Pergamon

Tetrahedron: *Asymmetry* 9 (1998) 581–587

TETRAHEDRON: *ASYMMETRY*

Enzymatic desymmetrization of prochiral 2,3-bis(acetoxymethyl)bicyclo[2.2.1]hepta-2,5-diene and 2,3-bis(hydroxymethyl)bicyclo[2.2.1]hepta-2,5-diene

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Received 14 November 1997; accepted 22 January 1998

Abstract

Enzymatic desymmetrization of the title compound **1** is reported using various commercially available lipases in hydrolysis and alcoholysis reactions or ester synthesis. In this area, lipase Amano AK (*Pseudomonas* sp.) proved to be the best lipase whatever the experimental conditions used. The monoacetate product **2** is indifferently obtained with more than 95% enantiomeric excess (ee) as the levorotatory enantiomer **2a** or the dextrorotatory one **2b**.

1. Introduction

Recently, we reported the synthesis of a novel polyfunctional bicyclic compound, 2,3-bis-(acetoxymethyl)bicyclo[2.2.1]hepta-2,5-diene **1** and its use in palladium catalyzed elimination.[1] Subtrate **1** may be considered as a useful substrate on the route toward natural products and compounds of pharmaceutical interest such as prostaglandins of the PGF series.[2,3] One of our aims was to use prochiral synthon **1** in order to obtain the chiral bicyclic aldehyde **4**, a potential precursor of β-santalene (Fig. 1).

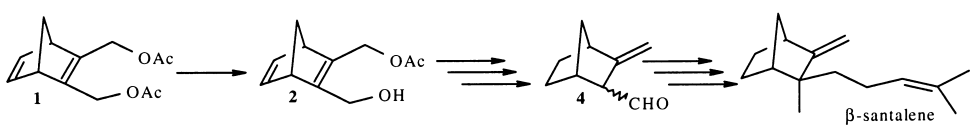

Fig. 1.

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0957-4166/98/$19.00
PII: S0957-4166(98)00027-5

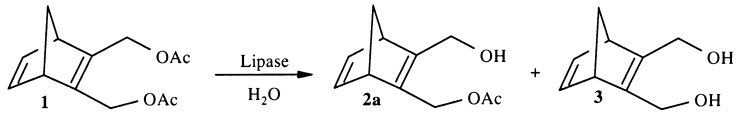

Fig. 2.

The synthesis of chiral aldehyde **4** may be envisioned from precursor **2** resulting from a chiral desymmetrization of prochiral compound **1**. A method of choice for the desymmetrization of prochiral alcohols or esters is the use of lipases and esterases. Numerous examples of such reactions have been described in the literature,[4] especially with substrates possessing a [2.2.1]-bicyclo heptane core.[5] In this work, we describe the enzymatic desymmetrization of substrate **1** using various commercially available lipases in either hydrolytic, alcoholytic or ester synthesis mode.

2. Results

Pure **1** is easily obtained in 65% chemical yield, resulting from a Diels–Alder reaction, by heating a mixture of dicyclopentadiene and 1,4-diacetoxy-2-butyne **2** (5:8 molar ratio) to 260°C for 1.5 h under 35 bar nitrogen pressure in a stainless steel autoclave, followed by two successive distillations.

*2.1. Enzymatic hydrolysis of compound **1***

Various lipases, known to have potential in the desymmetrization of prochiral compounds, have been tested with substrate **1** (Fig. 2 and Table 1).

The reactions were performed at 25°C using a pH stat apparatus maintaining the pH of the borate buffer (1 mM) at pH 7.5 (except for entry 3 where the reaction was performed at pH 6.5) by addition of a 0.1 N NaOH solution. Lipase Amano AK appeared to be the best enzyme tested, since (−)-bicyclo[2.2.1]-(methyleneacetoxy,methylenehydroxy)-2,3-heptadiene-2,5 **2a** was obtained with more than 95% ee in 82% chemical yield (entry 13). Nevertheless, all other enzymes tested gave poor results in terms of enantioselectivity. The influence of the experimental conditions has been investigated in detail in the case of *Mucor miehei* lipase (MML) since it gives the best result in terms of enantioselectivity (entry 1). In order to improve the enantiomeric excess, various water soluble cosolvents have been tested such as acetone, acetonitrile and tetrahydrofuran.[9] Acetone proved to be the best solvent since the enantioselectivity increased from 46 to 75% ee.

*2.2. Enzymatic alcoholysis of compound **1***

When possible, it is preferable to perform enzymatic reactions in organic solvents. Thus, many advantages are associated with such a procedure: better recovery of the products and enzymes by simple filtration, possible reuse of the enzyme and sometimes better stability of the substrate/product of the reaction in organic solvent versus water. For all these reasons, we have undertaken the study of the alcoholysis of **1** with butan-1-ol (Fig. 3) and the same set of enzymes previously used (except PLE and *Aspergillus* lipase). All the reactions were performed at 30°C and stopped after no evolution was encountered in consumption of **1** (Table 2).

Once again, lipase Amano AK proved to be the best lipase (entries 9 and 10), leading to (−)-**2a** with an enantiomeric excess of up to 95% ee, whatever the solvent used (n-hexane or THF). The isolated yields of (−)-**2a** are also excellent, varying respectively from 85% to 90%. The reaction performed with lipase

Table 1
Enzymatic hydrolysis of diacetate **1**

Entry	Enzyme[a]	Solvent system[b]	% of **2a**[d]	ee of **2a**[f] (%)
1	MML	BB	97	46
2	MML	BB/Acetone (75/25)	67(55)[e]	75
3	MML	BB/Acetone (75/25)[c]	45	61
4	MML	BB/CH_3CN (75/25)	98	51
5	MML	BB/Acetone (50/50)	66	55
6	PLE	BB	62	11
7	PLE	BB/THF (95/5)	57	16
8	PLE	BB/CH_3CN (90/10)	64	14
9	PPL	BB	64	40
10	CCL	BB	37	2
11	AL	BB	81	5
12	PFL	BB	52	12
13	**PL**	**BB**	**89(82)**[e]	**>95%**[g]

[a] MML : *Mucor miehei* lipase ; PLE : Pig liver esterase ; PPL : Porcine pancreatic lipase ; CCL : *Candida cylindracea* lipase ; AL : *Aspergillus* lipase ; PFL : *Pseudomonas fluorescens* (Amano P) lipase ; PL : *Pseudomonas sp.* (Amano AK) lipase. [b] BB : Borate Buffer, 1 mM, pH 7.5. [c] BB : Borate Buffer, 1 mM, pH 6.5. [d] Determined by GC on a BP 20 capillary column. [e] Isolated chemical yield of **2a**. [f] Ee determined by ^{19}F NMR on Mosher derivative of **2a** (See ref. 6-7). [g] $[\alpha]^{578}_{25} = -23.5$ (c = 1, CH_2Cl_2).

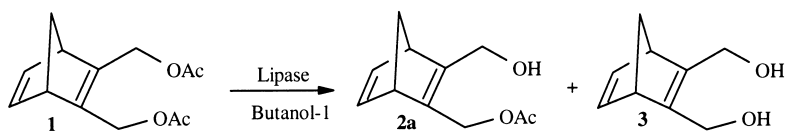

Fig. 3.

MML in a more polar solvent than hexane allowed the ee to be improved from 44% (entry 1) to 57% (entry 4). Subsequent addition of water increased the ee to 61% probing the influence of the polarity of the solvent used on the enantioselectivity. Except for MMLS leading to 75% ee (entry 5), the other tested lipases proved to be poor enantioselective catalysts for this desymmetrization.

*2.3. Esterification of compound **3***

In either hydrolytic or alcoholytic mode, lipase Amano AK afforded the levorotatory enantiomer of compound **2a**. In order to get access to the dextrorotatory enantiomer **2b**, we turned to the ester synthesis mode of lipase action. Thus, due to the microreversibility principle, the dextrorotatory enantiomer **2b** should be obtained from diol **3** (Fig. 4). Various enzymes have been used in various solvents and different irreversible acetyl donor compounds have also been tested (Table 3).

Table 2
Enzymatic alcoholysis of diacetate **1**

Entry	Lipase[a]	Solvent system	Reaction Time (d)	**1 : 2a :3**[b]	ee of **2a**[d] (%)
1	MML	Hexane	4	13 : 87 : 0	44
2	MML	THF	14	65 : 35 : 0	39
3	MML	THF + 250 µL H_2O	5	3 : 69 : 1	61
4	MML	MTBE + 250 µL H_2O	3	25 : 75 : 0	57
5	MMLS	MTBE + 250 µL H_2O	0.25	23 : 67 : 9	75
6	PPL	Hexane	5	25 : 75 : 0	35
7	CCL	Hexane	3	28 : 71 : 1	28
8	PFL	Hexane	3	6 : 93 : 1	2
9	**PL**	**Hexane**	**4**	**2 : 95 : 3**	**>95**[e]
10	**PL**	**THF + 250 µL H_2O**	**3**	**0 : 93 : 7**[c]	**>95**[e]

[a] See Table 1, MMLS : *Mucor miehei* supported lipase . [b] Ratio **1 : 2a : 3** determined by GC on a BP 20 capillary column. [c] Compound **2a** was obtained in 88% isolated yield. [d] Ee determined by ^{19}F NMR on Mosher derivative of **2a** (See ref. 6-7). [e] $[\alpha]^{578}_{25}$ = - 23.5 (*c* = 1, CH_2Cl_2).

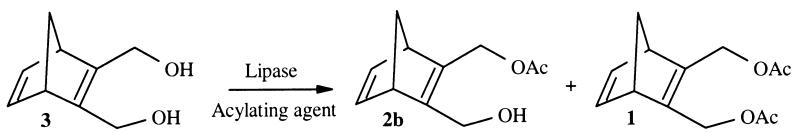

Fig. 4.

Generally speaking, in ester synthesis mode the ees are lower than in the case of hydrolysis or alcoholysis mode, whatever the tested lipase and the experimental conditions, except for lipase Amano AK which led exclusively to the formation of compound **2b** as the dextrorotatory enantiomer in >95% ee (entry 11) and 84% isolated yield.

3. Conclusion

In this study we have shown that the two enantiomers of the monoacetate **2** could be easily obtained through enzymatic desymmetrization of prochiral synthons **1** or **3**. Of all the enzymes tested, lipase Amano AK proved to be an outstanding catalyst. Thus, the two enantiomers **2a** and **2b** were obtained with enantiomeric excesses of up to 95% ee, by a hydrolysis or alcoholysis reaction for **2a** and an irreversible ester synthesis for **2b**, respectively, and in all cases with high chemical yields.

Table 3
Esterification of diol **3**

Entry	Lipase[a]	Acylating agent[b]	Solvent system	Reaction time	**1 : 2b :3**[c]	ee of **2b**[e] (%)
1	PFL	aa	THF	2 h	7 :87 :6	3
2	PFL	tcea	THF/NEt_3 (96/4)	8 h	0 :54 :46	10
3	CCL	tcea	THF/NEt_3 (96/4)	14 d	0 : 48 : 52	15
4	CCL	ippa	Heptane/THF (70/30)	25 h	0 : 39 : 61	14
5	PPL	tcea	THF/NEt_3 (96/4)	6 d	0 : 41 : 59	33
6	PPL	va	Heptane/THF (70/30)	4 d	20 : 64 : 16	4
7	MML	ippa	Heptane/THF (70/30)	1 h	85 : 13 : 2	2
8	MML	ippa	Heptane/THF (70/30)	78 h	0 : 49 : 51	10
9	MML	va	THF (MS 4Å)	2.5 h	46 : 47 : 7	11
10	MML	va	Heptane/THF (70/30)	3 h	26 : 54 : 20	19
11	**PL**	**va**	**Vinyl acetate**	**1 h**	**3 : 89 : 8**[d]	**>95**[f]

[a] See Table 1. [b] aa : acetic anhydride ; tcea : trichloroethyl acetate ; ippa : isopropenyl acetate ; va : vinyl acetate. [c] Ratio **1 : 2b : 3** determined by GC on a BP 20 capillary column. [d] Compound **2b** was obtained in 84% isolated yield. [e] Ee determined by ^{19}F NMR on Mosher derivative of **2b** (See ref. 6-7). [f] $[\alpha]^{578}_{25} = +23.4$ ($c = 1$, CH_2Cl_2).

4. Experimental

THF was distilled from potassium–benzophenone ketyl and degassed thoroughly with dry nitrogen directly before use. ^1H and ^{13}C NMR spectra were recorded in C_6D_6 solution at 100.00 MHz and 25.18 MHz on a Bruker AC100 instrument, respectively, when not indicated otherwise (the usual abreviations are used: s=singlet, d=doublet, t=triplet, q=quadruplet, m=multiplet). The positive chemical shift values are given in ppm. Gas chromatographic analyses were run on a BP 20 capillary column (25 m×0.32 mm; gas vector He, 1.0 bar). Porcine pancreatic lipase (PPL, type II) and Pig liver esterase (PLE) were purchased from Sigma. *Mucor miehei* lipase (MML) was from Biocatalysts. *Aspergillus niger* lipase was from Röhm. *Candida cylindracea* lipase (CCL, *Candida cylindracea*), *Pseudomonas fluorescens* lipase (PFL, Amano P) and *Pseudomonas* sp. lipase (PL, Amano AK) were from Amano pharmaceuticals. *Mucor miehei* supported lipase (MMLS) was from Novo industries.

*4.1. Preparation of 2,3-bis(hydroxymethyl)bicyclo[2.2.1]hepta-2,5-diene **3***

In a two-necked flask equipped with a dropping funnel and a condenser was placed 3.1 g (0.055 mol) of potassium hydroxide in anhydrous methanol (40 mL). A solution of **1** (6.2 g, 0.026 mol) in anhydrous methanol (15 mL) was slowly added at 0°C with vigorous stirring. The mixture was stirred overnight. Removal of the solvent *in vacuo* left an oily residue which was distilled to afford 3.5 g (89% yield) of **3** as a colorless liquid: bp 120°C/0.07 torr; ^1H NMR δ 1.98 (m, 2H), 3.47 (t, 2H), 4.25 (s, 4H), 4.63 (s,

2H), 6.83 (t, 2H); ^{13}C NMR δ 53.2, 59.3, 71.7, 142.8, 149.2; IR (cm^{-1}) 3320 (w), 3060 (s), 2975 (m), 2932 (m), 2861 (m), 1556 (s), 1289 (sh), 988 (m).

*4.2. General procedure for enzymatic hydrolysis of **1***

To 1.7 mmole of **1** (401 mg) placed in a beaker were added 20 mL of a 1 mM borate buffer solution, pH 7.5 and 100 mg of various commercial lipases. The evolution of the reaction was monitored by addition of 0.1 N NaOH solution using a pH stat apparatus (Methröm AG), the stirring being set at 250 rpm. At approximately 80% conversion, the reaction was stopped and the aqueous phase extracted three times with diethyl ether. The organic phases were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The monoacetate was then purified by flash chromatography on silica gel (eluent ethyl acetate:petroleum ether=1:4) to afford the desired product as an oil. ^{1}H NMR δ 1.94 (m, 2H), 2.02 (s, 3H), 3.18 (s, 1H), 3.49 (m, 2H), 4.28 (s, 2H), 4.74 (s, 2H), 6.75 (m, 2H); ^{13}C NMR δ 19.1, 51.7, 51.4, 58.1, 59.4, 70.3, 142.3, 142.5, 152.4, 169.7; IR (cm^{-1}) 3320 (w), 3080 (s), 2980 (m), 2870 (m), 1750 (m), 1380 (s), 1225 (sh), 960 (m).

*4.3. General procedure for enzymatic alcoholysis of **1***

2 mmoles of **1**, 30 mmoles of butan-1-ol and 9 mL of dried n-hexane, THF or MTBE were placed in a screw-cap tube. When using the latter two solvents, 250 μL of water were added at that time, followed by 200 mg of various lipases. The tube was placed in a reciprocal shaker. The evolution of the reaction was monitored by TLC (CH_2Cl_2:diethyl ether=9:1). When the formation of diol **3** was detected, the reaction was stopped by filtration of the enzyme on a plug of Celite. The solvents were evaporated under reduced pressure and the monoacetate was purified as described above.

*4.4. Enzymatic acylation of **3***

In a screw-cap tube were placed 2.5 mmole of **3**, 10 mmole of the acylating agent dissolved in different solvent systems and 100 mg of various lipases. The tubes were placed in a reciprocal shaker. After a classical treatment the monoacetate was purified as described above.

Acknowledgements

We are indebted to Amano Pharmaceutical Co. Ltd for the kind supply of lipases Amano AK and Amano P.

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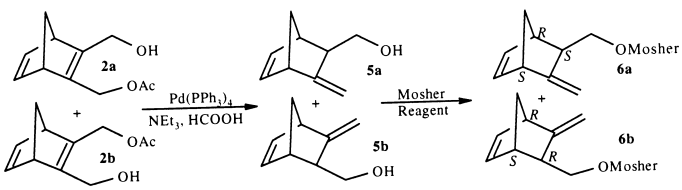
6. The determination of the enantiomeric excess of **2a** or **2b** has been realized by ^{19}F NMR spectroscopy after derivatization using (*R*)-(+)-α-methoxy trifluoromethylphenyl acetic acid chloride as derivatizing agent. In the presence of $Pr(fod)_3$, the spectra show two well-separated singlets at −73.2 and −71.2 ppm. Thus, the enantiomeric purity can be accurately measured and no kinetic resolution has been observed.
7. A racemic mixture of monoacetate **2** submitted to a hydrogenolysis reaction catalyzed by $Pd(PPh_3)_4$ in the presence of an excess of NEt_3 and formic acid led to the formation of compound **5**. Subsequent treatment of **5** by Mosher reagent led to the formation of a mixture of diastereomers **6a** and **6b**. By ^{19}F NMR spectroscopic analysis and according to the results previously described by Oppolzer et al.,[8] it appears that the signals observed at −71.6 ppm and −71.8 ppm correspond to diastereomers **6b** and **6a**, respectively. Moreover, according to this procedure it appears that treatment of a pure solution of levogyre **2** led to the obtention of diastereomer **6a** possessing an (*S*) absolute configuration at the carbon bearing the $CH_2OMosher$ group. A similar study on dextrogyre **2** led to the opposite conclusion (compound **6b** possesses an (*R*) absolute configuration at the carbon bearing the $CH_2OMosher$ group).



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