

<https://doi.org/10.15407/dopovidi2020.03.071>

UDC 547.783

**D.V. Prysiazhnuk, O.O. Kolodiazna, O.I. Kolodiaznyi**

V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry of the NAS of Ukraine, Kyiv  
E-mail: oikol123@bpci.kiev.ua

## Enzymatic deracemization of halogenated dihydroindenols and dihydroindenediols substituted in benzene ring

*Presented by Corresponding Member of the NAS of Ukraine O.I. Kolodiaznyi*

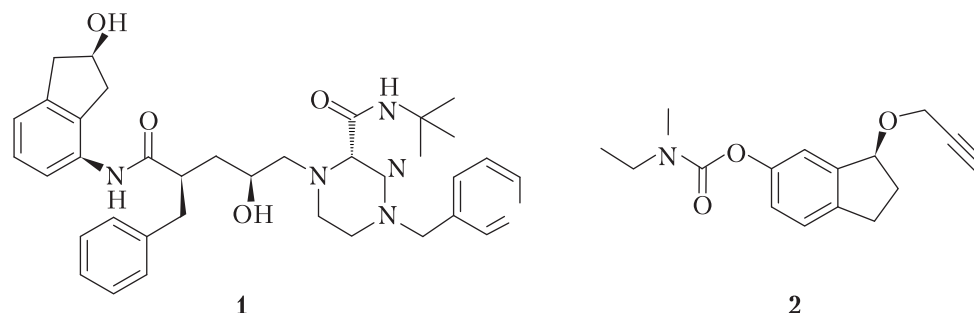
*The optically active halodihydroindenols and dihydroindenediols are components of many biologically active natural compounds, being important pharmacophore groups. In the present work, to obtain the above compounds with a high degree of optical purity, the resolution of racemates into enantiomers using enzymes is proposed. Dihydroindenones, which were reduced with sodium borohydride to dihydroindenols, were used as starting compounds. Burkholderia cepacia lipase (BCL) was used to separate racemic indenols. Racemic indenols were kinetically trans-esterified with vinyl acetate in organic media in the presence of a BCL biocatalyst. As a result, acylated indenols of (R)-absolute configuration and unreacted indenol of (S)-configuration, which were separated into individual compounds by column chromatography, were obtained. The enzymatic resolution of halodihydrinindene acetates by hydrolysis in the presence of Candida antarctica B lipase immobilized on diatomite was also investigated. Enantiomerically enriched halodihydroindenols and halodihydrinindene acetates were obtained when racemic halodihydrinindene acetates were treated with Novozym 435<sup>®</sup> in MTBE for 20–30 hours at 30–40 °C. The enantiomeric purity of the compounds was determined by the derivatization with Mosher acid. The absolute configuration of the compounds was established by the Kazlauskas method.*

**Keywords:** 1,2-aminocycloalkanols, 1,2-diaminocycloalkanes, enzymes, kinetic resolution, Burkholderia cepacia lipase, Candida antarctica lipase B.

The optically active halodihydroindenols and dihydroindenediols are components of many biologically active natural compounds. They attract close attention as synthetic blocks for the construction of important pharmaceutical preparations, peptide nucleic acids, and bioregulators. For example, indinavir is an oral drug used to treat human immunodeficiency virus infection [1]. This protease inhibitor, a highly active antiretroviral therapy product, is used to treat HIV infection and AIDS. *Cis*- and *trans*-indanedediols are the key chiral synthones in the synthesis of indinavir **1** (Scheme 1) [2–4]. Therefore, the chiral derivatives of indanes attract great interest of many chemists, and various approaches to their preparation were developed. Some chiral derivatives of indanes are produced in industry. For example, in the industrial method for producing the chiral synthetic block indinavir – (1*S*,2*R*)-*cis*-aminoindanol, the bacterial cells of *Rhodococ-*

Цитування: Prysiazhnuk D.V., Kolodiazna O.O., Kolodiaznyi O.I. Enzymatic deracemization of halogenated dihydroindenols and dihydroindenediols substituted in benzene ring. *Допов. Нац. акад. наук Укр.* 2020. № 3. С. 71–81. <https://doi.org/10.15407/dopovidi2020.03.071>

ISSN 1025-6415. *Допов. Нац. акад. наук Укр.* 2020. № 3: 71–81



Scheme 1

*cus* sp. Living cells of *Rhodococcus* Sp capable to catalyze the bioconversion of indene to chiral cis and trans indanediols [5] are used.

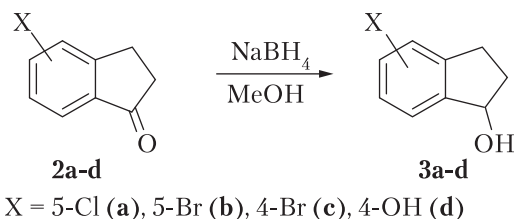
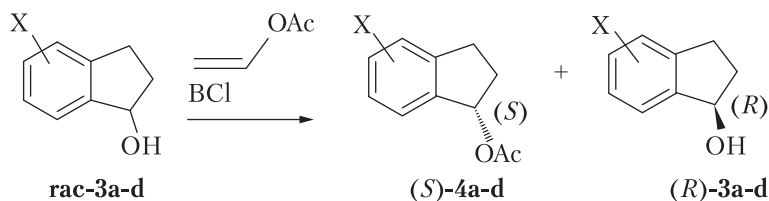
Another important pharmaceutical product based on indanes is Ladostigil **2**, a medicine for the treatment of Alzheimer's disease and Parkinson's disease, which can slow the development of clinical signs within a relatively short period [6–8]. It has a positive effect on symptoms of Alzheimer's disease such as depression, increased anxiety, and various forms of imbecility (Scheme 1).

In the chemical literature, information on the synthesis of halogenindanediols is extremely limited, although a number of patents were devoted to the synthesis and properties of these compounds. In chemical journals, there is only one work of Japanese authors devoted to the synthesis and properties of indanediol derivatives, however, only racemates [9]. Therefore, it is undoubtedly interesting to use the methods of biocatalysis to obtain enantiomerically pure representatives of halodihydroindenol and dihydroindendiols.

The oxidative kinetic resolution of racemic indenols was previously described using acetone as a hydrogen acceptor in the presence of the organometallic catalyst  $[\text{RuCl}_2\text{-(PPh}_3\text{)}_2\text{(ferrocenyl oxazolidine phosphine)}]$  [10]. The disadvantage of this method is the need to use a hard-to-reach and expensive ruthenium catalyst, as well as low yields and the insufficient enantiomeric purity of products. In addition, this method is carried out only on the milligram scale of starting reagents. In another method for the preparation of 1-indenol derivatives, the catalytic hydrogenation of indenones was applied with the use of the optically active Ru-PEG-BsDPEN catalyst [11] or the RuCl catalyst  $[(1S,2S)\text{-}p\text{-tosyl-N-CH(C}_6\text{H}_5\text{)CH(C}_6\text{H}_5\text{)-NH}_2\text{]}(\text{eta},6\text{-}p\text{-cymene})/\text{Bu}_4\text{NBr}$  [12]. The asymmetric hydroxylation of OH and  $\text{NH}_2$  substituted indenols with ethylbenzene dehydrogenase was studied in [13]. The resolution of racemic mixtures of indenols by means of preparative chromatography with the use of expensive precision instruments and chiral chromatographic columns [5], which are also hard-to-reach, was proposed in [6].

**Results and discussion.** The formulated problem was solved in that the kinetic resolution of racemic dihydro-1-indenols was carried out using the enzymatic trans-esterification with vinyl acetate in the presence of *Burkholderia cepacia* lipase [12].

An alternative method for the resolution of racemic mixtures into enantiomers of dihydro-1-indenols is the enzymatic hydrolysis of racemic dihydro-1-indenol acetates at the constant pH 7.2 buffer solution in the presence of *Candida Antarctica* lipase B. Therefore, we have developed a simple stereoselective method for the preparation of dihydro-1-indenols substituted in the aromatic ring, which provided the possibility of obtaining these substances in high yields and with high chemical purity.

**Scheme 2**

BCL – *Burkholderia cepacia* lipase

**Scheme 3**

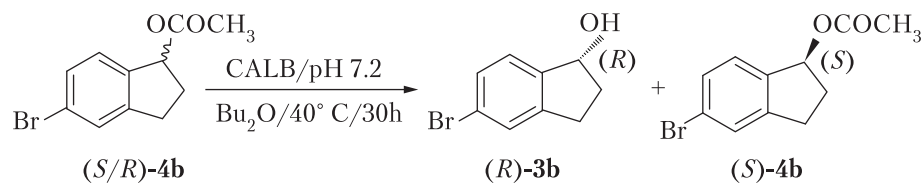
**Synthesis of initial compounds.** Starting from the halodihydroindenones **2a–c**, racemic halodihydroindenols **3a–c** were obtained. For this aim, halodihydroinden-1-ones were reduced with a insignificant excess of sodium borohydride in methanol, first with cooling to 0 °C and then at room temperature. The reaction was completed by a short reflux of the reaction mixture. As a result, **3a–c** halodihydroindenols were obtained as colorless crystalline substances in good yields (Scheme 2).

**Resolution of racemic indanols into stereoisomers.** Racemic halodihydroindenols were separated into enantiomers by the lipase-catalyzed transesterification with vinyl acetate in diisopropyl ether in the presence of *Burkholderia cepacia* lipase (BCL). In this case, (*R*)-halodihydroindenols **3** were isolated in 45 % yield with 100 % *ee* (enantiomeric excess), and (*S*)-halodihydroindenol acetates (+)-**4** were obtained in 40 % yield with 93 % *ee* after column chromatography.

The resolution of halodihydroinden-1-ol into enantiomers was carried out by acylation with vinyl acetate under kinetic control in the presence of *Burkholderia cepacia* lipase (BCL). The reaction was led to 50 % conversion, then the reaction was stopped by filtration of the biocatalyst. As a result, a mixture of diol and acylated diol was obtained. The mixture was separated by column chromatography (Scheme 3).

As a result the enantiomerically and diastereomerically pure haloidanols with 99 % *ee* and the corresponding halodihydroindenol acetates with 96 % *ee* were obtained. It turned out to be unexpected that dihydro-1-indenol substituted in the benzene ring was regioselectively acylated only at the hydroxyl group located in the cyclopentane ring.

It was found that the *tert*-butyl methyl ether (MTBE) as a solvent, the *Burkholderia cepacia* as a biocatalyst, and a temperature of 35 °C are the optimal conditions for the kinetic resolution of halogenindenols. In this case, the completion of the reaction at 50 % conversion provided a smooth and unambiguous reaction between the reagents, which led to the formation of only one (*R*)-enantiomer of indenol acetate. As a result, unreacted indenol of the absolute configuration (*S*)- and acyl indenol of the (*R*)-configuration were obtained, which ensured the resolution of the stereoisomers of this mixture with high chemical yield and high optical purity. Further



Scheme 4

hydrolysis of (*R*)-acyl indenol gives indenol of the (*R*)-configuration. Thus, it is possible to obtain both (*S*)- and (*R*)-enantiomers of indenol.

It is known that the solvent variation of the lipase-catalyzed kinetic resolution can influence the enantiomer or enantiotopic selectivity in many cases, as well as the reaction rate. Therefore, our next investigation was performed to find a more suitable solvent in this system. We found that dialkyl ethers were appropriate solvents for the enzymatic *trans*-esterification in the presence of the same lipase. Therefore, we have tested dibutyl ether (DBE), methyl *t*-butyl ether (MTBE), and toluene. Among the solvents tested, MTBE exhibited the best results. When MTBE was used as a solvent, enantiomeric excess of (+)-**3** was 96 % *ee* (45 % yield) and (–)-**4** was 92 % *ee* (48 % yield), respectively. So, this solvent was shown to be more suitable than the others, although DBE is not much inferior to MTBE in efficiency. Interestingly, it was found that toluene was also applicable to this resolution, though with slightly lower efficiency.

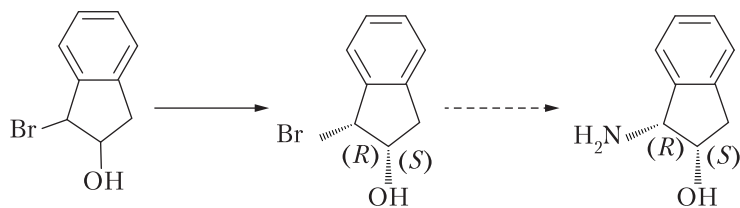
The reaction of kinetic enzymatic hydrolysis of halodihydrinindene acetates was carried out in a two-phase system with a pH 7.2 buffer solution in MTBE medium in the presence of a CALB biocatalyst at 45 °C. The reaction was completed upon reaching 50 % conversion. As a result, the (*R*)-enantiomer of indenol and unreacted (*S*)-indenol acetate were obtained. The methodology ensured the resolution of stereoisomers of a racemic mixture in high chemical yield and with high optical purity. Further hydrolysis of unreacted (*S*)-indenol acetate gave (*S*)-indenol (Scheme 4).

Conveniently, Novozym 435<sup>®</sup> (immobilized lipase from *Candida antarctica*) has heat-resistant properties, so we examined the influence of the reaction temperature on the enantiomeric excess of the product under the same conditions using MTBE as a solvent. Decreasing the reaction temperature (30 °C) did not markedly influence the enantioselectivity or reaction rate. However, the raise of the reaction temperature (60 °C) showed a remarkable increase in the reaction rate, although the progress of the reaction seemed to be halted after 8 hours.

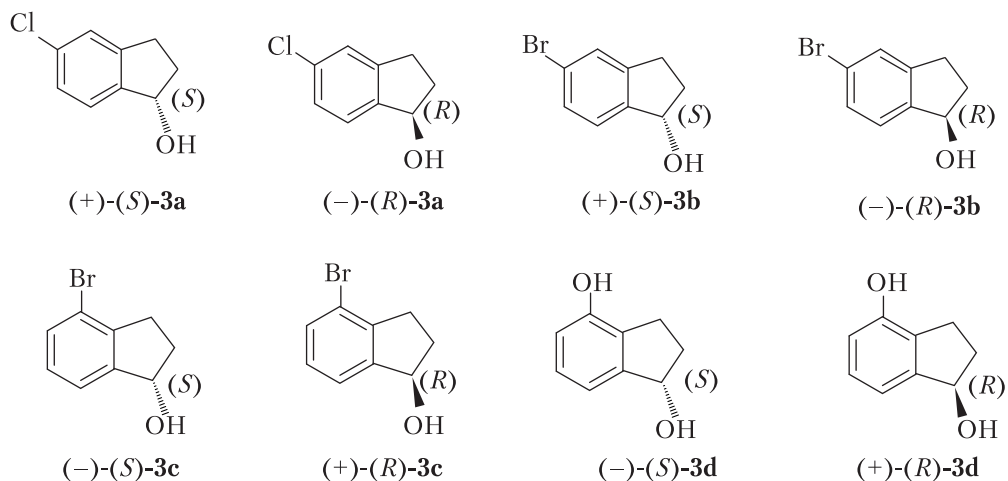
Since the reuse of a recovered enzyme is the most important problem to be overcome to establish a manufacturing process, the influence of the number of cycles on the resolution efficiency was investigated.

We found that lipase can be used up to three cycles without a noticeable decrease in the effectivity of resolution. Only in the fourth cycle, a decrease in the efficiency of the biocatalyst was noted.

Under the optimal conditions (treated with Novozym 435<sup>®</sup> and in dibutyl ether for 20–30 hours at 30–40 °C), (±)-**4** was converted to (1*S*)-**3** (yield: 47 %, 94 % *ee*) and (*R*)-**4** (yield: 49 %, 92 % *ee*), respectively (Scheme 2). The developed methodology provides the easy access to both (*S*)- and (*R*)-enantiomers of indeno-halohydrins with high optical purity. Using *Burkholderia cepacia* lipase and vinyl acetate, we resolved indandiol into enantiomers, which is a key intermediate in the synthesis of Ladostigil. These compounds can be easily converted to aminoinde-



Scheme 5



Scheme 6

nols by the reaction with acetonitrile under the conditions of the Ritter reaction, giving enantiomerically pure (1*S*, 2*R*)-*cis*-1-aminoindan-2-ol (Scheme 5) [14].

Chlorine and bromoindanol are colorless crystalline substances whose structure and chemical purity were confirmed by NMR spectra. Optical purity was established by the derivatization with Mosher acid, as well as by chiral HPLC analysis on a Chiralpak OD-3 column (hexane : IPA : MeOH = 95 : 2.5 : 2.5), flow rate = 0.6 mL/min,  $\lambda = 210$  nm). After recrystallization, the purity of synthesized bromoindanol was on the level of 99 % *ee* (Scheme 6).

The Kazlauskas rule was used to determine the absolute stereochemistry of enantiomerically pure products. The Kazlauskas rule is an empirical model based on the postulate that the enantioselectivity is proportional to the difference in size between large (L) and middle (M) substituents in the substrate. According to the Kazlauskas rule, these substituents are located in two different pockets of the active site of an enzyme, according to their size, which determines the absolute configuration of the products in the enzymatic reaction. Using this empiric rule, it was found that biocatalytic acylation produces (*R*)-esters and (*S*)-halogenindanol remain unreacted.

The experiments performed show that the proposed biocatalyst allows one to obtain both optical enantiomers of dihydro-1-indenols in high yields and high enantiomeric purity in a solution of methyl tert-butyl ether at room temperature and a moderate amount of the biocatalyst, using simple experimental conditions [5–8].

**Experimental part.**  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded in a  $\text{CDCl}_3$  solvent on a 500 MHz spectrometer at ambient temperature. Chemical shifts ( $\delta$ ) are shown in ppm in relation

to TMS as an internal standard. Signal multiplicity is shown as s – singlet; d – doublet; dd – doublet of a doublet; td – triplet of doublets; t – triplet; m – multiplet; br s – wide singlet. The spin-spin coupling constants,  $J$  are indicated in Hertz. Chiral HPLC analysis was done on an Agilent 1100 system equipped by a Chiralpak<sup>®</sup> OD-3 column or Chiracel<sup>®</sup> (Chiral Technologies) analytical columns with cellulose-based stationary phase. All reagents and solvents were used without special purification, unless otherwise indicated. Column chromatography was performed on silica gel 60 (70-230 mesh) using the indicated eluents. Optical rotations were measured on a Perkin-Elmer 241 polarimeter (D sodium line at 20 °C). Melting points were not corrected. All reactions were carried out in glassware dried on a fire or dried in a drying chamber with stirring on a magnetic stirrer. Lipase from *Burkholderia cepacia* (Amano PS) was purchased from Amano Pharmaceutical (Japan). The progress of the reactions was monitored by analytical thin layer chromatography (TLC) on glass plates of silica gel 60 F254 (Merck, Germany) and the products were visualized with anisaldehyde or UV. The purity of all compounds was checked using thin layer chromatography and NMR measurements.

(*S*)-5-Chloro-2,3-dihydro-1H-inden-1-ol ((*S*)-**3a**). A solution of racemic (*R/S*)-5-chloro-2,3-dihydro-1H-inden-1-ol (1.7 g, 0.01 mol), *Burkholderia cepacia* lipase (0.1 g), vinyl acetate (3 ml) in MTBE (3 ml) was stirred at 24 °C. Indenol acylation was monitored by TLC and NMR. The reaction mixture was stirred to 50 % conversion of the starting alcohol for 18 g. Then the reaction mixture was filtered, evaporated *in vacuo*, the resulting substance was chromatographed on column with silica gel using eluent hexane-ethyl acetate-ethanol (95 : 5–3 : 1). Indenol acetate of (*R*)-absolute configuration and unreacted indenol of (*S*)-absolute configuration were obtained. The resulting (*S*)-indenol was further recrystallized in toluene.

The yield of optically pure (*S*)-5-chloro-2,3-dihydro-1H-inden-1-ol is 50 %, mp 105 °C,  $[\alpha]_D^{20} +15$  (C = 3, CHCl<sub>3</sub>) or +9 (C = 3, ethanol).

<sup>1</sup>H NMR, (CDCl<sub>3</sub>),  $\delta$ , ppm ( $J$ , Hz): 1.95 m (1H, CH); 2.48 m (1H, CH); 2.80 m (1H, CH); 3.01 m (1H, CH); 5.19 t (1H,  $J = 6.0$  Hz, CHOH); 7.18 m (2H, H-Ar); 7.32 m (1H, H-Ar).

<sup>13</sup>C NMR, (CDCl<sub>3</sub>),  $\delta$ , ppm ( $J$ , Hz):  $\delta_C$  30.1; 35.9; 76.1; 126; 127; 128.9; 133.8; 143; 143.1.

Found, %: C, 64.35; H, 5.34; Cl, 21.25. C<sub>9</sub>H<sub>9</sub>ClO. Calculated, %: C, 64.11; H, 5.38; Cl, 21.02.

(*R*)-5-Chloro-2,3-dihydro-1H-inden-1-yl acetate ((*R*)-**4a**). Acetate obtained in the previous experiment was purified by vacuum distillation.

Yield 45 %, bp 75 °C,  $[\alpha]_D^{20} +76.36$  (C = 1, CHCl<sub>3</sub>).

<sup>1</sup>H NMR, (CDCl<sub>3</sub>),  $\delta$ , ppm ( $J$ , Hz):  $\delta_H$  2.15 s (3H, CH<sub>3</sub>CO); 2.13 m (1H, CH); 2.55 m (1H, CH); 2.9 m (1H, CH); 3.1 m (1H, CH); 6.13 s (1H, CHOH); 7.2 m (1H), 7.25 m (1H), 7.57 m (1H, H-Ar).

<sup>13</sup>C NMR, (CDCl<sub>3</sub>),  $\delta$ , ppm ( $J$ , Hz):  $\delta_C$  21.1; 30.3; 32.2; 79.7; 125.5; 126.7; 129.1; 134.2; 139.1; 144.1; 170.4.

Found, %: C, 62.72; H, 5.26. C<sub>11</sub>H<sub>11</sub>ClO<sub>2</sub>. Calculated, %: C, 62.72; H, 5.26.

(*R*)-5-Chloro-2,3-dihydro-1H-inden-1-ol ((*R*)-**3b**). Acetate **4a** (2.1 g, 0.01 mol) was hydrolyzed by treatment with a solution of potash (2 g) in 20 ml of methanol with stirring at room temperature. The progress of hydrolysis was monitored by TLC. The solvent was evaporated *in vacuo*, the residue was extracted with ethyl acetate. Then the extract was evaporated, and the residue was recrystallized in hexane. Pure (*R*)-indenol **3b** was obtained.

Yield 45 %, mp. 105 °C,  $[\alpha]_D^{20} = -15$  (C = 3, CDCl<sub>3</sub>).

$^1\text{H}$  NMR, ( $\text{CDCl}_3$ ),  $\delta$ , ppm ( $J$ , Hz):  $\delta_{\text{H}}$  1.95 m (1H, CH); 2.48 m (1H, CH); 2.80 m (1H, CH); 3.01 m (1H, CH); 5.19 t (1H, CHOH); 7.18 m (2H, H-Ar); 7.32 m (1H, H-Ar).

$^{13}\text{C}$  NMR, ( $\text{CDCl}_3$ ),  $\delta$ , ppm ( $J$ , Hz):  $\delta_{\text{C}}$  30.1; 35.9; 76.1; 126; 127, 128.9; 133.8; 143; 143.1.

Found, %: C, 64.02; H, 5.51.  $\text{C}_9\text{H}_9\text{ClO}$ . Calculated, %: C, 64.11; H, 5.38.

(*S*)-5-Chloro-2,3-dihydro-1H-inden-1-ol ((*S*)-**3a**). (*S/R*)-5-Chlor-2,3-dihydro-1H-inden-1-yl acetate was dissolved in MTBE, and phosphate buffer (0.05 M; pH = 7.2) and *Candida Antarctica* lipase B (20 % by weight of the substrate) were added. The reaction mixture was stirred for about 12–18 hours at room temperature. The progress of the reaction was monitored by NMR. When the reaction was completed, the biocatalyst was filtered off. The organic phase was separated from water. The water was washed 2 times with MTBE. The combined organic solutions were dried with sodium sulfate and evaporated. Optical purity was determined by NMR of the product derivatized with Mosher acid.

The yield of (*R*)-indenol is 45 %.  $[\alpha]_D^{20} - 25$  ( $C = 3$ ,  $\text{CDCl}_3$ ). The yield of unreacted (*S*)-acylindenol is 46 %  $[\alpha]_D^{20} + 81$  ( $C = 1$ ,  $\text{CDCl}_3$ ).

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.95–1.89 (m, 1H), 2.22 (s, 1H), 2.50–2.44 (m, 1H), 2.81–2.75 (m, 1H), 3.02–2.96 (m, 1H), 5.18–5.15 (m, 1H), 7.20–7.18 (m, 2H), 7.30–7.28 (m, 1H),

$^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  145.21, 143.36, 133.96, 126.86, 125.27, 124.99, 75.59, 35.96, 29.59.

(*S*)-5-Bromo-2,3-dihydro-1H-inden-1-ol ((*S*)-**3b**). The solution of (*S/R*)-5-Brom-2,3-dihydro-1H-inden-1-ol (2.3 g, 0.01 mol), *Burkholderia cepacia* lipase (0.2 g), and vinyl acetate (3 ml) in MTBE (10 ml) was stirred at +24 °C. The course of the reaction was monitored by TLC and NMR. The reaction mixture was stirred to 50 % conversion of the starting alcohol for 18 hours. The reaction mixture was filtered and evaporated *in vacuo*, the residue was separated by silica gel column chromatography, eluent hexane-ethyl acetate-ethanol (95 : 53 : 1). The isolated (*S*)-dihydro-1H-inden-1-ol was recrystallized from toluene.

Yield 50 %, mp 100 °C.  $[\alpha]_D^{20} + 20$  ( $C = 3$ ,  $\text{CDCl}_3$ ).

$^1\text{H}$  NMR, ( $\text{CDCl}_3$ ),  $\delta$ , ppm ( $J$ , Hz):  $\delta_{\text{H}}$  1.88–1.99 (m, 2H), 2.42–2.53 (m, 1H), 2.74–2.85 (m, 1H), 2.97–3.07 (m, 1H), 5.18 (t,  $J = 6.6$  Hz, 1H), 7.26 (d,  $J = 2.7$  Hz, 1H), 7.34 (s, 1H), 7.37 (d,  $J = 3.0$  Hz, 1H).

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$ : 145.6, 143.9, 129.7, 128.0, 125.7, 122.2, 75.6, 35.9, 29.6

Found, %: C, 50.44; H, 4.41.  $\text{C}_9\text{H}_9\text{BrO}$ : Calculated, %: C, 50.73; H, 4.26.

(*R*)-5-Bromo-2,3-dihydro-1H-inden-1-ol acetate ((*R*)-**4b**). The acetate obtained in the previous experiment was purified by vacuum distillation. Yield 40 %. Bp. 100 °C (0.01 mmHg).

$^1\text{H}$  NMR, ( $\text{CDCl}_3$ ),  $\delta$ , ppm ( $J$ , Hz):  $\delta_{\text{H}}$  2.05 c (3H,  $\text{CH}_3\text{CO}$ ); 2.55 m (1H, CH); 2.9 m (1H, CH); 3.1 m (1H, CH); 6.13 s (1H, CHO); 7.2 m (1H), 7.25 m (1H), 7.57 m (1H, H-Ar).

$^{13}\text{C}$  NMR, ( $\text{CDCl}_3$ ),  $\delta$ , ppm ( $J$ , Hz):  $\delta_{\text{C}}$  21.1; 29.5; 32.2; 79.7; 121.4; 123.2; 124.5; 131.6; 141.4; 143; 170.4.

(*R*)-5-Bromo-2,3-dihydro-1H-inden-1-ol ((*R*)-**3b**). The acetate (0.01 mol) obtained in the previous experiment was hydrolyzed by treatment with potash (2 g) in 20 ml of methanol with stirring at room temperature. The progress of hydrolysis was monitored by TLC analysis. The solvent was evaporated, and the residue was extracted with ethyl acetate. Then the extract was evaporated, and the residue was recrystallized from hexane.

Yield 50 %, mp. 105 °C.  $[\alpha]_D^{20} = -20$  ( $C = 3$ ,  $\text{CDCl}_3$ ).

$^1\text{H}$  NMR, ( $\text{CDCl}_3$ ),  $\delta$ , ppm ( $J$ , Hz):  $\delta_{\text{H}}$  1.69 s (1H, OH); 1.95 m (1H, CH); 2.48 m (1H, CH); 2.80 m (1H, CH); 3.01 m (1H, CH); 5.19 t (1H, CHOH); 7.18 m (2H, H-Ar); 7.32 m (1H, H-Ar).

$^{13}\text{C}$  NMR, ( $\text{CDCl}_3$ ),  $\delta$ , ppm ( $J$ , Hz):  $\delta_{\text{C}}$  30.1; 35.9; 76.1; 126; 127, 128.9; 133.8; 143; 143.1.

Found, %: C, 50.45; H, 4.29.  $\text{C}_9\text{H}_9\text{BrO}$ . Calculated, %: C, 50.73; H, 4.26.

(*R*)-5-Bromo-2,3-dihydro-1*H*-inden-1-ol ((*R*)-**3b**). (*R*)-5-Bromo-2,3-dihydro-1*H*-inden-1-yl acetate is dissolved in MTBE, and phosphate buffer (0.05 M; pH = 7.2) and CALB (20 % by weight of the substrate) were added. The reaction mixture was stirred for about 12–18 hours at room temperature. The progress of the reaction was monitored by NMR. When the reaction was completed the biocatalyst was filtered. The organic phase was separated from water. The aqueous phase was washed 2 times with MTBE. The combined organic solutions were dried with sodium sulfate and evaporated. The optical purity was defined by NMR of the product derivatized by Mosher acid. The yield 45 %,  $[\alpha]_D^{20} = -25$  ( $C = 3$ ,  $\text{CDCl}_3$ ).

The yield of unreacted (*S*)-acylindenol is 44 %  $[\alpha]_D^{20} + 75$  ( $C = 1$ ,  $\text{CDCl}_3$ ).

(*S*)-4-Bromo-2,3-dihydro-1*H*-inden-1-ol ((*S*)-**3c**). The solution of (*S*/*R*)-4-Bromo-2,3-dihydro-1*H*-inden-1-ol (2.3 g, 0.01 mol), *Burkholderia cepacia* lipase (0.2 g) and vinyl acetate (3 ml) in MTBE (10 ml) was stirred at +24 °C. The course of the reaction was monitored by TLC and NMR. The reaction mixture was stirred upto 50 % conversion of the starting alcohol for 18 hours. The reaction mixture was filtered and evaporated in vacuo. The residue was separated into unreacted (*S*)-indenol and (*R*)-indenyl acetate by column chromatography with silica gel, eluent hexane-ethyl acetate-ethanol (95 : 53 : 1). Isolated (*S*)-bromo-dihydro-1*H*-inden-1-ol was recrystallized from toluene.

Yield 50 %, mp 85 °C,  $[\alpha]_D^{20} = -19.00$  ( $C = 0.25$ , EtOH).

$^1\text{H}$  NMR, ( $\text{CDCl}_3$ ),  $\delta$ , ppm ( $J$ , Hz):  $\delta_{\text{H}}$  1.85–1.90 (m, 1H) 2.02–1.90 (m, 1H), 2.40–2.47 (m, 1H), 2.88–2.77 (m, 1H), 3.08 (ddd,  $J = 16.4$  Hz, 8.4 Hz, 4.8 Hz, 1H), 5.35–5.27 (m, 1H), 7.35 (d,  $J = 7.6$  Hz, 1H), 7.13 (t,  $J = 7.6$  Hz, 1H), 7.43 (d,  $J = 8.0$  Hz, 1H).

$^{13}\text{C}$  NMR, ( $\text{CDCl}_3$ ),  $\delta$ , ppm ( $J$ , Hz):  $\delta_{\text{C}}$  30.11, 35.82, 76.00, 120.20, 126.06, 127.25, 132.49, 142.55, 146.77.

(*R*)-4-Bromo-2,3-dihydro-1*H*-inden-1-ol ((*R*)-**3c**). Acetate **4c** (0.01 mol) obtained in the previous experiment was hydrolyzed by potash (2 g) in 20 ml of methanol with stirring at room temperature. The progress of hydrolysis was monitored by TLC. The solvent was evaporated, and the residue was extracted with ethyl acetate. Then the extract was evaporated, and the residue was recrystallized from hexane.

Yield 50 %, mp 85 °C,  $[\alpha]_D^{20} = +18.93$  ( $C = 0.25$ , EtOH).

$^1\text{H}$  NMR, ( $\text{CDCl}_3$ ),  $\delta$ , ppm ( $J$ , Hz):  $\delta_{\text{H}}$  1.86–1.77 (m, 1H) 2.02–1.90 (m, 1H), 2.58–2.47 (m, 1H), 2.88–2.77 (m, 1H), 3.08 (ddd,  $J = 16.4$  Hz, 8.4 Hz, 4.8 Hz, 1H), 5.35–5.27 (m, 1H), 7.35 (d,  $J = 7.6$  Hz, 1H), 7.13 (t,  $J = 7.6$  Hz, 1H), 7.43 (d,  $J = 8.0$  Hz, 1H),

$^{13}\text{C}$  NMR, ( $\text{CDCl}_3$ ),  $\delta$ , ppm ( $J$ , Hz): 31.1134.82, 77.00, 120.20, 123.06, 131.25, 143.49, 128.55, 146.77,

HRMS (EI) calculated for  $\text{C}_9\text{H}_9\text{BrO}^+$  ( $[\text{M}]^+$ ): 211.9837, found: 211.9836.

(*R*)-2,3-Dihydro-1*H*-inden-1,4-diol ((*R*)-**3d**). The solution of (*S*/*R*)-2,3-dihydro-1*H*-inden-1,4-diol **3d** (1.5 g, 0.01 mol), *Burkholderia cepacia* lipase (0.2 g), vinyl acetate (10 ml) and MTBE (3 ml) were stirred at 24 °C. The formation of the acetylated product was monitored by TLC



and NMR. The reaction mixture was stirred to 50 % conversion of the starting alcohol, which took 18 hours. The reaction mixture was filtered and evaporated *in vacuo*, the resulting oil was purified by silica gel chromatography using eluent hexane-ethyl acetate-ethanol (95 : 53 : 1). Indenediols was recrystallized from toluene.

Yield 50 %, mp 135 °C,  $[\alpha]_D^{20} = +42.95$  (C = 1, CDCl<sub>3</sub>).

<sup>1</sup>H NMR, (CDCl<sub>3</sub>), δ, ppm (*J*, Hz): δ<sub>H</sub> 2.0 m (1H, CH); 2.25 s (1H, OH); 2.5 m (1H, CH); 2.75 m (1H, CH); 3.0 m (1H, CH); 4.8 br (1H, OH); 5.25 m (1H, CHOH); 6.75 m (1H, H-Ar); 7.05 m (1H, H-Ar); 7.2 m (1H, H-Ar).

<sup>13</sup>C NMR, (CDCl<sub>3</sub>), δ, ppm (*J*, Hz): δ<sub>C</sub> 26.21 s (CH); 35.11 s (CH); 75.82 s (CHOH); 114.16 s (C-Ar); 115.39 s (C-Ar); 128.86 s (C-Ar); 128.99 s (C-Ar); 147.63 s (C-Ar); 153.25 s [C(OH)-Ar].

Found, %: C, 71.45; H, 6.82 C<sub>9</sub>H<sub>10</sub>O<sub>2</sub>. Calculated, %: C, 71.98; H, 6.71.

(*S*)-5-hydroxy-2,3-dihydro-1*H*-inden-1-yl acetate ((*S*)-**4d**). Acetate obtained in the previous example was purified by vacuum distillation. Yield 40 %.

<sup>1</sup>H NMR, (CDCl<sub>3</sub>), δ, ppm (*J*, Hz): δ<sub>H</sub> 2.1 s (3H, CH<sub>3</sub>CO); 2.2 m (1H, CH<sub>2</sub>); 2.4 m (1H, CH<sub>2</sub>); 2.7 m (2H, CH<sub>2</sub>); 5.7 br (OH); 6.12 s (CHO); 6.56 s; 6.65 s; 7.15 s; 7.16 s (3H, H-Ar).

Found, %: C 68.45; H 6.19. C<sub>11</sub>H<sub>12</sub>O<sub>3</sub>. Calculated, %: C 68.74; H 6.29.

(*S*)-2,3-Dihydro-1*H*-inden-1,4-diol ((*S*)-**3d**). (*S*)-2,3-dihydro-1*H*-indene-1,4-diol 1-acetate **4d** (5 mmol) was hydrolyzed in a phosphate-buffered aqueous solution at a constant pH 7.2 using *Burkholderia cepacia* lipase (0.2 g) with stirring and room temperature. Hydrolysis was monitored by TLC and <sup>1</sup>H NMR. Next, the lipase was filtered and washed with methylene chloride. The solvent was evaporated, and the residue was extracted with ethyl acetate. Then the extract was evaporated, and the residue was crystallized from hexane to give reduced alcohol.

Yield 42 %, mp 135 °C,  $[\alpha]_D^{20} = -42.95$  (C = 1, CDCl<sub>3</sub>).

<sup>1</sup>H NMR, (CDCl<sub>3</sub>), δ, ppm (*J*, Hz): δ<sub>H</sub> 2,0 m (1H, CH); 2,25 s (1H, OH); 2.5 m (1H, CH); 2.75 m (1H, CH); 3.0 m (1H, CH); 4.8 m (1H, OH); 5.25 m (1H, CHOH); 6.75 m (1H, H-Ar); 7.05 m (1H, H-Ar); 7.2 m (1H, H-Ar).

<sup>13</sup>C NMR, (CDCl<sub>3</sub>), δ, ppm (*J*, Hz): δ<sub>C</sub> 26,21 s (CH); 35.11 s (CH); 75.82 s (CHOH); 114.16 s (C-Ar); 115.39 s (C-Ar); 128.86 s (C-Ar); 128.99 s (C-Ar); 147.63 s (C-Ar); 153.25 s [C(OH)-Ar].

Found, %: C, 71.66; H, 6.92. C<sub>9</sub>H<sub>10</sub>O<sub>2</sub>: Calculated: C, 71.98; H, 6.71.

## REFERENCES

1. Shankar, S. S., Dubé, M. P., Gorski, J. C., Klaunig, J. E. & Steinberg, H. O. (2005). Indinavir impairs endothelial function in healthy HIV-negative men. *Am. Heart J.*, 150, pp. 933. <https://doi.org/10.1016/j.ahj.2005.06.005>
2. Liu, F., Boross, P. I., Wang, Y. F., Tozser, J., Louis, J. M., Harrison, R. W. & Weber, I. T. (2005). Kinetic, stability, and structural changes in high-resolution crystal structures of HIV-1 protease with drug-resistant mutations L24I, I50V, and G73S. *J. Mol. Biol.*, 354, pp. 789-800. <https://doi.org/10.1016/j.jmb.2005.09.095>
3. Eira, M., Araujo, M. & Seguro, A. C. (2006). Urinary NO<sub>3</sub> excretion and renal failure in indinavir-treated patients. *Braz. J. Med. Biol. Res.*, 39, pp. 1065-1070. <https://doi.org/10.1590/S0100-879X2006000800009>
4. Igarashi, Y., Otsutomo, S., Harada, M. & Nakano, S. (1997). Enzymatic resolution of indene bromohydrin acetate using immobilized lipase. *Tetrahedron: Asymmetry*, 8, No. 16, pp. 2833-2837. [https://doi.org/10.1016/S0957-4166\(97\)00351-0](https://doi.org/10.1016/S0957-4166(97)00351-0)

5. Luo, Z., Qin, F., Yan, S. & Li, X. (2012). An efficient and promising method to prepare Ladostigil (TV3326) via 25 asymmetric transfer hydrogenation catalyzed by Ru-Cs-DPEN in an HCOONa-H<sub>2</sub>O-surfactant system. *Tetrahedron: Asymmetry*, 23, pp. 333-338. <https://doi.org/10.1016/j.tetasy.2012.02.022>
6. Weinreb, O., Mandel, S. & Bar-Am, O. (2009). Multifunctional neuroprotective derivatives of rasagiline as anti-Alzheimer's disease drugs. *Neurotherapeutics*, 6, No. 1, pp. 163-174. <https://doi.org/10.1016/j.nurt.2008.10.030>
7. Weinstock, M., Luques, L., Bejar, C. & Shoham, S. (2006). Ladostigil, a novel multifunctional drug for the treatment of dementia co-morbid with depression. *J. Neural Transm. Suppl.*, 70, pp. 443-446. [https://doi.org/10.1007/978-3-211-45295-0\\_67](https://doi.org/10.1007/978-3-211-45295-0_67)
8. Yoshimatsu, S., Yamada, A. & Nakata, K. (2018). Silylative kinetic resolution of racemic 1-Indanol derivatives catalyzed by chiral guanidine. *J. Org. Chem.*, 83, pp. 452-458. <https://doi.org/10.1021/acs.joc.7b02493>
9. Murase, K., Nligata, K., Mase, T. & Murakami, M. (1972). Synthesis of new indent derivatives with  $\beta$ -adrenergic blocking properties. *Yakugaku Zasshi*, 92, Iss. 11, pp. 1358-1363. [https://doi.org/10.1248/yakushi1947.92.11\\_1358](https://doi.org/10.1248/yakushi1947.92.11_1358)
10. Pat. 2006/0199974 A1 US, IPC C07C 271/40, Process for the synthesis of enantiomeric indanylamine derivatives. Boulton, L.T., Lennon, I.C. & Bahar, E., Publ. 07.09.2006.
11. Kišić, A., Stephan, M. & Mohar, B. (2015). *ansa*-Ruthenium(II) complexes of R<sub>2</sub>NSO<sub>2</sub>DPEN-(CH<sub>2</sub>)<sub>n</sub> ( $\eta^6$ -aryl) conjugate ligands for asymmetric transfer hydrogenation of aryl ketones. *Adv. Synth. Catal.*, 357, pp. 2540-2546. <https://doi.org/10.1002/adsc.201500288>
12. Pat. 6,645,961 B1 US, IPC A61K 31/535, A61K 31/495, A61K 31/47, Dry granulation formulation for an HIV protease inhibitor. Lui, C.Y., Ostovic, D., Katdare, A.V. & Stelmach, C., Publ. 11.11.2003.
13. Pat. WO 2012/101011, IPC C07C 237/24, C07D 257/00, C07D 311/00, C07D 333/00, C07D 271/00, C07D 305/00, A61P 13/12, A61K 31/165, A61K 31/4245, A61K 31/41, New aryl-benzocycloalkyl amide derivatives, Aebi, J., Binggeli, A., Hertel, C., Konkar, A.A., Kuehne, H., Kuhn, B., Maerki, H.P. & Wang, H., Publ.02.08.2012.
- 14 Pat. 120692 UA, IPC C07C 27/08, C07C 27/22, Method of producing stereoisomers of dihydro-1-indoles of formula 1 using kinetic separation of racemates in the presence of Burkholderia cepacia lipase or Candida antarctica lipase B enzymes, Kolodiazhnyi, O.I., Kolodiazhna, A.O. & Kolodiazhna, O.O., Publ. 10.11.2017 (in Ukrainian).

Received 09.01.2020

Д.В. Присяжнюк, О.О. Колодяжна, О.І. Колодяжний

Інститут біоорганічної хімії та нафтохімії ім. В.П. Кухаря НАН України, Київ

E-mail: oikol123@bpci.kiev.ua

#### ФЕРМЕНТАТИВНА ДЕРАЦЕМІЗАЦІЯ ЗАМІЩЕНИХ У БЕНЗОЛЬНОМУ КІЛЬЦІ ГАЛОГЕНДИГІДРОІНДЕНОЛІВ І ДИГІДРОІНДЕНДІОЛІВ

Оптично активні галогендигідроінденоли і дигідроіндендіоли – компоненти багатьох біологічно активних природних сполук, є важливими фармакофорними групами. У представленій роботі для отримання вищевказаних сполук високого ступеня оптичної чистоти запропонований поділ рацематів на енантіомери за допомогою ферментів. Дигідроінденони, які відновлювали боргідридом натрію до дигідроінденолів, використовували як вихідні сполуки. Для поділу рацемічних інденолів використовували ліпазу *Burkholderia cepacia* (BCL). Рацемічні інденоли піддавали кінетичній переестерифікації вінілацетату в органічних середовищах у присутності біокатализатора BCL. У результаті отримано ацильований інденол з абсолютною конфігурацією (*R*) і інденол, що не прореагував, конфігурації (*S*), які були розділені на індивідуальні сполуки колонковою хроматографією. Досліджено також ферментативне розщеплення ацетатів інден-галогенгидринів гідролізом у присутності іммобілізованої на діатоміті ліпази *Candida Antarctica B*. У результаті отримано енантіомерно чисті (*S*)-галогенінданоли і (*R*)-ацетоксигалогеніндани. Запропонований біокатализатор дає можливість одержувати обидва оптичні енантіомери дигідро-1-інденолів з високими виходами і високою енантіомерною чистотою у розчині *метил-трет*-бутилового ефіру при кімнатній температурі і помірній кількості біокатализатора, що спрощує процес досягнення цих продуктів.

Енантіомерну чистоту сполук визначали шляхом дериватизації кислотою Мошера. Абсолютну конфігурацію сполук встановлювали за методом Казлаускаса.

**Ключові слова:** 1,2-аміноциклоалканоли, 1,2-діаміноциклоалкани, ферменти, кінетичне розділення, *Burkholderia cepacia* ліпаза, *Candida antarctica* ліпаза В.

Д.В. Присяжнюк, О.О. Колодяжна, О.И. Колодяжний

Институт биоорганической химии и нефтехимии им. В.П. Кухаря НАН Украины, Киев

E-mail: oikol123@bpci.kiev.ua

#### ФЕРМЕНТАТИВНАЯ ДЕРАЦЕМИЗАЦИЯ ЗАМЕЩЕННЫХ В БЕНЗОЛЬНОМ КОЛЬЦЕ ГАЛОГЕНДИГИДРОИНДЕНОЛОВ И ДИГИДРОИНДЕНДИОЛОВ

Оптически активные галогендигидроинденолы и дигидроиндендиолы — компоненты многих биологически активных природных соединений, являются важными фармакофорными группами. В представленной работе для получения вышеуказанных соединений высокой степени оптической чистоты предложено разделение рацематов на энантиомеры с помощью ферментов. Дигидроинденоны, которые восстанавливали боргидридом натрия до дигидроинденолов, использовали в качестве исходных соединений. Для разделения рацемических инденолов использовали липазу *Burkholderia cepacia* (BCL). Рацемические инденолы подвергали кинетической переэстерификации винилацетатом в органических средах в присутствии биокатализатора BCL. В результате получены ацилированный инденол, имеющий абсолютную конфигурацию (*R*), и непрореагировавший инденол конфигурации (*S*), которые были разделены на индивидуальные соединения колоночной хроматографией. Исследовано также ферментативное расщепление ацетатов инден-галогенгидринов гидролизом в присутствии иммобилизованной на диатомите липазы *Candida antarctica* В. В результате получены энантимерно чистые (*S*)-галогенинданолы и (*R*)-ацетокси-галогенинданы. Предложенный биокатализатор позволяет получать оба оптических энантиомера дигидро-1-инденолов с высокими выходами и высокой энантиомерной чистотой в растворе *метил-трет*-бутилового эфира при комнатной температуре и умеренном количестве биокатализатора, что упрощает процесс достижения этих продуктов. Энантиомерную чистоту соединений определяли путем дериватизации кислотой Мошера. Абсолютную конфигурацию соединений устанавливали по методу Казлаускаса.

**Ключевые слова:** 1,2-аміноциклоалканоли, 1,2-діаміноциклоалкани, ферменти, кінетичне розділення, *Burkholderia cepacia* ліпаза, *Candida antarctica* ліпаза В.