of N-phenylacetylated derivatives of 1-aminoalkanphosphonic acids **1-4**

Entry	*Conversion (%)*	*Substrate*		*Product*	
		ee (%)	*Yield (%)*	*ee (%)*	*Yield (%)*
1	42	70	40	95	55
2	42	66	37	96	54
3	40	59	38	96	58
4	45	72	41	95	52

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CONCLUSION

Penicillin G acylase from *E. coli* is a very powerful biocatalyst for the resolution of fluorinated 1-aminoalkanphosphonic acids. This readily available and inexpensive enzyme constitutes a very efficient tool in enantioselective organic chemistry.

EXPERIMENTAL SECTION

The structure of all the products was determined by means of proton NMR spectroscopy (Bruker WM 250 spectrometer) using D_2O as solvent and internal reference (δ = 4,6 ppm). The enantiomeric excesses were determined by means of ^{31}P NMR (Bruker 400 MHz) spectroscopy following the method developed by Glowacki *et al*[38].

E. coli Penicillin G acylase (EC 3.5.1.11) was purchased from Aldrich (0.1M solution in potassium phosphate buffer). The enzyme activity was determined with benzylpenicillin as a substrate.

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Synthesis of the 1-N-phenylacetylaminoalkanphosphonic acids.

1 mmol of the aminophosphonic acids **1-4** was dissolved in 2.3 mL of water with 3.62 mmol (3.04 g) of sodium hydrogen carbonate. 6.7 mL of acetone was added to this mixture. A solution of 7.03 g (4.6 mmol) of phenylacetyl chloride in 3.5 mL of acetone was slowly added at room temperature under magnetic stirring. Then, the mixture was warmed at 55°C for 24 hours. The acetone was removed under vacuum and the aqueous residue was extracted with chloroform in order to remove the phenylacetic acid. Treatment of the resulting water solution with ethyl acetate allowed the extraction of the l-N-phenylacetylaminophosphonic acids. This organic phase dried over anhydrous sulphate was evaporated under reduced pressure to give the solid N-phenylacylated derivatives. The NMR spectra of the compounds obtained showed that their purity was good enough, so they were used without further purification in the enzymatic resolutions.

N-phenylacetyl–1:NMR ^1H (D_2O), 3.39-3.55 (m, CH_2), 4.63 (m, CH), 7.01-7.19 (m, C_6H_5), yield 79 %; **N-phenylacetyl–2**:NMR ^1H (D_2O), 3.30–3.45 (m, CH_2), 4.90 (d, CH, J = 20.9), 6.75–7.12 (m, aromatic), yield 81%, **N-phenylacetyl–3**:NMR ^1H (D_2O), 3.33-3.52 (m, CH_2), 4.90 (d, CH, J = 20.9), 6.72-7.16 (m, aromatic), yield 76%, **N-phenylacetyl–4**:NMR ^1H (D_2O), 3.42-3.57 (m, CH_2), 4.92 (d, CH), 7.06-7.53 (m, aromatic), yield 83%.

Kinetic resolution by means of the Penicillin G acylase hydrolysis of 1-N-phenylacetylaminoalkanphosphonic acids.

0.4 mmol of (L,D)-1-N-phenylacetylaminoalkanphosphonic acid was dissolved in 1.5 mL of water. The pH was adjusted at 7 by means of a 1M NaOH solution. The enzyme preparation (167µL ; 232 units) was added and the mixture was stirred at room temperature. The pH was kept at 7 by addition of 1M NaOH. The reaction progress was calculated considering the amount of base added. When a conversion value of 40-45% was reached (1-2 hours), the reaction was quenched by acidification at pH 5 with 1M HCl. Then, the medium was warmed for 10 mn at 60–65°C in the presence of activated carbon. This mixture was filtered over fluorisil and the solution was extracted with ether. The aqueous phase, acidified at pH 2 with 1M HCl, was extracted with ethyl acetate. The organic layer dried over Na_2SO_4, afforded the pure unreacted substrate after distillation of the solvent. The aqueous layer was concentrated under vacuum and the free aminophosphonic acid was purified by elution (eluent:water) of the resulting solution on cationic exchange resin (type AG 50W-X8). The yields are given in Table I.

NMR ^1H (D_2O), **1:** 3.83–4.02 (m, CH); **2**:4.31–4.38 (d, CH, J = 15.8), 7.01-7.31 (m, aromatic); **3:** 4.21–4.27 (d, CH, J = 15.8), 6.92–7.29 (m, aromatic); **4**:4.46-4.53 (d, CH), 7.51-7.71 (m, aromatic).

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References

[1] P. Kafarsky and B. Lejczak; *Phosphorus, Sulfur and Silicon,* **63,** 193–215 (1991).
[2] V.P. Kukhar, N.M. Solodenko and V.A. Solodenko, *Ukr. Biokhim. Zh.*, **60**, 95 (1988).
[3] P.P. Giannousis and P.A. Bartlett, *J. Med. Chem.*, **30,** 1603–1609 (1987).
[4] L. Maier and P.L. Diel; *Phosphorus, Sulfur and Silicon,* **62,** 15–27 (1991).
[5] J.A. Colanduoni and J.J.Villafranca, *Bioorg. Chem.*, **14**, 163 (1986).
[6] F. Schwerdtle, H. Bieringer and M. Finke, Z. *Planzenkr., Planzenschutz.*, **9,** 431 (1981).
[7] D.T. Monaghan, R.J. Bridges and C.W. Cotman, *Ann. Rev. Pharmacol. Toxicol.*, **29,** 365 (1989).
[8] L. Maier, *Phosphorus, Sulfur and Silicon,* **14**, 295 (1983).
[9] P. Kafarsky, B. Lejczak and J. Szewczyk, *Can. J. Chem.*, **61**, 2425–2430 (1983).
[10] L. Maier and H. Spörri; *Phosphorus, Sulfur and Silicon,* **61**, 69–75 (1991).
[11] U. Schöllkopf and R. Schütze, *Liebigs Ann. Chem.*, 45–49 (1987).
[12] N. Minowa, M. Hirayama and S. Fukatsu S., *Bull. Chem. Soc. Jpn.*, **60,** 1761–1766 (1987).
[13] R. Jacquier, F. Ouazzani and M.L. Roumestant M.L., Viallefont P., *Phosphorus, Sulfur and Silicon,* **36**, 73–77 (1988).
[14] A. Togni and S.D.Pastor, *Tetrahedron Letters,* **30**, 1071–1072 (1989).
[15] S. Hannessian, Y.L. Bennani and D. Delorme, *Tetrahedron Letters,* **31**, 6461–6464 (1990).
[16] U. Groth, L. Richter and U. Schöllkopf, *Tetrahedron,* **48**, 117–122 (1992).

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[17] C Maury, J. Royer and H.P. Husson, *Tetrahedron Letters,* **33**, 6127–6130 (1992).
[18] H.J. Zeiss, *Tetrahedron,* **48**, 8263–8270 (1992).
[19] V.A. Soloshonok, Y.N. Belokon, N.A. Kuzmina, V.I. Maleev, N.Y. Svistunova, V.A. Solodenko and V.P. Kukhar, *J. Chem. Soc. Perkin Trans. I,*1525–1529 (1992).
[20] V.A. Solodenko, T.N. Kasheva, V.P. Kukhar, E.V. Kozlova, D.A. Mironenko and V.K. Svedas, *Tetrahedron,* **47**, 3989–3998 (1991).
[21] F. Hammerschmidt, H.Kählig and N.Müller, *J. Chem. Soc. Perkin Trans. I,* 365–369 (1991).
[22] N. Natchev, *Tetrahedron,* **47**, 1239–1248 (1991).
[23] A. Heisler, C. Rabiller, R. Douillard, N.Goalou, G. Hägele and F. Levayer, *Tetrahedron : Asymm.*, **4,** 959–960, (1993).
[24] A. Heisler, C. Rabiller and G. Hägele, *Phosphorus. Sulfur and Silicon,* **101,** 273–280 (1995).
[25] R.C. Corcoran and J.M. Green, *Tetrahedron Letters,* **31**, 6827–6830 (1990).
[26] D.S.C. Green, U. Gruss, G. Hägele, H.R. Hudson, L. Lindblom and M. Pianka, *Phosphorus, Sulfur and Silicon,* **113**, 179–207 (1996).
[27] A. Haas, Dissertation, Univ. Düsseldorf (1996).
[28] C. Fuganti, C.M. Rosell, S. Servi, A. Tagliani and M. Terreni, *Tetrahedron : Asymm.*, **3,** 383–386, (1992).
[29] C.M. Rosell, R. Fernandez-Lafuente and J.M. Guisan, *J. Molecular catalysis,* **84**, 365–371, (1993).
[30] M. Nettekoven, M. Psiorz and H. Waldmann, *Tetrahedron Letters,* **36,** 1425–1428 (1995).
[31] V.A. Soloshonok, N.A. Fokina, A.V. Rybakova, I.P. Shishkina, S.V. Galushko, A.E. Sorochinsky, V.P. Kukhar, M.V. Savchenko and V.K. Svedas, *Tetrahedron : Asymm.*, **6,** 1601–1610, (1995).
[32] A.G. Kirilenko, N.A. Fokina, I.P. Shishkina, S.V. Galushko, V.P. Kukhar, V.K. Svedas and E.V. Kozlova, *Tetrahedron : Asymm.*, **5,** 1119–1126, (1994).
[33] B. Herradon and S. Valverde, *Synlett,* 599, (1995).
[34] T. Pohl and H. Waldmann, *Tetrahedron Letters,* **36,** 2963–2966 (1995).
[35] V. Sakanian, L. Desmarez, C. Legrain, D. Charlier, I. Mett, A. Kochikyan, A. Savchenko, A. Boyen, P. Falmagne, A. Piérard and N. Glansdorff, *Appl. Environ. Microbiol.* **59,** 3878–3888, (1993).
[36] M. Dion, F. Loussouarn, N. Batisse, C. Rabiller and V. Sakanian, *Biotechnol. Letters,* **17**, 905-910, (1995).
[37] C. Rabiller, unpublished results.
[38] Z. Glowacki, M. Topolski, E. Matczak-Jon and M. Hoffmann, Magn. Res. Chem. **27,** 922–924 (1989).
[39] W. Marty, M.L. Pasquier and H. Gampp, Helv. Chem. Acta, **70,** 1774–1785, (1987).

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