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Graphical Abstract

Preparation of (1*R*,4*S*)-4-Hydroxycyclopent-2-en-1-yl Acetate via Novozym-435® Catalyzed Desymmetrization of ***cis*-3,5-Diacetoxy-1-cyclopentene.**

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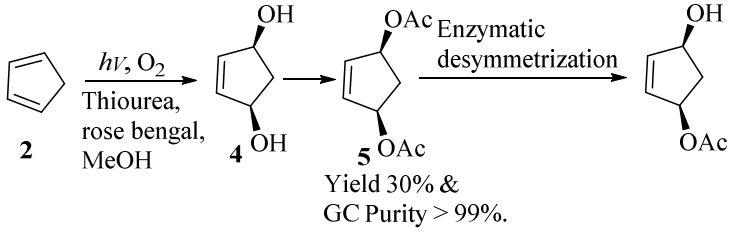

Yield 30% &
GC Purity > 99%.

(1*R*,4*S*)- **6**
Yield > 95 %;
e.e > 99% &
GC Purity > 99%.

Preparation of (1*R*,4*S*)-4-Hydroxycyclopent-2-en-1-yl Acetate via Novozym-435® Catalyzed Desymmetrization of *cis*-3,5-Diacetoxy-1-cyclopentene.

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ABSTRACT

Photooxidation of cyclopentadiene has been carried out in methanol using white light of LED lamp, rose bengal as photo initiator, and compressed air at 0 °C. Under conditions of [thiourea] >> [cyclopentadiene], the consumption of thiourea follows a pseudo-first-order reaction kinetics with half life of 75 ± 10 min; corr. coeff. r = 0.989. Slow addition of the monomer and maintaining excess thiourea concentration in reaction mass improves the yield. *cis*-3,5-Dihydroxy-1-cyclopentene is acetylated without isolation to obtain *cis*-3,5-Diacetoxy-1-cyclopentene of high purity (>99%) with overall isolated yield of 30%. Desymmetrization of the diacetate to (1*R*,4*S*)-4-hydroxycyclopent-2-en-1-yl acetate has been carried out via enzymatic transesterification with methanol in methyl *tert*-butyl ether (MTBE) at 5 °C using Novozym-435®. The enantiomerically pure monoacetate (*e.e.* >99%) was obtained in 95% isolated yield. The recovered enzyme was reused for more than 10 times without loss in yield and selectivity. The entire protocol does not require purification of final product by chromatography.

Keywords: cyclopentadiene, photooxidation, thiourea, rose bengal, enzyme, desymmetrization, transesterification, *cis*-3,5-Dihydroxy-1-cyclopentene.

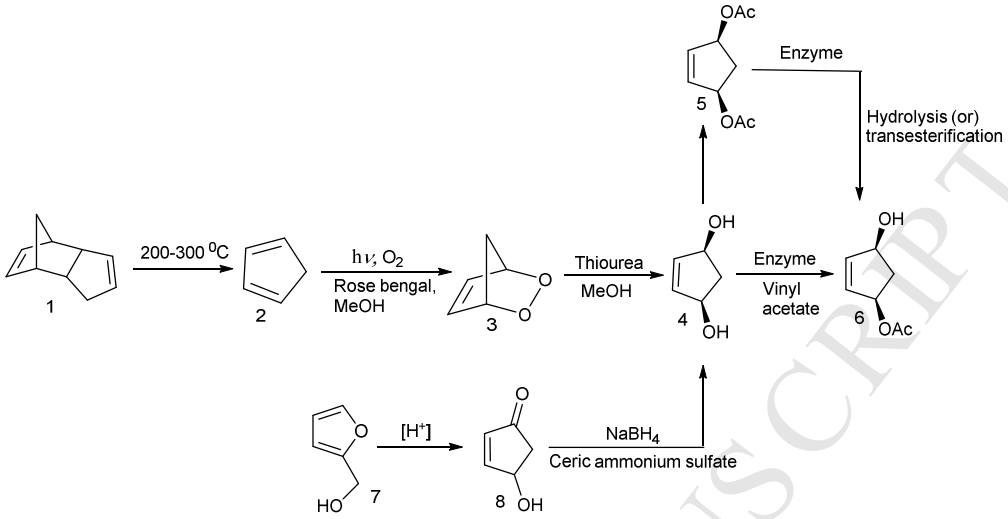
1. Introduction

Enantiomerically pure (1*R*,4*S*)-4-hydroxycyclopent-2-en-1-yl acetate **6** and its related isomers are common motifs in prostaglandins, antibiotics such as viridenomycin and pentenomycin, or antitumor compounds such as neocarzinostatin.[1-5] The required isomers can be prepared via chiral pool,[6] asymmetric reduction of hydroxycyclopent-2-enone,[7] Pd-catalyzed asymmetric allylic nucleophilic substitution,[8] or metathesis of chiral diol.[9] These methodologies are very interesting but not easy to scale-up.

For large scale preparation, the preferred routes are based on *meso*-diol **4** as starting point which is prepared by either photooxidation of cyclopentadiene (**2**)[10-16] or reduction of 3-hydroxy cyclopentenone **8**[17-18] (Scheme1). Previously, we had prepared the compound **8** starting with **7** in a microreactor,[19] but it would need several such reactors to make large quantities. Besides, the compound **8** is also unstable and highly water soluble. In comparison, the photochemical approach was easier. Commercially available dicyclopentadiene **1** is heated to 200-300 °C to prepare cyclopentadiene **2**. Photochemically generated singlet oxygen reacts with **2** to form an *endo*-peroxide **3** *via* a [4+2] cycloaddition reaction which is in situ reduced to diol **4** with thiourea.[10] The *meso*-diol is converted to enantiomerically pure monoacetate **6** using enzyme catalyzed desymmetrization[20-24] (Scheme 1).

Selective acylation of *meso*-diol **4** using vinyl acetate or related derivatives[24] or hydrolysis of the *meso*-diacetate **5**[14, 25-28] provide the monoacetate in good enantiomeric purity and high yields (Scheme 1). Enzymes employed are lipase from *Mucor* sp.,[25]*pancreatin*,[26] *Mucor miehei* lipase (Chirazyme),[27] *Pseudomonas fluorescens* lipase (PFL), *Candida rugosa* lipase[28] and lipase B from *Candida antarctica* (Novozym-435®).[26b]

Acetyl choline esterase from electric eel[29] and recombinant pig liver esterase[30] have also been used for the reaction.



Scheme 1. Preparation of enantiomerically pure (1*R*,4*S*)-4-hydroxycyclopent-2-en-1-yl acetate **6**.

In our laboratory, we required rather large quantities (>100 g) of (1*R*,4*S*)-**6** for our research purpose and considering the ease of preparation, we chose the route based on photooxidation of cyclopentadiene followed by enzymatic desymmetrization of diol **4** for preparation of **6**. According to available literature, the singlet oxygen is usually generated by employing rose bengal as photo initiator. The preferred reaction medium is methanol[31-32] but the reaction can be carried out efficiently in chlorinated solvents such as dichloromethane or carbon tetrachloride[14] where the life time of singlet oxygen is much longer.[33] The reported reaction temperatures ranging from -78° to + 25 °C with varied yields (20-90%). The reaction is usually performed in a stirred tank glass reactor irradiated with halogen or xenon lamp. A falling film microreactor has also been reported for the reaction[34] but it is not suitable for multigram scale synthesis. Recently this reaction has been carried out very efficiently in liquid and supercritical carbon dioxide at 0 °C with excellent yield of 94%.[35] Such high yields were attributed to longer life time of singlet oxygen[36-38] and stability of the *endo*-peroxide **3** over several minutes in this medium. However, the reaction was carried out

at high pressure of 120 bar and requires specially designed dedicated equipment as well as manpower skilled in handling such an equipment. Since our requirement did not warrant a huge investment of money and manpower, we decided to perform the preparation of **5** via **4** photooxidation of cyclopentadiene **2** using rose bengal as initiator. Since solubility of thiourea required for in situ conversion of the *endo*-peroxide to diol is very limited in chloroform or dichloromethane, methanol was used as solvent. Although the current trend in photochemical reactions is in favour of a continuous reactor[39] our reaction work-up involves removal of methanol from reaction mixture before acetylation of the diol **4** to get diacetate **5**. Since these operations are conveniently performed in a batch mode, we have studied photooxidation of cyclopentadiene in a jacketed glass reactor.

Among all the enzymatic processes described above, desymmetrization of **5** catalyzed by Novozym- 435® is very interesting since it is an immobilized form of *Candida Antarctica* lipase B available on commercial scale. Moreover, preparation of (1*R*,4*S*)-**6** in yields of over 96% with *e.e.* >98% have been reported.[14, 26b] This prompted us to explore this reaction in more detail.

2. Results and Discussion

After repeating the procedures reported in literature for photooxidation of cyclopentadiene using a halogen lamp, we realized that conducting the reaction on a small scale at -40 °C (1 g) was not difficult but on a larger scale, several problems were encountered. After careful examination, we realized the following: a) the capacity of temperature controller to maintain temperature diminished drastically as the reaction volumes increased. Heat emitted by the halogen lamp compounded the problem; b) the monomer tends to dimerize on long standing even at -20 °C and should be ideally used within few hours of making it; c) the reaction requires a continuous stream of oxygen to be bubbled in the reaction mixture. This causes the low boiling monomer (b.p. 41°C) getting carried over the

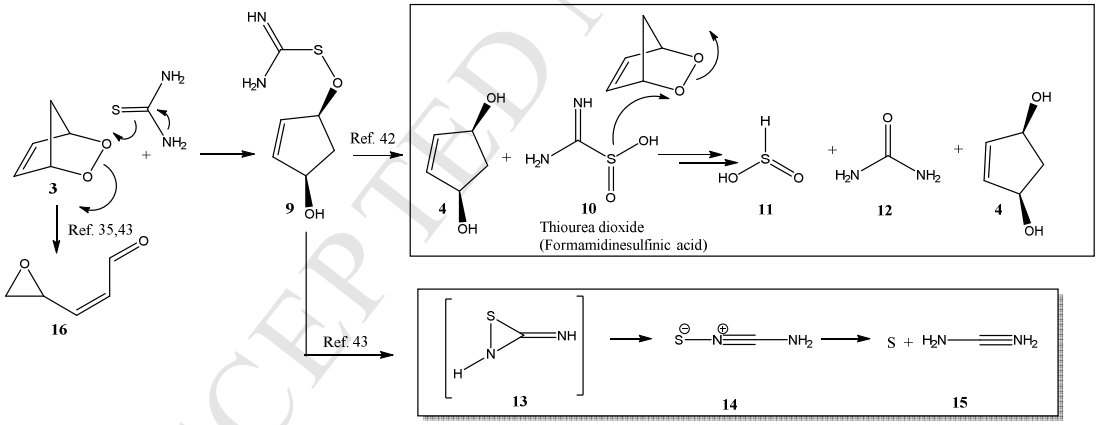
vent resulting in low yields. Safety issues also needed consideration. The use of pure oxygen and accumulation of *endo*-peroxide in the reactor is a dangerous combination. Present studies were directed towards prevention of the possible hazards and obtain a fair yield of the diacetate **5** on a preparative scale.

Cyclopentadiene monomer **2** was prepared by cracking dicyclopentadiene using equipment similar to that described by Magnusson[40] with modification. The dimer was added slowly to hot silicone oil maintained at 200 °C[41] and the monomer vapours were further passed through a glass column filled with Raschig rings and heated to 300 °C. A downward double walled condenser, maintained at 0 °C cooled the vapours and the monomer was collected in a receiver maintained at -20 °C with acetone-dry ice. This arrangement avoided wastage of the dimer and also allowed preparation of the monomer as and when required.

In the next stage, the photochemical reaction was performed in a 1 L glass reactor. We found that compressed air served the purpose of supplying oxygen without any change in product yield. This solved the problem of using pure oxygen in laboratory. Further experiments showed that commercially available white LED lamps used as flood lights were as good as halogen lamps for our purpose with advantage of not producing heat around the reactor. It was now possible to maintain temperature around 0 °C inside the reactor. Finally, by slow addition of the monomer to the reaction mixture (as a solution in 2-propanol) instead of one time addition in the beginning, the loss of monomer through evaporation was minimized. Interestingly, the reaction mass becomes turbid as the reaction proceeds indicating progress of the reaction. The purity of cyclopentadiene monomer is also indicated by clarity of its solution in 2-propanol. As the monomer starts dimerizing, the solution becomes cloudy. Although these signs are only rough indications, we hope that they would be helpful to the operator.

2.1. Effect of thiourea content on the reaction

Although there are several reports on the use of thiourea for hydrogenolysis of *endo*-peroxides, very little is known about the mechanism of the reaction. Arifoglu and co-workers[42] have studied the reaction of hydrogen peroxide with thiourea in water by ^{13}C-NMR. They have suggested that thiourea dioxide **10** formed in the first step in turn can react with a second molecule of hydrogen peroxide (*endo*-peroxide in our case) and further decompose to sulfenic acid **11** and urea **12**. This mechanism suggests consumption of half mole of thiourea per mole of *endo*-peroxide. On the other hand, Spivey and co-workers have suggested fragmentation of the sulfenate intermediate **9** to give the product diol **4** and thiazirine **13**. Ring opening of **13** followed by decomposition of nitrile sulphide **14** produces cyanamide and elemental sulphur.[43] This mechanism supports consumption of one mole of thiourea per mole of *endo*-peroxide (Scheme 2).



Scheme 2. Effect of thiourea content on the reaction

We have studied the consumption of thiourea during the course of reaction. Reaction was carried out with 0.2 moles of thiourea in methanolic solution and 0.18 moles of monomer dissolved in 2-propanol were slowly added over 2 h. A slow stream of air was passed through the reaction mass which was illuminated with 100 watts LED lamp. Samples were collected at regular intervals and analysed for thiourea content by reverse phase HPLC. The curve for

decrease in thiourea concentration (peak area) as a function of time is shown as curve A (Fig. 1).

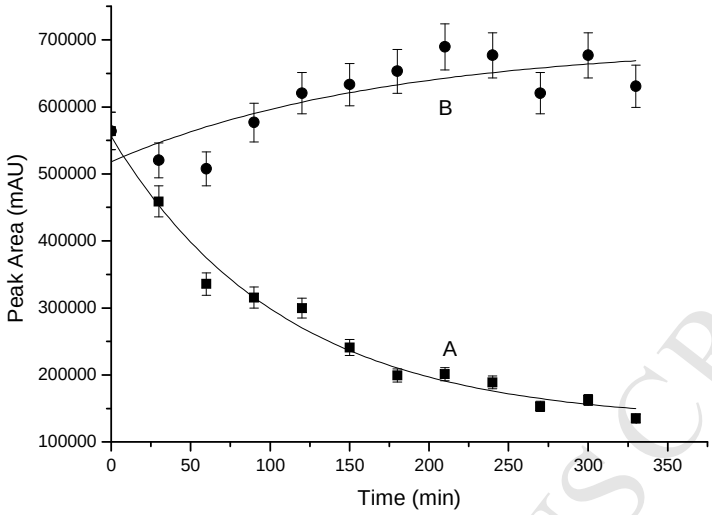


Fig.1. (A) [Thiourea] = 0.2 moles (one-time addition), [rose bengal] = 0.5 g; [Cyclopentadiene] = 0.18 mole; Temp. 5 °C. Reaction volume 560 mL. (B) Initial [Thiourea] = 0.1 mole followed by continuous addition of thiourea, total 0.2 moles. Total reaction volume 760 mL.

Interestingly, the thiourea concentration decreases logarithmically and follows a pseudo-first-order kinetics. The apparent pseudo-first-order rate constant was found to be $0.55 \pm 0.07\ h^{-1}$, half life 75 ± 10 min; Corr. coeff. r = 0.989. Surprisingly, only about 0.5 moles of thiourea were consumed per mole of cyclopentadiene used for the reaction as evidenced from the concentration of thiourea in reaction mass at the beginning and end of reaction. The isolated yield of diacetate **5** in this experiment was 6 g (18%) which was somewhat better than our first experiment but still less than expected.

To understand the pathway towards formation of **4**, the reaction mass was analyzed for sulphur content since the mechanism suggested by Spivey and co-workers predicts formation of 1 equivalent of elemental sulphur per mole of thiourea consumed. Only traces of sulphur were found in the reaction mass suggesting that the reaction follows the mechanism suggested by Arifoglu and co-workers.

Although less than 0.5 equivalents of thiourea were consumed, the product yield was low. Apart from possibilities of incomplete acetylation, dimerization of monomer, formation of polymeric products etc., one major reason for the low yield of the reaction could be the spontaneous decomposition of the *endo*-peroxide (**3**) to (*Z*)-3-(oxiran-2-yl)acrylaldehyde **16**.[35,43] If a competition exists between formation of **16** and **4** from the *endo*-peroxide **3**, the rate of formation of **4** would decrease as thiourea in reaction mass is consumed. One way to overcome the problem would be to maintain the thiourea concentration via a continuous addition of thiourea to the reaction mass. Thus the reaction was performed by using two dosing pumps. In the beginning, the reactor contained a solution of thiourea (0.1 mole) and rose bengal in methanol. One dosing pump added the monomer solution at a rate of 0.5 mL/min while the second dosing pump added thiourea solution in methanol at a rate of 2 mL/min. At any given stage, the concentration of thiourea was always much higher than the monomer. At the end of addition, a total of 0.2 moles of thiourea and 0.18 moles of cyclopentadiene were added to the reaction mass. HPLC analysis showed that thiourea concentration in reaction mass was maintained (curve B, Fig.1) but again, only half mole of thiourea was consumed for one mole of monomer. However, above strategy lead to an improved yield of 10.2 g (31%). Although we are pretty sure that it would increase with further lowering of reaction temperature and reducing the formation of **16**, the current experimental setup is limited by temperature maintenance near 0-5 °C. Moreover, the low temperatures caused severe frosting of the glass reactor through which light could not pass. A configuration where the lamp is inside the reactor is being studied.

2.2. Product Isolation

The reaction mass mostly contained methanol, 2-propanol, unreacted thiourea, thiourea oxidation products, rose bengal, the diol **4**, some dimerized cyclopentadiene and the aldehyde **16** at the end of reaction. Isolation of pure **5** without chromatography was another

challenge. The solvents, methanol and 2-propanol, were easily recovered by distillation under reduced pressure. Extraction of the residue with *n*-heptane removed cyclopentadiene dimer. The residue was then suspended in ethyl acetate and acetylated using triethylamine-acetyl chloride protocol to prepare diacetate **5**. The solvent was removed at this stage on a rotavapor and the residue was repeatedly extracted with *n*-heptane. The *n*-heptane extracts were washed with sodium carbonate solution to remove traces of rose bengal and dried over anhydrous magnesium sulphate. Removal of *n*-heptane gave the final diacetate **5** in 30% overall yield and >99% purity (GC analysis).

The reaction was further scaled up in a 20 L glass reactor. Here, 4 LED lamps were placed around the reactor. The general experimental conditions were similar to those in 1 L reactor (see experimental section), only multiplied by factor of 10 except the aeration rate which was maintained at 1 L/min to minimize solvent evaporation. We were able to obtain 100 (± 10) g of product from 120 g of cyclopentadiene in 3 repeated batches.

2.3. Hydrolysis in aqueous buffer

Hydrolysis of the diacetate **5** in aqueous buffer catalyzed by Novozym-435® was performed as per literature procedure [26b] using 100 g of substrate in 1 L of 0.1 M phosphate buffer at pH 7.5 and room temperature. During enzymatic hydrolysis of the acetate group, acetic acid is released in the reaction mass as the reaction proceeds. This lowers the pH below pH-optimum (7.0-7.5) for the enzyme and reaction slows down considerably. The pH of reaction mass was therefore maintained by intermittent addition of 2N NaOH. Although the reaction did proceed smoothly, a few problems were encountered. In our hands, the yield was only 70% and *e.e.* of crude (1*R*,4*S*)-**6** was 95%. We also observed that the product (1*R*,4*S*)-**6** was soluble in aqueous buffer and several extractions with ethyl acetate were needed to obtain a good yield. The reaction was accompanied by formation of diol **4** as a side product

(GC analysis of the reaction mass). This was either due to self hydrolysis of **5** or **6** or due to inefficient mixing of NaOH added during the reaction which causes non-selective alkaline hydrolysis of the substrate. Another drawback of the procedure was the loss of enzyme activity after each recycle. Since the enzyme is immobilized via adsorption,[44] it tends to leach out in aqueous buffer and consequently the expensive enzyme cannot be reused many times. It is also difficult to scale-up a three-phase: immobilized enzyme-water immiscible substrate-aqueous buffer system.

2.4. Enzymatic transesterification in organic solvents

The use of an organic solvent instead of aqueous medium for enzymatic reactions offers advantages such as increased solubility of hydrophobic substrates and easy recovery of reaction products. The biocatalyst is also recovered by simple filtration. However, a solvent can markedly affect enzyme reactivity. A major factor is solvent hydrophobicity, generally measured as logP_{octanol} which increases with increase in value of logP_{octanol}. Enzyme activity is generally higher in hydrophobic solvents (log P >1.0) than hydrophilic solvents (log P < 1.0). The hydrophilic solvents apparently “strip” tightly bound water essential for enzyme catalytic activity causing decrease of the enzyme activity while the hydrophobic solvents show no obvious effect on the enzyme structure.[45] However, functional groups and molecular structure of hydrophobic organic solvents do exert significant influences on enzymes activity. Although simple alkanes such as hexane do not significantly change the enzyme 3-D structure, solvents with functional groups such as -OH, -CO, -CN etc. have polar interactions with the enzyme surface that may cause "stripping off" the essential water from enzymes and reduce the enzyme activity.[46] Interestingly, a solvent with its functional group in the terminal carbon atom has higher denaturation capacity than that possessing functional group elsewhere.[47] It has been also reported that lipases recognize not only the structure of the substrate, but also that of solvent[48] primarily due to changes in the relative solvation

modes of the substrate in the transition state.[49] A preliminary screening was thus performed in various organic solvents routinely employed in Novozym-435® catalyzed reactions. Alcohols such as methanol, ethanol, *n*-propanol and *n*-butanol were used for transesterification at 30 °C. Surprisingly, methanol and ethanol were found to be effective while *n*-butanol, which is the most preferred alcohol in lipase catalyzed transesterification reactions, did not cause formation of product. The reactions with methanol and ethanol occurred in most solvents except dichloromethane and dimethyl carbonate, but no enantioselectivity was observed. Apart from formation of monoacetates (1*R*,4*S*)-**6**, (1*S*,4*R*)-**6** and diol **4** were also formed (Scheme 1).

It is well known that reaction temperature has a significant effect on enzyme enantioselectivity.[50-53] Increase in enantioselectivity is generally observed with decrease in temperature. This is mostly due to difference in Gibbs free energy of activation, $\Delta_{RS}\,\Delta G^{\ddagger}$ for stereoisomeric transition states formed by two enantiomers *R* and *S* in the rate-limiting step with respect to the ground state**.** The transesterification reactions were thus carried out at lower temperature of 5 °C using methanol. Although the reaction rate decreased, a dramatic increase (*e.e.* 97-99%) in enantiomeric purity of the product was observed. The conversion was low (15%) in toluene, possibly due to high solubility of the substrate in the reaction medium and preferential partitioning of the substrate in bulk solvent phase rather than enzyme active site. The lower conversion in hexane (56%) is mostly due to low solubility of the substrate (from the peak area in GC analysis, it was apparent that some of the substrate had deposited on the polymer and did not react) although the enantioselectivity was high. For cyclic ethers such as THF and 2-MeTHF, the conversions remained low. A possible competition of such solvents (5-membered ring) at the active site of the enzyme with the substrate, which is also constituted by a 5-membered ring may cause this effect since

noncyclic ethers such as MTBE and DIPE apparently do not compete for the active site with the substrate and increase the conversion (Table 1).

Table 1

Effect of solvents and Temperatures on enzymatic desymmetrization

Entry	Solvent	Conversion at 30 °C. [substrate:enzyme ratio (w/w) 5:1]	*e.e.* of **6** at 30 °C	Conversion at 5 °C [substrate:enzyme ratio (w/w) 5:1]	*e.e.* of **6** at 5 °C
1	Hexane	56	No selectivity	10	98
2	Toluene	15		5	96
3	Tetrahydrofuran	70		15	97
4	2-Methyl tetrahydrofuran	82		12	99
5	Dichloromethane	0		0	0
6	Methyl *tert*-butyl ether (MTBE)	100		100	>99
7	Diisopropyl ether	100		100	>99
8	Methyl isobutyl ketone (MIBK)	85		100	>99
9	Dimethyl carbonate	0		0	-

Reaction conditions: **5** (1 g, 5.43 mmol), Solvent (10 mL), Methanol (0.44 mL, 0.01 mmol), Enzyme (200 mg), 16h.

For optimum reaction conditions, MTBE was chosen as solvent and methanol as reagent for transesterification. The effect of methanol concentration on product distribution was studied at 5 °C. Optimum ratio of molar concentration of methanol to substrate was found to be 2.5. Above this concentration, diol formation was quite evident.

2.5. Enzymatic resolution

Novozym-435® catalyzed desymmetrization of *cis*-3,5-Diacetoxy-1-cyclopentene **5** was finally carried out in MTBE at 5 °C in a jacketed stirred vessel. At 10% (w/v) loading of substrate, mole ratio of methanol to substrate at 2.5 and substrate to enzyme ratio of 20% (w/w), the reaction was complete in 16 h. GC analysis of the reaction mass did not show presence of any other component other than the product **6**, methanol, methyl acetate and the solvent. Enzyme was separated by filtration and after concentrating the filtrate on rotavapor, the residue was stored in refrigerator where it crystallized slowly to a white solid. The recovered enzyme was reused 10 times without any appreciable change in conversion rate and enantioselectivity. The combined reaction mass after 10 recycles provided 73 g of **6** (yield 95%, *e.e.* >99%).

3. Conclusion

In conclusion, we have developed an optimized methodology for preparation of *cis*-3,5-Diacetoxy-1-cyclopentene of high purity with overall yield of 30% without chromatographic purification. The procedure described herein uses air instead of pure oxygen and continuous feeding of the reactants with in situ reduction of *endo*-peroxide which reduces the peroxide build-up in the reactor. The overall process is safer compared to earlier reports. Novozym-435® catalyzed desymmetrization via transesterification in methyl *tert*-butyl ether (MTBE) is an improvement over reported procedures in aqueous buffer. The methodology does not involve solvent extraction. The recovered enzyme and the solvent can be recycled

and the product with excellent enantioselectivity is easily isolated. Since the procedure involves a simple two-phase system of immobilized enzyme and substrate solution in organic solvent, it is easy to scale-up.

4. EXPERIMENTAL SECTION

4.1. General methods

HPLC analysis was carried out on Shimadzu LC-1080 VP system with SPD-M10A VP diode array detector and SIL-10 AD VP auto injector. HPLC pump LC-10AT and LC-20 AP, Shimadzu, Japan were used for dosing. GC analysis was performed on Shimadzu QP2010 Plus GC-Mass unit equipped with Mass detector.

Jacketed glass reactor Model RS-37, capacity 1 L, was obtained from Radleys, UK. The 20 L jacketed reactor was manufactured locally. 100 Watts LED lamps were purchased from local market. A Poly Science Digital Temperature Controller with 40 % ethylene glycol-water (75 L capacity) was used for maintaining the temperature of the reactor. Novozym-435® was purchased from M/s Brenntag Ingredients (India) Private Limited, Mumbai and was used as received. All reagents and solvents used were of analytical grade obtained from Alfa Aesar and Qualigens, India.

4.2. Preparation of cyclopentadiene monomer **2**

Dicyclopentadiene (300 g) was slowly added (2 mL/min) with a dosing pump to a 1L, 2-necked round bottom flask, containing magnetically stirred silicone oil (250 mL) heated at 200 °C. The monomer vapors formed due to cracking of the dimer were further passed through a glass column filled with Raschig rings and heated to 300 °C. A downward double walled condenser, maintained at 0 °C cooled the vapors and the monomer was

collected in a receiver maintained at -20 °C with acetone-dry ice. Thus 255 mL (200 g) of monomer was collected in 3 h.

4.3. Photochemical reaction

The reaction was performed in a 1 L jacketed glass reactor maintained at 0-5 °C using an ethylene glycol-water circulating water bath. A solution of thiourea (7.6 g, 100 mmol) and rose bengal (0.5 g) in methanol (500 mL) was introduced and stirred at 150 rpm with aeration (10 L/h) using an air compressor. A 100 watts LED lamp was used for illumination. The monomer (25% (v/v) solution in 2-propanol maintained at -20 °C was continuously added to the reactants at a rate of 0.5 mL/min with a dosing pump. Simultaneously, a solution of thiourea (3 % w/v) in methanol was also pumped separately at the rate of 2 mL/min into the reaction mass. Aliquots of reaction mass were collected at regular intervals of 30 min and stored in refrigerator. The addition of monomer and thiourea was stopped after 2 h. The total amount of monomer added to the reactor at this point was 12.0 g (180 mmol) and thiourea (14.8 g, 195 mmol). The reaction was continued for 4 h more with the light switched on. Afterwards the cooling and illumination were switched off and the reactants were stirred overnight.

The reaction mass was then collected, the reactor was carefully rinsed with methanol and combined methanolic solution was evaporated on rotavapor. The residue was extracted with *n*-heptane (2 × 100 mL). After removal of the solvent, the residue was suspended in dichloromethane (250 mL) in a 500 mL round bottom flask. The flask was placed in an ice bath, the contents were stirred on a magnetic stirrer and triethylamine (40.4 g, 400 mmol) was slowly added. After stirring for 15 min, acetyl chloride (31.4 g, 400 mmol) was added dropwise with cooling. After stirring for 6 h, water (250 mL) was added to dissolve solids and the layers were separated. The aqueous layer was extracted with dichloromethane (2 × 100 mL), solvent was removed from combined organic extracts on rotavapor and the residue

was repeatedly extracted with *n*-heptane (4 × 100 mL). The pale yellow *n*-heptane extract was washed with 10% sodium carbonate solution till the aqueous layer was colourless (3 × 25 mL) to remove last traces of rose bengal. The organic layer was dried over anhydrous magnesium sulphate and evaporated on rotavapor. *cis*-3,5-Diacetoxy-1-cyclopentene **5** was obtained as a pale-yellow oil. Yield 10 g, 30%. Purity >99%, GC. ^{1}H NMR ($CDCl_3$, δ) 6.09 (d, 2H), 5.54 (qd, 2H), 2.86 (quin, 1H), 2.05 (s, 6H), 1.73 (dt, 1H). ^{13}C NMR ($CDCl_3$, δ) 170.61, 134.54, 76.54, 37.07, 21.06. MASS: $[M+Na]^+$ 207; IR [KBR]: 3451, 2948, 1737, 1434 cm^{-1}.

4.4. Analysis of thiourea content by HPLC

Aliquots of samples collected at regular intervals of 30 min were diluted with mobile phase (10 µL to 10 mL) and analysed for thiourea content by reverse phase HPLC. Column: Phenomenex Luna C-18, 5 µ, 250 × 4.5 mm; injection volume 20 µL; mobile phase 10% (v/v) methanol-water, flow rate 1 mL/min, detection at 245 nm. Retention time: 3.1 min.

4.5. Analysis of sulphur content by HPLC

The reaction mass (1 g) was stirred with dichloromethane (100 mL) for 15 min, centrifuged and the supernatant was analyzed by HPLC. Column: ZodiacSil 120-5-CN 5 µ, 250 × 4.5 mm, injection volume 20 µL; mobile phase: *n*-heptane-dichloromethane-2-propanol-acetonitrile (80:6:10:4), flow rate 1 mL/min, detection at 240 nm. Retention time: 3.1 min. A standard sample of sulphur (0.1 mg/mL in mobile phase) was used for comparison.

4.6. Enzymatic transesterification of cis-3,5-Diacetoxy-1-cyclopentene

cis-3,5-Diacetoxy-1-cyclopentene (10 g, 54.3 mmol) dissolved in MTBE (100 mL, 10% w/v) and MeOH (4.4 mL, 100 mmol) were stirred at 150 rpm with immobilized enzyme

(2 g, w/w) at 5 °C in a double walled glass reactor using an overhead mechanical stirrer. The reaction was followed by analysis on TLC and chiral GC. The reaction was complete in 16 h.

4.7. Enzyme recycle

After 12 h of reaction (>99% conversion & >99% *e.e*), the enzyme was separated by filtration and washed twice with MTBE (2 × 5 mL). The combined organic portion was collected separately. The used enzyme was found to be recyclable for up to 10 times without significant change in productivity and activity of the enzyme.

4.8. Product separation

The combined reaction mass after 10 recycles was concentrated on rotavapor and the residue was stored in refrigerator where it crystallized slowly to a white solid (yield 73 g, 95 %, *e.e* >99%). $[\alpha]_D^{25}$ = + 65.80 (*c* = 1, $CHCl_3$), (lit[26b] $[\alpha]_D^{20}$ = + 66 (*c* = 1, $CHCl_3$, *e.e* 99%). The Mass and NMR spectra were comparable with literature report.[27]

4.9. GC analysis

Analysis was performed on Shimadzu QP2010 Plus GC-Mass unit using mass detector. Retention time **4**: 47.97 min; **5**: 55.85 min; (1*R*,4*S*)-**6**: 48.88 min; (1*S*,4*R*)-**6**: 50.69 min. Column SUPELCO β-Dex 120, length 30 mt, Thickness: 0.25 µm; Dia: 0.25 mm. Linear velocity: 49.0 cm/sec; Column oven temp. 64°C, Injection temperature 230 °C, Carrier gas: Helium. Initial column oven temperature was 64 °C hold 2 min and @ 1 °C/min to 130 °C, hold 5 min and increased up to 200 °C at 10 °C/min hold 1 min.

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Notes

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