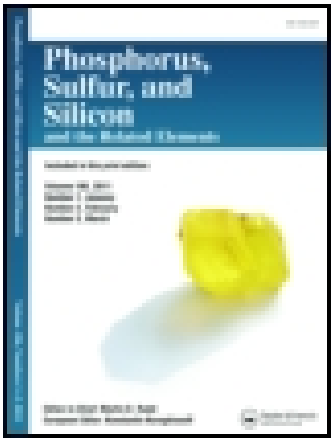

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ENZYMES IN ORGANIC CHEMISTRY, 8.[11] PROTEASE-CATALYZED KINETIC RESOLUTION OF α-CHLOROACETOXYPHOSPHONATES IN A BIPHASIC SYSTEM

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ENZYMES IN ORGANIC CHEMISTRY, 8.[1] PROTEASE-CATALYZED KINETIC RESOLUTION OF α-CHLOROACETOXYPHOSPHONATES IN A BIPHASIC SYSTEM

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Protease Chirazyme® P-2 hydrolyses racemic α-chloroacetoxyphosphonates (±)-**1** enantioselectively to furnish α-hydroxyphosphonates (*R*)-(–)-**2** with ee's ranging from 31 to 97% at a conversion of 45% and unreacted esters (*S*)-(+)-**1**.

Keywords: α-hydroxyphosphonates/chiral, nonracemic; α-chloroacetoxyphosphonates/enantioselective hydrolysis; proteases; Mosher esters; subtilisin; kinetic resolution

INTRODUCTION

In recent years enzyme-catalyzed reactions have found an astonishingly broad application for the preparation of chiral, nonracemic compounds in organic chemistry.[2] Especially lipases and proteases are most widely used to hydrolyse esters and amides enantioselectively on a laboratory and on an industrial scale. α-Hydroxyphosphonates are base-labile alcohols, which are easily prepared as racemates by the Abramov reaction.[3] The synthesis of chiral, nonracemic α-hydroxyphosphonates[4] has become an important area of research, particularily in connection with the search for

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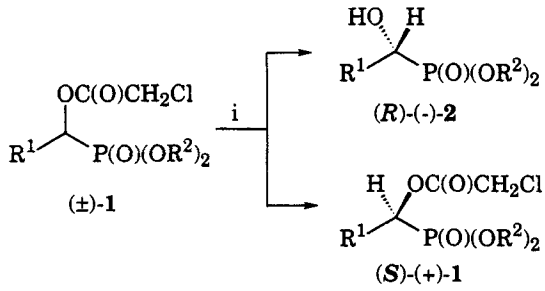
biologically active surrogates[5] for the corresponding carboxylic acids and phosphoric acid esters. α-Hydroxyphosphonates are starting materials for other α-substituted phosphonates and phosphonic acids, noteably α-aminophosphonic acids.[6]

We have found that α-acetoxy- and α-chloroacetoxyphosphonates are resolved kinetically by lipases AP 6 (from *Aspergillus niger)* and FAP 15 (from *Rhizopus oryzae*) with good to high enantiomeric excesses depending on the protecting groups at phosphorus, the acyl (acetyl or chloroacetyl) and the alkyl group of the underlying aldehyde.[7] Both enzymes hydrolyse the *(S)* esters preferentially and lipase AP 6 has the much broader substrate specificity.

RESULTS AND DISCUSSION

In an effort to find *(R)* selective hydrolytic enzymes, we tested commercially available proteases. Preliminary experiments showed that racemic diisopropyl 1-acetoxyethylphosphonate was not hydrolysed in a biphasic system by proteases B, M, N, and S from Amano, but only very slowly by protease Chirazyme® P-2 (an alcaline endoprotease of serine type). This last enzyme accepted also racemic diisopropyl 1-acetoxy-(2-thienyl)methylphosphonate as substrate. 120 mg of enzyme were sufficient to hydrolyse 45% of 1 mmol of substrate in 16 h at room temp. and the ee of the isolated α-hydroxyphosphonate having *(R)* configuration was 71%. These two substrates are easily transformed by lipases AP 6 and FAP 15 giving *(S)* α-hydroxyphosphonates.[7,8] This result was encouraging and we decided to study the more reactive chloroacetates (±)-**1** of representative α-hydroxyphosphonates, as the low reaction rate of the two compounds was at least in part attributed to the acetate group. All chloroacetates, except (±)-**1b** prepared by esterification of diethyl 1-hydroxyhexylphosphonate using chloroacetic anhydride/pyridine,[7] are known compounds.

The protease was applied in the same way as the lipases (Scheme 1).[7] Briefly, 1 mmol of (±)-**1** was hydrolysed at constant pH 7.0 at room temp. in a vigorously stirred biphasic system consisting of a sterile phosphate buffer, *t*-butyl methyl ether, and hexanes with an appropriate amount of enzyme (Scheme 1, Table I). At a conversion of 45% (by consumption of base) 1 M HCl was added to bring pH to 4.0. Extractive workup and flash



Reagents and conditions: i, 1 mmol (±)-**1**, 17 ml phosphate buffer (50 mM), 2 ml *t*-BuOMe, 2 ml hexanes, protease, room temp., pH 7.0.

1,2	R^1	R^2
a	C_5H_{11}	*i*-Pr
b	C_5H_{11}	Et
c	C_9H_{19}	Et
d	$(CH_3)_2CH$	*i*-Pr
e	$(CH_3)_2CHCH_2$	*i*-Pr
f	$(CH_3)_3C$	*i*-Pr
g	*c*-C_4H_7	*i*-Pr
h	*c*-C_6H_{11}	*i*-Pr
i	*c*-C_7H_{13}	*i*-Pr
j	*c*-$C_6H_{11}CH_2CH_2$	Et
k	$PhCH_2$	*i*-Pr

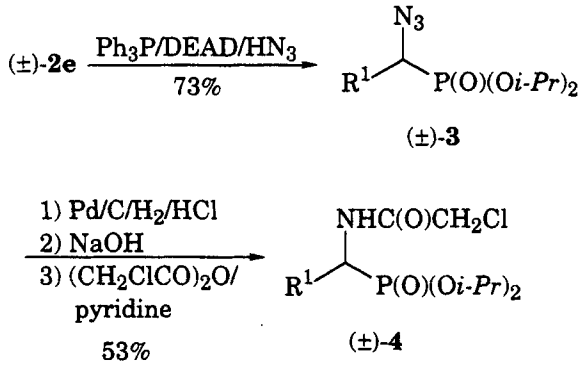
SCHEME 1 Protease-catalyzed hydrolysis of 1-chloroacetoxyphosphonates (±)-**1**

chromatography furnished **1** and **2**. The ee and absolute configuration of (-)-**2** were determined by NMR spectroscopy of the Mosher ester. Chloroacetates (+)-**1** can be hydrolysed without racemisation under mild conditions (Et_3N/MeOH) if desired. The data of the twelve experiments and for comparison also the data from the literature[7] obtained with lipase AP 6 for the same substrates are compiled in Table I.

Protease Chirazyme® P-2 hydrolysed in all cases tested preferentially the *(R)* enantiomer, lipase AP 6 the opposite enantiomer, except for **1k** (entries 1 – 11). Increasing the length of the alkyl group R^1 of (±)-**1** from n-pentyl to n-nonyl leads to a decrease in the reaction rate and the ee as with lipase AP 6 (entry 3). Chloroacetates (±)-**1** having branched (isopropyl, isobutyl, *t*-butyl, entries 4–6) or cyclic (cyclobutyl, cylcohexyl, entries

7 and 8) residues for R^1 are hydrolysed with excellent enantioselectivity ranging from 85–97% by protease Chirazyme® P-2 compared to lipase AP 6, which does not accept (±)-**1f** as substrate. The active site of the protease cannot accommodate a cycloheptyl ring as evidenced by (±)-**1i** (entry 9). (±)-**1k** is resolved by both enzymes, but the optical purities of the isolated α-hydroxyphosphonates (-)-**2** are just 48 and 52%, respectively (entry 11). The latter substrate was also hydrolysed by cross linked microcrystalline subtilisin[9] (ChiroCLEC™-BL) in the phosphate buffer without organic solvent (entry 12). The results are very similar to those obtained with protease Chirazyme® P-2.

Chloroacetoxyphosphonate (±)-**1e** is an excellent substrate for protease Chirazyme® P-2. Finally, the corresponding α-chloroacetamidophosphonate (±)-**4** was prepared according to Scheme 2. The α-hydroxyphosphonate[11] (±)-**2e** was transformed[12] into azide (±)-**3**, which was reduced catalytically to the amine and acylated to give α-chloroacetamidophosphonate (±)-**4**. It was not hydrolysed by Chirazyme® P-2, even if 216 mg of enzyme were used for 1 mmol of substrate at a reaction time of 24 h.



SCHEME 2 Synthesis of chloroacetamide (±)-**4**

In summary, α-choroacetoxyphosphonates (±)-**1** are resolved by protease Chirazyme® P-2 in a biphasic system at pH 7.0 to afford (*R*) configurated α-hydroxyphosphonates (-)-**2** with an ee of up to 97%. The best results in terms of enantiomeric excess are obtained, if R^1 of (±)-**1** is a short straight chain alkyl group, a short branched or a cyclic (with up to six carbon atoms) alkyl group.

TABLE I Data for protease-catalyzed hydrolysis of (±)-**1**

Entry	*Substrate*	*Enzyme*[a] *(mg)*	*Temp. (°C)*	*Time (h); Conv.*[b] *(%)*	*Produced alcohol 2*			*Recovered ester 1*			*Alcohol produced with AP 6*			
					Yield (%)	*Ee*[c] *(%)*	$[\alpha]_D^d$ *(c)*	*E*[e]	$[\alpha]_D^d$ *(c)*	*Yield (%)*	*AP 6 (mg)*	*E*[e]	*Time (h)*	*Ee (%)*
1	**1a**	53	22	18.1; 45/45	35	83 (84)	-14.2 (0.9)	24	+18.6 (1.6)	38	57	48	3	92
2	**1b**	52	22	3.4; 45/46	37	70 (70)	-14.5 (1.0)	10	+18.0 (1.0)	44	41	9	16	69[f]
3	**1c**	163	21	69.6; 44/42	33	32 (31)	-4.8 (1.0)	2	+5.9 (3.1)	47	163	2	70	63
4	**1d**	61	23	14.0; 45/47	37	84 (85)	-9.0 (1.4)	28	+20.6 (2.2)	49	106	8	6	64
5	**1e**	68	24	3.3; 45/46	37	96 (96)	-23.9 (1.9)	125	+30.9 (2.4)	50	58	3	20	35
6	**1f**	162	29	50.3; 43/32	25	98 (97)	-4.3 (1.1)	103	+9.1 (2.3)	54	no reaction			
7	**1g**	5	23	7.4; 45/47	32	92 (92)	-18.6 (1.0)	61	+14.9 (2.2)	40	26	14	10	75
8	**1h**	144	22	44.5; 43/47	40	95 (94)	-8.8 (1.1)	85	+8.6 (1.1)	45	28	29	14	86
9	**1i**	70	23	17.4; 0	not worked up						110	14	20	75
10	**1j**	172	20	44.2; 45/49	38	39 (40)	-6.6 (1.6)	3	+7.1 (2.0)	41	47	20	4	82
11	**1k**	40	22	21.0; 45/-	36	50 (48)	-16.2 (1.2)	4	+17.4 (1.2)	42	32	5	19	52[g]
12	**1k**	[h]	19	35.5; 45/48	43	44[i]	-14.8 (1.0)	4	+17.6 (1.3)	47	32	5	19	52[g]

[a] Enzyme for entries 1–11: protease Chirazyme® P-2; for entry 12: ChiroCLEC™-BL. [b] Conv. = conversion determined from 0.5 M NaOH consumed / conversion by ^{1}H NMR. [c] Ee by ^{1}H NMR (by ^{31}P NMR). [d] In acetone solution at 20 °C; concentration was rounded to the nearest tenth. [e] Enantiomeric ratio[10] calculated from the conversion (^{1}H NMR) and the ee (^{31}P NMR) of alcohol **2**. [f] The data with AP 6 were obtained for the acetate. [g] *(R)*alcohol. [h] 0.4 ml of aqueous suspension of ChiroCLEC™-BL; the enzyme was removed by filtration for workup after bringing pH to 4.0. [i] Calculated from value of entry 11.

EXPERIMENTAL

For general remarks see ref. 1

(±)-Diisopropyl 1-azido-3-methylbutylphosphonate [(±)-3]

2.52 g (10 mmol) of hydroxyphosphonate[11] (±)-**2e** were transformed into azide (±)-**3** by a literature procedure[12], using a 0.91 M solution of HN_3 in toluene. The crude product obtained after removal of diethyl hydrazodicarboxylate was purified by flash chromatography (petroleum ether : acetone = 5 : 1; $R_f = 0.35$) to furnish 2.02 g (73%) of azide (±)-**3** as a viscous oil.

IR (Si): $\nu = 2980\ cm^{-1}$, 2112, 1387, 1261, 1106, 989. – ^1H NMR (400.13 MHz, $CDCl_3$); δ = 0.90 [d, J = 6.9 Hz, 3H, $CH(CH_3)_2$], 0.95 [d, J = 6.7 Hz, 3H, $CH(CH_3)_2$], 1.33 [d, J = 6.4 Hz, 12H, $CH(CH_3)_2$], 1.60 (m, 2H, CH_2), 1.82 (m, 1H, CH), 3.34 (ddd, J= 3.4, 11.8, 12.8 Hz, 1H, CHP), 4.75 (m, 2H, OCH). – ^{13}C NMR (100.61 MHz, $CDCl_3$): δ = 20.85 (CH_3), 23.15 (CH_3), 23.93 and 23.97 [2xd, J_{PC} = 4.2 Hz, $OCH(CH_3)_2$], 24.11 [d, J_{PC} = 3.4 Hz, $OCH(CH_3)_2$], 24.14 [d, J_{PC} = 3.6 Hz, $OCH(CH_3)_2$], 25.04 [d, J_{PC} = 13.6 Hz, $CH(CH_3)_2$], 36.80 (CH_2), 55.83 (d, J_{PC} = 156.7 Hz, CHP), 71.59 (d, J_{PC} = 7.2 Hz. OCH), 71.72 (d, J_{PC} = 7.3 Hz, OCH). – $C_{11}H_{24}N_3O_3P$ (277.30); calcd. C 47.64, H 8.72, N 15.15; found C 47.91, H 8.53, N 14.88.

(±)-Diisopropyl 1-chloroacetamido-3-methylbutylphosphonate [(±)-4]

1.98 g (7.14 mmol) of azide (±)-**3** were hydrogenated in a mixture of 60 ml of dry ethanol and 4 ml of concentrated hydrochloric acid on 0.4 g of palladium (10%) on charcoal in a Parr apparatus for 5 hrs at 3.4 atm at room temperature. The catalyst was removed and the solution was concentrated in vacuo. 10 ml of water were added to the residue and the pH was adjusted to 9 – 10 using 2 N NaOH, followed by extraction with chloroform (3 × 20 ml). The combined organic layers were dried with Na_2SO_4 and concentrated in vacuo to give 1.43 g (5.69 mmol) of the α-aminophosphonate as an oil.

A solution of 1.46 g (8.53 mmol) of chloroacetic anhydride in 10 ml of dry dichloromethane was added dropwise to a stirred and cooled (0 °C) solution of 1.43 g (5.69 mmol) of the above α-aminophosphonate and 1.35 g (17.1 mmol, 1.38 ml) of pyridine in 30 ml of dry dichloromethane

under argon. After stirring for two hrs at 0°C (TLC: dichloromethane : ethyl acetate = 5 : 3), 9 ml of water were added and stirring was continued for another 10 min. Then 0.9 ml of concentrated hydrochloric acid were added and the organic phase was removed. The aqueous phase was extraced twice with dichloromethane. The combined organic layers were washed with water and a saturated solution of sodium hydrogen carbonate, dried with $MgSO_4$, and concentrated in vacuo. The residue was crystallised from dichloromethane/hexanes to give 1.25 g (53%, starting from azide) of chloroacetamide (±)-**4**; mp. 92–96 °C.

IR (nujol): ν =3218 cm^{-1}, 3056, 1687, 1556, 1320, 1230, 1159, 1105, 994, 890. – ^1H NMR (400.13 MHz, $CDCl_3$): δ = 0.86 [d, J = 5.9 Hz, 3H, $CH(CH_3)_2$], 0.88 [d, J = 6.4 Hz, 3H, $CH(CH_3)_2$], 1.24, 1.25, 1.26 and 1.27 [4xd, J = 6.4 Hz, each 3H, $OCH(CH_3)_2$], 4.01 (AB-system, J_{AB} = 15.0 Hz, 2H, CH_2Cl), 4.35 (m, 1H, CHP), 4.64 [non, J = 6.4 Hz, 2H, $OCH(CH_3)_2$], 6.64 (d, J = 9.8 Hz, 1H, NH). – ^{13}C NMR (100.61 MHz, $CDCl_3$): δ = 21.17 (CH_3), 23.31 (CH_3), 23.87 [d, J_{PC} = 4.9 Hz, $OCH(CH_3)_2$], 24.04 [d, J_{PC} = 2.5 Hz, $OCH(CH_3)_2$], 24.05 [d, J_{PC} = 3.7 Hz, $OCH(CH_3)_2$], 24.56 [d, J_{PC} = 13.3 Hz; $CH(CH_3)_2$], 38.54 (CH_2), 42.48 (CH_2Cl), 44.94 (d, J_{PC} = 157.7 Hz, CHP), 71.30 (d, J_{PC} = 7.3 Hz, OCH), 71.34 (d, J_{PC} = 6.9 Hz, OCH), 165.37 [d, J_{PC} = 5.2 Hz, CO). – $C_{13}H_{27}ClNO_4P$ (327.79); calcd. C 47.63, H 8.30, N 4.27; found C 47.70, H 8.28, N 4.54.

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