ARTICLE

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Enzymatic synthesis of phosphocarnitine, phosphogabob and fosfomycin †

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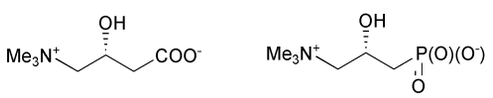
Phosphocarnitine was conveniently obtained from easily available diethyl 3-chloro-2-oxopropanephosphonates, followed by subsequent reduction, *Mucor miehei* lipase (IM) mediated resolution, amination and dealkylation. *Candida antarctica* lipase B (CALB) served as an effective biocatalyst in the resolution of several 1- or 2- hydroxyalkanephosphonates. The chlorine atom in different positions on the molecules greatly affected their enantioselectivity. CALB also showed satisfactory enantioselectivity toward those molecules bearing an azido moiety. Both enantiomers of phosphogabob and fosfomycin were also prepared *via* CALB-mediated resolution as the key step.

Introduction

Synthesis of optically active hydroxyalkanephosphonates exhibiting biological activity or synthetic use has found considerable attention during the past years.[1] Biocatalysis is now well recognized as an excellent strategy for the synthesis of enantiomers.[2] Biocatalytic reactions are environmentally friendly in contrast to conventional chemical catalytic reactions, especially when these make use of heavy-metal catalysis. Aldolases and baker's yeast have been exploited in the enantioselective synthesis of hydroxyalkanephosphonates.[3] Lipase-mediated resolution of alcohols and related compounds is a well-established method in chemical laboratories. Hammerschmidt and Frank have used such a hydrolase for enantioselective hydrolysis of a series of 1-acyloxyalkanephophonates.[4] Based on the pioneering work of Martinek and coworkers on non-aqueous enzymatic reactions,[5a] Klibanov extensively developed the field of applications of enzymology in organic solvents.[5b,5c] The first lipase-catalyzed kinetic resolution of hydroxyalkanephosphonates in organic media was achieved by Khushi *et al*.[5d] Based on the study of the relationship between the substrates' structure and lipase's catalytic activity, as well as its enantioselectivity, we have developed a *Candida antarctica* lipase B (CALB)-catalyzed acetylation, a *Candida rugosa* lipase (CRL)-catalyzed hydrolysis in organic media, and an immobilized *mucor miehei* lipase (IM)-mediated alcoholysis for the preparation of optically pure hydroxyalkanephosphonates and their derivatives.[6] In this article, we wish to disclose in detail our efforts to synthesize some biologically interesting phosphonic acid derivatives *via* lipase-mediated kinetic resolution as the key step.

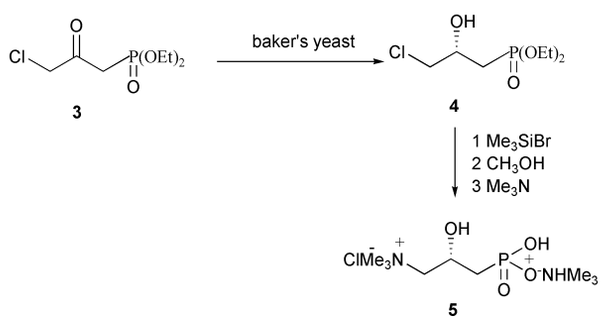
Results and discussion

Vitamin-like (*R*)-Carnitine **1** is responsible for the metabolism of long-chain fatty acids by regulating their transport through mitochondrial membranes and has been applied in therapy as a stimulator of fatty acid degradation and also in treatment of heart disease and other disorders.[7] The (*S*)-enantiomer is a competitive inhibitor of carnitine acyltransferase, causing depletion of (*R*)-carnitine levels in heart tissue. Various analogues of carnitine have been synthesized in order to study their mode of action and the structural features of the binding sites.[8] It is known that phosphorus analogues of naturally occurring amino acids are produced by certain organisms and are of great interest in bioorganic and medicinal chemistry. These molecules could act as enzyme inhibitors due to the mimetic behaviour of the unstable tetrahedral carbon intermediates formed in the enzymatic processes. Thus, the synthesis of a phosphorus analogue of carnitine, namely, phosphocarnitine **2**, is of great interest.



1 **2**

The first enantioselective synthesis of (*R*)-phosphocarnitine was achieved by chemical conversion of (*R*)-(−)-epichlorohydrin as reported by Michalski's group.[9] Our laboratory disclosed the first enzymatic synthesis of (*R*)-phosphocarnitine using baker's yeast mediated bio-reduction of readily available 3-chloro-2-oxopropanephosphonate **3** as the key step (Scheme 1).[3f] Wróblewski and Halaajewska-Wosik reported the synthesis of enantiomeric (*S*)-phosphocarnitine from (*S*)-2,3-epoxypropanephosphonate, followed by a C-3 regioselective opening of the oxirane ring with $MgBr_2$, quantitative bromide displacement with trimethylamine, and ester hydrolysis.[10] Mikolajczyk and coworkers also recently reported their chemoenzymatic synthesis of phosphocarnitine enantiomers, which also used baker's yeast-mediated bioreduction and lipase-catalyzed enantioselective acetylation strategy.[11]



3 **4** **5**

Scheme 1

Among all the above reported protocols, the key intermediate of phosphocarnitine was (*S*)-dialkyl 3-chloro (or bromo)-2-hydroxypropanephosphonate. We have established lipase-catalyzed resolution for the preparation of optically active

† Studies on organophosphorus compounds. Part 128. For Part 127, see C. Yuan, C. Xu and Y. Zhang, *Tetrahedron*, 2003, **59**, 6095.

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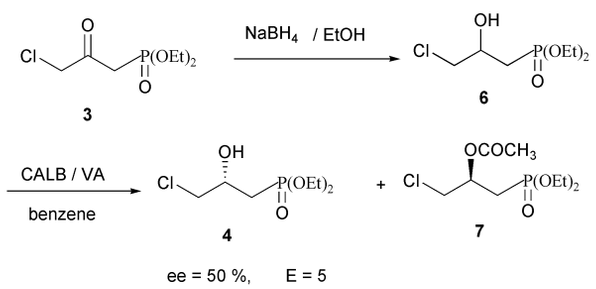
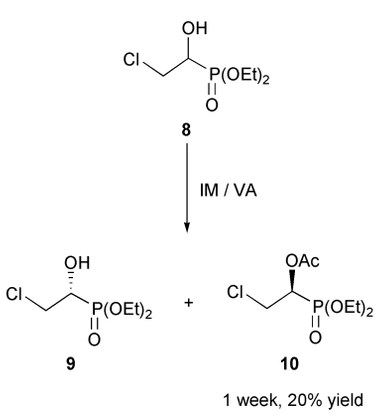
hydroxyalkanephosphonates in organic media. *Candida antarctica* lipase B showed high selectivity in the resolution of hydroxyalkanephosphonates with medium substituent smaller than a propyl moiety based on Kazlauskas model.[6a] We had attempted the CALB-catalyzed acetylation for the preparation of optically enriched (*R*)-3-chloro-2-hydroxypropanephosphonate **4** (Scheme 2). The racemic **6** could be easily prepared by reduction of diethyl 3-chloro-2-oxopropanephosphonate **3** with $NaBH_4$ in 93% yield, which could be easily synthesized by reaction of the carbanion of diethyl methylphosphonate with ethyl chloroacetate in 75% yield. However, it is found that the chlorine atom in the medium substituent greatly inhibited CALB's selectivity[12] (Scheme 2) and the enantiomeric ratio *E* is only 5 under optimal conditions. According to Kazlauskas rule,[13] the unreacted chlorohydrin should be of the (*S*)-configuration, that was further confirmed by Mosher's method.



Scheme 2

Hammerschmidt has reported the resolution of diisopropyl 2-azido-1-hydroxyethanephosphonates using lipase SP524 (*mucor miehei* lipase).[14] Among the lipases screened for the resolution of diethyl 2-chloro-1-hydroxyethanephosphonate **8**, we also found immobilized *mucor miehei* lipase (IM) which showed high enantioselectivity but poor reactivity in the acetylation of 2-chlorine-1-hydroxyethanephosphonate (Scheme 3).

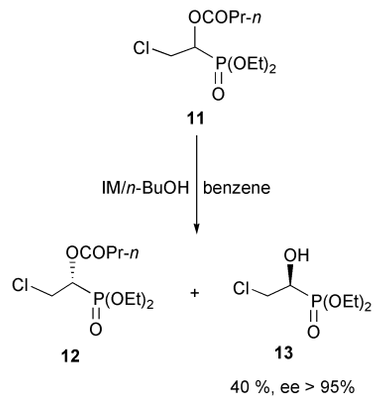


Scheme 3

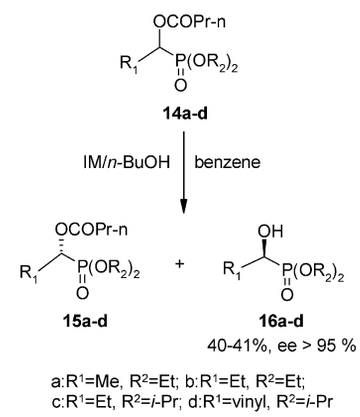
Alcoholysis of the butyryloxy derivative **11** in anhydrous benzene using *n*-butanol as the nucleophile, however, afforded optically pure **13** in 40% yield (Scheme 4). This enzymatic reaction, performed in organic media, avoided boring work-up procedures related to extraction and thus, improved the yield.

IM-catalyzed alcoholysis of other 1-butyryloxyalkanephosphonates also gave satisfactory results (Scheme 5).

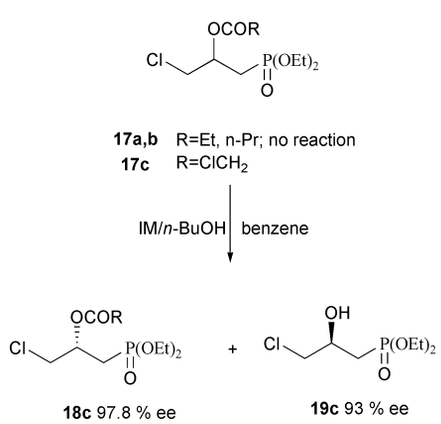
Inspired by the excellent results of IM-catalyzed alcoholysis for the preparation of optically active hydroxyalkanephosphonates, it was reasonable for us to apply this method for the preparation of **5**, however we pitifully failed. The phenomenon may be ascribed to the fact that the electron-withdrawing phosphoryl group is located far away from the chiral center which may reduce the reactivity. Instead of the butyryloxy group, however, the chloroacetyloxyl derivative has successfully solved this problem (Scheme 6).



Scheme 4



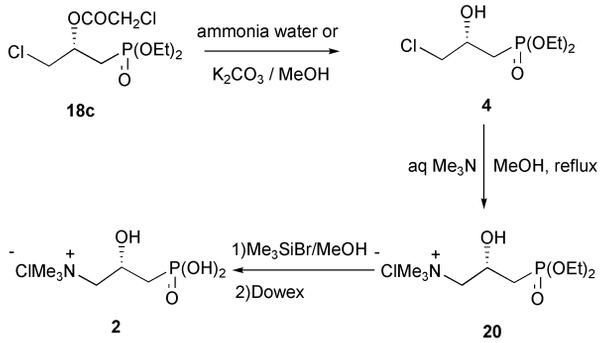
Scheme 5



Scheme 6

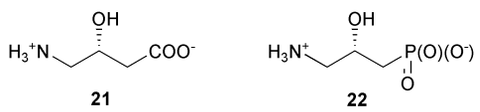
The starting diethyl 3-chloro-2-chloroacetyloxypropanephosphonate **17c** can be easily prepared from **6**, followed by subsequent reactions with DCC, chloroacetic acid and DMAP in 93% yield. Under optimized conditions, **18c** and **19** resulted in 97.8 and 93% ee respectively. As observed by us and indicated by Mikolajczyk and coworkers,[11] direct chemical hydrolysis of **17a**,**b** was not very successful, while **18c** could be easily converted to corresponding alcohol **4** in satisfactory yield using an amonia–water–methanol system or a potassium carbonate–methanol system (Scheme 7). The amination of **4** with aqueous Me_3N, followed by dealkylation with Me_3SiBr–MeOH, and ion change afforded the phosphocarnitine with a total yield up to 45%. Analogously, its enantiomer could be synthesized from **19** in the same manner.

Having established a convenient enzymatic approach to phosphocarnitine and its enantiomer in high optical purity, we turned our attention to the enantiomeric synthesis of phosphogabob. γ–Amino-β-hydroxybutyric acid **21** (GABOB) has an application as an antipipetic and hypotensive drug.[15] It is a well known medicament that functions as an antagonist of

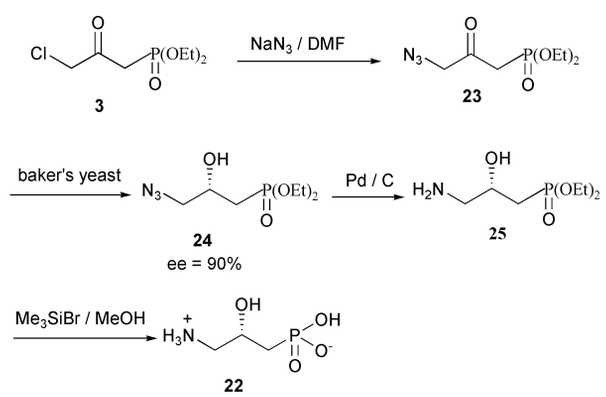


Scheme 7

γ-aminobutyric acid. It has been demonstrated to be effective in managing a variety of clinical conditions including schizophrenia and other character-based disorders including epilepsy and other illnesses that result in severe convulsions. Its use for the correction of some clinical conditions observed in children has also been reported.



Our group has also reported baker's yeast-mediated bioreduction for the first enantioselective synthesis of the phosphorus analogue of Gabob, namely, phosphogabob **22** (Scheme 8).[3f] The (*S*)-diethyl-3-azido-2-hydroxypropane phosphonate **24**, from bioreduction of diethyl 3-azido-2-oxo propanephosphonate **23**, underwent hydrogenation and subsequent dealkylation, affording the target molecule conveniently.

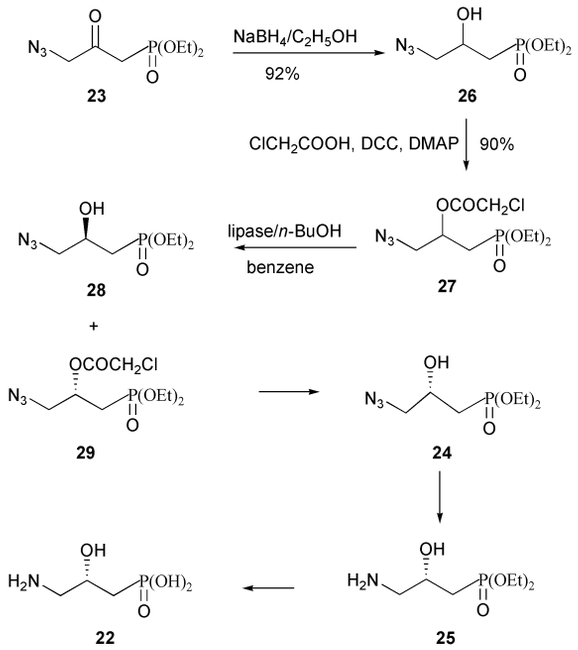


Scheme 8

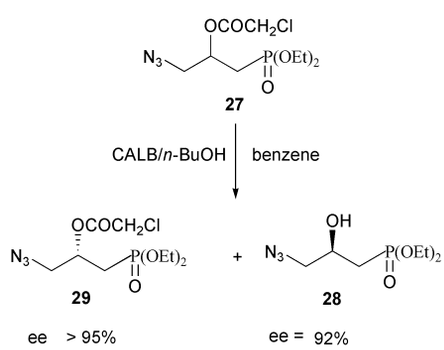
Our strategy for the lipase-mediated enantioselective synthesis of **22** is illustrated in Scheme 9.

Ring opening of racemic diethyl 2,3-oxiranypropanephosphonate with NaN_3–NH_4Cl–MeOH–H_2O afforded racemic **26** with 10% C-2 opening product (as shown by ^{31}P NMR), while direct reduction of **23** gave **26** in 92% yield. IM could also resolve **26** *via* alcoholysis with satisfactory enantioselectivity and reactivity. This enzymatic process provided alcoholyzed **28** and (*S*)-**29** in 43% yield, 93% ee and 44% yield, 90% ee respectively. Although CALB-catalyzed acetylation of racemic **26** showed poor reactivity, CALB-catalyzed alcoholysis of **27**, exhibited high activity and even higher enantioselectivity than that of IM (Scheme 10). (*S*)-**29**, Treated with K_2CO_3–MeOH, and the procedures shown in Scheme 8, led to phosphogabob with high optical purity.

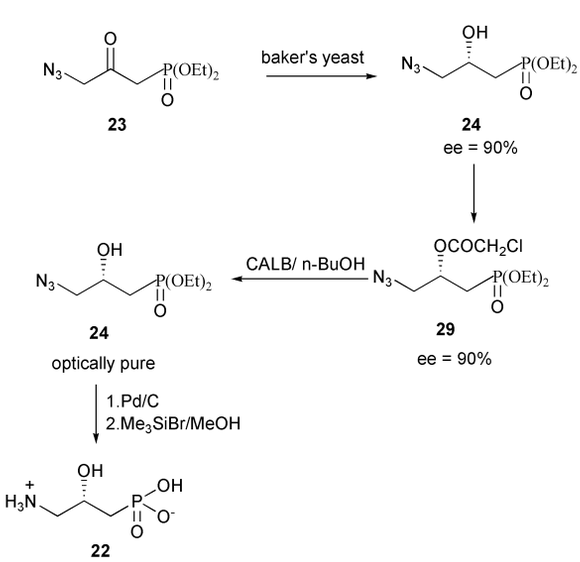
Another strategy for baker's yeast-mediated bio-reduction for enantioselective synthesis of phosphogabob **22** is illustrated in Scheme 11. **24** was formed with 90% ee, by bioreduction of **3** using baker's yeast. The resulting chiral hydroxy compound



Scheme 9



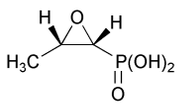
Scheme 10



Scheme 11

was subjected to further CALB-mediated kinetic resolution, affording optically pure **22** in high yield.

Having established the convenient access to phosphocarnitine (**2**), phosphogabob (**22**)and their enantiomers, we turned our attention to fosfomycin which was isolated from *Streptomyces fradiae*.[16] It has been used as an antibacterial antibiotic in the clinic and its enantioselective total synthesis has attracted great attention during the past decade.[17] For a recent comprehensive review, see Savignac *et al.*[17a]



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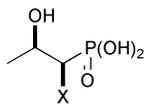
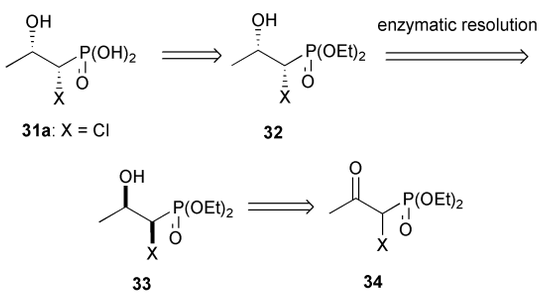


31a: X = Cl
31b: X = Br

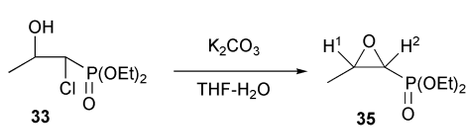
The 1-halo-2-hydroxypropanephosphonic acid acted as an important precursor of fosfomycin. Noyori[18] and Girotra[19] and their coworkers had adopted (1*R*,2*S*)-1-chloro-2-hydroxy propanephosphonic acid **31a** and (1*R*,2*S*)-1-bromo-2-hydroxypropanephosphonic acid **31b** respectively as the precursor of fosfomycin, which, subjected to basic cyclization, afforded the target fosfomycin in satisfactory yield. Based on our study associated with enzymatic kinetic resolution of hydroxyalkanephosphonates, we constructed the synthetic route shown in Scheme 12.



31a: X = Cl **32** **33** **34**

Scheme 12

The diethyl 2-chloro-3-oxopropanephosphonates **34** could be easily prepared from diethyl 2-oxopropanephosphonate. Upon treatment with sulfuryl chloride, and Na_2SO_3, **34** was obtained in 75% yield. The racemic diethyl 1-chloro-2-hydroxypropanephosphonate **33** was obtained by reduction with sodium borohydride, giving the predominant isomer in 75%, yield as illustrated by ^{31}P NMR. The *syn* configuration of the main isomer was assigned by its chemical conversion to diethyl 2,3-epoxypropanephosphonates (Scheme 13). The ^{1}H NMR of the mixture clearly indicated the main product was the *cis*-isomer.[20]



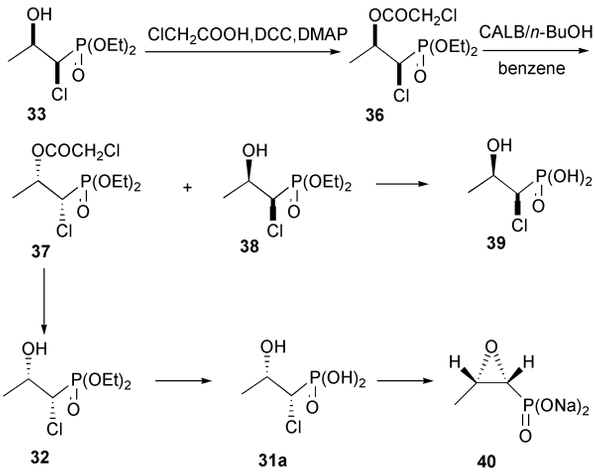
33 **35**

Scheme 13

The presence of a chelating agent such as $CaCl_2$ enhanced the *syn* : *anti* ratio to 6.3. These two isomers could be carefully separated by column chromatography. We had tried several lipase-catalyzed acetylations, but none of them were proven to be sufficiently reactive.

In many cases, enhancement of the difference of the electronic effect between the two substituents of the chiral center often brings enhanced enantioselectivity of a lipase-mediated resolution. Although CALB-mediated resolutions of secondary alcohols bearing a chlorine atom in the medium substituent showed poor selectivity, the chlorine atom in the larger sized substituent may be helpful for CALB-mediated resolutions. Bearing this in mind, we attempted CALB-catalyzed alcoholysis of the chloroacetyloxy derivative of **33** (Scheme 14).

Fortunately, as predicted, CALB-catalyzed alcoholysis of racemic **36** provided both enantiomers with ee > 95% (determined by ^{19}F NMR and ^{31}P NMR of the Mosher esters). It was also found that IM also gave the satisfactory results we observed. Optically pure **37**, treated with ammonia–water–CH_3OH, afforded **32** in 85% yield. Both **32** and **38**, subjected to dealkylation, gave the enantiomers of **31a**, which ensured the synthesis of the fosfomycin disodium salt (**40**) under basic conditions.



33 **36** **37** **38** **39** **32** **31a** **40**

Scheme 14

In summary, we have demonstrated that CALB and IM-catalyzed alcoholysis serves as an efficient methodology for the preparation of some optically pure hydroxyalkanephosphonates, which provide convenient access to both enantiomers of phosphocarnitine, phosphogabob and fosfomycin.

Experimental

General

IR spectra were recorded on a Shimadzu IR-440 spectrometer. EI mass spectra (MS) were run on a HP-5989A mass spectrometer. ^{1}H NMR spectra were recorded on a Bruker AMX-330 (300 MHz) spectrometer in $CDCl_3$ and chemical shifts were reported in ppm downfield relative to TMS (internal standard); ^{31}P NMR spectra were taken on the same spectrometer using 80% phosphorus acid as external standard. Optical rotations were measured on a Perkin-Elmer 241MC polarimeter. The specific optical rotations were measured in units of 10^{-1} deg cm^2 g^{-1}. The melting point was not corrected. *Candida rugosa* lipase (901 units mg^{-1}) was purchased from Sigma Chemical Co. The enantiomeric excess value was mainly determined by ^{31}P NMR and measurement of the solution made from 20 mg hydroxyalkanephosphonate and 1.5 equivalents quinine in 0.5 cm^3 $CDCl_3$.

(*S*)-Diethyl 1-acetyloxy-2-chloroethanephosphonate (10). To anhydrous diisopropyl ether (2 cm^3) was added **8** (100 mg),[21] vinyl acetate (0.5 cm^3) and IM (100 mg). The mixture was stirred at 30 °C for one week. The enzyme was filtered off and washed with acetone (4 cm^3). The solvent was removed under reduced pressure and the residue was subjected to flash column chromatography to furnish **10** (23 mg, 20%) as a colorless oil. $[\alpha]_D^{18}$ + 11.7 (*c* 0.9, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$):δ 5.43–5.51 (1H, m), 4.11–4.22 (4H, m), 3.75–3.94 (2H, m), 2.18 (3H, s,), 1.29–1.37 (6H, m); IR (neat): 2987, 1758, 1372, 1263, 1217, 1025, 976 cm^{-1}; (EI) *m*/*z*: 259(M$^+$ + 1) (23.13), 213 (5.65), 181 (21.30), 152 (27.98), 138 (100), 111 (49.47), 82 (23.42), 43 (47.10). Anal. calcd for $C_8H_{16}ClO_5P$: C, 37.15; H, 6.24; found: C, 37.47; H, 6.51.

(*S*)-Diethyl 1-hydroxy-2-chloroethanephosphonate (13). To anhydrous toluene (2 cm^3) was added **11** (prepared by butyrylation of **10** with *n*-PrCOOH, DCC, DMAP[22]) (1 mmol), *n*-butanol (0.3 cm^3) and IM (100 mg). The mixture was stirred at 30 °C for 40 hours. The enzyme was filtered and washed with acetone (3 cm^3). After romoval of the solvent, the concentrated oil was subjected directly to flash chromatography to furnish **13** (95 mg, 44%, ee > 95%) as a colorless oil. $[\alpha]_D^{18}$ +15.4 (*c* 0.8, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.81(1H, br s), 4.04–4.17 (4H, m), 3.66–3.81 (2H, m), 1.31 (6H, t, *J* = 7.1 Hz); IR

(film): 3285, 2986, 1243, 1211, 1093, 1051, 1026, 974 cm^{-1}; (EI) *m*/*z*: 217 (M^+ + 1), 189, 138, 111, 82, 81; Anal. calcd for $C_6H_{14}ClO_4P$: C, 33.27; H, 6.51; found: C, 33.02; H, 6.71.

16a–d Were synthesized in the same manner, *i.e.*, alcoholysis of the butyryloxy derivatives of the 1-hydroxyalkanephosphonates.[22]

(*S*)-Diethyl-1-hydroxyethanephosphonate (16a).[6a] (41% Yield); $[\alpha]_D^{25}$ −4.6 (*c* 1.5, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ4.10–4.17 (4H, m), 4.00 (1H, dq, *J* = 3.5 Hz, 7.1 Hz), 3.98 (1H, s), 1.40 (3H, dd, *J* = 7.1 Hz, 17.5Hz), 1.28–1.33 (6H, m); ^{31}P NMR (120 MHz, $CDCl_3$) δ26.6; IR (film) 3317, 2984, 1220, 1053, 1029 cm^{-1}; (EI) *m*/*z*: 183 (M^+ + 1), 165, 155, 43, 138, 111, 82, 45.

(*S*)-Diethyl-1-hydroxypropanephosphonate (16b).[6a] ^1H NMR (300 MHz, $CDCl_3$) δ4.08–4.23 (5H, m), 3.66 (1H, br s), 1.96 (2H, dd, *J* = 6.3 Hz, 17.1 Hz), 1.36–1.38 (6H, m), 1.31 (3H, dd, *J* = 2.4 Hz, 5.9Hz); ^{31}P NMR (120 MHz, $CDCl_3$) δ30.4; IR (film): 3399, 2983, 1234, 1048 cm^{-1}; (EI) *m*/*z*: 197 (M^+ + 1), 179.

(*S*)-Diisopropyl-1-hydroxypropanephosphonate (16c).[23] ^1H NMR (300 MHz, $CDCl_3$): δ 4.68–4.79 (2H, m), 3.64–3.70 (2H, m), 1.64–1.80 (2H, m), 1.23–1.33 (12H, m), 1.05 (3H, t, *J* = 7.4Hz); ^{31}P NMR (120 MHz, $CDCl_3$) δ 24.9; IR (film): 3307, 2981, 1214, 1012, 991cm^{-1}; (EI) *m*/*z*: 225 (M + 1) (100), 183 (60.66), 141 (48.58), 124 (73.85), 109 (26.92), 82 (57.48), 59 (33.19).

(*S*)-Diisopropyl-1-hydroxy-2-propenylphosphonate (16d).[6a] ^1H NMR (300 MHz, $CDCl_3$):δ5.27–6.01 (3H, H of vinyl), 4.71–4.79 (2H, m), 4.43 (1H, dd, *J* = 5.4 Hz, 13.3 Hz), 3.96 (1H, br s), 1.26–1.44 (12H, m); ^{31}P NMR (120 MHz, $CDCl_3$) δ 20.7; IR (film): 3297, 2970, 1645, 1240 cm^{-1}; (EI) *m*/*z*: 223 (M^+ + 1), 193, 181, 165, 139, 109, 43.

IM-catalyzed alcoholysis for the preparation of (*R*)-3-chloro-2-hydroxy propanephosphonate (19c).[3d] To anhydrous benzene (1.5 cm^3) was added 1 mmol racemic **17c** (prepared by reaction with $ClCH_2COOH$, DCC, DMAP[22])(1 mmol), *n*-butanol (0.3 cm^3) and IM (*mucor miehei* lipase) (100 mg). The reaction mixture was stirred at 30 °C, and IM was filtered off. After removal of the solvent, the residue was subjected to flash chromatography to provide**19c** as a colorless oil (93% ee). $[\alpha]_D^{25}$ −13.3 (*c* 3.0, $CHCl_3$) ^1H NMR (300 MHz, $CDCl_3$) δ 4.14–4.31 (5H, m), 3.85 (1H, br s), 3.61 (d, *J* = 2H, 5.2Hz), 2.04–2.17 (2H, m), 1.25–1.36 (6H, m); ^{31}P NMR (120 MHz,$CDCl_3$) δ 29.2; IR (film): 3384, 2965, 1220, 1025 cm^{-1}; (EI) *m*/*z*: 231 (M^+ + 1), 181, 139, 125, 111, 93.

(*R*)-Diethyl 3-chloro-2-chloroacetyloxypropanephosphonate (18c). $[\alpha]_D^{25}$ + 4.1 (*c* 1.05, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ5.41–5.45 (1H, C*H*CH_2P), 4.11–4.21 (6H, m), 3.75–3.89 (2H, m), 2.23–2.34 (2H, m), 1.34 (6H, t, *J* = 7.2 Hz); IR (film):2987, 1743, 1372, 1240, 1026, 965, 826 cm^{-1}; EIMS (*m*/*z*): 279 (12.35), 213 (70.05), 195 (74.48), 185 (75.38), 177 (99.85), 157 (100), 121 (58.42), 81 (42.60); Anal. calcd for $C_9H_{17}Cl_2O_5P$: C, 35.20; H, 5.58; found: C, 35.26; H, 5.83.

(*S*)-3-Chloro-2-hydroxypropanephosphonate (4).[3d] To methanol (1 cm^3) was added **18c** (100 mg) and K_2CO_3 (10 mg) and the reaction mixture was stirred at room temperature until **18c** disappeared as monitored by TLC. Filter off K_2CO_3 through a short colum padded with silica. Direct flash chromatography of the concentrated oil furnished **4** as a pale yellow oil (85%). $[\alpha]_D^{25}$ −10.7 (*c* 1.8, $CHCl_3$).

Phosphocarnitine (2).[11] To **4** (200 mg) was added aq. Me_3N (30%, 3 cm^3) and methanol (5 cm^3). The reaction mixture was refluxed overnight. After removal of the solvent, the residue (**20**) was treated with Me_3BrSi (1 cm^3) in CH_2Cl_2 (3 cm^3) overnight. After removal of CH_2Cl_2 under reduced pressure, methanol (2 cm^3) was added to the residue and the mixture was stirred for 2 h. The solvent was removed under reduced pressure and the residue was subjected to a Dowex ion exchange column to furnish **2** as a white solid. $[\alpha]_D^{25}$ −15.1 (*c* 2.1, H_2O) ^1H NMR (300 MHz, CD_3OD) δ 4.17 (1H, C*H*OH), 3.54–3.73 (2H, m, CH_2N), 3.34 (9H, s), 1.72–2.01 (2H, m); ^{31}P NMR (120 MHz, $CDCl_3$) δ 21.7; IR (film): 3379, 2701, 1653, 1129, 1057, 987, 923cm^{-1}; MS(CI): 234.1 (M + 1), 201.2, 199.1 (M + 1 − Cl).

Diethyl 3-azido-2-hydroxypropanephosphonate (26).[3d] To ethanol (40 cm^3) was added **23**[3d] (1 mmol), $NaBH_4$ (0.7 mmol) was slowly added at 0 °C and the mixture was then warmed up to rt and stirred for another hour. After the usual work up, **26** (216 mg, 91%) was obtained as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$) δ 4.10–4.31 (5H, m), 3.30–3.40 (2H, m), 1.97–2.10 (2H, m), 1.31–1.37 (6H, m); ^{31}P NMR (120 MHz, $CDCl_3$) δ 29.6; IR (film): 3350, 2985, 2104, 1223, 1050, 1028, 966 cm^{-1}; (EI) *m*/*z*: 238 (M^+ + 1) (1.18), 181 (57.37), 153 (22.15), 125 (100), 107 (29.52).

Diethyl 3-azido-2-chloroacetyloxypropanephosphonate 27. To DCC (1.2 mmol) and $ClCH_2COOH$ (1.2 mmol) in CH_2Cl_2 (40 cm^3) was added **26** (1 mmol) and DMAP (20 mg), the reaction mixture was stirred at room temperature overnight. After removal of the precipitate, ether (20 cm^3) was added. After filtration, the solvent was removed under reduced pressure. The residue was subjected to flash chromatography to afford **27** (290 mg, 92%) as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$) δ 5.31–5.35 (1H, m), 4.08–4.18 (m, 6H), 3.52–3.68 (2H, m), 2.21 (2H, dd, *J* = 6.9 Hz, 19.35 Hz), 1.35 (3H, t, *J* = 7.5Hz); ^{31}P NMR (120 MHz, $CDCl_3$) δ 27.46; IR (film): 2986, 2110, 1766, 1253, 1166, 1052, 1025, 966 cm^{-1}; (EI) *m*/*z*: 314 (M + 1) (9.46), 213 (14.06), 181 (83.95), 153 (45.52), 125 (100), 109 (28.49). Anal. calcd for $C_9H_{17}ClN_3O_5P$: C, 34.46; H, 5.47; N, 13.40; found: C, 34.81; H, 5.83; N, 13.81. The procedure of IM-catalyzed alcoholysis of **27** is as above.

(*S*)-Diethyl 3-azido-2-chloroacetyloxypropanephosphonate 28. (Yield 43%) as a colorless oil; ee = 93%, $[\alpha]_D^{18}$ −3.3 (*c* 0.95, $CHCl_3$).

(*R*)-Diethyl 3-azido-2-hydroxypropanephosphonate 29. (Yield 44%) as a colorless oil, ee = 90%, $[\alpha]_D^{17}$ −6.8 (*c* 1.75, $CHCl_3$).

(*S*)-Diethyl 3-azido-2-hydroxypropanephosphonate 24. To methanol (1 cm^3) was added **29** (100 mg) and K_2CO_3 (10 mg) and the reaction mixture was stirred at room temperature until **29** was consumed (monitored by TLC). K_2CO_3 was filtered off through a short colum padded with silica. Direct flash chromatography of the concentrated oil furnished **24** as a colorless oil (64 mg, 85%). Phsophogabob was prepared according to reference,[3e] and the data was identical to that reported. $[\alpha]_D^{17}$ +15.2 (*c* 1.3, H_2O). CALB-catalyzed kinetic resolution of **27** was in a similar manner. The baker's yeast-mediated bioreduction of **23** was reported before.[3d] The other procedure for the synthesis of **22** is the same as above.

Diethyl 1-chloro-2-hydroxypropanephosphonate 33. To anhydrous methanol (100 cm^3) was added **34** (4.65 g, 20 mmol) and $CaCl_2$ (2.22 g, 20 mmol),the reaction mixture was stirred for half of an hour to dissolve $CaCl_2$. $NaBH_4$ (15 mmol) was slowly added at 0 °C. The reaction mixture was slowly warmed to room temperature and was stirred overnight. Work-up gave **33** as a colorless oil (3.68g, 80%). ^1H NMR (300 MHz, $CDCl_3$) δ 4.20–4.34 (5H, m), 3.87–3.92 (1H, dd, *J* = 2.4 Hz, *J* = 12.4 Hz), 3.04 (1H, br s), 1.37–1.43 (9H, m); ^{31}P NMR (120 MHz, $CDCl_3$) δ 19.7, 19.5 (6.3 : 1). IR (film): 3391, 2985, 1248, 1026

cm^{-1}; (EI) *m*/*z* :233 (M^+ + 3) (33.09), 232 (M^+ + 2) (17.30), 231 (M^+ + 1) (100), 186 (14.32), 159 (18.16), 130 (16.52), 45 (15.24); Anal. calcd for $C_7H_{16}ClO_4P$: C, 36.40; H, 6.99; found: C, 35.78; H, 6.86.

Diethyl 1-chloro-2-chloroacetyloxypropanephosphonate 36. The procedure for the preparation of **36** is similar to that of **27**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.58–5.62 (C*H*CHP), 4.17–4.30 (4H, m), 4.14 (2H, d, J = 2.7 Hz, ClCH_2), 3.93–3.99 (1H, dd, J = 3.6 Hz, 14.4 Hz), 1.48 (3H, d, J = 6.9 Hz), 1.38 (5H, t, J = 7.2 Hz), 1.28 (1H, t, J = 7.2 Hz); ^{31}P NMR (120 MHz, $CDCl_3$) δ 14.3; IR (film): 2987, 1763, 1308, 1162, 1185, 1134, 1096, 1050, 1024, 969 cm^{-1}; (EI) *m*/*z*: 307 (M + 1) (1.73), 213 (34.46), 213 (14.75), 186 (85.69), 177 (22.70), 155 (100), 109 (72.07), 81 (42.19); Anal. calcd for $C_9H_{17}Cl_2O_5P$: C, 35.20; H, 5.58. Found: C, 35.10; H, 5.83.

CALB-catalyzed alcoholysis of 36. To anhydrous benzene (3 cm^3) was added **36** (614 mg, 2 mmol) and *n*-butanol (1 cm^3) and CALB (200 mg) or IM (150 mg). The reaction mixture was stirred at 30 °C. After approx. 50–60 h, the enzyme was filtered off and washed with ethyl acetate (5 cm^3). Direct flash chromatography afforded:

(1*R*,2*S*)-Diethyl 1-chloro-2-chloroacetyloxypropanephosphonate 37. (275 mg, 45%), ee > 95%. $[a]_D^{20}$ −25.2 (*c* 1.3, $CHCl_3$).

(1*S*,2*R*)-Diethyl 1-chloro-2-hydroxypropanephosphonate 38. (202 mg, 44%). ee > 95%. $[a]_D^{20}$ +2.7 (*c* 3.1, $CHCl_3$).

(1*R*,2*S*)-Diethyl 1-chloro-2-hydroxypropanephosphonate 32. To methanol (1 cm^3) was added **37** (100 mg) and K_2CO_3 (5 mg) and the reaction mixture was stirred at room temperature until **37** was consumed (monitored by TLC). Filter off K_2CO_3 through a short colum padded with silica. Direct flash chromatography of the concentrated oil furnished **32** as a colorless oil (90%). $[a]_D^{20}$ −2.6 (*c* 3, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.40 (5H, m), 3.87 (2H, dd, $^3J_{H-H}$ = 2.4 Hz, $^3J_{P-H}$ = 13 Hz), 3.05 (1H, OH), 1.40 (6H, t, J = 7.9 Hz). IR(film): 3391, 2985, 1248, 1026, 975, 795 cm^{-1}. ^{31}P NMR (120 MHz, $CDCl_3$) δ 20.0; (EI) *m*/*z*: 231(M^+, 100), 213 (6.48), 186 (10.37), 159 (14.48), 130 (16.81), 109 (4.61), 45 (22.62). Anal. calcd. for $C_7H_{16}ClO_4P$: C, 36.46; H, 6.99; found C, 36.73; H, 7.28.

(1*R*,2*S*)-1-Chloro-2-hydroxypropanephosphonic acid 31a.[19] **32** (100 mg) was treated with Me_3BrSi (0.5 cm^3) in CH_2Cl_2 (3 cm^3) overnight. After removal of CH_2Cl_2 under reduced pressure, methanol (2 cm^3) was added to the residue and the mixture was stirred for 2 h. The solvent was removed under reduced pressure and the residue was subjected to a Dowex ion exchange column to furnish **31a** as a white solid (65 mg, 87%). ^{1}H NMR (300 MHz, D_2O) δ 4.04–4.13 (1H, m, CHOH), 3.67–3.77 (1H, dd, J = 3.3 Hz, 9 Hz, CHP), 1.09–1.11 (3H, m, CH_3); ^{31}P NMR (120 MHz, D_2O) δ 17.2; IR (film): 3337, 1009 cm^{-1}; (EI) *m*/*z*: 175 (M^+ + 1) (11.71), 157 (13.77), 130 (40.20), 76 (58.52), 41 (100).

Fosfomycin

31a (90 mg) was added to 10 M aqueous sodium hydroxide (1 cm^3). At complete conversion, the pH of the solution was adjusted to 9–10 with aqueous HCl. The mixture was concentrated under reduced pressure, methanol (5 cm^3) was added to the residue and the mixture was stirred and filtered and the solvent was removed under reduced pressure. The fosfomycin, disodium salt (49 mg, 53%) was obtained. $[a]_D^{20}{}_{365}$ −16.8 (*c* 2, H_2O), (lit.[17d] $[a]_D^{20}$ −19.0 for fosfomycin, disodium salt); ^{1}H NMR (300 MHz, D_2O) δ 1.29 (3H, d, J = 6 Hz, CH_3), 2.64 (1H, dd, J = 6, 18 Hz, C*H*P), 3.08 (1H, m, C*H*CH_3); ^{31}P NMR (120 MHz, D_2O) δ 10.3; ^{13}C NMR (D_2O) δ 13.67 (s, *C*H_3), 54.60(d, J = 1.8 Hz, *C*HCH_3), 55.04 (d, J = 175 Hz, *C*HP).

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