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TETRAHEDRON: *ASYMMETRY*

Evaluation of lipase from *Candida rugosa* in the resolution of *endo*-(±)-1,8,9,10,11,11-hexachloropentacyclo-[6.2.1.1^{3,6}.0^{2,7}.0^{5,9}]dodecan-4-alcohol

Valentim E. U. Costa,* João Alifantes and Jose E. D. Martins

Instituto de Química, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, 91501-970 Porto Alegre-RS, Brazil

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Abstract

endo-(±)-1,8,9,10,11,11-Hexachloropentacyclo[6.2.1.1^{3,6}.0^{2,7}.0^{5,9}]dodecan-4-ol (±)-**7** and *exo*-(±)-1,8,9,10,11,11-hexachloropentacyclo[6.2.1.1^{3,6}.0^{2,7}.0^{5,9}]dodecan-4-ol (±)-**4** have been prepared and the enantiomeric enrichment capacity of the lipase from *Candida rugosa* in the transesterification with vinyl acetate of these compounds was evaluated. It was verified that the lipase recognize only the alcohol (±)-**7**, producing *endo*-(+)-1,8,9,10,11,11-hexachloropentacyclo[6.2.1.1^{3,6}.0^{2,7}.0^{5,9}]dodecan-4-yl acetate (+)-**8** with ee >95% and conversion of 44% as the only product.

1. Introduction

The synthesis of hexachlorinated polycyclic systems, the insecticides isodrin, endrin and its derivatives have been reported in the early 1950s.[1] Soloway et al.[2] verified that chlorinated compounds with the 1,4,5,8-dimethanonaphthalene nucleus undergo unusual skeletal rearrangements in their reactions. Winstein et al.[3] observed Wagner–Meerwein rearrangements in solvolysis reactions of polycyclic derivatives, whose mechanisms involve the formation of non-classical ions. The degradation of chlorinated polycyclic systems, usually involving hydrolytic, oxidative and intramolecular rearrangement reactions, leaves the basic carbon skeletal system intact.[4]

The investigation of rigid cyclic systems with unique conformations have contributed to the understanding of several aspects of NMR spectroscopy in relation to geometry and steric parameters. Long-range steric effects of tetracyclododecanes *endo–endo* and *endo–exo* have been studied in our group by means of ^{13}C and ^{1}H NMR.[5–7]

* Corresponding author. E-mail: valentim@if.ufrgs.br

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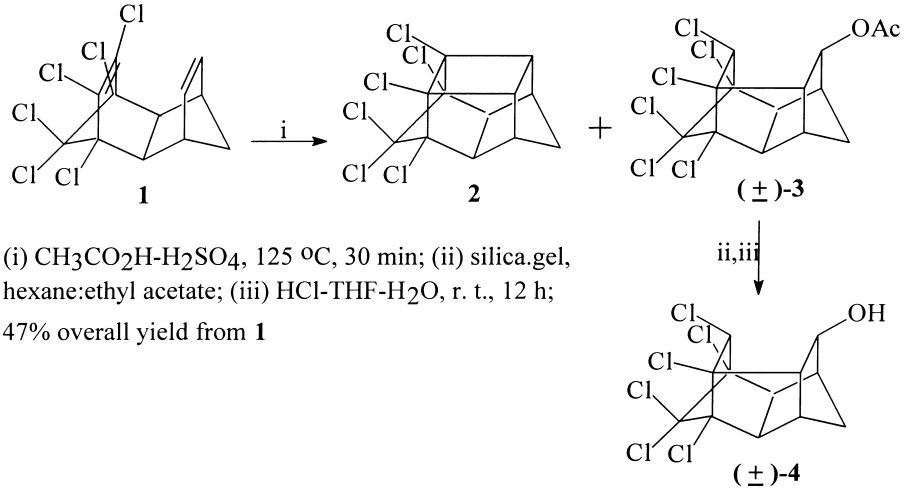
Although some of these structures possess chirality, these studies were carried out with racemic compounds. Efficient methodologies for enantiomeric analysis and resolution of these compounds are lacking. Recently our work group has demonstrated that chiral and achiral chemical shift reagents can be good analytical alternatives for determination of enantiomeric purity.[8]

In this paper, we describe the preparation of the hexachlorinated *endo*- and *exo*-pentacyclododecanols (±)-**4** and (±)-**7**, their transesterification reaction with vinyl acetate catalyzed by lipase from *Candida rugosa* and the resolution of *endo*-(+)-1,8,9,10,11,11-hexachloropentacyclo[6.2.1.1^{3,6}.0^{2,7}.0^{5,9}]dodecan-4-yl acetate (+)-**8** with high optical purity.

2. Results and discussion

The racemic alcohol (±)-**4** was obtained as shown in Scheme 1. Following the modified Lidov[2] method, the isodrin **1** is completely consumed in 5 min by treatment with acetic acid and catalytic sulfuric acid, giving a mixture of four products: *exo*-(±)-1,8,9,10,11,11-hexachlorotetracyclo[6.2.1.3^{3,6}.0^{2,7}]dodecan-4-yl acetate, 1,8,9,10,11,11-hexachlorohexacyclo[6.2.1.1^{3,6}.0^{2,7}.0^{4,9}.0^{5,9}] dodecane **2**, *exo*-(±)-1,8,9,10,11,11-hexachloropentacyclo[6.2.1.1^{3,6}.0^{2,7}.0^{5,9}]dodecan-4-yl acetate (±)-**3** and acetate (±)-**8**[9]. The chlorinated birdcage hydrocarbon **2** and chlorinated tetracyclic acetate can be separated by column chromatography, but chlorinated pentacyclic *exo*-acetate (±)-**3** and acetate (±)-**8** are very difficult to separate by this procedure. We have observed that the proportions of derivatives depend on reaction time and temperature. In 30 min, only the chlorinated birdcage hydrocarbon **2** and acetate pentacyclic *exo*-(±)-**3** compounds are produced, which are easily separated by column chromatography. By hydrolysis of the chlorinated pentacyclic *exo*-acetate with hydrochloric acid in tetrahydrofuran the alcohol (±)-**4** was obtained.

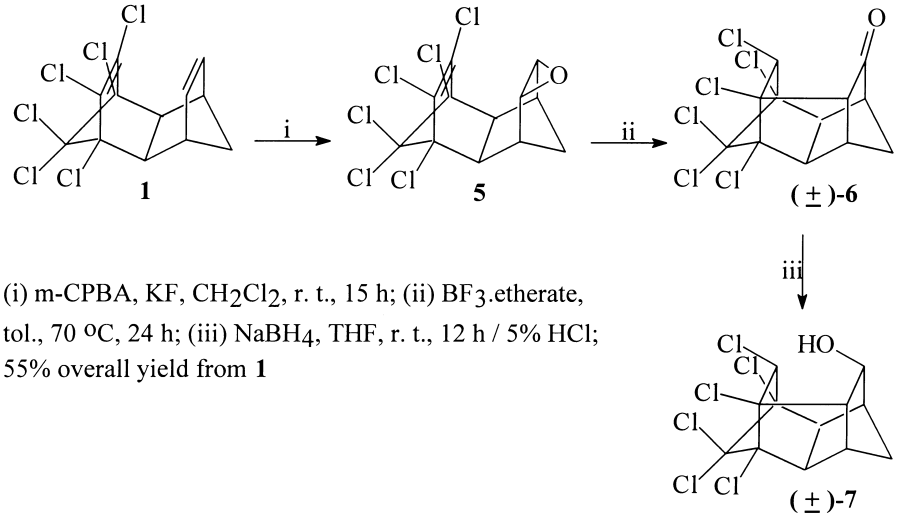


(i) CH_3CO_2H-H_2SO_4, 125 °C, 30 min; (ii) silica.gel, hexane:ethyl acetate; (iii) HCl-THF-H_2O, r. t., 12 h; 47% overall yield from **1**

Scheme 1.

The racemic alcohol (±)-**7** was prepared according to Scheme 2. By epoxidation of isodrin **1** with 3-chloroperoxybenzoic acid in the presence of potassium fluoride,[10] the 1,8,9,10,11,11-hexachlorotetracyclo[6.2.1.3^{3,6}.0^{2,7}]dodecan-4,5-epoxi-9-eno (endrin) **5** was obtained. The treatment of endrin with boron trifluoride etherate, following the methodology of Fukanaga,[11] produces the

1,8,9,10,11,11-hexachloropentacyclo[6.2.1.1^{3,6}.0^{2,7}.0$^{5-9}$]dodecan-4-one (±)-**6**, which was reduced by sodium borohydride giving exclusively the alcohol (±)-**7**.



(i) m-CPBA, KF, CH_2Cl_2, r. t., 15 h; (ii) BF_3.etherate, tol., 70 °C, 24 h; (iii) $NaBH_4$, THF, r. t., 12 h / 5% HCl; 55% overall yield from **1**

Scheme 2.

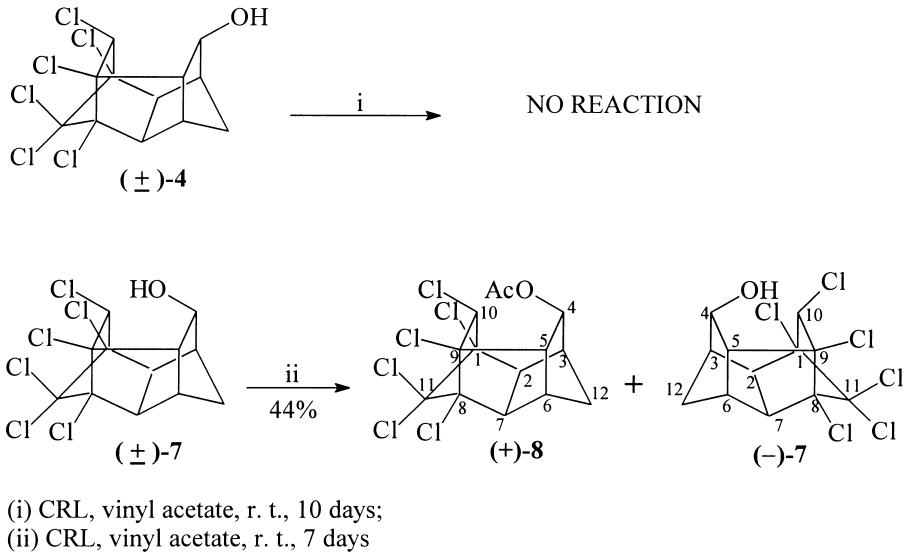
Faber et al.[12–14] studied the enzymatic resolution of *endo*-bicyclo[2.2.1]hept-2-yl and *endo*-bicyclo[2.2.2]oct-2-yl butyrates and observed that systems with *endo*-configurations exhibit enantioselectivity in reactions catalyzed by lipase from *Candida rugosa*. The *R*-configuration is preferentially transesterified.

Lightner et al.[15] dechlorinated the tetracyclic alcohol obtained by hydroboration of isodrin, prepared the half-acid phthalate and resolved it by fractional crystallization of the brucine salt. However, the enantiomeric excess was only 41.9%. Otherwise, we have observed that the use of enzymes to resolve dechlorinated polycyclic alcohols does not give good results. The same observation has been found for alcohols with the *exo*-configuration. However, enzymatic resolution of 7,7-disubstituted 1,4,5,6-tetrachlorobicyclo[2.2.1]hept-5-en-2-ols[16] using the lipase from *Candida rugosa* in vinyl acetate was achieved with complete specificity conversion (~50%) and high selectivity to the alcohol (ee 98%) and to the ester (ee >99%).

Encouraged by these findings, we selected the lipase from *Candida rugosa* for resolving racemic alcohol (±)-**4** and alcohol (±)-**7** by transesterification with excess vinyl acetate (Scheme 3).

As the enzymatic transesterification of alcohol (±)-**4** did not occur, even with a reaction time of 10 days, the hydrolysis reaction of the respective chlorinated pentacyclic *exo*-acetate, in aqueous solution of monobasic potassium orthophosphate (0.01 M) was tested for 7 days, without success. On the other hand, testing the alcohol (±)-**7** under the same conditions in transesterification (reaction with vinyl acetate, 7 days), a high degree of enantioselectivity of the enzyme was found (E>100)[17] and the optically pure acetate (+)-**8** was obtained, as well as the alcohol (−)-**7** with high enantiomeric excess. The reaction yield of enantiomeric conversion to the acetate (+)-**8** was 44% and these data are shown in Table 1.

For the determination of the enantiomeric excess of chiral compounds, two techniques are normally used: (a) chromatography (GC and HPLC) on chiral columns, and (b) NMR spectroscopy. Since chlorinated compounds have high boiling points, the use of GC on chiral columns with cyclodextrin is impossible and the use of HPLC on chiral columns with cyclodextrin did not result in satisfactory separation. Consequently, we have chosen ^{1}H NMR as the analytical method, using chiral chemical shift reagents. A high resolution of the signals has been achieved for the enantiomeric protons (α-Cl) of both,



(i) CRL, vinyl acetate, r. t., 10 days;
(ii) CRL, vinyl acetate, r. t., 7 days

Scheme 3.

Table 1
Results of transesterification catalyzed by lipase from *Candida rugosa*

alcohol (−)-**7**		acetate (+)-**8**			
$[\alpha]^{20}_{D}$ [a]	ee[b]	$[\alpha]^{20}_{D}$ [a]	ee[b]	conv[c]	E[d]
− 0.86	77%	+ 0.75	>95%	44%	>100

a. optical rotation (*c* 2, ethyl acetate) to acetate (+)-**8** and (*c* 0.6, ethyl acetate) to alcohol (−)-**7**.
b. enantiomeric excess (in %) obtainned from NMR using chiral chemical shift reagents.
c. conversion (in %) determinated by GC.
d. enantiomeric ratio (E) calculated according to the formula[17] $E = \ln[1- conv(1 + ee_p)] / \ln[1- conv(1- ee_p)]$.

alcohol (±)-**7** and acetate (±)-**8** and the CH_3 signal of the ester. Sequential addition of 5 mg of tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato] europium(III) ($Eu(hfc)_3$) to $CDCl_3$ solutions of these compounds (10 mg) in a 5 mm NMR tube, achieved the best results with 20 mg of the chiral chemical shift reagent. The differences in chemical shifts ($\Delta\Delta\delta$) of the enantiomeric hydrogens H_{10} (α-Cl) were 0.0074 ppm for the alcohol (±)-**7**, 0.041 ppm for the H_{10} and 0.09 ppm for the CH_3 of acetate (±)-**8** (Fig. 1a, b and c).

3. Experimental section

The melting points were measured on an Electrothermal IA 9100 digital melting point apparatus. NMR spectra were measured with a Varian VXR-200 spectrometer at a magnetic field of 4.7 T and a temperature of 22°C. Chemical shifts are expressed as δ (ppm) relative to TMS as an internal standard. Elemental analyses were recorded on a Perkin–Elmer 2400 CHN elemental analyzer apparatus. Products were analyzed by GC using capillary columns (25 m×0.2 mm (i.d.)×0.11 μm) packed with SE-30 and a Varian Model star 3400 CX chromatograph equipped with an FID. Optical rotations were measured with an Acatec PDA 8200 digital polarimeter with a 1 dm cell at a temperature of 20°C.

Lipase, type VII (from *Candida rugosa*); tris[3-(heptafluoropropylhydroxymethylene)-(+)-

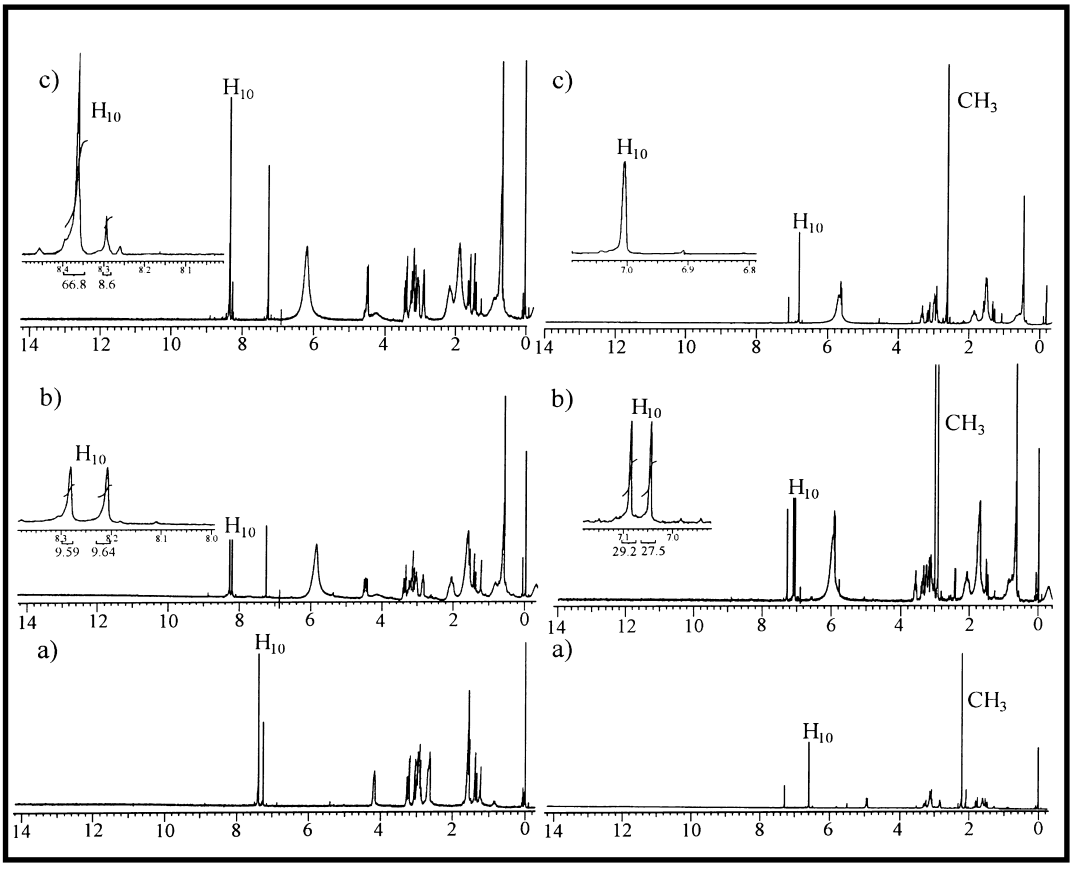


Fig. 1. Spectra: (a) alcohol (±)-**7** and acetate (±)-**8**; (b) alcohol (±)-**7** and acetate (±)-**8** in the presence of (+)-Eu(hfc)$_3$; (c) alcohol (−)-**7** and acetate (±)-**8** in the presence (+)-Eu(hfc)$_3$

camphorato] europium(III); chloroform-d_1 (99.8 atom% D 98%); 3-chloroperoxybenzoic acid (50–60%) and isodrin (98%) were purchased from the Aldrich Chemical Co. Vinyl acetate (>99%), silica gel 60 (230–400 mesh ASTM), sodium borohydride, ethyl acetate (99.5%) and hexane (99%) were purchased from Merck.

3.1. exo-*(±)-1,8,9,10,11,11-Hexachloropentacyclo[6.2.1.1^{3,6}.0^{2,7}.0^{5,9}]dodecan-4-yl acetate (±)-**3***

Concentrated sulfuric acid (1 mL) was added to a solution of isodrin (3.0 g, 8.2 mmol) in acetic acid (12 mL) at a temperature of 125°C under magnetic stirring. After 30 minutes, the system was cooled down and neutralized with a 10% $NaHCO_3$ solution. A precipitate was formed and separated. An additional extraction with chloroform was undertaken. The extract was combined with the precipitate. This solution was dried with $MgSO_4$, and after solvent evaporation from the filtrates, a white solid was obtained as a mixture of two compounds. These products were separated on a silica gel column (hexane:ethyl acetate, 0 to 20%). The obtained compounds were the chlorinated birdcage hydrocarbon **2** (0.8 g, 2.2 mmol), 27% yield, and chlorinated pentacyclic *exo*-acetate (±)-**3**, (1.77 g, 4.1 mmol), 50% yield.

Chlorinated birdcage hydrocarbon **2**: mp 290°C (lit.[2] 287–289°C). ^{13}C NMR (50 MHz, $CDCl_3$): δ 39.8 (CH_2), 43.4 (2CH), 54.4 (2CH), 58.4 (2CH), 78.2 (2C), 83.5 (2C), 97.5 (C).

Chlorinated pentacyclic *exo*-acetate (±)-**3**: mp 193–194 (lit.[9] 194–195). FTIR (film, $CHCl_3$): ν (cm^{-1})

1735 (C=O). ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.1 (s, 3H, methyl), 5.5 (s, 1H, Hα-Cl) and 5.8 (s, 1H, Hα-O). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.1 (CH_3), 36.5 (CH_2), 41.6 (CH), 43.4 (CH), 55.6 (CH), 58.8 (CH), 60.0 (CH), 64.7 (CH), 74.0 (C), 76.4 (CH), 79.6 (C), 84.8 (C), 99.5 (C), 169.8 (C=O).

3.2. exo-*(±)-1,8,9,10,11,11-Hexachloropentacyclo[6.2.1.1^{3,6}.0^{2,7}.0^{5,9}]dodecan-4-ol (±)-**4***

To a chlorinated pentacyclic *exo*-acetate (±)-**3** solution (1 g, 2,31 mmol) in THF (50 mL) an aqueous solution of 15% HCl was added at room temperature under magnetic stirring for 12 hours. The mixture was neutralized with 10% $NaHCO_3$, extracted with chloroform and this solution was dried with $MgSO_4$. Evaporation of the solvent gives the respective alcohol (±)-**4** (0.83 g, 2.2 mmol), mp 217 (lit.[9] 218°C), 95% yield. FTIR (KBr): ν (cm^{-1}) 3471 (C–OH). ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.0 (s, 1H, Hα-O) and 5.4 (s, 1H, Hα-Cl). ^{13}C NMR (50 MHz, $CDCl_3$): δ 36.0 (CH_2), 41.2 (CH), 45.3 (CH), 55.6 (CH), 58.9 (CH), 62.3 (CH), 64.6 (CH), 73.7 (CH), 74.3 (C), 79.6 (C), 85.1 (C), 98.6 (C).

*3.3. 1,8,9,10,11,11-Hexachlorotetracyclo[6.2.1.3^{3,6}.0^{2,7}]dodecan-4,5-epoxi-9-eno (endrin) **5***

To an isodrin solution (5.00 g, 13.7 mmol) in CH_2Cl_2 (15 mL), *m*-CPBA acid (4.74 g, 27.4 mmol) and KF (1.6 g, 27.4 mmol) were added. After stirring for 15 hours at r.t., activated KF (1 hour at 120°C/0.1 torr) (1.6 g, 27.4 mmol) was added up to a total 1:2 *m*-CPBA:KF molar ratio and the reaction mixture was stirred for 1 hour at r.t. The crude mixture was then filtered off and the solvent was removed by distillation to give the respective epoxide **5** (4.83 g, 12.69 mmol), mp 246°C dec. (lit.[1] 245°C dec.), 92% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.4 (s, 2H, Hα-O). ^{13}C NMR (50 MHz, $CDCl_3$): δ 29.8 (CH_2), 39.2 (2CH), 47.0 (2CH), 54.5 (2CH), 79.5 (2C), 108.7 (C), 132.3 (2C).

*3.4. (±)-1,8,9,10,11,11-Hexachloropentacyclo[6.2.1.1^{3,6}.0^{2,7}.0^{4,5}]dodecan-4-one (±)-**6***

A solution of endrin **5** (5.27 g, 13.8 mmol) in toluene (20 mL) was heated to reflux (70°C) and then boron trifluoride etherate (2 mL) in toluene (10 mL) was added. Refluxing was continued for 24 hours. After cooling a solid precipitated from the reaction mixture. The product was removed by filtration and cleaned by flash column chromatography (chloroform). Evaporation of the solvent sets free the pentacyclic ketone (±)-**6** (4.2 g, 11.04 mmol), 80% yield, mp 284–285°C dec. (lit.[2] 285–286°C dec.). FTIR (KBr): ν (cm^{-1}) 1750 (C=O). ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.0 (s, 1H, Hα-Cl). ^{13}C NMR (50 MHz, $CDCl_3$): δ 34.3 (CH_2), 41.0 (CH), 49.4 (CH), 53.7 (CH), 59.2 (CH), 65.1 (C), 69.0 (CH), 73.3 (C), 79.7 (C), 87.1 (C), 98.8 (C), 205.2 (C=O).

3.5. endo-*(±)-1,8,9,10,11,11-Hexachloropentacyclo[6.2.1.1^{3,6}.0^{2,7}.0^{5,9}]dodecan-4-ol (±)-**7***

Under an inert gas atmosphere, a mixture of pentacyclic ketone (±)-**6** (4.0 g, 10.47 mmol) and sodium borohydride (0.70 g, 20.94 mmol) in dry tetrahydrofuran (100 mL) was stirred for 12 hours at r.t. The resulting anionic complex was decomposed with dilute HCl. The mixture was neutralized with 10% $NaHCO_3$ and extracted with chloroform. Evaporation of the solvent produces the corresponding alcohol (±)-**7** (3.16 g, 8.28 mmol), 75% yield, mp 258–260°C dec. (lit.[2] 258–260°C dec.). FTIR (KBr): ν (cm^{-1}) 3341 (C–OH). ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.2 (d, J=6.05 Hz, 1H, Hα-OH) and 7.4 (s, 1H, Hα-Cl). ^{13}C NMR (50 MHz, $CDCl_3$): δ 38.0 (CH_2), 42.9 (CH), 44.1 (CH), 56.0 (CH), 56.3 (CH), 58.5 (CH), 64.6 (CH), 72.7 (C), 79.8 (CH), 81.1 (C), 83.3 (C), 99.3 (C).

3.6. endo-*(+)-1,8,9,10,11,11-Hexachloropentacyclo[6.2.1.1^{3,6}.0^{2,7}.0^{5,9}]dodecan-4-yl acetate (+)-**8***

Lipase (50% w/w of substrate) was added to a solution of pentacyclic alcohol (±)-**7** (0.38 g, 1 mmol) in vinyl acetate (10 mL) and the suspension was shaken at 250 rpm at 25°C. When the appropriate degree of conversion was achieved (7 days), the enzyme was filtered and the excess vinyl acetate was evaporated. The products were separated by flash column chromatography (hexane:ethyl acetate=4:1), giving the alcohol (−)-**7** and acetate (+)-**8** (0.19 g, 0.44 mmol), 44% yield.

Alcohol (−)-**7**: mp 258–260°C dec., $[\alpha]_D^{20}$ −0.86 (*c* 0.6, ethyl acetate). FTIR (KBr): ν (cm^{-1}) 3341 (C–OH). ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.2 (d, *J*=6.05 Hz, 1H, Hα-OH) and 7.4 (s, 1H, Hα-Cl). ^{13}C NMR (50 MHz, $CDCl_3$): δ 38.0 (CH_2), 42.9 (CH), 44.1 (CH), 56.0 (CH), 56.3 (CH), 58.5 (CH), 64.6 (CH), 72.7 (C), 79.8 (CH), 81.1 (C), 83.3 (C), 99.3 (C). Anal. calcd: C, 37.60; H, 2.61. Found: C, 37.58; H, 2.59.

Acetate (+)-**8**: mp 153–154°C, $[\alpha]_D^{20}$ +0.75 (*c* 2, ethyl acetate). FTIR (KBr): ν (cm^{-1}) 1731 (C=O). ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.19 (s, 3H, CH_3), 4.92 (d, *J*=6.05 Hz, 1H, Hα-OAc) and 6.57 (s, 1H, Hα-Cl). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.0 (CH_3), 37.4 (CH_2), 42.5 (CH), 43.1 (CH), 54.8 (CH), 56.1 (CH), 58.6 (CH), 64.8 (CH), 72.4 (C), 79.5 (CH), 80.6 (C), 83.4 (C), 99.0 (C), 169.4 (C=O). Anal. calcd: C, 39.53; H, 2.82. Found: C, 39.77; H, 2.79.

Acknowledgements

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