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Efficient synthesis and physicochemical characterization of natural danshensu, its *S* isomer and intermediates thereof

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ABSTRACT

The synthesis and molecular structure details of *R*- 3,4-dihydroxyphenyl lactic acid (danshensu) and related compounds, i.e. *S* isomer and the key intermediates have been described. Danshensu is an important water soluble phenolic acid of *Salvia miltiorrhiza* herb (danshen or red sag) with numerous applications in traditional Chinese medicine (TCM). Our synthetic approach was based on the Knoevenagel condensation of the protected 3,4-dihydroxybenzaldehyd and Meldrum acid derivative, followed by asymmetric Sharples dihydroxylation, reductive mono dehydroxylation and final deprotection. All compounds were characterized by various spectroscopic techniques: ^{1}H-, ^{13}C- magnetic resonance (NMR); Fourier-transformed infrared (FTIR); Raman, HR mass spectroscopy. For the determination of compound optical purities original HPLC methods were developed which allowed for the efficient resolution of danshensu *R* and *S* enantiomers as well as its intermediate enantiomers, using commercially available chiral stationary phases. Furthermore, in order to better understand danshensu specificity as a potential API in drug formulation, the physicochemical properties of the compounds were studied by thermal analysis, including differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA).

1. Introduction

Salvia miltiorrhiza, known as danshen or red sage, is a common plant used in traditional Chinese and Japan medicine for the treatment of cardiovascular diseases [1]. Water and ethanolic extracts of danshen are the components of Compound Danshen Formula (CDF) which is officially listed in the *Chinese* Pharmacopoeia [2]. Up to now more than seventy compounds have been isolated from danshen and characterized. The compounds with hydrophilic properties, including specific mono- and polyphenolic acids, comprise one of the sub-groups present in the plant. Danshensu (*R*-DSS), i.e. *R*-3,4-dihydroxyphenyl lactic acid (Fig. 1), seems to be its major active representative [3]. Manufacturing of sodium *R*-danshensu is generally based on the alkaline extraction of *salvia miltiorrhiza* herbs with ethanol precipitation to remove impurities, followed by adsorptive (non-ionic) macroporous resins separation [4]. This *R* isomer of danshensu (DSS) from the natural source reveals multiple pharmacological effects on the cardiovascular system: vasorelaxant action on the coronary arteries through the inhibition of calcium channels [5], anticoagulation [6], cardioprotection while ischemia reperfusion injury [7], antiarrhythmia and antihypertensive action [8]. Moreover, sodium danshensu and derivatives thereof have shown neuroprotective effect against neuronal loss and cerebral ischemia injury [2,9]. Although the major source of *R*-3,4-dihydroxyphenyl lactic acid is herbal material, there are several literature methods of the danshensu synthesis. This compound or its O-protected derivatives are mostly used as building blocks in the organic synthesis of natural polyphenolic acids i.e. rosmarinic acid [10–12], (+)-salvianolic acid A [13], (+)- lithospermic acid [14], as well as novel biologically active entities [9,15–19].

While synthetizing natural organic compounds, one of the major problems is to obtain the preferred optical isomer. Thus, the synthesis of chiral polyphenolic acid present in herbal material requires the use of substrates with predetermined configuration at the chiral carbon. This approach can be achieved using the chiral catalysts [17,18], enzymes [10] or Sharpless asymmetric dihydroxylation [14,16]. In the known syntheses of polyphenolic acids mainly the ester derivatives of *R* and *S* danshensu were described as intermediates. These compounds were used for further chemical

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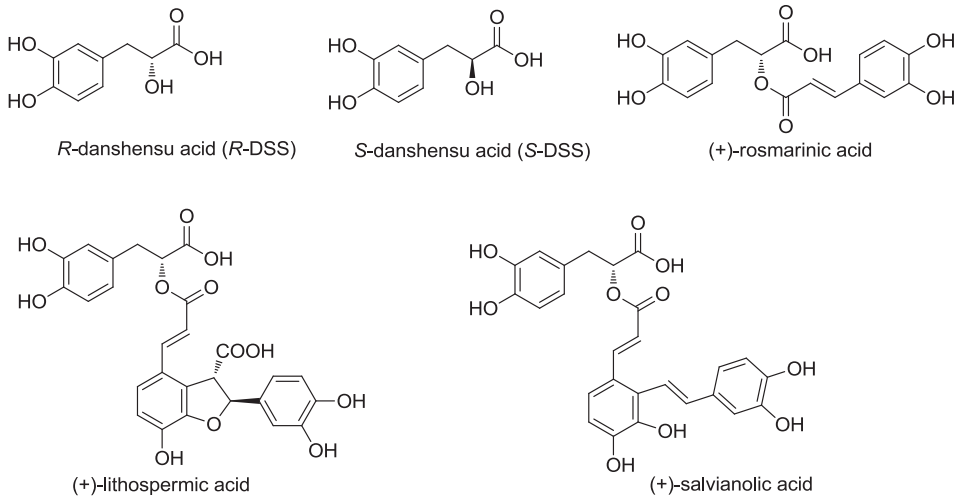


Fig. 1. Structures of the polyphenolic acids.

transformations without removing ester protecting groups. There is no data on the synthesis of naturally occurring *R*-isomer of danshensu. Although its corresponding *S*-isomer (Fig. 1) has been mentioned in Bogucki's work [11], the chiral purity of the compound nor its physical and chemical properties have been determined.

Herein, we have presented the synthesis of *R* and *S* danshensu acids from commercially available 3,4-dihydroxybenzaldehyde and Meldrum acid. These substrates, after simple derivation, were transformed into final compounds *via* the Knoevenagel condensation, asymmetric Sharples dihydroxylation, regioselective dehydroxylation followed by deprotection. The *R* and *S* danshensu and their key intermediates were characterized by infrared, Raman and nuclear magnetic resonance spectroscopy (IR, Raman, NMR), mass spectrometry (MS), high-performance liquid chromatography (HPLC) and differential scanning calorimetry (DSC) techniques.

2. Results and discussion

2.1. Chemistry

The synthesis of both *R* and *S* isomers of danshensu was completed through intermediate **4**. This compound was alternatively prepared in two routes with commercially available 3,4-dihydroxybenzaldehyde, **1**.

In the first method (Scheme 1), 3,4-dihydroxybenzaldehyde **1** was quantitatively *O*-alkylated to **2** according to the known procedure [10]. The reaction was carried out in ethanol with benzyl bromide and potassium carbonate. Then the Knoevenagel condensation of **2** with malonic acid in the presence of pyridine and piperidine, followed by the esterification of thus-obtained **3**, led to particular benzyl ester **4** with the total yield 67%.

In the alternative method the synthesis of **4** was accomplished in a convenient one-pot procedure. At the beginning, commercially available Meldrum acid was reacted with benzyl alcohol to give benzyl ester of malonic acid (Scheme 2). This compound, without isolation, was reacted with **2** by Knoevenagel conditions. The obtained crude **4** was purified by column chromatography in the total yield of 93%. This efficient and faster, alternative approach allowed us to obtain a benzyl derivative of caffeic acid (**4**) with a better yield in comparison to Scheme 1 method. However, during this process the formation of small amount of an impurity was observed. The impurity was isolated and characterized by NMR and HRMS techniques as 4-(3,5-dimetoxyphenyl)-but-3-en-2-one (**5**). The compound forms as a by-product of the aldol condensation of aldehyde (**2**) and acetone which is released in the first phase of the one-pot procedure from Meldrum's acid.

Asymmetric Sharples dihydroxylation was carried out according to the known procedure [20]. Respective dihydoxy enantiomers (**6a**, **6b**) were synthetized using AD-mixα or AD-mixβ, asymmetric catalysts which contain an osmium tetraoxide source, a re-oxidant and a chiral ligand (Scheme 3). In order to find effective conditions for our substrate conversion, we started with verifying literature data. We experimentally confirmed that the reaction in *tert*-butanol/water (1:1 v/v) in the presence of methanesulfonamide resulted in the 35% yield of **6a** and **6b**. Then it was found that the increase in temperature from ambient to 50 °C and the modification of the solvent system for acetone/acetonitrile/water (3: 3: 1, v/v/v) improved the yield of dihydroxylation to 75%. This result was achieved after the purifications of enantiomers by column chromatography. The purities of **6a** and **6b** were verified by the HPLC RP18 and chiral analytical analyses. In the next step, the regioselective removal of one hydroxy group with triethylsilane in the presence of trifluoroacetic acid in DCM was applied. While verifying the work-up procedure, it was noticed that repeated evaporation of crude oily products with methanol effectively removed undesirable residual solvents and volatiles. Finally, the compounds were purified by crystallization from methanol to give *R* and *S* isomers of perbenzylated danshensu (**7a** and **7b**) as white powders with the yields of 60–85% (Scheme 3).

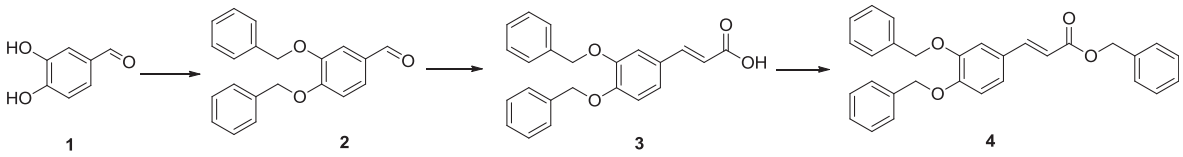
The *R* and *S* danshensu acid isomers (***R*-DSS** and ***S*-DSS**) were obtained by the complete removal of benzyl protecting groups *via* Pd/C-catalyzed hydrogenation at room temperature under continuous flow conditions, using hydrogen generator. Strongly hydrophilic compounds were purified by column chromatography, followed by lyophilization/or precipitation to obtain both isomers as hygroscopic off-white foam or solid with 95% yields (Scheme 4).

The structures of all compounds were confirmed by NMR experiments as well as mass spectroscopy.

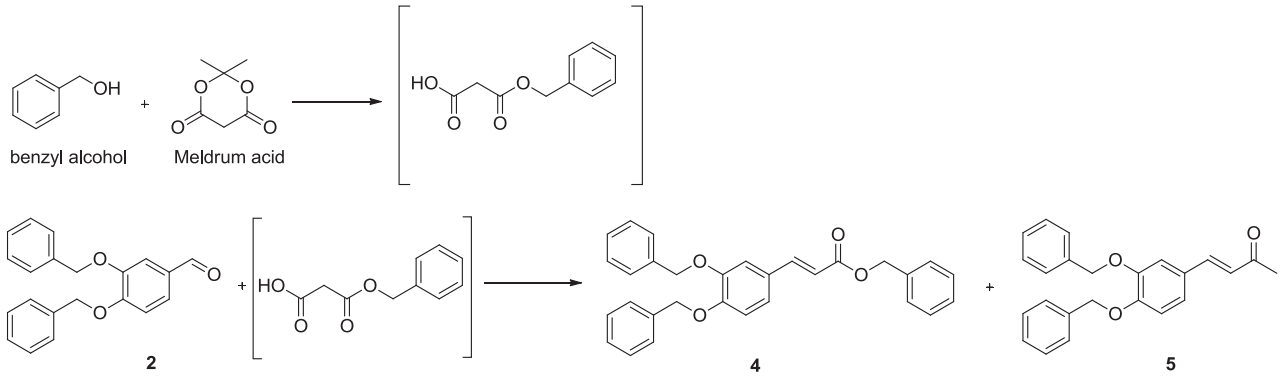
2.2. HPLC studies

HPLC methods were developed for the efficient resolution of enantiomers: **6a**/**6b**, **7a**/**7b** and ***S*-DSS**/***R*-DSS**, using commercially available chiral stationary phases and organic solvents.

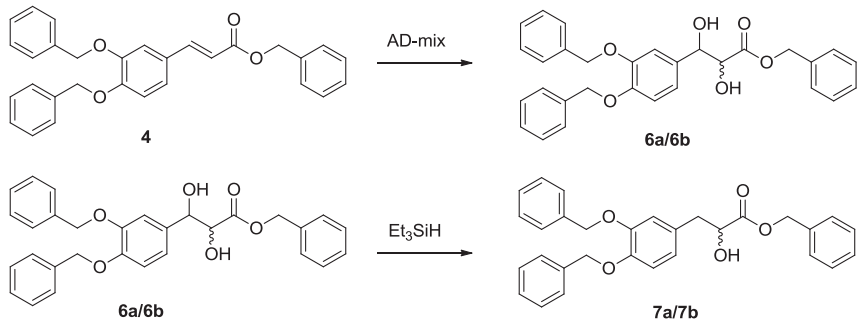
For **6a/6b** a typical normal phase eluent, n-hexane:ethanol (80/



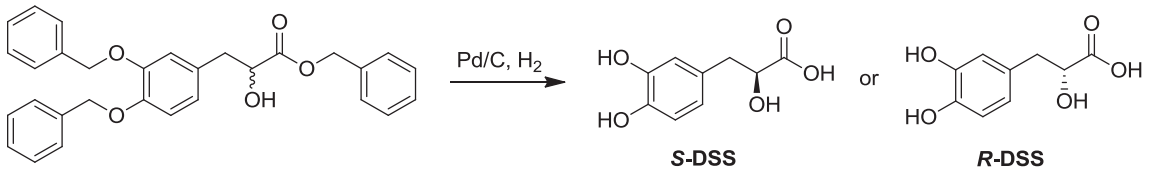
Scheme 1. Method with benzylation of **3.**



Scheme 2. Synthesis of **4** with Meldrum's acid.



Scheme 3. Asymmetric Sharples dihydroxylation and regioselective dehydroxylation.



Scheme 4. Deprotection of benzyl groups.

20, v/v), was used in combination with a Chiralcel OD-H column. A good baseline separation was observed (Fig. 2), therefore, this system was used for the enantiomeric excess determination of dihydroxy enantiomers. The enantiomeric excess of the corresponding enantiomers **6a** ($t_R = 17.4$ min) and **6b** ($t_R = 19.4$ min) was 99.6% and 99.8%, respectively (Figs. 3 and 4).

As it was mentioned earlier, the removal of one hydroxyl group from **6a** and **6b** led to the corresponding *S* and *R* isomers of perbenzylated danshensu (**7a** and **7b**). The development of the enantioseparation method for these chiral compounds turned out to be quite challenging. Various columns (Chirlapak IA, Chiralpak AD, LuxCellulose, Chiralcel OD-H) and mobile phases (hexane:2-propanol, hexane:ethanol, hexane:2-propanol:methanol, hexane:2-propanol: ethanol) had been tested, as well as other factors that could influence the retention and enantioresolution (flow rate, column temperature). The most promising, almost baseline enantioseparation was achieved on the Chiralcel OD-H column using hexane:2-propanol:ethanol (8:1:1). In the subsequent steps the flow rate and composition of the mobile phase were optimized. The best chromatographic conditions for the enantiomeric purity determination of **7a** and **7b** were then selected (see Figs. 5–7).

For *R* and *S* danshensu acid (***R*-DSS** and ***S*-DSS**) enantiomers determination was performed using a Chiralpak AD column (see Figs. 8–10). The mobile phase consisted of the mixture of n-hexane, 2-propanol and methanol (90:5:5, v/v/v), with 0.1% of an acidic additive (TFA), and was pumped at the constant flow-rate of 1.2 mL/min. The detection was performed at 254 nm.

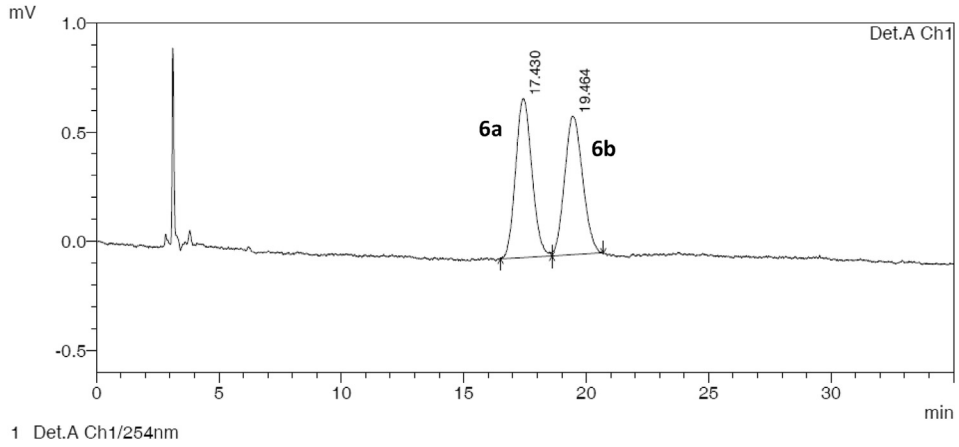


Fig. 2. Chromatogram of the racemic mixture of **6a** and **6b.**

2.3. IR and Raman spectroscopy

A comparison of IR and Raman data has showed differences in the spectra of **6a** and **6b** enantiomers (Supporting info: Figs. 1 and 2). This resulted from the presence of polymorphic mixtures in both samples, as it was additionally demonstrated by a subsequent DSC analysis. The IR and Raman spectra (Figs. 11 and 12) confirmed the presence of characteristic functional groups in the structures of intermediates **6a** and **6b** as it was summarized in Table 1. Contrary to the data collected for dihydroxyl analogues, while analyzing the IR and Raman spectra of **7a** and **7b**, a strong similarity effect was observed (Supporting info: Figs. 3 and 4). The analysis of IR and Raman spectra (Fig. 13) proved the presence of characteristic functional groups in the structures of this enantiomer pair (Table 1).

The comparison of the IR-ATR and Raman spectra of ***S*-DSS** and ***R*-DSS** final isomers revealed some differences (supporting info: Figs. 5 and 6). The broad bands in ***S*-DSS** spectra pointed at the amorphous structure of the analyzed sample. All characteristic functional groups in the structures of ***S*-DSS** and ***R*-DSS** isomers were identified (Figs. 15 and 16) and the bands assignment was collected in Table 1.

In addition the IR and Raman spectra of process impurity **5** were analyzed (Fig. 14, Table 1).

2.4. DSC analysis

The DSC curves of **6a** and **6b** enantiomers were characterized by two melting effects at about 77 and 87 °C (Fig. 17). This indicated the presence of the polymorphs mixture in the analyzed samples.

Only single melting effects were visible on the DSC curves of **7a**, **7b** enantiomers and impurity **5** (Fig. 18). While comparing the **7a**, **7b** pair, a slight increase in the characteristic thermal parameters: temperature and heat of fusion was observed for compound **7a** (95.27 °C, −118.67 J/g) in comparison to **7b** (onset = 92.58 °C, −101.30 J/g). It may have indicated a better chemical purity of **7a** enantiomer. Impurity **5** melted at 106.81 °C.

The DSC curve of ***R*-DSS** were characterized by two endothermic effects (Fig. 19). The first one, in the temperature range from 60 to 120 °C, comes from the solvents evaporation. The mass loss of −7.7% was evaluated from the TGA curve for this effect. The second endothermic effect at 229.24 °C comes from the substance melting. The DSC curve of ***S*-DSS** was characterized by a broad endothermic effect from 100 to 190 °C which comes from the solvents evaporation (Fig. 19). For this effect the mass loss of −9.1% was calculated from the TGA curve as well. On the DSC curve of ***S*-DSS** no melting effect was observed. It pointed at the amorphous structure of the compound which was consistent with the IR and Raman data.

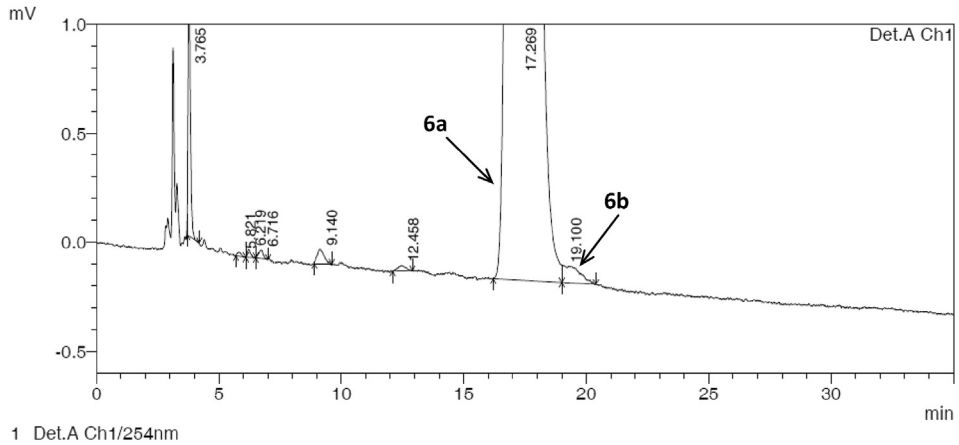


Fig. 3. Chromatogram of virtually enantiopure **6a.**

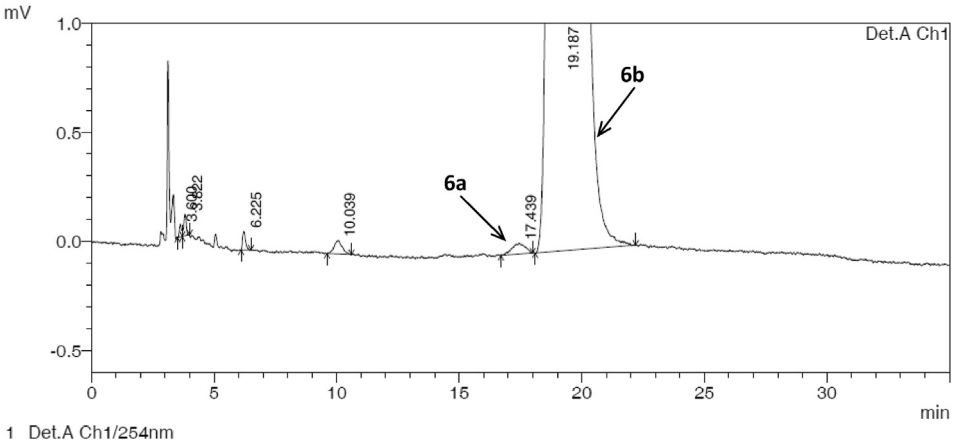


Fig. 4. Chromatogram of virtually enantiopure **6b.**

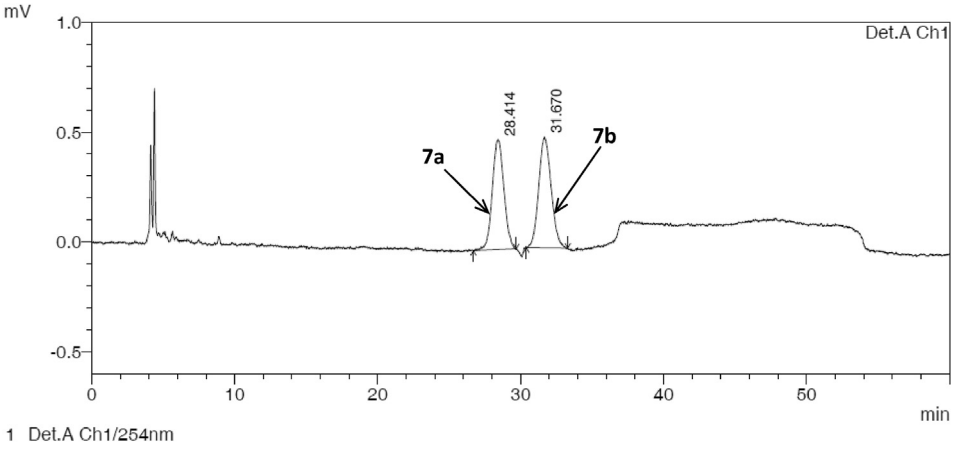


Fig. 5. Chromatogram of the racemic mixture of **7a** and **7b** (Chiralcel OD-H column, 80:18:2 hexane:2-propanol:ethanol, flow rate 0.7 mL/min).

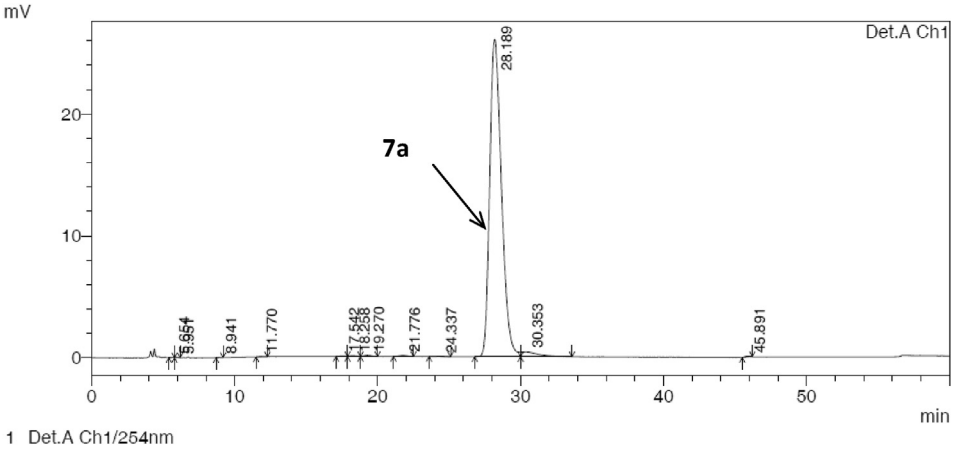


Fig. 6. Chromatogram of virtually enantiopure **7a.**

3. Conclusions

The therapeutics of plant origin or their modified analogues seem to be an important group of drugs on the pharmaceutical market. In many cases the reason for their introduction was the common knowledge about the therapeutic properties of raw plant material which stemmed from the ethnopharmacological tradition. Danshensu (DSS) is a water soluble phenolic acid component of danshen. Extracts from this plant have long been used in traditional Chinese medicine (TCM) for the treatment of coronary heart diseases like angina pectoris or myocardial infarction. As one of the active danshen ingredients, danshensu acid is generally obtained from natural material by multi-stage extraction combined with a chromatographic technique. In this paper we propose an

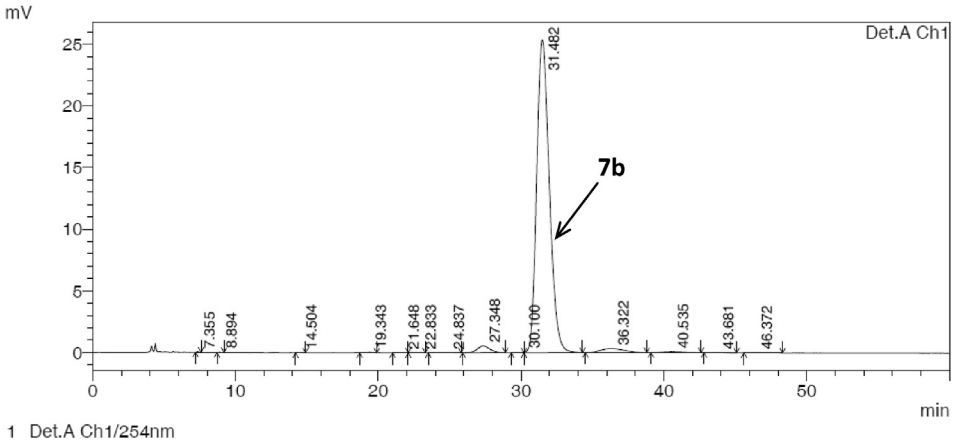


Fig. 7. Chromatogram of virtually enantiopure **7b.**

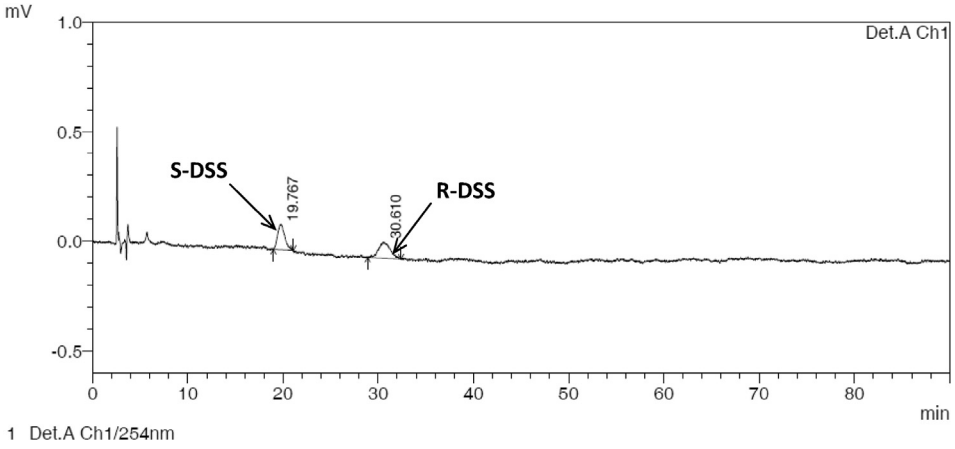


Fig. 8. Chromatogram of the racemic mixture of ***S*-DSS** and ***R*-DSS**.

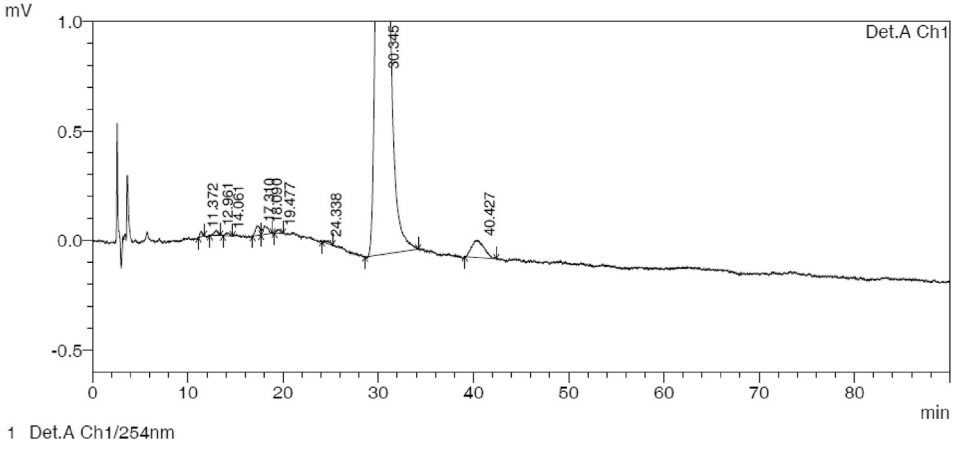


Fig. 9. Chromatogram of the ***R*-DSS** sample.

alternative method of obtaining an active substance of herbal origin by applying a chemical synthesis from commercial substrates. This type of approach may be advantageous for obtaining high quality danshensu in the course of an optimized and repeatable manufacturing procedure. We believe that only compounds obtained as a result of a well-characterized process, with the correct level of defined impurities and in accordance with ICH requirements, may be considered as an API for a future drug composition. Furthermore, synthetic danshensu and its related compounds appear to be attractive precursors or building blocks for further chemical modifications to give novel entities with interesting biological profiles. Although the current state of

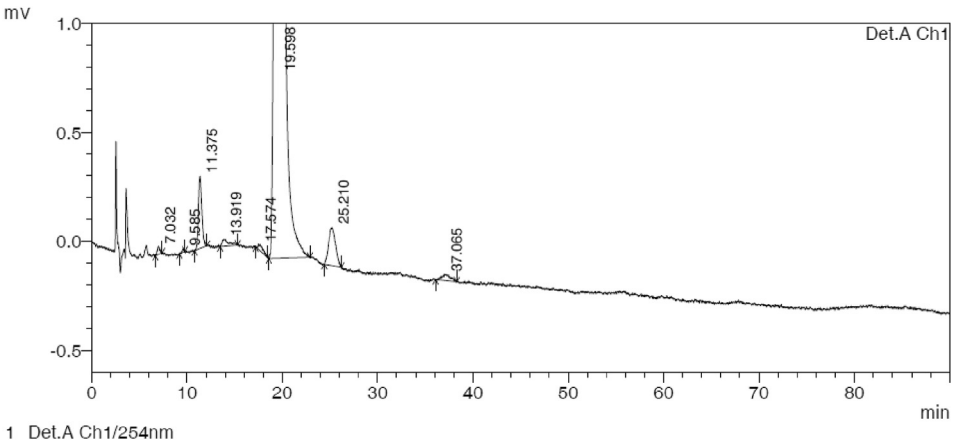


Fig. 10. Chromatogram of the ***S*-DSS** sample.

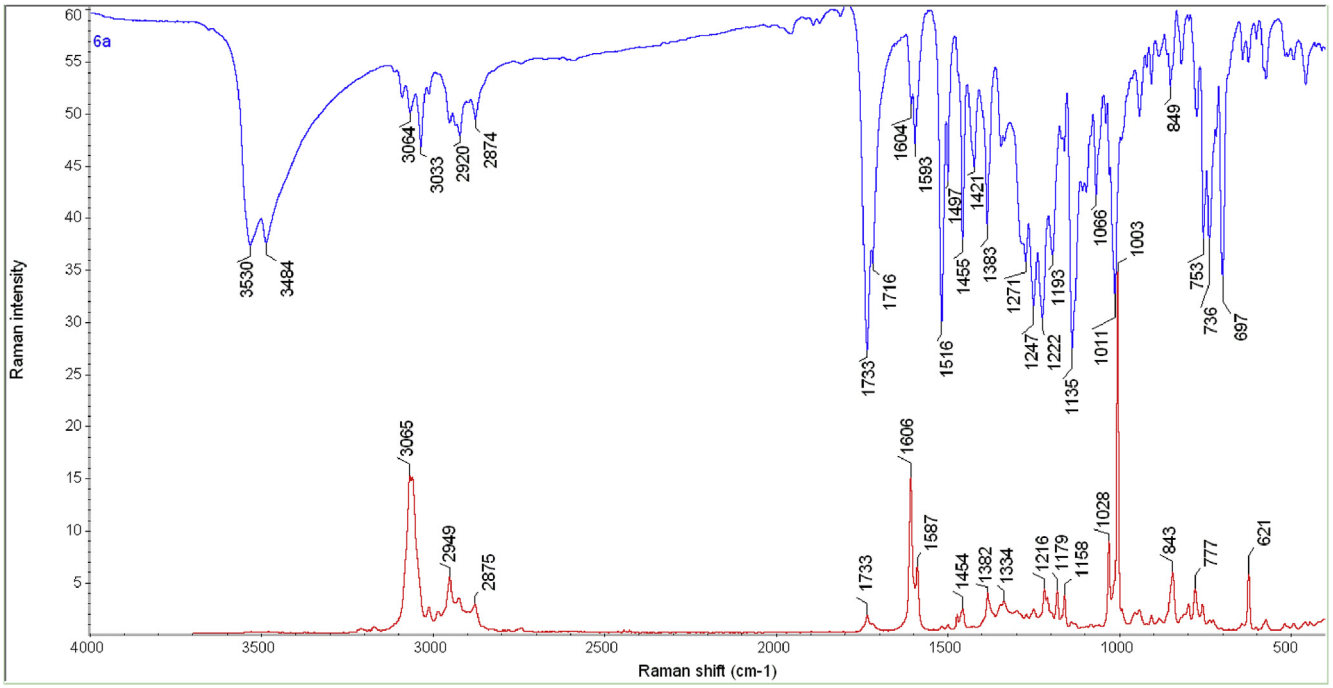


Fig. 11. IR and Raman spectra of **6a** enantiomer.

pharmaceutical sciences and medicine allows for effective testing of the active substances such as danshensu (DSS), knowledge about their physical and chemical properties is still incomplete. Thus, for the first time, we have fully characterized danshensu and its related compounds (isomers, key intermediates) in the course of systematic analytical studies, including the development of an efficient HPLC protocol for the separation of specific isomers.

4. Experimental

4.1. General

Melting points (m.p.) were determined using a Mettler Toledo MP90 apparatus and were uncorrected. The ^{1}H and ^{13}C NMR spectra of all compounds studied were measured in $CDCl_3$, DMSO-D_6 or D_2O using Varian-NMR-vnmrs500, Varian-NMR-vnmrs600 and Varian Gemini 200 spectrometers at the temperature of 298 K. Standard experimental conditions and standard Varian programs were used. To assign the structures under consideration the following 2D experiments were employed: 2D gradient selected COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC. The ^{1}H and ^{13}C NMR chemical shifts relate to the TMS (for compounds dissolved in $CDCl_3$ or DMSO-D_6) and DSS (for compounds dissolved in D_2O). The concentration of all solutions used in the measurements was about 10–20 mg of the compounds in 0.6–0.8 cm^3 of the solvent. Optical rotations were measured with a Jasco P-2000 automatic polarimeter.

The ESI-MS spectra were recorded on a PE Biosystems Mariner mass spectrometer. The progress of the reaction was monitored by thin layer chromatography (TLC) with Merck DC-Alufolien Kieselgel 60 F_{254}. The chemicals and solvents were purchased from Fluka Company. Column chromatography was performed on Merck silica gel 60 (230–400 mesh).

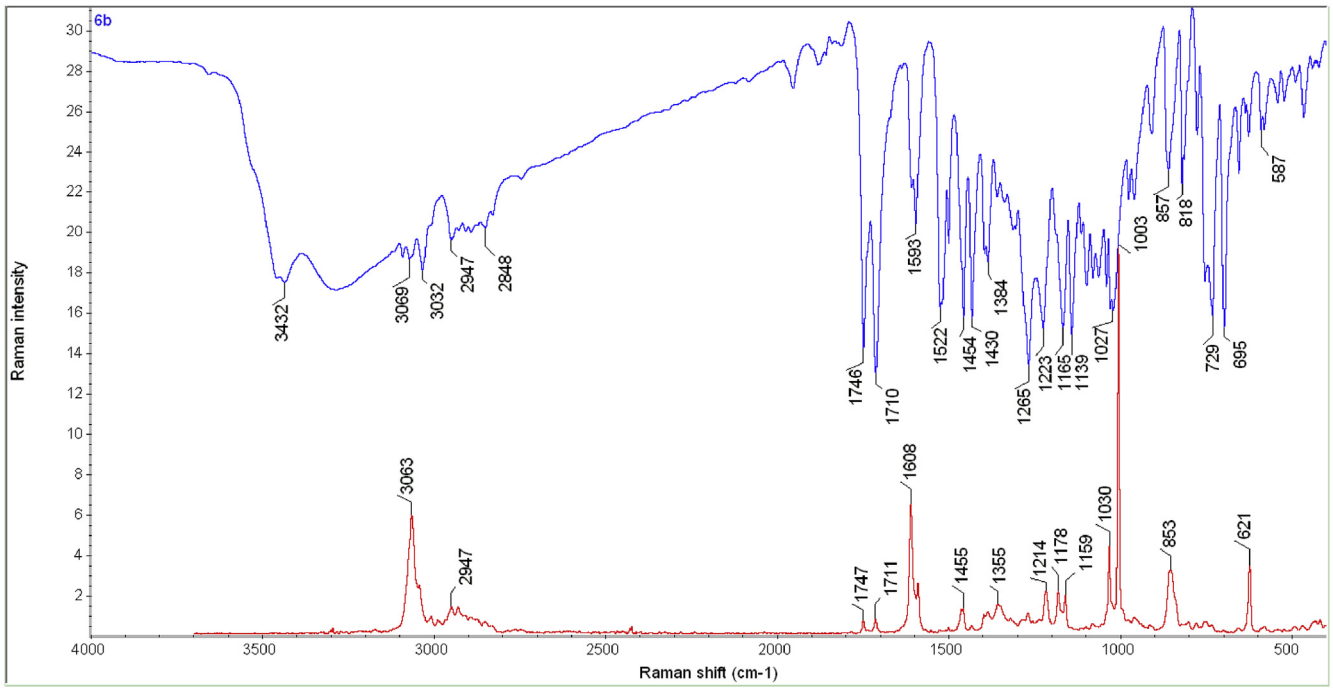


Fig. 12. IR and Raman spectra of **6b** enantiomer.

Table 1
The bands description of intermediate **6a**, **6b**, **7a**, **7b** and danshensu isomers ***S*-DSS**, ***R*-DSS** and impurity **5** from IR and Raman spectra.

Wavenumbers[a], [cm^{-1}]						
6a	6b	7a 7b	5	*S*-DSS	*R*-DSS	Bands description
3530, 3484	3432	3396	–	broad band	broad band	O-H stretching
3064, 3033,2920, 2874	3069, 3032,2947, 2848	3063, 3034, 2948, 2863	3063, 3030,2903, 2860	**3070, 3030, 2927**	**3071, 3033, 2915**	C-H stretching
1733, 1716	1746, 1710	1724	1663	1713	1717	C=O stretching
1606, 1587	**1608, 1587**	**1605, 1587**	**1621, 1594**	**1610**	**1623, 1606**	C=C stretching
1516	1522	1521	1516	1525	1526	Ar ring
–	–	–	1434	–	–	C-CH_3 deformation
1383	1384	1380	–	1359	1370	O-H deformation
1271–1222	1265–1223	1270–1242	1275–1228	–	–	C-*O*-C stretching asymmetric
1135	1139	1139	–	1197	1195	C-O stretching
1028	**1030**	**1028**	**1030**	**–**	**–**	C-*O*-C stretching symmetric
1003	**1003**	**1002**	**1003**	**1018**	**1010**	Ar ring
753, 736,697	729, 695	730, 695	806, 731,694	803	809	C-H out of plane from Ar ring

[a] Wavenumbers from the Raman spectra are included in bold.

4.2. HPLC analysis

HPLC separation of the enantiomers was performed using a Shimadzu LC-2010A HT HPLC system (Shimadzu, Kyoto, Japan) equipped with the autosampler, column thermostat, and UV detector. The data collection and treatment were performed with LCsolution (version 1.24 SP1) software (Shimadzu Kyoto, Japan). Various types of Chiralcel and Chiralpak columns (250 × 4.6 mm, packed with 5 μm particles; Daicel Corporation, Tokyo, Japan), as well as the LuxCellulose column (150 × 4.6 mm, 3 μm particles; Phenomenex, Torrence, USA), were tested to achieve the most effective chiral separation. The following methods for the determination of enantiomeric purity of all three pairs of isomers were developed: (1) for **6a/6b** – Chiralcel OD-H column (250 × 4.6 mm, 5 μm particles), 80:20 hexane:ethanol, flow rate 1 mL/min, column temperature 20 °C; (2) for **7a/7b**– Chiralcel OD-H column (250 × 4.6 mm, 5 μm), 80:18:2 hexane:2-propanol:ethanol, flow rate 0.7 mL/min, column temperature 30 °C; (3) for ***S*-DSS/*R*-DSS** - Chiralpak AD column (250 × 4.6 mm, 5 μm), 90:5:5 hexane:2-propanol:methanol + 0.1%TFA, flow rate 1.2 mL/min, column temperature 30 °C. In all cases the detection was performed at 254 nm.

4.3. Infrared spectroscopy

IR spectra were recorded on the Nicolet iS10 instrument (Thermo Scientific, Waltham, MA, USA). Solid samples were measured in KBr pellets (about 200 mg of KBr/1.5 mg of the studied sample) and by means of the ATR (Attenuated Total Reflectance) technique with the use of Di crystal in the range from 4000 to 400 cm^{-1} and 4000-650 cm^{-1}, respectively, with the spectral resolution of 4 cm^{-1}. For one spectrum performed for the KBr pellet 16 scans were recorded. For one spectrum performed by means of the ATR technique 64 scans were recorded.

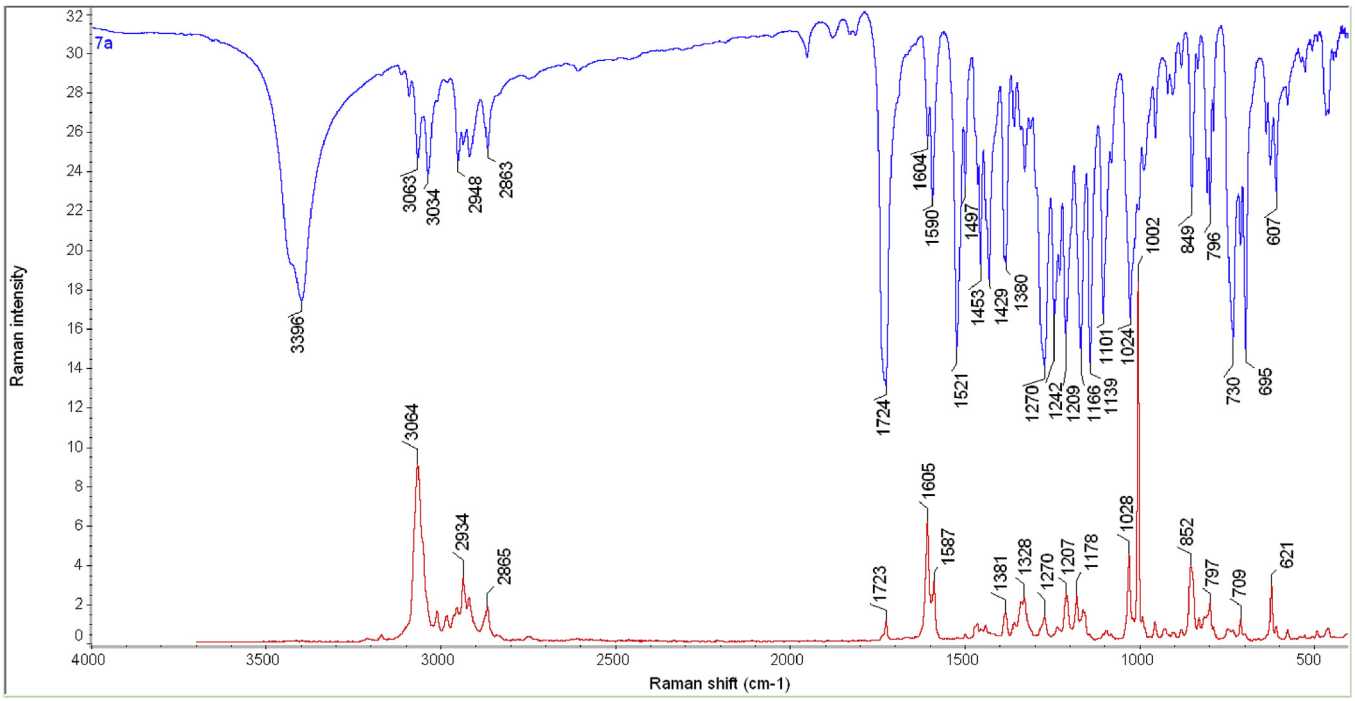


Fig. 13. IR and Raman spectra of **7a** enantiomer.

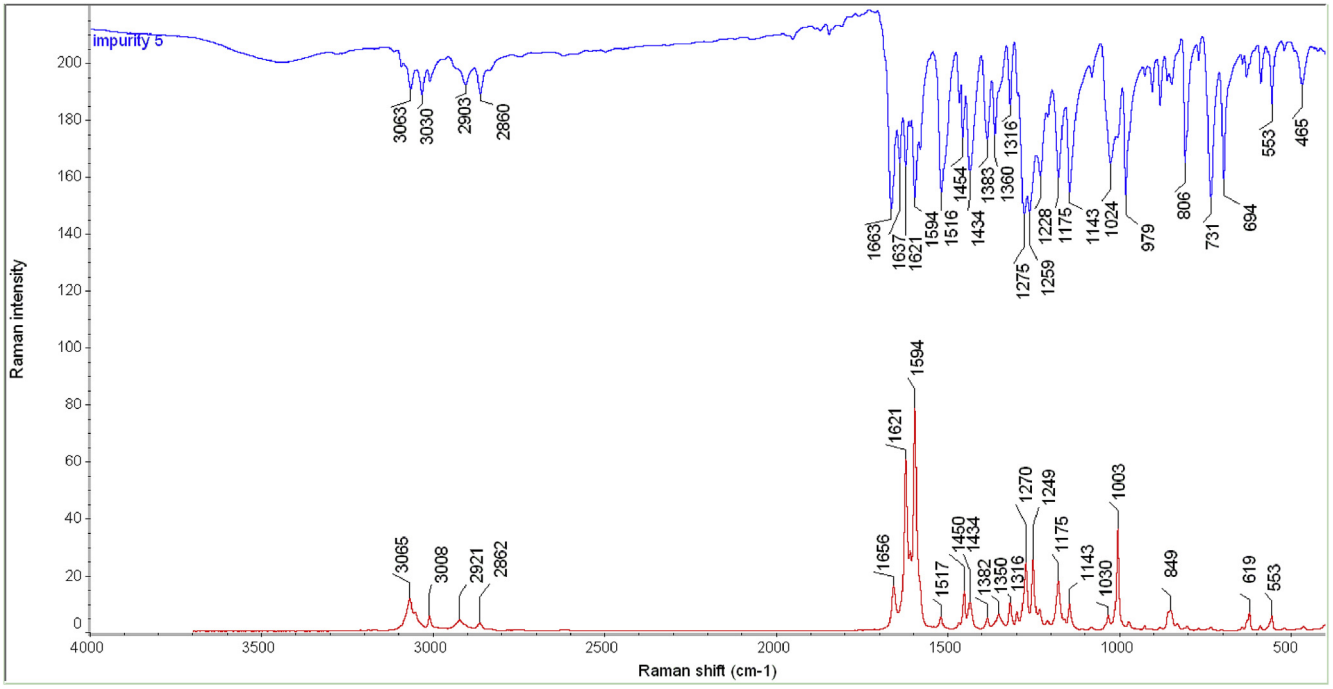


Fig. 14. IR and Raman spectra of impurity **5**.

4.4. Raman spectroscopy

The FT Raman spectra were recorded on the Nicolet NXR 9650 instrument (Thermo Scientific, Waltham, MA, USA) using 1064 nm excitation from the Nd:YVO4 laser in the range from 3700 to 150 cm^{-1} with the spectral resolution of 4 cm^{-1}. For one spectrum from 128 to 300 scans were recorded with the laser power from 0.8 to 1.0 W.

4.5. Differential scanning calorimetry

The DSC measurements were performed by means of the DSC822e cell with an IntraCooler (Mettler Toledo GmbH, Schwerzenbach, Switzerland). About 7 mg of the studied samples was weighed into standard aluminium crucibles (40 μL). The crucibles were hermetically sealed and perforated before the measurements. The samples were heated up from 25 to 300 °C at 10 °C/min. The

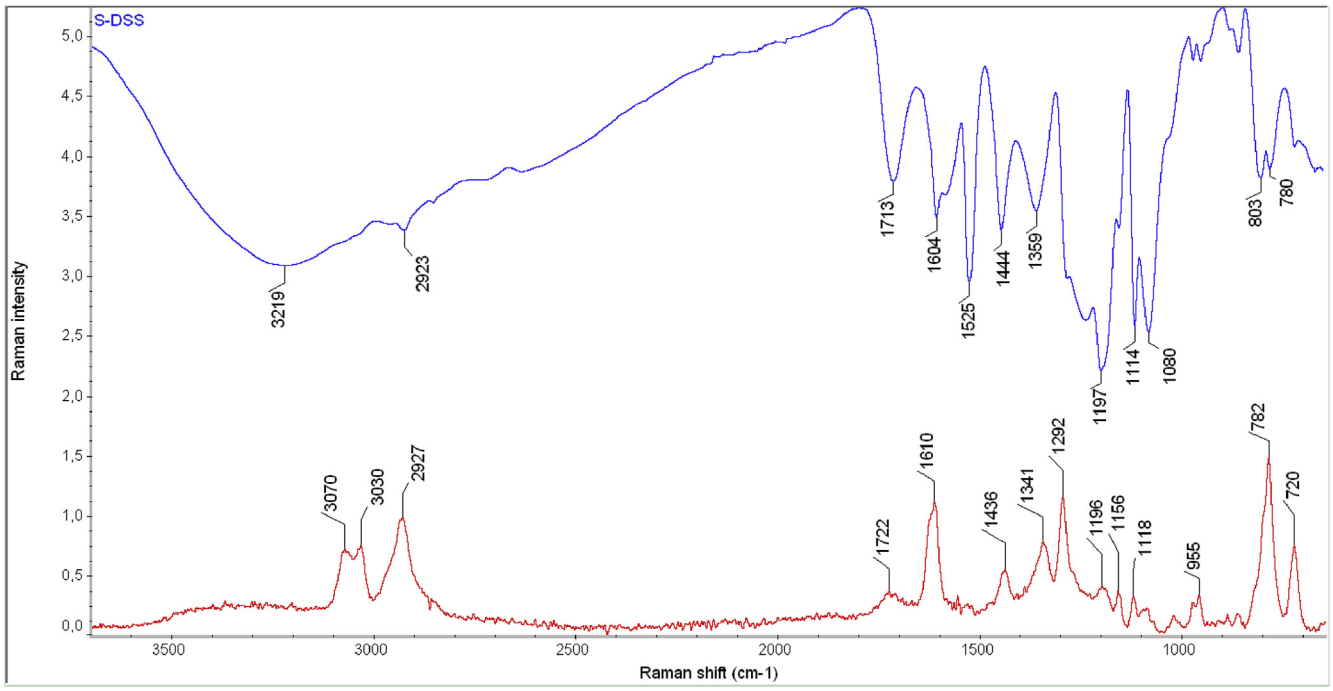


Fig. 15. IR-ATR and Raman spectra of ***S*-DSS** isomer.

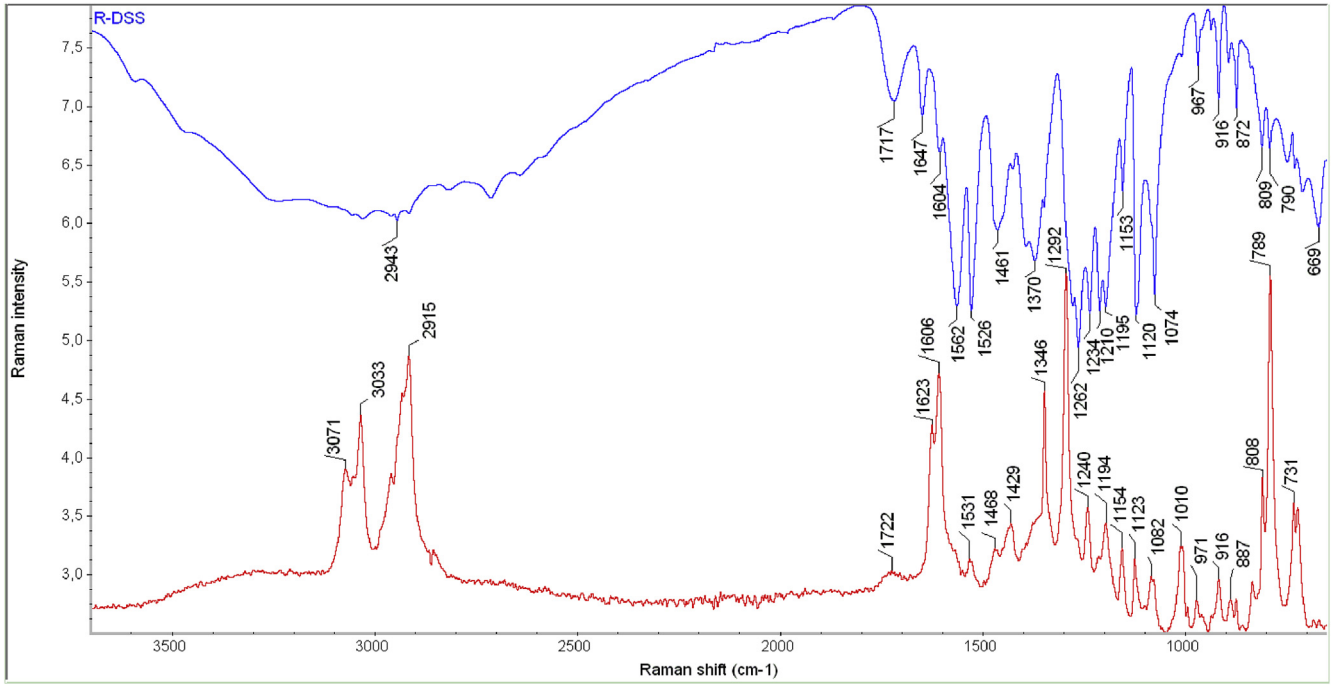


Fig. 16. IR-ATR and Raman spectra of ***R*-DSS** isomer.

measurements were performed in the nitrogen atmosphere at the flow rate of 60 mL/min.

4.6. Thermogravimetry

The TGA measurements were performed by means of the TGA/SDTA851 cell (Mettler Toledo GmbH, Schwerzenbach, Switzerland). About 2 mg of the studied samples was weighed into standard aluminium crucibles (40 μL). The crucibles were hermetically sealed and perforated before the measurements. The samples were heated up from 25 to 300 °C at 10 °C/min. The measurements were performed in the nitrogen atmosphere at the flow rate of 60 mL/min.

The measurements were blank curve corrected.

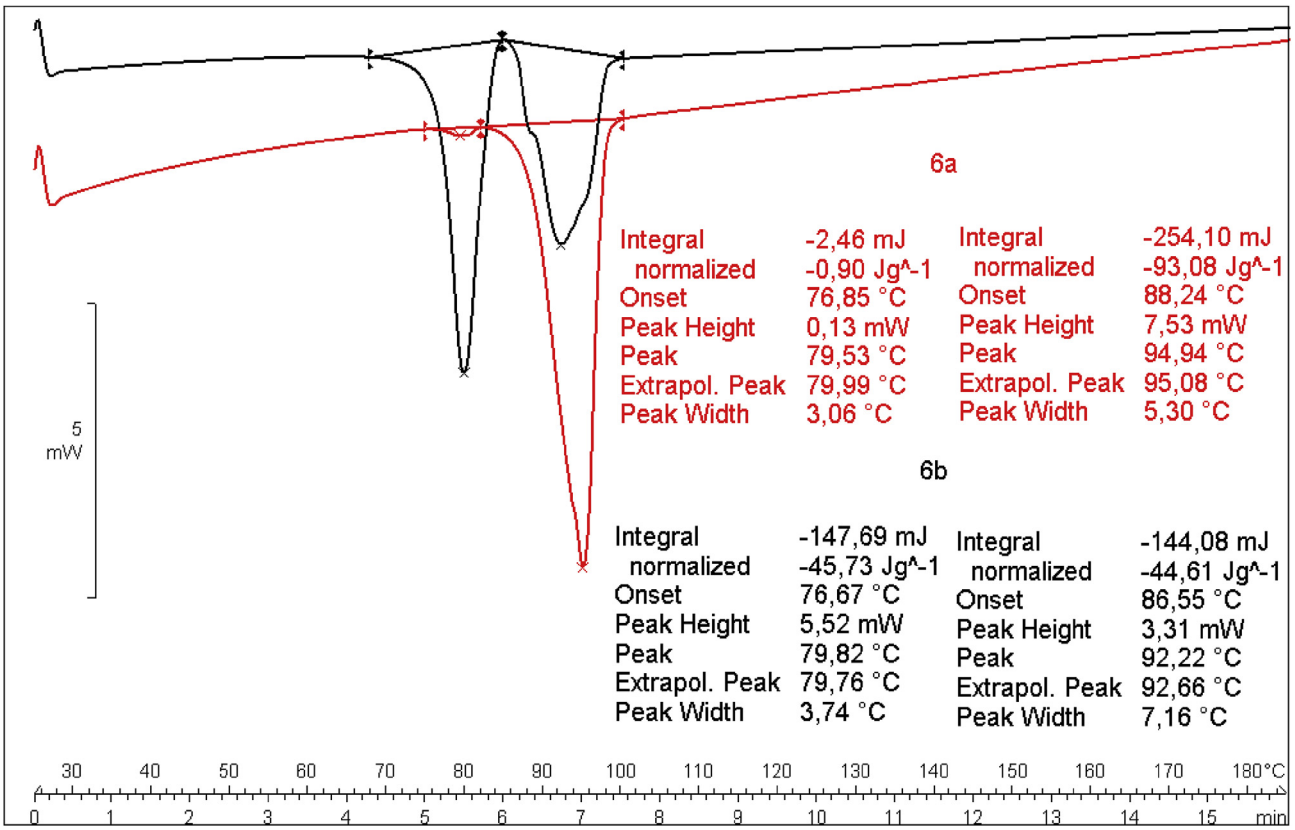


Fig. 17. DSC curves of **6a** and **6b** enantiomers.

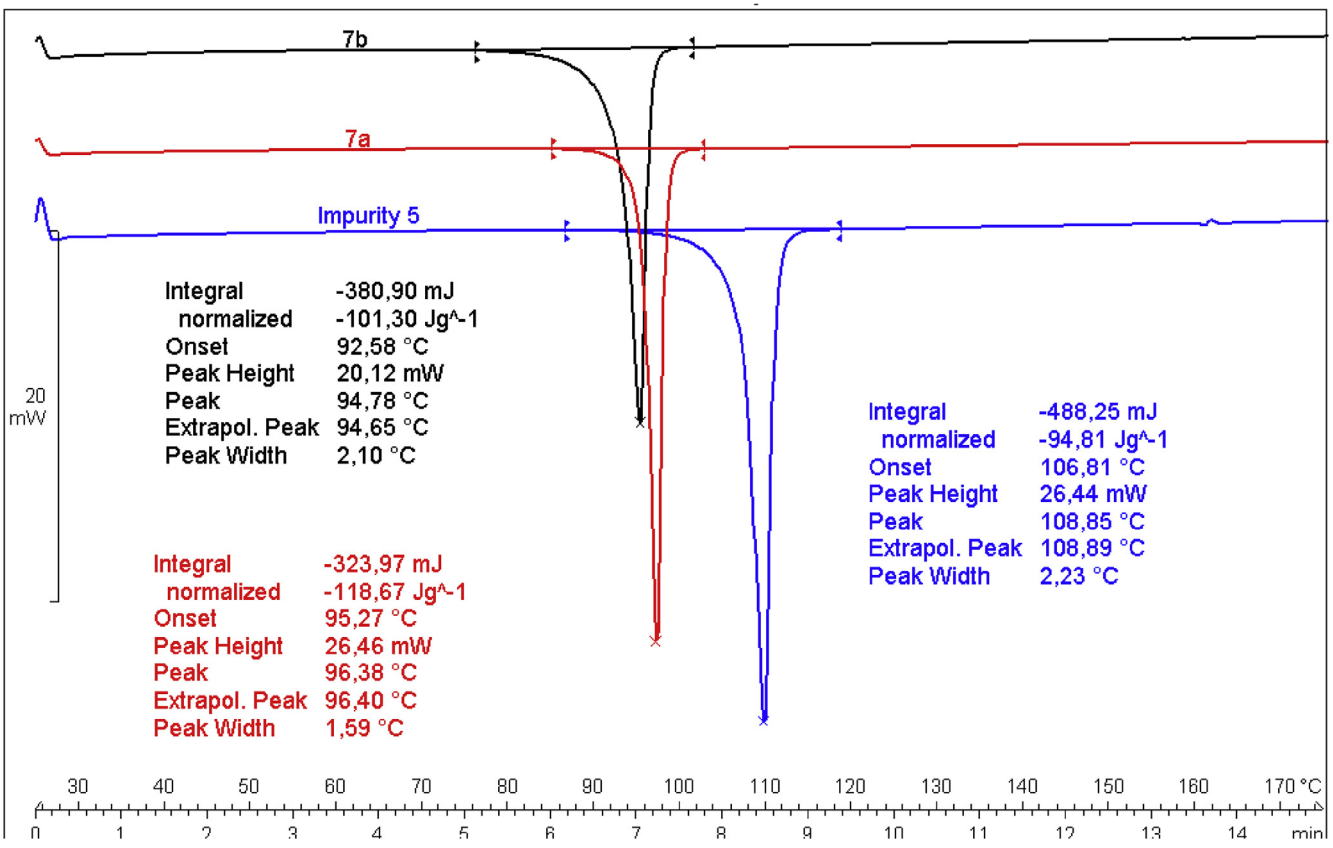


Fig. 18. DSC curves of **7a** and **7b** enantiomers and impurity **5**.

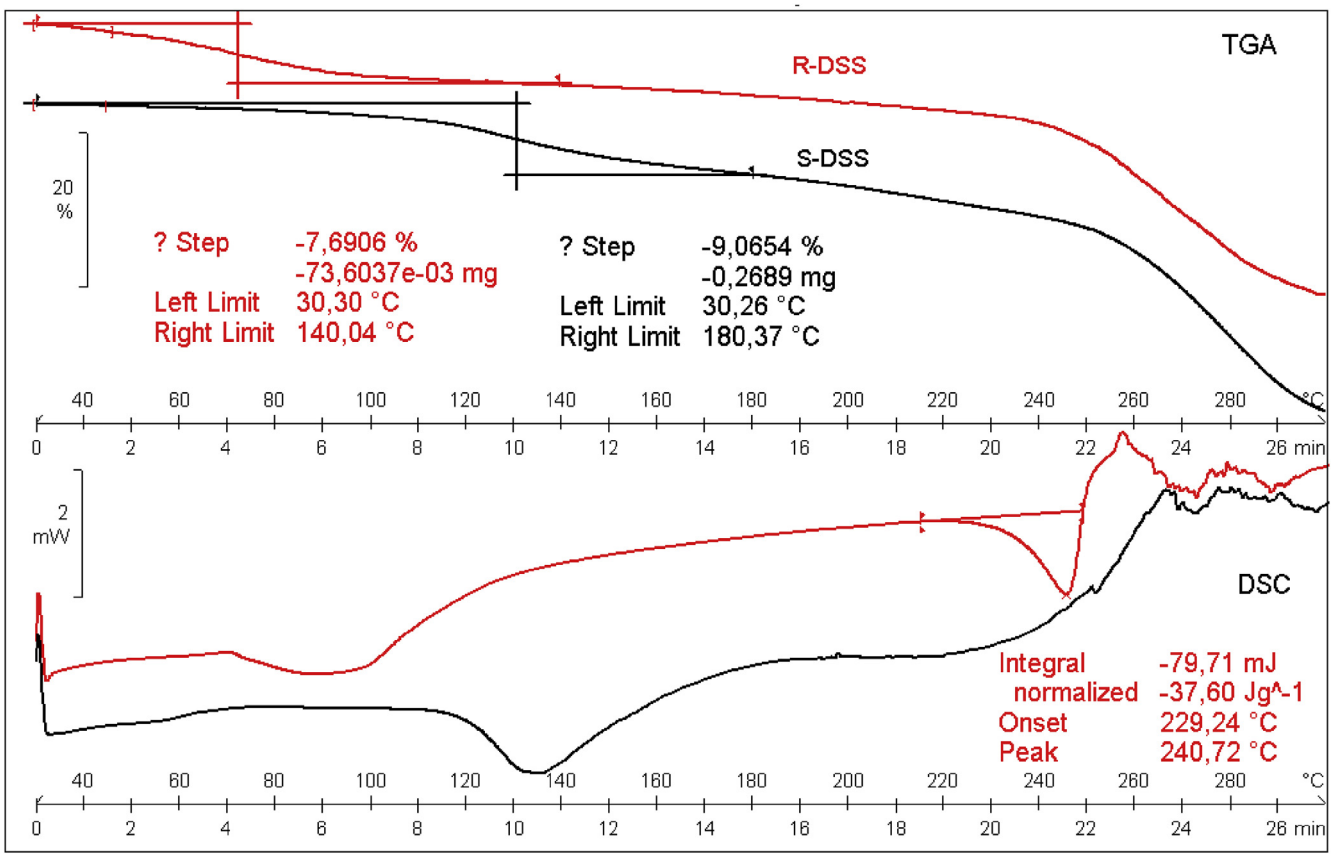


Fig. 19. DSC and TGA curves of ***S*-DSS** and ***R*-DSS** isomers.

4.7. 3,4-Dibenzyloxybenzaldehyde 2

Benzyl bromide (52 mL, 434.6 mmol), K_2CO_3 (36 g, 260.5 mmol) were added to the solution of 3,4-dihydroxybenzaldehyde **1** (15 g, 108.7 mmol) in ethanol (225 mL). The reaction was stirred and heated for 24 h at reflux. After cooling to the ambient temperature, the K_2CO_3 was filtered, washed with ethanol, and the residue was evaporated to dryness. The residue was crystallized from diethyl ether to afford a white solid; yield 29.8 g (86%); m.p. 82–85 °C; mp 88 °C [16]; ^{1}H NMR ($CDCl_3$; 200 MHz) δ 9.80 (s, 1H), 7.49–7.34 (m, 12H), 7.04 (d, 1H, J = 8.4 Hz), 5.25–5.21 (m, 4H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 190.2, 153.6, 148.5, 135.9, 135.6, 129.6, 128.0, 127.9, 127.5, 127.4, 126.6, 126.4, 126.1, 112.4, 111.6, 99.9, 70.3, 70.2.

4.8. (E)-3,4-(dibenzyloxy)cinnamic acid 3

Piperidine (0.1 mL) was added to the solution of 3,4-dibenzyloxybenzaldehyde **2** (0.3 g, 0.94 mmol), and malonic acid (0.147 g, 1.41 mmol) in pyridine (5 mL), and the reaction was heated for 5 h at reflux, and next for 24 h at room temperature. After the reaction had been completed, 2 M HCl was added to the mixture. The precipitate was filtered, and washed with water. The crude product was crystallized from ethyl acetate to give the title compound as a white solid (0.25 g, 74%) m.p. 196–199 °C; mp 201–202 °C [16]; ^{1}H NMR ($CDCl_3$; 200 MHz) δ 7.44 (d, 1H, J = 15.7 Hz), 7.42–7.26 (m, 10H), 7.19–7.14 (m, 2H), 6.96 (d, 1H, J = 8.1), 6.21 (d, 1H, J = 16.1 Hz), 5.22–5.18 (m, 4H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 171.2, 148.9, 146.7, 136.6, 128.6, 127.9, 127.4, 127.3, 127.1, 123.3, 114.7, 114.2, 113.8, 71.3, 70.9.

4.9. (E)-Benzyl 3-(3,4-dibenzyloxyphenyl)-propenate 4

Benzyl bromide (0.079 mL, 0.66 mmol), K_2CO_3 (91.2 mg, 0.66 mmol), KI (5 mg), TBAI (7 mg) were added to the solution of **3** (200 mg, 0.55 mmol) in acetone (2 mL). The reaction was stirred and heated for 24 h at room temperature. After the reaction had been completed, the solvent was evaporated under reduced pressure at ca. 40 °C. The column chromatography of the residue (hexane – ethyl acetate, 20:1 → 3:1) afforded the title compound (225 mg, 90%) as a white solid; m.p. 68–72 °C, m.p. 80–82 °C [20].

4.10. (E)-Benzyl 3-(3,4-dibenzyloxyphenyl)-propenate 4

Benzyl alcohol (15.8 mL, 153.3 mmol) was added to the solution of Meldrum's acid (22.1 g, 153.3 mmol) in toluene (250 mL). The reaction was stirred and heated for 6 h at reflux, and next in room temperature for 18 h. 3,4-Dibenzyloxybenzaldehyde **2** (20 g, 62.8 mmol), pyridine (5 mL), piperidine (5 mL) were added to the solution, and the reaction was heated for 2 h at reflux. After the reaction had been completed, the solvent was evaporated under reduced pressure at ca. 40 °C. The resulting oil was treated with ethyl acetate (200 mL), the organic layer was washed successively with the $NaHCO_3$ aq. solution, 1 M HCl and water to pH 6. The extract was dried over anhydrous $MgSO_4$, filtered and evaporated to dryness. The column chromatography of the residue (hexane – ethyl acetate, 20:1 → 2:1) afforded the title compound (26 g, 93%) as a white solid. m.p. 68–72 °C; mp 80–82 °C [20]; ^{1}H NMR ($CDCl_3$; 600 MHz) δ 7.62–7.59 (d, 1H, J = 15.8 Hz), 7.43–7.31 (m, 15H), 7.12 (d, 1H, J = 1.86 Hz), 7.05 (dd, 1H, J = 1.87 Hz, J = 8.33 Hz), 6.90 (d, 1H, J = 8.33 Hz), 6.29 (d, 1H, J = 15.8 Hz), 5.23 (s, 2H), 5.18 (s, 2H), 5.16 (s,

2H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 166.9, 151.1, 148.9, 144.9, 136.8 (C), 136.7 (C), 136.1 (C), 128.57, 128.56, 128.55, 128.3, 128.26, 128.2, 127.9, 127.8, 127.3, 127.1, 122.9, 115.9, 114.2, 113.7, 71.3 (CH_2), 70.9 (CH_2), 66.2 (CH_2).

4.11. (3E)-4-[3,4-bis(phenylmethoxy)phenyl]-3-buten-2-one 5

m.p. 99–101 °C; ^{1}H NMR ($CDCl_3$; 600 MHz) δ 7.45–7.44 (m, 4H), 7.40–7.35 (m, 5H), 7.32–7.29 (m, 2H), 7.13 (d, 1H, J = 2 Hz), 7.07 (dd, 1H, J = 2 Hz, J = 8.4 Hz), 6.92 (d, 1H, J = 8.4 Hz), 6.52 (d, 1H, J = 16 Hz), 5.19–5.17 (m, 4H), 2.33 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 198.3, 151.3, 149.0, 143.3, 136.8, 136.6, 128.5, 127.9, 127.7, 127.3, 127.1, 125.4, 123.2, 114.2, 113.7, 71.3 (CH_2), 70.9 (CH_2), 27.3; HR-MS (ESI) calc. for $C_{24}H_{23}O_3$ $[M+H]^+$: 359.1647. Found: 359.1649.

4.12. Benzyl (2S, 3R)-2,3-dihydroxy-3-(3,4-dibenzyloxyphenyl)-propionate 6a

AD-mix β (16 g) and methanosulponamide (1.1 g) were added to the suspension of **4** (5.0 g, 11.09 mmol) in the mixture of acetone/acetonitile/water (3:3:1, v/v, 80 mL). The suspension was stirred for 24 h at room temperature. The reaction was quenched with the saturated solution of Na_2SO_3, concentrated, and extracted with ethyl acetate. The solvents were evaporated under diminished pressure. Column chromatography of the residue (hexane – ethyl acetate, 6:1 → 1:1) afforded the title compound as a white solid (3.1 g, 58%); HPLC purity 99.22%; m.p. 86–88 °C; mp. 97–100 °C [20]; $[\alpha]_D^{20}$ = - 1.2 (*c* 0.75, $CHCl_3$); ^{1}H NMR (CD_3OD; 500 MHz) δ 7.44–7.42 (m, 4H), 7.35–7.31 (m, 9H), 7.24–7.23 (m, 2H), 7.08 (d, 1H, J = 1.5 Hz), 6.90–6.89 (m, 2H), 5.11–5.04 (m, 6H), 4.85 (d, 1H, J = 4.5 Hz), 4.27 (d, 1H, J = 4.5 Hz); ^{13}C NMR (CD_3OD, 125 MHz) δ 173.0, 149.3, 149.0, 137.7, 137.6, 135.8, 134.4, 128.94, 128.9, 128.8, 128.7, 128.6, 128.3, 128.0, 127.8, 120.4, 115.3, 114.2, 76.3, 75.1, 71.8, 67.4.

4.13. Benzyl (2R, 3S)-2,3-dihydroxy-3-(3,4-dibenzyloxyphenyl)-propionate 6b

AD-mix α (33 g) and methanosulponamide (2.2 g) were added to the suspension of **4** (10.0 g, 22.19 mmol) in the mixture of acetone/acetonitile/water (3:3:1, v/v, 160 mL). The suspension was stirred for 24 h at room temperature. The reaction was quenched with the saturated solution of Na_2SO_3, concentrated, and extracted with ethyl acetate. The solvents were evaporated under diminished pressure. Column chromatography of the residue (hexane – ethyl acetate, 6:1 → 1:1) afforded the title compound as a white solid (7.37 g, 69%); HPLC purity 99.72%; m.p. 84–89 °C; 88–91 °C [20]; $[\alpha]_D^{20}$ = + 1.2 (*c* 0.75, $CHCl_3$); ^{1}H NMR (CD_3OD; 500 MHz) δ 7.42–7.41 (m, 4H), 7.33–7.25 (m, 9H), 7.20–7.18 (m, 2H), 7.10 (d, 1H, J = 1.9 Hz), 6.93 (d, 1H, J = 8.3 Hz), 6.85 (dd, 1H, J = 0.4 Hz, J = 1.9 Hz), 5.07–4.97 (m, 6H), 4.81 (d, 1H, J = 4.9 Hz), 4.23 (d, 1H, J = 4.9 Hz); ^{13}C NMR (CD_3OD, 125 MHz) δ 173.6, 150.1, 149.8, 137.8, 138.7, 137.0, 135.5, 129.5, 129.42, 129.41, 129.2, 128.8, 128.7, 128.6, 121.2, 115.9, 115.1, 77.3, 76.1, 72.3, 67.6.

4.14. Benzyl (2S)-hydroxy-3-(3,4-dibenzyloxyphenyl)-propionate 7a

To the ice-cooled solution of **6a** (2.41 g, 4.97 mmol) in anhydrous DCM (25 mL), Et_3SiH (7.94 mL, 49.74 mmol) and TFA (4.44 mL, 59.64 mmol) were added dropwise and stirred for 30 min at 0 °C. Next, the whole mixture was stirred at room temperature until no more starting material was detected on TLC (approx. 5 h). The solution was evaporated to dryness, then CH_3OH was added and evaporated once more to dryness. The residue was crystallized from CH_3OH to afford a white solid (1.96 g, 84%); HPLC purity 97.00%; m.p. 88–90 °C; $[\alpha]_D^{20}$ = - 44.6 (*c* 0.75, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 500 MHz) δ 7.44–7.25 (m, 15H), 6.81–6.80 (m, 2H), 6.64–6.63 (m, 1H), 5.13–5.09 (m, 6H), 4.43 (m, 1H), 3.01 (dd, 1H, J = 4.5 Hz, J = 14 Hz), 2.89 (dd, 1H, J = 6.4 Hz, J = 14 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.8, 148.7, 148.0, 137.4, 137.2, 135.0, 129.4, 128.7, 128.6, 128.5, 128.4, 127.7, 127.4, 127.3, 122.5, 116.6, 115.1, 71.4, 71.2 (CH_2), 67.3 (CH_2), 39.9 (CH_2); HR-MS (ESI) calc. for $C_{30}H_{28}O_5Na$ $[M+Na]^+$: 491.1834. Found: 491.1837.

4.15. Benzyl (2R)-hydroxy-3-(3,4-dibenzyloxyphenyl)-propionate 7b

To the ice-cooled solution of **6b** (7.0 g, 14.45 mmol) in anhydrous DCM (70 mL), Et_3SiH (23.1 mL, 144.46 mmol) and TFA (12.92 mL, 173.4 mmol) were added dropwise and stirred for 30 min at 0 °C. Next, the whole mixture was stirred at room temperature until no more starting material was detected on TLC (approx. 6 h). The solution was evaporated to dryness, then CH_3OH was added and evaporated once more to dryness. The residue was crystallized from CH_3OH to afford a white solid (4 g, 60%); HPLC purity 94.91%; m.p. 86–89 °C; $[\alpha]_D^{20}$ = + 44.8 (*c* 0.75, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 500 MHz) δ 7.44–7.28 (m, 15H), 6.81–6.79 (m, 2H), 6.64–6.62 (dd, 1H, J = 2 Hz, J = 8.1 Hz), 5.12–5.08 (m, 6H), 4.42 (m, 1H), 3.01 (dd, 1H, J = 4.5 Hz, J = 14 Hz), 2.89 (dd, 1H, J = 6.4 Hz, J = 14 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.8, 148.7, 148.0, 137.4, 137.3, 135.0, 129.4, 128.7, 128.6, 128.5, 128.4, 127.7, 127.4, 127.3, 122.5, 116.6, 115.1, 71.4, 71.2 (CH_2), 67.3 (CH_2), 39.9 (CH_2); HR-MS (ESI) calc. for $C_{30}H_{28}O_5Na$ $[M+Na]^+$: 491.1834. Found: 491.1819.

4.16. 3-(3,4-dihydroxyphenyl)-(S)-lactic acid S-DSS

The solution of **7a** (0.5 g, 1.06 mmol) and 10% Pd/C (100 mg) in the mixture of THF/C_2H_5OH (1:1, v/v, 30 mL) was placed under hydrogen and stirred for 1 h at room temperature. The mixture was filtered through a Celite which was washed with ethanol (5 mL). The filtrate was concentrated in vacuo at 40 °C to give brown oil. Column chromatography of the crude product (chloroform – methanol, 10:1 → 1:1) was performed to afford the title compound as yellow oil. The lyophilization of oil afforded the product as yellow foam (0.201 g, 95%); HPLC purity 94.91%; $[\alpha]_D^{20}$ = - 24.0 (*c* 0.5, H_2O); ^{1}H NMR (D_2O; 600 MHz) δ 6.86 (d, 1H, J = 8.1 Hz), 6.82 (d, 1H, J = 2 Hz), 6.72 (dd, 1H, J = 2 Hz, J = 8.1 Hz), 4.39–4.36 (m, 1H), 3.01 (dd, 1H, J = 4.5 Hz, J = 14.2 Hz), 2.83 (dd, 1H, J = 7.6 Hz, J = 14.2 Hz); ^{13}C NMR (D_2O, 150 MHz) δ 181.1, 146.4, 145.3, 132.6, 124.5, 119.8, 118.8, 74.7, 41.7; HR-MS (ESI) calc. for $C_9H_9O_5$ $[M-H]^-$: 197.0450. Found: 197.0454.

4.17. 3-(3,4-dihydroxyphenyl)-(R)-lactic acid R-DSS

The solution of **7b** (0.4 g, 0.854 mmol) and 10% Pd/C (80 mg) in the mixture of THF/C_2H_5OH (1:1, v/v, 20 mL) was placed under hydrogen and stirred for 1.5 h at room temperature. The mixture was filtered through a Celite which was washed with ethanol (5 mL). The filtrate was concentrated in vacuo at 40 °C to give brown oil. Column chromatography of the crude product (chloroform – methanol, 10:1 → 1:1) was performed to afford the title compound as yellow oil. The solid product was precipitated from hexanes (0.201 g, 95%); HPLC purity 96.33%; $[\alpha]_D^{20}$ = + 25.4 (*c* 0.5, H_2O); ^{1}H NMR (D_2O; 600 MHz) δ 6.88 (d, 1H, J = 8.1 Hz), 6.84 (d, 1H, J = 2 Hz), 6.73 (dd, 1H, J = 2 Hz, J = 8.1 Hz), 4.29–4.25 (m, 1H), 3.00 (dd, 1H, J = 4.2 Hz, J = 14.2 Hz), 2.79 (dd, 1H, J = 7.7 Hz, J = 14.2 Hz); ^{13}C NMR (D_2O, 150 MHz) δ 182.3, 146.1, 144.8, 132.9, 124.2, 119.5, 118.5, 75.3, 41.7; HR-MS (ESI) calc. for $C_9H_9O_5$ $[M-H]^-$: 197.0450. Found: 197.0455.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.molstruc.2017.09.118.

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