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Enzymatic desymmetrization of *meso*-2,3-bis(acetoxymethyl) and bis(hydroxymethyl) substituted hexachloronorbornadiene derivatives

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Abstract—*meso*-Bis(acetoxymethyl)-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hepta-2,5-diene and *meso*-bis(hydroxymethyl)-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hepta-2,5-diene were transformed into chiral monoesters with 65–92% ee via enzymatic desymmetrization using various lipases. The absolute configuration was determined by transforming 2-acetoxymethyl-3-hydroxymethyl-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hepta-2,5-diene into the 2-acetoxymethyl-3-hydroxymethylbicyclo[2.2.1]hepta-2,5-diene of known absolute configuration.

1. Introduction

Experimental studies on the syntheses of complex targets have resulted in the development of reactions, which emphasize chemo-, regio- and stereoselectivity.[1] In defining strategies and reactions to construct complex chiral molecules, in particular, high stereoselectivity is required. In a wide variety of asymmetric methods, enzymatic asymmetric hydrolysis has been a powerful method in the development of strategies for the asymmetric synthesis of various natural products.[2] In particular, hydrolase type enzymes are among those enzymes most widely applied in organic synthesis. Desymmetrization of *meso*-diesters using hydrolase type enzymes is an important concept since it potentially affords only one enantiomer in quantitative yield without wasting the other enantiomer.[3]

The optically active norbornadienes are key intermediates in the synthesis of biologically active analogues of the prostaglandin endoperoxides PGH_2 and PGG_2 and β-santalol used by Corey et al.[4] and Ogasawara and co-workers.[5] Evans et al. has studied the synthesis of optically active norbornadienes using α,β-unsaturated *N*-acyloxazolidinones as chiral auxiliaries in Diels–Alder reactions of cyclopentadiene.[6] Yamamoto and co-workers achieved the first enantioselective catalytic Diels–Alder reaction of cyclopentadiene and acetylenic aldehydes.[7] Bis(acetoxymethyl) substituted norbornadiene can be considered as a useful substrate for biologically important compounds such as prostaglandins of the PGF series.[3a,8] In the literature, there are only a few examples of enzymatic resolution of hexachlorinated norbornene derivatives.[9] Recently, we reported the lipase and esterase-catalyzed resolution of (±)-2-hydroxymethylbicyclo[2.2.1]hepta-2,5-diene, (±)-2-hydroxymethyl-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hepta-2,5-diene and (±)-2-acetoxymethylbicyclo[2.2.1]hepta-2,5-diene, (±)-2-acetoxymethyl-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hepta-2,5-diene, respectively.[10] In connection to these biotransformations, during the course of enzymatic desymmetrization of *meso*-bis(acetoxymethyl)-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hepta-2,5-diene **1** and *meso*-bis(hydroxymethyl)-1,4,5,6,7,7-hexachlorobicyclo-[2.2.1]hepta-2,5-diene **3**, screening reactions were first done with various hydrolases (i.e., CCL, PPL, PL, PLE and HLE) using substrate/enzyme ratios from 1:1 to 1:0.5. Among the hydrolases studied, PLE, CCL, PPL and PL proved to be suitable for the desymmetrization of these substrates. In particular, PL showed some interesting enzyme- and substrate-dependant reversals of enantioselectivity with the substrates *meso*-**1** and *meso*-**3**. The observed promising preliminary results prompted us to undertake a thorough catalytic study. PLE-, CCL- and PPL-catalyzed reactions of diester substrate **1** and diol substrate **3** afforded half ester (−)-**2a**. In contrast to this, PL yielded monoester (+)-**2b** with the diol substrate **3** whereas the same enzyme afforded

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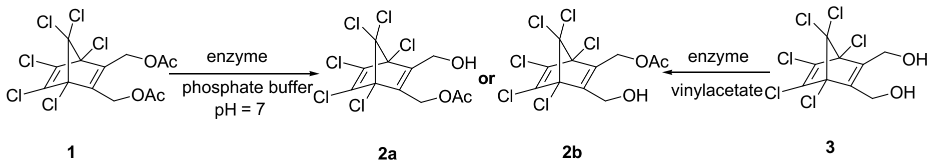


Scheme 1.

monoester (−)-**2a** with diester substrate **1**. CCL in particular, exhibited higher ee's and PL showed unusual versatility and diversity in the enzymatic desymmetrization of the hexachlorinated substrate.

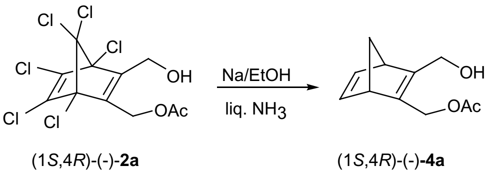
Herein, we describe the highly efficient resolution of the *meso*-substrates **1** and **3** with PLE, CCL, PPL and PL (Scheme 1).

2. Results and discussion

Prochiral **1** was obtained in 85% chemical yield, using a Diels–Alder reaction by heating a mixture of hexachlorocyclopentadiene and 2-butyne-1,4-diol diacetate (1:2 molar ratio) to 150 °C for 8 h in a sealed tube.[11] Hydrolysis of prochiral **1** afforded 97% of the prochiral substrate **3**.[11]

2.1. Enzymatic hydrolysis of prochiral substrate 1

Various lipases were tested with prochiral substrate **1** (Table 1). The first bioconversion was performed using PLE according to the following general procedure. To a stirred solution of **1** (500 mg) in phosphate buffer (pH 7.00, 50 mL), PLE (100 µL) was added in one portion and the reaction mixture was stirred at 20 °C in a pH stat unit. The conversion was monitored by TLC. After 24 h, (−)-2-acetoxymethyl-3-hydroxymethyl-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hepta-2,5-diene **2a** was obtained with 65% ee in 48% chemical yield (entry 1, Table 1). The next attempts involved substrate **1** using CCL, PPL and PL under the same conditions as above. CCL appeared to be the best enzyme tested, since (−)-2-acetoxymethyl-3-hydroxymethyl-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hepta-2,5-diene **2a** was obtained with 92% ee in 81% isolated yield (entry 2). All other enzymes tested afforded **2a** with poor results in terms of enantioselectivity (entries 3 and 4). In order to improve the enantiomeric excess, experimental conditions have been tested in detail using various co-solvents such as isopropanol, MTBE and *n*-butanol in the case of CCL, since it gives the best result in terms of enantioselectivity. No increase in the enantioselectivity was observed (entries 5–7). The absolute configuration of the isolated product (−)-**2a** was determined by transforming it into the corresponding (1*S*,4*R*)-(−)-**4a** by reaction with Na in liquid NH_3 (Scheme 2).[3a]



Scheme 2.

2.2. Esterification of prochiral substrate 3

In prochiral ester hydrolysis studies, all enzymes used afforded the (1*S*,4*R*)-(−)-**2a**. Related to this study, the enzyme catalyzed acetyl transfer to *meso*-diol **3** with CCL, PPL and PL was examined. The bioconversion of *meso*-**3** was performed by CCL according to the following general procedure. To a stirred solution of *meso*-**3** (500 mg) in vinyl acetate (5 mL), CCL (10 mg) was added in one portion and the reaction mixture was shaken at 20 °C. The conversion was monitored by TLC. After 21 h, the products were separated using flash column chromatography and compound (1*S*,4*R*)-(−)-**2a** was isolated with 86% ee in 73% yield (entry 1 in Table 2). PPL gave the same compound (1*S*,4*R*)-(−)-**2a** as above with 68% ee in 65% yield (entry 2). Among the enzymes used in esterification of *meso*-**3**, PL afforded

Table 1. Results of the enzyme-catalyzed hydrolysis of *meso*-**1**

Entry	Substrate	Enzyme	Solvent system	Time (h)	Monoester	Yield (%)[a]	$[\alpha]_D^{20}$	Ee (%)[b]
1	*meso*-**1**	PLE	PB[c]	28	(1*S*,4*R*)-**2a**	48	−16.1	65
2	*meso*-**1**	CCL	PB	23	(1*S*,4*R*)-**2a**	81	−22.8	92
3	*meso*-**1**	PPL	PB	24	(1*S*,4*R*)-**2a**	78	−21.3	86
4	*meso*-**1**	PL	PB	24	(1*S*,4*R*)-**2a**	83	−21.8	88
5	*meso*-**1**	CCL	Isopropanol	20	(1*S*,4*R*)-**2a**	79	−18.8	76
6	*meso*-**1**	CCL	MTBE	21	(1*S*,4*R*)-**2a**	85	−19.5	79
7	*meso*-**1**	CCL	*n*-Butanol	20	(1*S*,4*R*)-**2a**	80	−16.6	67

[a] Yields (%) are given as the isolated half esters.

[b] Enantiomeric excess values are determined by the Chiralcel ODH chiral column HPLC analysis.

[c] PB: phosphate buffer.

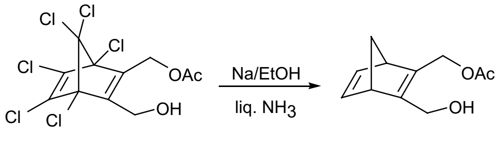
Table 2. Results of enzyme-catalyzed esterification of *meso*-**3**

Entry	Substrate	Enzyme	Solvent system	Time (h)	Monoester	Yield (%)[a]	$[\alpha]_D^{20}$	Ee (%)[b]
1	*meso*-**3**	CCL	Vinyl acetate	21	(1*S*,4*R*)-**2a**	73	−21.3	86
2	*meso*-**3**	PPL	Vinyl acetate	24	(1*S*,4*R*)-**2a**	65	−16.8	68
3	*meso*-**3**	PL	Vinyl acetate	24	(1*R*,4*S*)-**2b**	82	+20.8	84

[a] Yields (%) are given as the isolated half esters.

[b] Enantiomeric excess values are determined by the Chiralcel ODH chiral column HPLC analysis.

(1*R*,4*S*)-(+)-**2b** with 84% ee in 82% yield (entry 3). In order to control the efficiency of the method applied in the absolute configuration determination of (1*S*,4*R*)-(−)-**2a**, the isolated product (+)-**2b** was transformed into the corresponding (1*R*,4*S*)-(+)-**4b** by reaction with Na in liquid NH_3 as above (Scheme 3).[3a]



(1*R*,4*S*)-(+)-**2b** (1*R*,4*S*)-(+)-**4b**

Scheme 3.

3. Conclusion

In this study, we have shown that the two enantiomers of half ester **2a** and **2b** could be obtained through enzymatic desymmetrization of prochiral substrates **1** and **3**. Among the enzymes used in both hydrolysis and esterification conditions, CCL showed the best enantioselectivity. The two enantiomers **2a** and **2b** were obtained only with PL by a hydrolysis and an esterification, respectively. The absolute configuration of both **2a** and **2b** were determined by transforming them into the corresponding nonchlorinated norbornadiene derivatives with Na in liquid NH_3. Commercially available and inexpensive enzymes, in particular, CCL and PL used in catalytic levels, renders the process very attractive for large scale preparations.

4. Experimental

^1H NMR spectra were recorded in $CDCl_3$ on Bruker Spectrospin Avance DPX 400 spectrometer. Chemical shifts are given in ppm downfield from tetramethylsilane. IR spectra were obtained from a Perkin–Elmer Model 1600 series FT-IR spectrometer and are reported in cm^{-1}. Optical rotations were measured in $CHCl_3$ solution in a 1 dm cell using a Bellingham & Stanley P20 polarimeter at 20 °C. Elemental analyses were performed on a LECO 932. PLE (pig liver esterase) and PL (lipase, *Pseudomonas* species) were purchased from Sigma as a suspension in ammonium sulfate solution (3.2 mol/L) and as a powder, respectively. CCL (lipase, Type VII, from *Candida rugosa*), PPL (lipase, Type II, from porcine pancreas) and PFL (lipase, from *Pseudomonas fluorescens*) were purchased from Aldrich.

4.1. Synthesis of *meso*-bis(acetoxymethyl)-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hepta-2,5-diene 1

A mixture of 2-butyne-1,4-diol diacetate (3.40 g, 20 mmol) and hexachlorocyclopentadiene (2.73 g, 10 mmol) containing few crystals of hydroquinone was sealed under vacuum in a thick-walled Pyrex tube. The mixture was heated 8 h at 150 °C. The crude product was purified by flash column chromatography to afford *meso*-**1** (EtOAc/hexane, 1:5) (3.76 g, 85% yield). Mp: 54 °C (lit.,[11] mp: 54–55 °C). ^1H NMR: δ 2.01 (s, 6H, O_2CCH_3), 4.79 (d, 2H, AB system CH_aH_bOAc, $J = 13$ Hz), 4.88 (d, 2H, AB system CH_aH_bOAc, $J = 13$ Hz). ^{13}C NMR: δ 21.4, 57.1, 85.2, 114.1, 137.6, 143.5, 170.3. IR (KBr): 1726, 1680, 750 cm^{-1}. Anal. Calcd for $C_{13}Cl_6H_{10}O_4$ (442.94): C, 35.25; Cl, 48.02; H, 2.28. Found: C, 35.21; Cl, 48.04; H, 2.27.

4.2. Synthesis of *meso*-bis(hydroxymethyl)-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hepta-2,5-diene 3

A mixture of *meso*-**1** (2.00 g, 4.5 mmol), concd HCl (0.25 mL) and methanol (10 mL) was stirred for 3 h at reflux. The reaction vessel was cooled down to 25 °C and diluted with EtOAc (25 mL), extracted with saturated $NaHCO_3$ (3 × 50 mL) and brine (2 × 50 mL), dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by flash column chromatography to afford *meso*-**3** (EtOAc/hexane, 2:3) (1.57 g, 97% yield). Mp: 155 °C (lit.,[11] mp: 155–156 °C). ^1H NMR: δ 2.10–2.15 (br s, 2H, OH), 4.34 (d, 2H, AB system CH_aH_bOH, $J = 14$ Hz), 4.50 (d, 2H, AB system CH_aH_bOH, $J = 14$ Hz). ^{13}C NMR: δ 54.9, 84.7, 112.7, 136.8, 143.2. IR (KBr): 3410, 3425, 1630, 745 cm^{-1}. Anal. Calcd for $C_9Cl_6H_6O_2$ (358.87): C, 30.12; Cl, 59.27; H, 1.69. Found: C, 30.12; Cl, 59.24; H, 1.67.

4.3. General procedure for enzymatic hydrolysis of 1

To a stirred solution of 500 mg *meso*-**1** in 50 mL pH 7.00 phosphate buffer, 100 μL PLE (or 10 mg of lipase) was added in one portion and the reaction mixture was stirred at 20 °C in a pH stat unit. The conversion was monitored by TLC. The reaction mixture was extracted with ethyl acetate, dried over $MgSO_4$ and concentrated under reduced pressure. The product (1*S*,4*R*)-(−)-**2a** was purified by flash column chromatography (EtOAc/hexane, 1:5). $[\alpha]_D^{20} = -22.8$ (*c*, 4.17, MeOH). ^1H

NMR: δ 1.99 (s, 3H, O_2CCH_3), 2.71–2.76 (br s, 1H, O*H*), 4.29 (d, 1H, AB system CH_aH_bOH, $J = 15$ Hz), 4.53 (d, 1H, AB system CH_aH_bOH, $J = 15$ Hz), 4.61 (d, 2H, AB system CH_aH_bOAc, $J = 13$ Hz), 5.12 (d, 2H, AB system CH_aH_bOAc, $J = 13$ Hz). ^{13}C NMR: δ 21.5, 57.2, 57.7, 85.2, 85.3, 113.7, 137.4, 137.7, 139.0, 148.2, 171.9. IR (neat): 3300, 1730, 1681, 1433, 751 cm^{-1}. Anal. Calcd for $C_{11}Cl_6H_8O_3$ (400.90): C, 32.96; Cl, 53.06; H, 2.01. Found: C, 32.95; Cl, 53.07; H, 2.03.

4.4. General procedure for enzymatic acetylation of 3

To a stirred solution of 500 mg *meso*-**3** in 5 mL vinyl acetate, 10 mg of CCL (or lipase) was added in one portion and the reaction mixture was stirred at 20 °C (TLC monitoring). The reaction mixture was filtered and vinyl acetate was evaporated under reduced pressure. The product (1*S*,4*R*)-(−)-**2a** or (1*R*,4*S*)-(+)-**2b** were purified by flash column chromatography (EtOAc/hexane, 1:5). (1*R*,4*S*)-(+)-**2b**: $[\alpha]_D^{20} = +20.8$ (*c*, 0.83, MeOH).

4.5. Dechlorination of (1*S*,4*R*)-(−)-2-acetoxymethyl-3-hydroxymethyl-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]-hepta-2,5-diene (1*S*,4*R*)-(−)-2a

To a stirred solution of metallic sodium (2.05 g, 89.5 mmol) in liquid NH_3 (80 mL), (1*S*,4*R*)-(−)-**2a** (1.46 g, 3.64 mmol) in absolute EtOH/ether (25 mL, 1:1 ratio) was added dropwise under argon atmosphere over 20 min. The resultant mixture was stirred for additional 20 min and then solid NH_4Cl was added in small portions until the solution turned to be colourless. NH_3 was removed by passing N_2 through the mixture and ice water was added. The resultant mixture was acidified with 2 M HCl and extracted with ether (3 × 50 mL). Organic phase was washed with saturated $NaHCO_3$ (3 × 50 mL), brine (2 × 50 mL), and dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by flash column chromatography to afford (1*S*,4*R*)-(−)-**4a** (EtOAc/hexane, 1:5). $[\alpha]_D^{20} = -19.8$ (*c* 2, CH_2Cl_2) (0.61 g, 86% yield). All ^1H NMR, ^{13}C NMR and IR data are in accordance with the literature data.[3a]

4.6. Dechlorination of (1*R*,4*S*)-(−)-2-acetoxymethyl-3-hydroxymethyl-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]-hepta-2,5-diene (1*R*,4*S*)-(+)-2b

To a stirred solution of metallic sodium (2.05 g, 89.5 mmol) in liquid NH_3 (80 mL), (1*R*,4*S*)-(+)-**2b** (1.00 g, 2.50 mmol) in absolute EtOH/ether (25 mL, 1:1 ratio) was added dropwise under argon atmosphere over 20 min. The resultant mixture was stirred for additional 20 min and then solid NH_4Cl was added in small portions until the solution turned to be colourless. NH_3 was removed by passing N_2 through the mixture and ice water was added. The resultant mixture was acidified with 2 N HCl and extracted with ether (3 × 50 mL). Organic phase was washed with saturated $NaHCO_3$ (3 × 50 mL), brine (2 × 50 mL) and dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by flash column chromatography to afford (1*R*,4*S*)-(+)-**4b** (EtOAc/hexane, 1:5). $[\alpha]_D^{20} = +17.8$ (*c* 2, CH_2Cl_2) (0.39 g, 81% yield).

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