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Synthesis and enzymatic resolution of methyl ferrocenylpentanoate using *Thermomyces lanuginosus* lipase for construction of ferrocene alkyl thiols

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Racemic methyl 3-methyl-5-ferrocenylpentanoate was synthesized by the acylation of ferrocene with 3-methylglutaric anhydride under Friedel-Crafts conditions followed by the Clemmensen reduction of the obtained (±)-3-methyl-4-ferrocenylbutanoic acid and subsequent esterification. The kinetic resolution of the obtained compound in phosphate buffer, pH 7.0 using *Thermomyces lanuginosus* lipase was studied. After two consecutive steps of hydrolysis the unaltered methyl (+)-3-methyl-5-ferrocenylpentanoate with enantiomeric excess (ee) up to 87% was isolated. The other enantiomer, methyl (–)-3-methyl-5-ferrocenylpentanoate with ee 73%, was synthesized by the esterification of (–)-3-methyl-5-ferrocenylpentanoic acid formed in the hydrolysis reaction. The enantiomeric purity of the obtained esters was determined by NMR using a chiral shift reagent. These enantiomerically enriched compounds could be used as starting materials in the synthesis of chiral (S)- and (R)-ferrocene electroactive substrates containing terminal mercapto functions.

Key words: methyl 3-methyl-5-ferrocenylpentanoate, enzymatic resolution, *Thermomyces lanuginosus* lipase

INTRODUCTION

In the previous papers [1–4] we have described the synthesis and investigation of a number of electrochemically active dicyclopentadienylirons (ferrocenes), containing homologous alkylalkanoate and other functionalized chains. Au electrodes coated with these compounds or modified by the respective self-assembled monolayers were found to be suitable in the construction of amperometric (bio) sensors for the detection and analysis of hydrolytic enzymes including *Thermomyces lanuginosus* lipase (TLL, EC 3.1.1.3) and bovine intestinal mucosa alkaline phosphatase, as well as for practically important derivatives of vitamin C, such as ascorbic acid palmitate and ascorbic acid phosphate.

It is well known that the reactions proceeding with the use of enzymes are sensitive not only to the structure of the substrate but also to its stereoconfiguration. Substrates having a chiral carbon atom could be resolved into enantiomers by enzymes. Consequently, chiral substrates could be used for the measuring of the activity of the enzymes with higher accuracy as well as for the evaluation of their enantioselectivity.

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Thus, the aim of the present work was the enzymatic synthesis of enantiomerically enriched ferrocene compounds that could be used as chiral building blocks in the synthesis of relevant substrates, containing the ferrocene function. The starting compounds could be branched chain carboxylic acids and / or their esters containing a ferrocene moiety.

In order to obtain enantiomers of acids and / or respective esters, the kinetic enzymatic resolution of enantiomers based on the difference in the hydrolysis or esterification rates is one of the most convenient methods. Various esterases and lipases are used for this purpose. Enantioselectivity of the reaction depends on the structure of the substrate, the reaction conditions and the enzyme used special additives like detergents and even on the lipase immobilization method [5–8, and references cited herein]. The hydrolysis can be carried out both in aqueous conditions and in organic solvents.

Thermomyces lanuginosus lipase hydrolyses long chain acylglycerides, functions at the lipid / water interface and therefore is expected to be appropriate to use in water-oil emulsions. TLL appeared to be highly enantioselective in the hydrolysis of diethyl phenylmalonate [9]. Therefore we selected the latter enzyme for the enzymatic resolution of racemic methyl 3-methyl-5-ferrocenylpentanoate into enantiomers. The consecutive approach consisting of the enzymatic hydrolysis of the remaining ester was used in order to increase enantiomeric purity of the resolved compounds.

EXPERIMENTAL

The solvents used were dried and purified by the routine laboratory methods. The reagents were purchased from Aldrich, Sigma, and Fluka and were used without further purification. Melting points were determined in an open capillary using a Mel-Temp apparatus. Flash column chromatography was performed on silica gel 60 (0.035–0.070 nm) purchased from Fluka. *Thermomyces lanuginosus* lipase was obtained from Sigma, batch No. 110K1357.

IR spectra of thin films or pellets with KBr were recorded on a Perkin-Elmer Model Spectrum GX FT-IR spectrometer. ^{1}H NMR spectra in chloroform-d ($CDCl_3$) were recorded on a Varian Unity Inova 300 spectrometer (300 MHz) with tetramethylsilane (TMS) as an internal standard, and ^{13}C NMR spectra at 75 MHz using $CDCl_3$ (δ = 77.0 ppm) as an internal standard. Chemical shifts are reported in ppm (δ), multiplicity is indicated as s – singlet, d – doublet, t – triplet, q – quadruplet, m – multiplet, br – broad; the ferrocene moiety – as Fc. The coupling constants, *J*, are given in Hz. Optical rotations were measured in EtOH with a Perkin-Elmer Model 343 polarimeter at 25 °C. Concentration of the solutions was in the limits of c = 4–22 mg/ml, measured angles (α_D^{25}) were 0.060–0.150° (±0.003).

Preparation of racemic 3-methyl-5-ferrocenylpentanoates

(±)-3-Methyl-4-ferrocenylbutanoic acid ((±)-1). To a suspension of 4.8 g (36 mmol) $AlCl_3$ in 50 ml of dichloromethane (CH_2Cl_2) a solution of 6.7 g (36 mmol) ferrocene and 1.7 g (13 mmol) 3-methylglutaric anhydride in 100 ml CH_2Cl_2 was added within 45 min at room temperature. The reaction mixture was stirred for additional 2 h and then poured on ice. The pH was adjusted to 4, and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2, the organic layer was concentrated to approximately 200 ml and extracted with a 2 *N* aqueous solution of NaOH (100 ml). Traces of the organic solvent were removed from the aqueous phase *in vacuo*, and the product was precipitated by slow addition of phosphoric acid (50%) at 5 °C. The orange precipitate was filtered off, washed with water and dried to yield 2 g (50%) of (±)-**1**. M. p.: 96–98 °C. ^{1}H NMR, δ (ppm): 1.11 (d, J = 6.5, 3H, CH_3), 2.62–2.82 (m, 5H, CH_2CHCH_2), 4.20–4.80 (m, 9H, Fc); ^{13}C NMR, δ (ppm): 20.45, 26.76, 40.86, 45.68, 69.35 (2C), 69.81 (5C), 72.35 (2C), 79.04, 178.23 (CO), 203.63 (CO).

Methyl (±)-3-methyl-5-ferrocenylpentanoate ((±)-2). To the solution of (±)-**1** (3.14 g, 10 mmol) in 50 ml of MeOH, 10 ml benzene, 16 ml of H_2O and 16 ml of concentrated HCl, freshly prepared zinc amalgam (from 14 g of Zn dust, 1.7 g of $HgCl_2$, 0.6 ml of conc. HCl and 17 ml of H_2O) was added, and the mixture was refluxed for 6 h. After cooling to room temperature, the reaction mixture was diluted with benzene; the amalgam was filtered off and washed with benzene. The organic layer was washed with water and brine, dried over $MgSO_4$. The solvent was removed *in vacuo*, and the residue was chromatographed on silica gel (eluent CH_2Cl_2) to yield 2.5 g (80%) of (±)-**2** as yellow oil. ^{1}H NMR, δ (ppm): 0.98 (d, J = 6.6, 3H, CH_3), 1.35–1.63 (m, 2H, CH_2), 1.99 (m, 1H, CH), 2.12–2.42 (m, 4H, $FcCH_2$, CH_2CO), 3.67 (s, 3H, CH_3O), 4.02–4.12 (m, 9H, Fc); ^{13}C NMR, δ (ppm): 19.7, 26.8, 30.3, 37.8, 41.5, 51.4, 67.1, 67.9, 68.4, 89.1, 173.5 (C=O); IR (film, ν, cm^{-1}): 1 738 (C=O).

(±)-3-Methyl-5-ferrocenylpentanoic acid ((±)-3). A solution of (±)-**2** (3.14 g, 10 mmol) in 100 ml of MeOH, 50 ml of H_2O and 2.8 g (50 mmol) of KOH was refluxed for 3 h and evaporated to approximately 50 ml. The remaining solution was poured on ice (200 g) and the pH was adjusted to 4 with concentrated HCl. The mixture was extracted with CH_2Cl_2, the organic layer was washed with water and brine. After drying over $MgSO_4$ and removal of the solvent, the residue was purified by column chromatography (eluent – CH_2Cl_2/EtOAc, 3:1 v/v) to yield 2.1 g (70%) of (±)-**3** as yellow solid. M. p. 62–65 °C. ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 1.01 (br. d, J = 6.6 Hz, 3H, CH_3), 1.35–1.70 (m, 2H, CH_2), 2.00 (br. m, 1H, CH), 2.12–2.5 (m, 4H, $FcCH_2$, CH_2CO), 4.02–4.12 (m, 9H, Fc); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 19.6, 26.8, 30.1, 37.7, 41.4, 67.1, 67.8, 68.4, 88.9, 179.2 (C=O).

9-Bromononyl (±)-3-methyl-5-ferrocenylpentanoate ((±)-4). A mixture of (±)-3-methyl-5-ferrocenylpentanoic acid ((±)-**3**, 600 mg, 2.0 mmol), 9-bromononanol (446 mg, 2.0 mmol), and *p*-toluenesulfonic acid (344 mg, 2.0 mmol) in benzene (60 ml) was refluxed for 12 h in a flask with a Dean-Stark trap under Ar. After cooling to room temperature, the

reaction mixture was washed with water and the organic phase was dried over $MgSO_4$. After evaporation under reduced pressure the residue was purified by column chromatography eluting with CH_2Cl_2/*n*-C_6H_{14} (3:1 v/v) to yield 660 mg (65.3%) of (±)-**4** as yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 0.98 (d, *J* = 6.6 Hz, 3H, CH_3), 1.22–1.91 (m, 16H, 7CH_2, CH_2), 2.10–2.44 (m, 4H, FcCH_2, CH_2CO), 3.40 (t, *J* = 6.8 Hz, 2H, CH_2Br), 4.02–4.11 (m, 11H, CH_2O, Fc); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 19.7, 25.8, 26.8, 28.1, 28.6, 29.1, 29.2, 30.3, 32.7, 34.0, 37.8, 41.8, 64.3, 67.0, 67.8, 67.8, 68.4, 89.0, 173.2 (CO). IR (KBr, ν, cm^{-1}): 1 732 (C=O).

9-Mercaptononyl (±)-3-methyl-5-ferrocenylpentanoate ((±)-5). 9-Bromononyl (±)-3-methyl-5-ferrocenylpentanoate ((±)-**4**, 505 mg, 1.0 mmol) and thiocarbamide (304 mg, 4.0 mmol) in dry acetone (30 ml) were refluxed for about 90 h under Ar. The solvent was evaporated *in vacuo* and the crude isothiouronium salt was poured into the mixture of $CHCl_3$ (40 ml) and water (20 ml). After addition of solid $Na_2S_2O_5$ (380 mg, 2 mmol), the reaction mixture was vigorously stirred and refluxed for 4 h under Ar. The organic phase was washed with water, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was dissolved in CH_2Cl_2/*n*-C_6H_{14} (1:1 v/v) and chromatographed over silica gel. Elution with CH_2Cl_2/*n*-C_6H_{14} (1:1 v/v) gave (±)-**5** as yellow oil (190 mg, 41.5%). ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 0.98 (d, *J* = 6.6 Hz, 3H, CH_3), 1.22–1.70 (m, 17H, 7CH_2, CH_2, SH), 1.99 (m, 1H, CH), 2.10–2.42 (m, 4H, FcCH_2, CH_2CO), 2.52 (q, *J* = 7.5 Hz, 2H, CH_2S), 4.03–4.12 (m, 11H, CH_2O, Fc); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 19.7, 24.6, 25.9, 26.8, 28.3, 28.6, 28.9, 29.1, 29.3, 30.3, 33.9, 37.8, 41.8, 64.3, 67.0, 67.8, 68.4, 89.0, 173.2 (CO); IR (KBr, ν, cm^{-1}): 1 570 (SH), 1 732 (C=O). As a by-product 9-(3'-methyl-5'-ferrocenylpentanoyloxy)nonyl disulfide ((±)-**6**) was eluted from the column with CH_2Cl_2 to yield 50 mg (11%) of yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 0.98 (d, *J* = 6.6 Hz, 6H, 2CH_3), 1.22–1.72 (m, 32H, 14CH_2, 2CH_2), 1.99 (m, 2H, 2CH), 2.11–2.44 (m, 8H, 2FcCH_2, 2CH_2CO), 2.67 (t, *J* = 7.4 Hz, 4H, 2CH_2S), 4.02–4.11 (m, 22H, 2CH_2O, 2Fc); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 19.7, 25.9, 26.8, 28.4, 28.6, 29.1, 29.3, 30.3, 37.8, 39.0, 41.8, 64.3, 67.0, 67.8, 68.4, 89.0, 173.2 (2CO). IR (KBr, ν, cm^{-1}): 1 732 (C=O).

Enzymatic hydrolysis of methyl (±)-3-methyl-5-ferrocenylpentanoate ((±)-2)

General procedure. To a suspension of (±)-**2** or to the ester recovered after the first enzymatic hydrolysis ((+)-**2a**, (+)-**2c**) in the phosphate buffer (PB, pH 7.0) *Thermomyces lanuginosus* lipase was added (1 mmol of methyl ester (±)-**2**, (+)-**2a** or (+)-**2c**, 10 ml of 10 mM PB, and 19.5 mg of TLL lipase in 1 ml of 10 mM PB). The mixture was stirred for 3 h or 24 h, diluted with water (30 ml) and extracted with CH_2Cl_2. The combined organic layers were washed with water and brine, dried over $MgSO_4$. The solvent was evaporated and the residue was purified by column chromatography on silica gel. Elution of the column with CH_2Cl_2/*n*-C_6H_{14} (3:1) gave methyl (+)-3-methyl-5-ferrocenylpentanoate ((±)-**2a**–(±)-**2d**). (–)-3-Methyl-5-ferrocenylpentanoic acid ((–)-**3**) was eluted with CH_2Cl_2/$CH_3COOC_2H_5$ (5:2 v/v).

Methyl (±)-3-methyl-5-ferrocenylpentanoate ((+)-2b): yellow oil, yield 17%, ee 74%, $[\alpha]_D^{25}$ = +7.6° (c 0.0225); ^{1}H NMR, δ (ppm): 0.98 (d, *J* = 6.6, 3H, CH_3), 1.35–1.63 (m, 2H, CH_2), 1.99 (m, 1H, CH), 2.12–2.42 (m, 4H, FcCH_2, CH_2CO), 3.67 (s, 3H, CH_3O), 4.02–4.12 (m, 9H, Fc); ^{13}C NMR, δ (ppm): 19.7, 26.8, 30.3, 37.8, 41.5, 51.4, 67.1, 67.9, 68.4, 89.1, 173.5 (C=O); IR (film, ν, cm^{-1}): 1 738 (C=O).

(–)-3-Methyl-5-ferrocenylpentanoic acid ((–)-**3**): orange crystals, yield 20% (60 mg), m. p. 62–65 °C, $[\alpha]_D^{25}$ = –9.0° (c = 0.0150); ^{1}H NMR, δ (ppm): 1.01 (d, *J* = 6.6, 3H, CH_3), 1.35–1.70 (br. m, 2H, CH_2), 2.00 (br. m, 1H, CH), 2.12–2.5 (m, 4H, FcCH_2, CH_2CO), 4.02–4.12 (m, 9H, Fc); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 19.6, 26.8, 30.1, 37.7, 41.4, 67.1, 67.8, 68.4, 88.9, 179.2 (C=O).

Preparation of methyl (–)-3-methyl-5-ferrocenylpentanoate ((–)-2e)

A solution of (–)-**3** (isolated after 3 h enzymatic hydrolysis, 70 mg, 0.23 mmol) in 3 ml of MeOH and 0.5 ml of concentrated HCl was refluxed for 3 h. After cooling to room temperature, CH_2Cl_2 was added. The organic layer was washed with water and brine, dried over Na_2SO_4. The solvent was removed *in vacuo*, and the residue was chromatographed on silica gel with CH_2Cl_2/*n*-C_6H_{14} (3:1) to yield 55 mg (82%) of (–)-**2e** as yellow oil, ee 73%, $[\alpha]_D^{25}$ = –9.1° (c = 0.0160); ^{1}H NMR, δ (ppm): 0.98 (d, *J* = 6.6, 3H, CH_3), 1.35–1.63 (m, 2H, CH_2), 1.99 (m, 1H, CH), 2.12–2.42 (m, 4H, FcCH_2, CH_2CO), 3.67 (s, 3H, CH_3O), 4.02–4.12 (m, 9H, Fc); ^{13}C NMR, δ (ppm): 19.7, 26.8, 30.3, 37.8, 41.5, 51.4, 67.1, 67.9, 68.4, 89.1, 173.5 (C=O); IR (film, ν, cm^{-1}): 1 738 (C=O).

^{1}H NMR determination of enantiomeric purity

Enantiomeric excess was determined by ^{1}H NMR spectroscopy using Europium(III) tris-[3-(heptafluoropropylhydroxymethylene)-*d*-camphorate (Eu(hfc)$_3$) as a shift-reagent. Addition of Eu(hfc)$_3$ to the solution of ester (±)-**2** in $CDCl_3$ results in the shift of all NMR signals to higher ppm values and their duplication. For example, when the molar ratio of ester (±)-**2** and Eu(hfc)$_3$ was in the range 1:0.2 to 1:0.4, the NMR signal of the OCH_3 group (δ = 3.67 ppm) splits into two components with the peak-to-peak separation Δδ = 0.006–0.01 ppm, and each component of the doublet belonging to the 3-CH_3 group (δ = 1.14 and 1.16 ppm) splits into two components with Δδ = 0.005–0.006 ppm (Fig. 1A, 1A'). The intensities of signals of the individual peaks are equal for racemic ester (±)-**2**, whereas the signals, shifted downfield of the esters recovered after the enzymatic hydrolysis of (+)-**2a** (Fig. 1B), are more intensive than the signals shifted upfield and belong to the (+)-enantiomer. In the ^{1}H NMR spectra of the (–)-**2e** solution with Eu(hfc)$_3$ in $CDCl_3$, the ratio of the intensities of split OCH_3 signals is opposite in comparison with the (+)-**2a**–(+)-**2c** signals (Fig. 1C), indicating that the more intensive peak belongs to the (–)-enantiomer. The same tendency of

the intensity ratio remains in the case of the doublet of the 3-CH_3 group. The signals were used for the determination of ee by dividing them into components (Fig. 2) and calculating the areas under the curves with the GRAMS software. Curve fitting was performed using mixed Lorentzian and Gaussian functions. The calculation of ee was performed using the equation:

$$ee\ (\%) = [(S_1 - S_2)/(S_1 + S_2)] \times 100,$$

where S_1 and S_2 are the areas of the individual peaks, and $S_1 > S_2$.

RESULTS AND DISCUSSION

Synthesis of alkyl (±)-3-methyl-5-ferrocenylpentanoates

Racemic alkyl 3-methyl-5-ferrocenylpentanoates were prepared via several-step synthesis (Scheme 1) in the similar manner as described in [10, 11] and adapted by us previously [12] for the synthesis of functionalised alkyl 5-ferrocenylpentanoates. In the first step ferrocene was acylated with 3-methylglutaric anhydride under Friedel-Crafts conditions to form racemic 3-methyl-4-ferrocenylbutanoic acid ((±)-**1**) bearing the fragment with a chiral carbon atom. In the next step the Clemmensen reduction of the obtained ketoacid (±)-**1** with the freshly prepared zinc amalgam in conc. HCl and MeOH and subsequent esterification yielded methyl (±)-3-methyl-5-ferrocenylpentanoate ((±)-**2**). Saponification of (±)-**2** resulted in (±)-3-methyl-5-ferrocenyl pentanoic acid ((±)-**3**) that was esterified with 9-bromononanol to give (±)-**4**. Conversion of bromide (±)-**4** into isothiouronium salt and conducting the decomposition of the latter in the water / chloroform heterogeneous phase containing $Na_2S_2O_5$ leaded to the mixture of racemic Fc-terminated mercapto derivative (±)-**5** and its disulfide analogue (±)-**6** which were separated by chromatography on silica gel.

Enzymatic hydrolysis of methyl (±)-3-methyl-5-ferrocenylpentanoate

Enzymatic hydrolysis of racemic methyl 3-methyl-5-ferrocenylpentanoate ((±)-**2**) proceeds with the formation of (–)-3-methyl-5-ferrocenylpentanoic acid ((–)-**3**), and unreacted ester remains in the reaction mixture (Scheme 2).

The reaction was carried out in 10 mM phosphate buffer (PB), pH 7.0, using 0.68 μM of TLL (activity was 0.1 lipase unit (LU)**) for 1 mM of the ester (±)-**2**, (+)-**2a** or (+)-**2c** at room temperature. In order to obtain enantiomerically enriched compounds the hydrolysis was carried out in two steps: the formed acid (–)-**3** was separated after 3 h or 24 h and the remaining ester (+)-**2a** or (+)-**2c** was repeatedly hydrolyzed 24 h under the identical conditions to give enantiomerically enriched ester (+)-**2b** or (+)-**2d**. Measurements of optical rotation show that remaining esters are enriched with (+)-enantiomer, while acid **3** is enriched with (–)-enantiomer, and consecutive hydrolysis increases the quantity of (+)-enantiomer in the remaining ester (Table).

** LU is defined as the quantity lipase (mg) liberating 1 μmol of p-nitrophenol in the reaction with p-nitrophenylpalmitate in 50 mM PB containing 0.5% Triton X-100, pH 7.0, at 50 °C temperature per 1 min (μmol/min · mg).

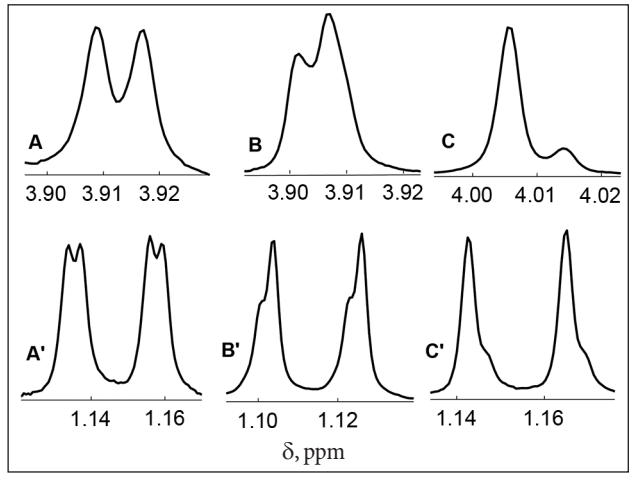


Fig. 1. Signals of protons of O$\underline{CH_3}$ (A, B, C) and C*H-$\underline{CH_3}$ (A′, B′, C′) groups in the ^{1}H NMR spectra of the racemic ester (±)-**2** (A, A′), remaining ester (+)-**2a** (B, B′), and the ester (–)-**2e** obtained by esterification of acid (–)-**3** (C, C′)

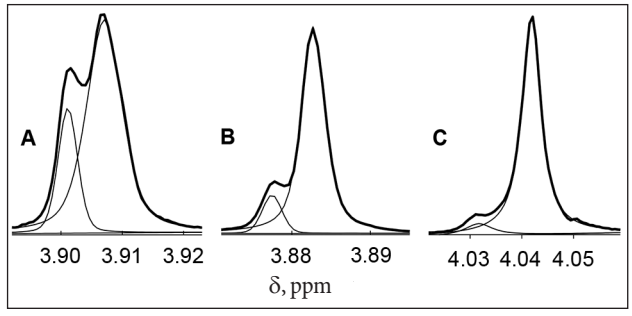


Fig. 2. Signals of protons of O$\underline{CH_3}$ group of esters (+)-**2a** with ee = 51% (A), (+)-**2b** with ee = 74% (B), and (+)-**2c** with ee 87% (C)

Thus, after two consecutive steps of the hydrolysis we obtained methyl (+)-3-methyl-5-ferrocenylpentanoate with enantiomeric excess (ee) up to 87% of (+)-**2d**. All samples of the remaining ester had identical NMR and IR spectra implying the absence of side reactions.

The other enantiomer of the ester (–)-**2e** with ee 73% was obtained by esterification of (–)-**3** isolated after 3 h of enzymatic hydrolysis of the compound (±)-**2** (Scheme 2). Enantiomeric purity of the resolved compounds was estimated by NMR spectroscopy using the chiral shift reagent Eu(hfc)$_3$. Integration of the O$\underline{CH_3}$ and C*H-$\underline{CH_3}$ signals in ^{1}H NMR spectra of the resolved ester (+)-**2a**, and the ester (–)-**2e** obtained by esterification of acid (–)-**3** were compared to the duplication of signals of the racemic ester (±)-**2**.

Optical rotations of the enantiomeric samples measured in EtOH and calculated for ee 100%, $[\alpha]_D^{25}$, gave quite close values: +(10.3 ± 0.6)° for methyl (+)-3-methyl-5-ferrocenylpentanoate, and –(13.1 ± 0.9)° for methyl (–)-3-methyl-5-ferrocenylpentanoate.

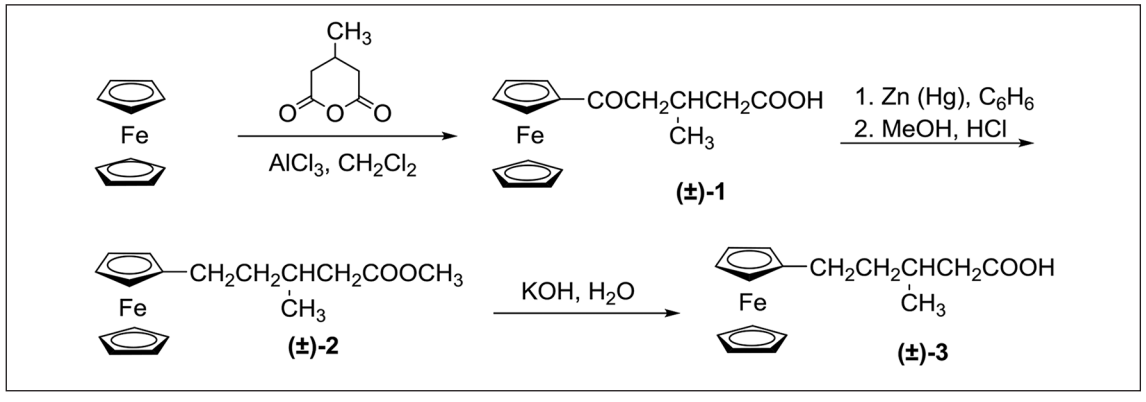
Table. **Dependence of yields (%) and enantiomeric composition of the hydrolysis products on the reaction conditions**

Compound under hydrolysis	Reaction time, h	Recovered ester				Yield of (–)-3, %
		No. of compound	Yield, %	ee, %	$[\alpha]_D^{25}$	
(±)-**2**	3	(+)-**2a**	43	51	not measured	10
(+)-**2a**	24	(+)-**2b**	17	74	+7.6° (c 0.0225)	20
(±)-**2**	24	(+)-**2c**	44	53	+5.8° (c 0.0150)	9*
(+)-**2c**	24	(+)-**2d**	16	87	not measured	3

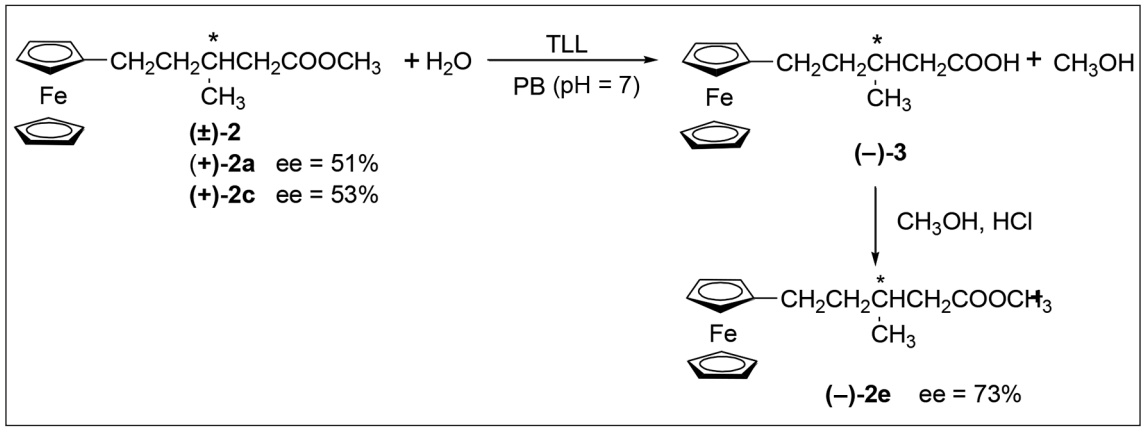
*$[\alpha]_D^{25} = -9.0°$ (c 0.0150).

Determination of the absolute configuration of the resolved (+)- and (–)-enantiomers by circular dichroism spectroscopy is complicated due to weak absorption of the ester chromophore in the UV-Vis region (ε_{438} = 93 M^{-1} cm^{-1}, ε_{325} = 56 M^{-1} cm^{-1} in EtOH). However, we could make the assignment of the configuration on the basis of literature data about the enzymatic hydrolysis of branched chain fatty acids. It was established that in the hydrolysis of methyl branched alkanoic acid esters enantiopreference of the hydrolysis, among other factors, depends on the position of the chiral atom in the chain relative to the reaction center [7, 13, 14]. For instance, in the hydrolysis of racemic thiolesters of 3-methyl octanoic acids the faster reacting enantiomer has (R)-configuration, whereas an analogous 2-isomer shows (S)-enantiopreference [13]. It is noteworthy that lipase used in the latter work was *Rhizomucor miehei* lipase, which is similar in the structure to TLL [15]. The same was also observed in the case of the hydrolysis of 3-hydroxy-3-methylalkanoic acid esters [14] catalyzed by pig liver esterase. Moreover, the substituents in the 5-position of these compounds as well as in the carboxylate group did not change the enantiopreference of the reaction [14]. Therefore, based on these considerations, we assume that in the hydrolysis of methyl 3-methyl-5-ferrocenylpentanoate catalyzed by TLL lipase the hydrolyzed enantiomer has (R)-configuration, whereas the remaining one has (S)-configuration (Fig. 3).

Thus, using enzymatic kinetic resolution followed by esterification of the obtained acid, racemic methyl 3-methyl-5-ferrocenylpentanoate was resolved into S-(+)- or R-(–)-enantiomers of ester **2** with high ee. However, the yields of the both desirable products were rather low possibly due to the loss in the separation of substrate from lipase. We expect that using immobilized lipase could solve the problem, and therefore optimisation of reaction conditions is aimed at it.



Scheme 1. Synthesis of racemic alkyl 3-methyl-5-ferrocenylpentanoates



Scheme 2. Enzymatic hydrolysis of racemic methyl 3-methyl-5-ferrocenylpentanoate

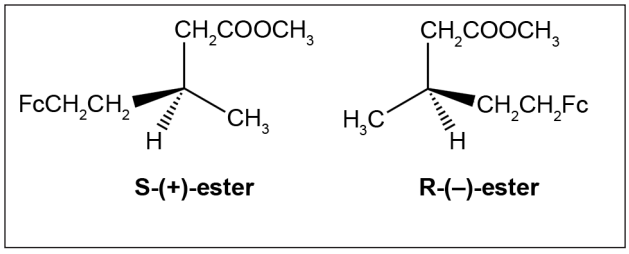


Fig. 3. Absolute configuration of resolved enantiomers of methyl 3-methyl-5-ferrocenylpentanoate

CONCLUSIONS

Racemic 3-methyl-5-ferrocenylpentanoates were synthesized by the acylation of ferrocene with 3-methylglutaric anhydride to form 3-methyl-4-ferrocenylbutanoic acid, its reduction to 3-methyl-4-ferrocenylbutanoic acid, esterification with methanol or 9-bromononyl alcohol and conversion of the obtained bromide into 9-mercaptononyl (±)-3-methyl-5-ferrocenylpentanoate in the water / chloroform heterogeneous phase containing $Na_2S_2O_5$. The hydrolysis of methyl 3-methyl-5-ferrocenylpentanoate in the phosphate buffer, pH 7.0 using *Thermomyces lanuginosus* lipase as a biocatalyst was studied. After two consecutive steps of hydrolysis the remaining methyl (+)-3-methyl-5-ferrocenylpentanoate with ee up to 87% was isolated**.** The other enantiomer, methyl (–)-3-methyl-5-ferrocenylpentanoate, with ee 73% was synthesized by the esterification of (–)-3-methyl-5-ferrocenylpentanoic acid formed in the hydrolysis reaction. The enantiomeric purity of the obtained esters was determined by NMR using chiral shift reagent $Eu(hfc)_3$. (+)-Enantiomer showed larger induced chemical shifts than (–)-enantiomer. These enantiomerically enriched compounds could be used as starting materials in the synthesis of (S)- and (R)-substrates containing a ferrocene moiety.

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References

1. B. Kazakevičienė, G. Valinčius, G. Niaura, Z. Talaikytė, M. Kažemėkaitė, V. Razumas, *J. Phys. Chem. B*, **107**, 6661 (2003).
2. G. Valinčius, G. Niaura, B. Kazakevičienė, et al., *Langmuir*, **20**, 6631 (2004).
3. G. Valinčius, I. Ignatjev, G. Niaura, et al., *Anal. Chem*., **77**, 2632 (2005).
4. B. Kazakevičienė, G. Valinčius, G. Niaura, et al., *Langmuir*, **23**, 4965 (2007).
5. U. T. Bornschauer, R. J. Kazlauskas, *Hydrolases in Organic Synthesis. Regio- and Stereoselective Biotransformations*, 2nd edn., Wiley, Weinheim (2006).
6. A. Ghanem, H. Y. Aboul-Enein, *Chirality*, **17**, 1 (2005).
7. N. W. J. T. Heinsman, M. C. R. Franssen, A. van der Padt, R. M. Boom, K. Vant Riet, A. E. de Groot, *Biocat. Biotransform*., **20**, 297 (2002).
8. L. Nini, L. Sarda, L.-C. Comeau, E. Boitard, J.-P. Dubes, H. Chahinian, *Biochim. Biophys. Acta*, **1534**, 34 (2001).
9. Z. Cabrera, J. M. Palomo, G. Fernandez-Lorente, R. Fernandez-Lafuente, J. M. Guisan, *Enzyme Microb. Technol*., **40**, 1280 (2007).
10. K. L. Rinehart, R. J. Curby, P. E. Sokol, *J. Am. Chem. Soc.*, **79**, 3420 (1957).
11. K. L. Rinehart, R. J. Curby, D. H. Gustafson, K. G. Harrison, R. E. Bozak, D. E. Bublitz, *J. Am. Chem. Soc.*, **84**, 3263 (1962).
12. M. Kažemekaitė, A. Bulovas, V. Smirnovas, G. Niaura, E. Butkus, V. Razumas, *Tetrahedron Lett.*, **42**, 7691 (2004).
13. P. E. Sonnet, M. W. Baillargeon, *Lipids*, **26**, 295 (1991).
14. W. K. Wilson, Sh. B. Baca, Y. J. Barber, T. J. Scallen, C. J. Morrow, *J. Org. Chem.*, **48**, 3960 (1983).
15. S. S. Chimni, S. Singh, S. Kumar, S. Mahajan, *Tetrahedron: Asymmetry*, **13**, 511 (2002).

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METILFEROCENILPENTANOATO SINTEZĖ IR FERMENTINIS ENANTIOMERŲ ATSKYRIMAS *THERMOMYCES LANUGINOSUS* LIPAZE FEROCENO ALKILTIOLIŲ GAVIMUI

S a n t r a u k a

Raceminis metil (3-metil-5-ferocenil) pentanoatas buvo susintezuotas acilinant feroceną 3-metilglutaro anhidridu Friedel-Crafts'o reakcijos sąlygomis, po to atlikus Clemmensen'o redukciją ir susidariusios (±)-3-metil-4-ferocenoilbutano rūgšties esterinimą. Tirtas gauto junginio racemato kinetinis atskyrimas naudojant lipazę *Thermomyces lanuginosus* fosfatiniame buferyje (pH 7,0). Po dviejų hidrolizės reakcijų išskirtas nesureagavęs metil (+)-3-metil-5-ferocenilpentanoatas, kurio enantiomerinis perteklius (ep) yra 87 %. Kitas enantiomerinis esteris, (–)-metil (3-metil-5-ferocenil) pentanoatas, kurio ep 73 %, buvo gautas esterinant hidrolizės reakcijoje susidariusią (–)-3-metil-5-ferocenilpentano rūgštį. Gautų esterių enantiomerinis grynumas buvo nustatytas BMR spektroskopijos metodu naudojant chiralinį poslinkio reagentą. Gauti enantiomeriškai praturtinti junginiai gali būti naudojami chiralinių (S)- ir (R)-feroceno darinių sintezėje.