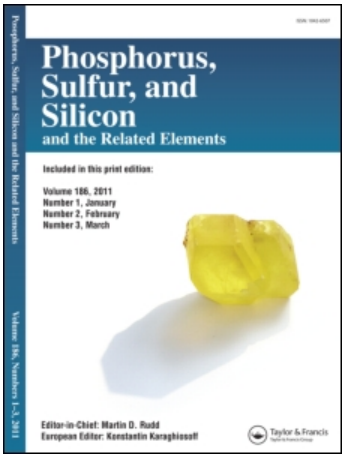

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ENZYME CATALYSED TRANSESTERIFICATION OF AMINOPHOSPHONIC ACIDS. II—ISOTHREONINE-P ANALOGUES

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ENZYME CATALYSED TRANSESTERIFICATION OF AMINOPHOSPHONIC ACIDS. II—ISOTHREONINE-P ANALOGUES

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In this work, we used lipases to resolve kinetically the four possible configurations of an important aminophosphonic acid: isothreonine-P (isoTHR-P). We synthesized a diastereomeric mixture of N-Cbz-isoTHR-P(O)$(OEt)_2$ and also each diastereomer of isoTHR-P(O)$(OEt)_2$. Several enzymatic reactions were then performed (hydrolysis and transesterification) with these compounds. The lipases used (mainly *Pseudomonas fluorescens* and *Candida rugosa*) proved to be diastereoselective and chimioselective, but no enantioselectivity was observed.

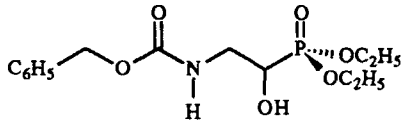
Dans ce travail, on utilise les lipases en vue d'effectuer la résolution cinétique des quatre configurations des dérivés d'un important acide aminophosphonique: l'isothréonine-P (isoTHR-P). Après avoir réalisée la synthèse du mélange des deux diastéréomères de la N-Cbz-isoTHR-P(O)$(OEt)_2$, puis celle de chacun des diastéréomères de l'isoTHR-P(O)$(OEt)_2$, diverses réactions enzymatiques sont mises en œuvre (transestérification, hydrolyse). Si les lipases utilisées (essentiellement *Pseudomonas fluorescens* et *Candida rugosa*) font preuve de diastéréosélectivité et de chimiosélectivité, aucune énantiosélectivité n'est par contre détectée.

Key words: Aminophosphonic acids, isothreonine-P, lipases, kinetic resolution.

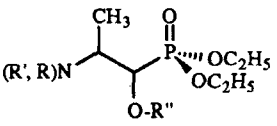
INTRODUCTION

Aminophosphonic acids are a very important class of compounds because of their structural similarity to natural carboxylic aminoacids. Furthermore, their tetrahedral phosphore structure can mimic the transition state of the hydrolysis reaction of esters and amides and they can therefore act as inhibitors of hydrolases.[1–4] As a consequence, aminophosphonic acids have found many industrial applications as pharmaceuticals and agrochemicals.[5–8] The bioactivity of these compounds is known to be strongly dependent on their enantiomeric structure, but up to now only a few methods such as diastereomeric resolutions[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 recently described a very efficient method for the resolution of the N-protected isoserine-P diethyl ester 1 [N-Cbz-isoSER-P(O)$(OEt)_2$] using enzymatic reac-

tions.[23] In this paper, we present our results concerning the selectivity of the lipases towards isothreonine-P derivatives 2, 3, 4 (see scheme below).



N-CBZ-isoSER-P(O)(OEt)$_2$

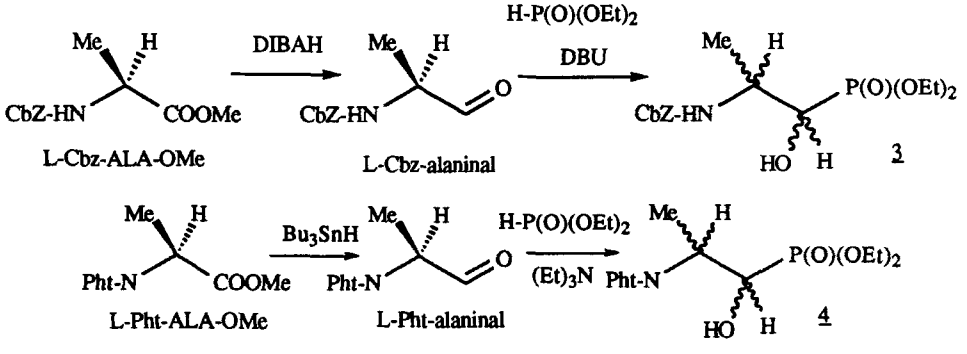


2 R = R' = R" = H
3 R = Cbz, R' = R" = H
4 R, R' = N-Pht, R" = H
5 R = Cbz, R' = H, R" = Ac

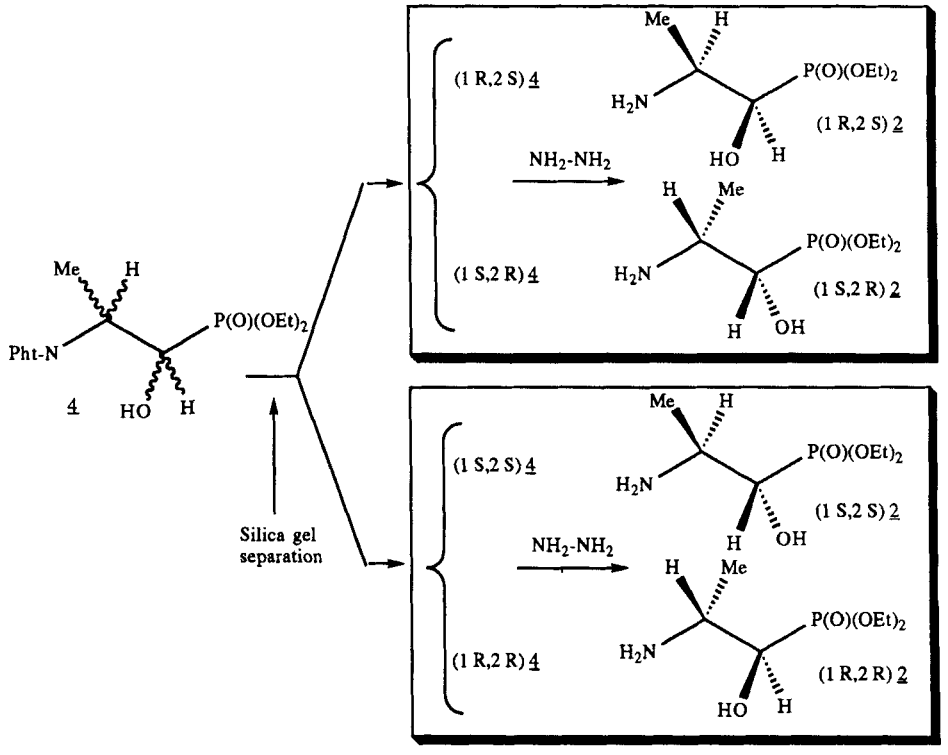
iso-THR-P(O)(OEt)$_2$ and derivatives

RESULTS AND DISCUSSION

The resolution of the phosphonate analogue of isothreonine is much more complicated than the resolution of the analogous isoserine-P. This is due to the fact that two diastereomeric forms can theoretically be present and also because the presence of the methyl group which makes the access to the hydroxyl group more difficult. In effect, our previous studies on compound 1[23] showed that the lipase catalysed transesterification using vinyl acetate as an acyl donor was a relatively slow reaction. The application of such a methodology to N-Cbz-isoTHR-P(O)(OEt)$_2$ 3 or N-Pht-isoTHR-P(O)(OEt)$_2$ 4 was totally unsuccessful and after one month of incubation with the classic triad of lipases (*Candida Rugosa, Porcine pancreatic and Pseudomonas fluorescens*) we were unable to detect any conversion. Therefore, we decided to replace vinyl acetate by acetic anhydride. Under these conditions, the acylation proceeds much more rapidly especially with *Pseudomonas fluorescens lipase*, but no stereoselectivity is observed because the uncatalysed reaction, although proceeding at a lower rate than the enzymatic one also takes place. Thus, very good yields (>95%) were obtained in the synthesis of the diastereomeric mixture of the O-Acetyl derivative: N-Cbz,O-Ac-isoTHR-P(O)(OEt)$_2$ 5. We decided to move towards the lipase catalysed hydrolysis of these compounds and the acylation of isoTHR-P(O)(OET)$_2$ 2 with vinyl acetate. Due to the presence of the two diastereomers and in order to facilitate the study of the enantioselectivity, we tried to prepare isoTHR-P derivatives with a given configuration at carbon C-2 according to the following scheme:

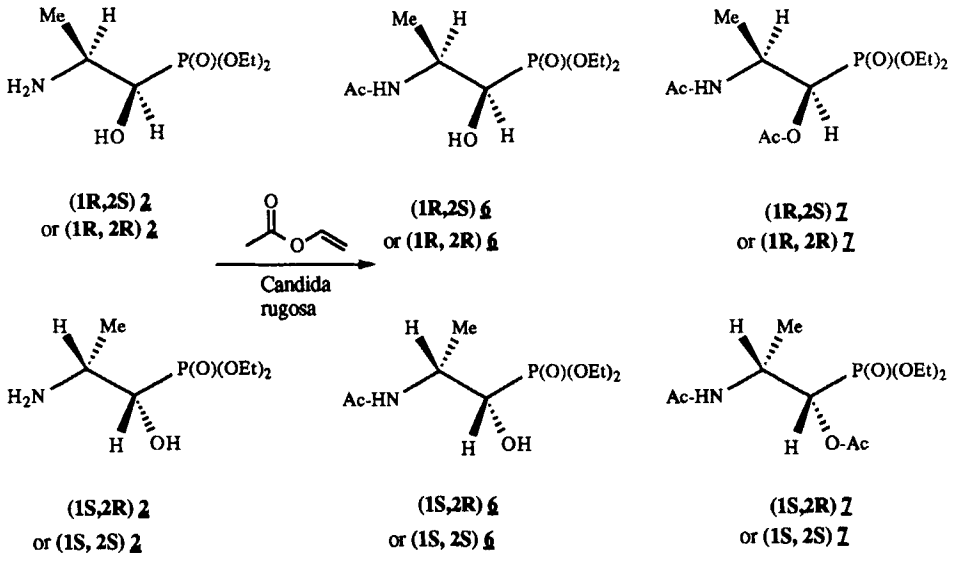


Unfortunately, the addition, under basic conditions, of the phosphite to the aldehyde group completely racemized the chiral carbon of the protected alaninal and the separation of the diastereoisomers of 3 thus synthesized proved difficult. We had more luck with type 4 derivatives for which it was possible to separate efficiently the erythro from the threo racemate by liquid chromatography (see experimental section). Subsequent deprotection of the phtalimido group by means of hydrazine treatment afforded the pure diastereomers of 2. The configuration of each diastereomer was assigned after the complete removal of the protections and by comparison with the known NMR parameters for these compounds[26,27] (see also experimental section).



For the hydrolysis of the N-Cbz,O-Ac-isoTHR-P(O)(OEt)$_2$ 5, *Candida rugosa* lipase (CRL) was shown to be the best catalyst and after 15 days a conversion of 25% was observed starting from a diastereomeric mixture of 64% (1R,2R/1S,2S) and 36% (1R,2S/1S,2R). At this point the composition of the diastereomeric products 4 determined by means of the NMR spectra (^{1}H and ^{13}C) was 31% (1R,2R/1S,2S) and 69% (1R,2S/1S,2S) (see Table I). Thus CRL was able to induce a good diastereoselectivity (de = 60%). As this mixture showed no optical rotation, we concluded that this lipase was unable to recognize efficiently the enantiomers during this hydrolysis. In that case also, the reaction was a slow one and one might assume that the use of the less hindered derivatives 2 should enhance the rate of the acylations. Furthermore, as we were able to synthesize separately the two D,L pairs of 2 the situation became more clear. Unfortunately, we have encountered

another problem with the chimioselectivity. Lipases are known to catalyse not only transesterification but also transamidation reactions. In our case, using vinyl acetate, CRL induces first the N-acylation reaction. For each diastereomer the N-acyl 6 derivatives were obtained first showing a very good chimioselectivity. After five days of incubation of 2(1R,2S/1S,2R) or 2(1S,2S/1R,2R) with CRL as a catalyst, a conversion of c.a. 100% was measured and the proton NMR spectra revealed the presence of the N-acetylated 6 compounds as a single product. The latter compounds were obtained in high yields after purification. This reaction which proceeds without any enantioselectivity was followed by the slow transesterification leading to the of N,O-diacetylated 7 compound. Small amounts of other polyacetylated compounds (probably the N,N-diacetylated and the triacetylated products) were also present.



The question was to know whether or not the O-acylation was enantioselective. Derivatives 6 were incubated two weeks in the conditions described above. For each diastereomer of 6 a conversion of c.a. 20% was observed. The N,O-diacetylated 7 derivative was separated from the remaining N-acetylated compound over a silica gel column. It was hydrolysed in order to obtain the isothreonin-P. Optical activity measurements performed on each diastereomer gave optical rotation values close to zero showing once again no enantioselectivity for CRL in the studied acylations.

CONCLUSION

In summary, we can say that the resolution of each enantiomeric pair of iso-THR-P is totally inefficient when using lipases (particularly *Candida Rugosa* lipase). Conversely, this enzyme induces a good diastereoselectivity and when the hydroxyl

and amino groups are in competition in the acylation reaction, a very high preference (chimioselectivity) for the last function is observed.

EXPERIMENTAL

The structure of all the products was determined by means of proton and carbon NMR spectroscopy (Bruker WM 250 spectrometer). The solvents used were $CDCl_3$ in the case of any protected aminophosphonic acid and D_2O in the case of isothreonine-P.

The products were usually purified over a silica gel column (Merck 60-60–70 mesh) and the purification or the advancement of a given reaction were followed by means of TLC plates (Merck silica gel 60 F254 plates).

Synthesis of the N-Cbz-isoTHR-P(O)(OEt)$_2$ 3 (mixture of the two diastereomers): To a solution of 16 mmoles of (R/S)-N-Cbz-alaninal in 30 ml of CH_2Cl_2 was added 32 mmoles (4.4 g, 4.1 ml) of diethylphosphite at room temperature. Then, six drops of DBU were added to the mixture at 0°C. The mixture was allowed to react for 18 hours. The solvent and the excess of phosphite were eliminated under reduced pressure (0.1 Torr, 100°C). Compound 3 was purified over silica gel (eluent: SH_2Cl_2/acetone 1/1) in order to eliminate the impurities and then 4/1 to elute the product, R_f = 0.25 with the last eluent). Yield = 50%, mixture of diastereomers: 80% 3 (1R,2R/1S,2S), 20% 3 (1R,2S/1S,2R) determined from the integration of the NH ^{1}H NMR signal.

Compound 3 (1R,2R/1S,2S): NMR ^{1}H ($CDCl_3$), ≈1.2 (t, 6H, *J* n.d., CH_3—CH_2—O), ≈1.2 (d, 3H, *J* n.d., CH_3—CH), 3.84 (2d, 1H, *J* = 3.3 Hz, J_{PH} = −9.4 Hz, CH—P), ≈4.1 (m, 4H, *J* n.d., CH_3—CH_2—O), ≈4.1 (m, 1H, *J* n.d., CH—N), 5.05 (s, 2H, CH_2—C_6H_5), 6.12 (d, 1H, *J* = 8.4 Hz, NH), 7.25 (s, 5H, CH_{arom}); NMR ^{13}C, 16.4 and 16.5 (2q, CH_3—CH_2—O), 18.4 (q, J_{CP} = 11 Hz, CH_3—CH), 47.9 (2d, CH—N), 62.7 and 62.8 (t, CH_3—CH_2—O), 66.6 (t, CH_2—C_6H_5), 71.0 (2d, CH—P, J_{CP} = 161.1), 127.9 (d, 2 CH_{arom}), 128.4 (d, 2CH_{arom}), 128.8 (s, C_{arom}), 136.6 (s, C_{arom}), 156.0 (s, O—CO—N).

Compound 3 (1R,2S/1S,2R): NMR ^{1}H ($CDCl_3$), 1.12 (d, 3H, *J* n.d., CH_3—CH), ≈1.2 (t, 6H, *J* n.d., CH_3—CH_2—O), ≈4.1 (m, 4H, *J* n.d., CH_3—CH_2—O), ≈4.1 (m, 2H, *J* n.d., CH—N and CH—P), 5.05 (s, 2H, CH_2—C_6H_5), 5.62 (d, 1H, *J* = 8.4 Hz, NH), 7.25 (s, 5H, CH_{arom}). NMR ^{13}C, 16.4 and 16.5 (2q, CH_3—CH_2—O), 16.1 (q, CH_3—CH), 49.0 (2d, CH—N, J_{CP} = 7.3 Hz), 62.9 and 63.0 (2t, CH_3—CH_2—O), 66.8 (t, CH_2—C_6H_5), 70.9 (2d, CH—P, J_{CP} = 157.5 Hz), 128.1 (d, 2CH_{arom}), 128.3 (d, 2 CH_{arom}), 128.5 (s, C_{arom}), 136.4 (s, C_{arom}), 156.2 (s, O—CO—N).

Synthesis of rac-N-phtalimido-alaninal: A solution of 2.0 g of rac-N-phtalimidoalanine acid chloride in 17 ml of dry toluene was degazed three times with argon. Then, 100 mg (0.84 mmole) of tetrakis(triphenylphosphine)palladium(0) were added and the solution was degazed once again with argon. The mixture was vigorously stirred and 4.9 g (16.9 mmoles, 4.6 ml) of tributyltin hydride was added. The mixture was kept at room temperature under stirring for 5 hours. Afterwards, the solution was filtered and the solvent was evaporated. The residue redissolved in 20 ml of acetone was stirred vigorously for 3 hours with 20 ml of a saturated solution of sodium fluoride. The precipitate (tributyltin fluoride) was filtered and washed with ether (3 × 100 ml). The solvents were eliminated under reduced pressure and the crude product was redissolved in 100 ml of acetonitrile. This solution was washed three times with petroleum ether in order to precipitate the remaining tributyltin fluoride. The combined solutions were then dried over sodium sulfate and the solvents were distilled. The white solid product thus obtained was found pure enough (1.15 g, yield = 68%).

Synthesis of each diastereomer of the N-Pht-isoTHR-P(O)(OEt)$_2$ 4: To a solution of 3.7 mmoles (750 mg) of rac-N-Phtalimido-alaninal and triethylphosphite (7.4 mmoles, 1 g, 0.9 ml) in 10 ml of dry CH_2Cl_2 were added 7.4 mmoles (1 ml) of triethylamine. The mixture was stirred for 4 days at 50°C. The solvent and the excess of phosphite were then eliminated under vacuum (0.1 Torr, 100°C). The mixture was chromatographed rapidly on silica gel (eluent: CH_2Cl_2/acetone 1/1 in order to eliminate the impurities). The diastereomers thus obtained as a clear oil were sufficiently pure [752 mg, yield = 60%, diastereomeric composition: 34% 4 (1R,2R/1S,2S) and 66% 4 (1S,2R/1R,2S). Then, a second chromatography using the eluent CH_2Cl_2/acetone 5/1 allowed the separation of the two isomers: R_f = 0.68 for 4 (1R,2R/1S,2S) and R_f = 0.45 for 4 (1S,2R/1R,2S)]. The treatment of these compounds with hydrazine and HCl 6 N afforded the pure diastereomers of isothreonine-P. The comparison of the NMR parameters with the literature data (the R,R isomer is known) allowed the identification.[26,27]

Compound 4 (1R,2R/1S,2S): NMR ^1H ($CDCl_3$), 1.11 (t, 6H, J = 6.9 Hz, CH_3—CH_2—O), 1.41 (d, 3H, J = 6.9 Hz, CH_3—CH), ≈4.0 (m, 4H, J = 6.9 Hz, CH_3—CH_2—O), 4.12 (t, 1H, J and J_{PH} n.d., OH), 4.68 (m, 1H, J and J_{PH} n.d., CH—P), 4.75 (m, 1H, J and J_{PH} n.d., CH—N), 7.61 (m, 2H, CH_{arom}), 7.71 (m, 2H, CH_{arom}); NMR ^{13}C, 15.2 (2q, J_{CP} = 5.5 Hz, CH_3—CH), 15.9 (q, CH_3—CH_2—O), 47.6 (2d, J_{CP} = 7.3 Hz, CH—N), 62.1 and 62.5 (2t, J_{CP} = 7.3 Hz, CH_3—CH_2—O), 68.8 (2d, J_{CP} = 163.0 Hz, CH—P), 122.7 (d, 2 CH_{arom}), 131.3 (s, C_{arom}), 133.6 (d, 2 CH_{arom}), 168.1 (s, CO—N).

Compound 4 (1R,2S/1S,2R): NMR ^1H ($CDCl_3$), 1.13 (t, 6H, J = 6.9 Hz, CH_3—CH_2—O), 1.52 (d, 3H, J = 6.2 Hz, CH_3—CH), ≈4.1 (m, 4H, J = 6.9 Hz, CH_3—CH_2—O), 4.52 (m, 1H, J = 4.6 Hz, J = 5.5 Hz, J_{PH} = −8.2 Hz, CH—P), 4.63 (m, 1H, J = 4.6 Hz, J = 6.2 Hz, J_{PH} n.d., CH—N), 5.25 (2d, 1H, J = 5.5 Hz, J_{PH} = 7.3 Hz, OH), 7.65 (m, 2H, CH_{arom}), 7.75 (m, 2H, CH_{arom}); NMR ^{13}C, 15.4 (2q, J_{CP} = 9.2 Hz, CH_3—CH), 16.1 and 16.3 (4q, J_{CP} = 5.5 Hz, CH_3—CH_2—O), 47.5 (d, CH—N), 68.1 (2d, J_{CP} = 161.1, CH—P), 123.2 (d, 2 CH_{arom}), 131.9 (s, C_{arom}), 134.0 (d, 2 CH_{arom}), 168.0 (s, CO—N).

Isothreonine-P (1R,2R/1S,2S): NMR ^1H (D_2O, pH =), 1.42 (d, 3H, J = 6.8 Hz, CH_3—CH), 3.61 (m, 1H, J = 4.9 Hz, J = 6.8 Hz and J_{PH} = 5.3 Hz, CH—N), 3.72 (m, 1H, J = 4.9 Hz, J_{PH} = −9.3 Hz, CH—P); NMR ^{13}C, 20.1 (2q, J_{CP} = 9.1 Hz, CH_3—CH), 53.7 (d, CH—N), 73.1 (2d, J_{CP} = 153.8 Hz, CH—P).

Isothreonine-P (1R,2S/1S,2R): NMR ^1H (D_2O, pH =), 1.44 (d, 3H, J = 7.0 Hz, CH_3—CH), 3.72 (m, 1H, J = 4.1 Hz, J = 7.0 Hz and J_{PH} = 7.8 Hz, CH—N), 3.94 (m, 1H, J = 4.1 Hz, J_{PH} = −11.4 Hz, CH—P); NMR ^{13}C, 17.8 (q, CH_3—CH), 53.6 (2d, J_{CP} = 5.5 Hz, CH—N), 72.8 (2d, J_{CP} = 152.0 Hz, CH—P).

Synthesis of each diastereomer of isoTHR-P(O)(OEt)$_2$ 2: 629 mg (1.84 mmoles) of 4 (1R,2R/1S,2S) were treated with 50 ml of a 1 M methanolic solution of hydrazine. The solution was stirred for two days. The white precipitate was filtered and the solution was extracted with chloroform (3 × 10 ml). After elimination of the solvent under reduced pressure, 2 was dried in a dessicator. 300 mg of pure product was obtained as a clear oil (yield: 77%). A similar procedure was applied to the synthesis of 2 (1S,2R/1R,2S) giving 343 mg of this compound (yield = 87%).

Compound 2 (1R,2R/1S,2S): NMR ^1H ($CDCl_3$), 1.15 (d, 3H, J = 6.2 Hz, CH_3—CH), 1.29 (t, 6H, J = 7.1 Hz, CH_3—CH_2—O), 3.23 (m, 1H, J = 4.9 Hz, J = 6.2 Hz, J_{PH} = 6.9 Hz, CH—N), 3.52 (broad s, 2H, OH and NH_2), 3.56 (2d, 1H, J = 4.9 Hz, J_{PH} = −7.7 Hz, CH—P), ≈4.2 (m, 4H, J = 7.0 Hz, CH_3—CH_2—O); NMR ^{13}C, 16.0 and 16.1 (2q, CH_3—CH_2—O), 19.6 (2q, J_{CP} = 9.2 Hz, CH_3—CH), 46.9 (2d, J_{CP} = 3.7 Hz, CH—N), 61.7 and 62.2 (4t, 2C, J_{CP} = 5.5 Hz, CH_3—CH_2—O), 70.7 (2d, J_{CP} = 161.1 Hz, CH—P).

Compound 2 (1R,2S/1S,2R): NMR ^1H ($CDCl_3$), 0.95 (d, 3H, J = 6.6 Hz, CH_3—CH), 1.12 (t, 6H, J = 7.0 Hz, CH_3—CH_2—O), 2.97 (m, 1H, J = 5.2 Hz, J = 6.6 Hz, J_{PH} = 11.8 Hz, CH—N), 3.32 (broad s, 2H, OH and NH_2), 3.53 (2d, 1H, J = 5.2 Hz, J_{PH} = −8.1 Hz, CH—P), ≈3.9 (m, 4H, J = 7.0 Hz, CH_3—CH_2—O); NMR ^{13}C, 16.0 and 16.1 (2q, CH_3—CH_2—O), 18.6 (2q, J_{CP} = 3.7 Hz, CH_3—CH), 48.2 (2d, J_{CP} = 3.7 Hz, CH—N), 61.9 and 62.2 (4t, 2C, J_{CP} = 7.3 and 9.2 Hz, CH_3—CH_2—O), 71.5 (2d, J_{CP} = 159.3 Hz, CH—P).

Enzymatic synthesis of the acetates 5 from the diastereomeric mixture of 3: A mixture of 687 mg (1.99 mmole) of 3 [77% (1R,2R/1S,2S), 23% (1S,2R/1R,2S)] and of 1.02 g (10 mmole, 0.94 ml) of acetic anhydride in 15 ml of dry toluene was incubated at room temperature in the presence of 400 mg of a commercial preparation containing PFLipase (Amano). The reaction was magnetically stirred during 3 weeks and was followed by means of TLC (eluent: CH_2Cl_2/acetone 3/1, R_f = 0.37 for 3 and R_f = 0.65 for the acetates 5). At this time, a conversion of more than 95% was observed (determined from the NMR spectra of the mixture). The lipase was filtered and washed with chloroform. The solvents were removed and the mixture was chromatographed over a silica gel column (eluents: CH_2Cl_2/acetone 5/1 at the beginning to remove impurities, 3/1 to elute the acetate and 5/1 to elute the remaining alcohol. 677 mg of the O-acetylated derivatives 5 were obtained as a clear oil (yield = 88%). The diastereomeric composition of the acetates 5 was the same as the one of the substrates 3.

Compound 5 (1R,2R/1S,2S): NMR ^1H ($CDCl_3$) ≈1.2 (t, 6H, J n.d., CH_3—CH_2—O), ≈1.2 (d, 3H, J = 7.0 Hz, CH_3—CH), 1.92 (s, 3H, CH_3CO), ≈4.0 (m, 4H, J n.d., CH_3—CH_2—O), ≈4.1 (m, 1H, J = 5.5 Hz, CH—N), 4.95 (s, 2H CH_2—C_6H_5), 5.08 (2d, 1H, J = 5.5 Hz, J_{PH} = −9.9 Hz, CH—P), 5.51 (d, 1H, J = 9.1 Hz, NH), 7.21 (s, 5H, CH_{arom}); NMR ^{13}C, 15.9 and 16.0 (4q, 2 CH_3—CH_2—O, J_{CP} = 5.5 Hz), 17.9 (2q, CH_3—CH, J_{CP} = 7.3 Hz), 20.0 (q, CH_3CO), 46.8 (d, CH—N), 62.5 (2t, CH_3—CH_2—O, J_{CP} = 7.3 Hz), 66.2 (t, CH_2—C_6H_5), 69.8 (2d, CH—P, J_{CP} = 166.6 Hz), 128.2 (d, 2 CH_{arom}), 128.5 (d, 2CH_{arom}), 128.5 (s, C_{arom}), 136.4 (s, C_{arom}), 155.4 (s, O—CO—N), 169 (s, COO).

Compound 5 (1R,2S/1S,2R): NMR ^1H ($CDCl_3$) ≈1.2 (d, 3H, $C\underline{H}_3$—CH), ≈1.2 (t, 6H, J n.d., $C\underline{H}_3$—CH_2—O), 1.93 (s, 3H, $C\underline{H}_3CO$), ≈4.0 (m, 4H, J n.d., CH_3—$C\underline{H}_2$—O), ≈4.1 (m, 1H, J = 3.8 Hz, CH—N), 4.95 (s, 2H $C\underline{H}_2$—C_6H_5), 5.21 (2d, 1H, J = 3.8 Hz, J_{PH} = −11.4 Hz, CH—P), 5.78 (d, 1H, J = 8.8, NH), 7.20 (s, 5H, CH_{arom}); NMR ^{13}C, 15.9 and 16.0 (4q, $\underline{C}H_3$—CH_2—O, J_{CP} = 5.5 Hz), 16.5 (q, $\underline{C}H_3$—CH), 20.1 (q, $\underline{C}H_3CO$), 47.3 (d, CH—N), 62.3 and 62.8 (4t, CH_3—CH_2—O, J_{CP} = 7.3 Hz), 66.2 (t, $C\underline{H}_2$—C_6H_5), 69.8 (2d, J_{CP} = 166.6 Hz, CH—P), 128.2 (d, 2 CH_{arom}), 128.5 (d, 2 CH_{arom}), 128.5 (s, C_{arom}), 136.4 (s, C_{arom}), 155.6 (s, O—$\underline{C}$O—N), 169.2 (s, $\underline{C}$OO).

Enzymatic hydrolysis of the acetates 5: An emulsion of the mixture of 366 mg (0.85 mmole) of the acetates 5 [64% (1R,2R/1S,2S) and 36% (1S,2R/1R,2S)] in 10 ml of distilled water was incubated in the presence of 300 mg of the *Candida rugosa* lipase (Amano AY 30) under a vigorous stirring during 2 weeks. The pH was maintained at 7 by means of the continuous addition of a 1 M soda solution. The mixture was then extracted three times with ether (1 × 50 ml and 2 × 25 ml). The organic layers were dried over Na_2SO_4. The solvent was removed and the products were dried in a dessicator under reduced pressure (0.01 torr). The compounds were purified as described above. Thus, 68 mg of diastereomers 3 were obtained [yield 22%, 31% (1R,2R/1S,2S) and 69% (1S,2R/1R,2S)] and 198 mg of the starting material were recovered.

Enzymatic transesterification of each diastereomer of isoTHR-P(O)(OEt)$_2$ 2: A solution of 302 mg (1.43 mmoles) of 2 (1R,2R/1S,2S) in 10 ml of vinyl acetate (acyl donor and solvent) was incubated in the presence of 500 mg of the CRL preparation from Amano (AY30). The mixture was stirred and warmed at 40°C during 5 days. The solution contained only the N-acetylated 6 derivative (100% conversion). The latter was purified by means of liquid chromatography (326 mg, 1.29 mmoles of pure 6 product). A further incubation of 14 days in the same conditions afforded a mixture of 80% of the N-acylated 6 and 20% of the N,O-diacetylated 7 compounds. The reaction was followed by means of TLC (eluent: CH_2Cl_2/acetone 4/1, 2: R_f = 0.10, 5: R_f = 0.37, 6 and 7: R_f = 0.57). The products were separated on a silica gel column using a mixture of CH_2CL_2/acetone 1/1 as an eluent. 248 mg (1.22 mmoles) of 6 and 72 mg (0.24 mmole) of 7 were thus obtained (total yield: 85%).

Starting from 343 mg (1.63 mmoles) of the D,L pair 1S,2R/1R,2S of 2 in similar conditions, 357 mg (1.41 mmoles of the corresponding N-acetylated 6 derivative was obtained after 5 days of incubation. A further incubation of 14 days afforded a mixture of 83% of the N-acetylated 6 and of the N,O-diacetylated 7 products. The separation of the compounds achieved by means of liquid chromatography gave 298 mg (1.18 mmoles) of 6 (1S,2R/1R,2S) and 68 mg (0.23 mmole) of 7 (1S,2R/1R,2S).

Compound 6 (1R,2R/1S,2S): NMR ^1H ($CDCl_3$), 1.22 (d, 3H, J = 6.5 Hz, $C\underline{H}_3$—CH), 1.25 (t, 6H, J = 6.8 Hz, $C\underline{H}_3$—CH_2—O), 1.95 (s, $C\underline{H}_3CO$), 3.85 (m, 1H, J = 3.4 Hz, J_{HP} n.d., CH-P), ≈4.1 (m, 4H, J = 6.8 Hz, CH_3—$C\underline{H}_2$—O), 4.26 (m, 1H, J = 8.2 Hz, J = 3.4 Hz, J_{HP} n.d., CH—N), 7.05 (d, 1H, J = 8.2 Hz, NH); NMR ^{13}C, 16.4 and 16.5 (2q, $\underline{C}H_3$—CH_2—O), 17.6 (2q, J_{CP} = 11.0 Hz, $\underline{C}H_3$—CH), 23.2 (q, CH_3CO), 46.6 (1d, CH—N), 62.9 and 63.0 (4t, J_{CP} = 7.3 Hz, CH_3—$C\underline{H}_2$—O), 70.8 (2d, J_{CP} = 161.1, CH—P), 170.2 (s, COO).

Compound 6 (1R,2S/1R,2S): NMR ^1H ($CDCl_3$), 1.18 (d, 3H, J = 6.4 Hz, $C\underline{H}_3$—CH), 1.23 and 1.24 (2t, 6H, J = 6.8 Hz, $C\underline{H}_3$—CH_2—O), 1.89 (s, $C\underline{H}_3CO$), 3.95 (2d, 1H, J = 2.1 Hz, J_{PH} = −9.8 Hz, CH—P), ≈4.1 (m, 4H, J = 6.8 Hz, CH_3—$C\underline{H}_2$—O), 4.21 (m, 1H, J = 2.1 Hz, J = 7.3 Hz, J = 7.8 Hz, J_{PH} = 6.4 Hz, CH—N), 7.03 (d, 1H, J = 7.8 Hz, NH); NMR ^{13}C, 15.4 (q, $\underline{C}H_3$—CH), 16.2 and 16.3 (2q, $\underline{C}H_3$—CH_2—O), 22.8 (s, $\underline{C}H_3CO$), 47.4 (2d, J_{CP} = 7.3 Hz, CH—N), 62.7 and 62.9 (4t, J_{CP} = 5.5 and 9.2 Hz, CH_3—$C\underline{H}_2$—O), 70.3 (2d, J_{CP} = 158.6 Hz, CH—P), 170.8 (s, CO—N).

Compound 7 (1R,2R/1S,2S): NMR ^1H ($CDCl_3$), 1.22 (d, 3H, J = 7.0 Hz, $C\underline{H}_3$—CH), 1.30 and 1.31 (t, 6H, J = 7.0 Hz, $C\underline{H}_3$—CH_2—O), 1.95 (s, $C\underline{H}_3COO$), 2.15 (s, $C\underline{H}_3CO$—N), ≈4.15 (m, 4H, J = 7.0 Hz, CH_3—$C\underline{H}_2$—O), 4.47 (m, 1H, J = 4.4 Hz, J = 7.0 Hz, J = 8.7 Hz, J_{CP} = 14.5 Hz, CH—N), 5.23 (2d, 1H, J = 4.4 Hz, J_{CP} = −9.9 Hz, CH—P), 6.20 (d, 1H, J = 8.7 Hz, NH); NMR ^{13}C, 16.4 and 16.5 (2q, $\underline{C}H_3$—CH_2—O), 17.9 (2q, $\underline{C}H_3$—CH, J_{CP} = 7.3), 20.7 (q, $\underline{C}H_3COO$), 23.5 (q, $\underline{C}H_3CO$—N), 45.0 (1d, CH—N), 63.1 and 63.2 (4t, CH_3—$\underline{C}H_2$—O, J_{CP} = 7.3 Hz), 69.4 (2d, J_{CP} = 164.8 Hz, CH—P), 169.3 and 169.4 (2s, $\underline{C}$OO, $\underline{C}$O—N).

Compound 7 (1R,2S/1S,2R): NMR ^1H ($CDCl_3$), ≈1.2 (d, 3H, J = 6.8 Hz, $C\underline{H}_3$—CH), ≈1.3 (t, 6H, J = 6.9 Hz, $C\underline{H}_3$—CH_2—O), 1.95 (s, $C\underline{H}_3COO$), 2.05 (s, $C\underline{H}_3CO$—N), ≈4.15 (m, 4H, J = 6.9 Hz, CH_3—$C\underline{H}_2$—O), 4.41 (m, 1H, J = 4.4 Hz, J = 6.8 Hz, J = 8.0 Hz, J_{HP} n.d., CH—N), 5.18 (2d, 1H, J = 4.4 Hz, J_{HP} = −11.4 Hz, CH—P), 6.52 (d, 1H, J = 8.0 Hz, NH); NMR ^{13}C, 16.3 and 16.4 (2q, $\underline{C}H_3$—CH_2—O), 16.8 (q, $\underline{C}H_3$—CH), 20.6 (q, $\underline{C}H_3COO$), 23.2 (q, $\underline{C}H_3CO$—N), 45.4 (1d, CH—N), 63.1 and 63.2 (4t, CH_3—$\underline{C}H_2$—O, J_{CP} = 7.3 Hz), 69.6 (2d, CH—P, J_{CP} = 164.8 Hz), 169.7 (2s, $\underline{C}$OO and $\underline{C}$O—N).

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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, *Bull. Chem. Soc. Jpn.*, **60,** 1761–1766 (1987).
13. R. Jacquier, F. Ouazzani, M. L. Roumestant and P. Viallefont, *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).
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. R. C. Corcoran and J. M. Green, *Tetrahedron Letters*, **31,** 6827–6830 (1990).
25. F. Hammerschmidt and H. Völlenke, *Liebigs Ann. Chem.*, 577, 583 (1989).
25. C. S. Chen, Y. Fujimoto, G. Girkaudas and C. J. Sih, *J. Am. Chem. Soc.*, **104,** 7294–7299 (1982).
26. G. Hägele, H. Heine Universität Düsseldorf, personal communication.
27. F. Hammerschmidt, G. Bovermann and K. Bayer, *Liebigs Ann. Chem.*, 1055–1061 (1990).