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Arylacetic acid derivatization of 2,3- and internal *erythro*-squalene diols. Separation and absolute configuration determination

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Abstract—We have studied a new approach for the resolution and absolute configuration determination of the enantiomers of squalene diols as intermediate precursors in the chemical synthesis of different squalene oxides (SOs); (3*R*)- and (3*S*)-2,3-SO, (6*R*,7*R*)- and (6*S*,7*S*)-6,7-SO, and (10*R*,11*R*)- and (10*S*,11*S*)-10,11-SO. Monoderivatization of the corresponding racemic squalene diol intermediates with pure stereoisomers of (*S*)-(+)-methoxyphenyl acetic acid ((*S*)-(+)-MPA), (*S*)-(+)-9-anthrylmethoxyacetic acid ((*S*)-(+)-9-AMA) and (*S*)-(+)-acetoxyphenylacetic acid ((*S*)-(+)-APA) afforded the diastereomeric esters which could be easily separated by column flash chromatography with silica gel. In addition, the absolute configuration for these diastereoisomers of the derivatized diols was advantageously inferred from ^{1}H NMR data according to the models depicted for these derivatizing chiral agents. In order to demonstrate the absolute configuration assignment of the different stereoisomers, (*S*)-(+)-AMA showed the larger $\Delta\delta$ by ^{1}H NMR, however, (*S*)-(+)-MPA esters were much more stable derivatives.

1. Introduction

In the past years, we developed a route to synthesize and determine the absolute configuration of the different enantiomers of the squalene oxides using 2,3- and *erythro*−6,7- and 10,11-squalene diols (Sdiols) as intermediates. In this case, the resolution of the corresponding Sdiols was achieved by derivatization with MTPA (Mosher's acid, α-methoxy-α-trifluoromethylphenylacetic acid). In addition, we demonstrated that these enantiomers of the internal SOs were valuable substrates for inhibitory studies of squalene epoxidase (SE) leading to the epoxidation at both terminal double bonds of the squalene chain but affording regioselective and asymmetric ratios of enantiomers of squalene dioxides.[1] Likewise, we studied the activity and interactions of the resulting squalene dioxides with oxidosqualene-lanosterol cyclase (OSLC), another key enzyme in the cholesterol biosynthesis.[1]

In this paper, we report a practical new approach to resolve both enantiomers of the different squalene diols with high enantiomeric excesses by simple flash chromatography using as derivatizing agents chiral aryl acetic acids currently applied to determine the absolute configuration of secondary alcohols. For this purpose, the (*S*)-(+)-MPA, (*S*)-(+)-APA, and (*S*)-(+)-9-AMA diastereomeric esters from the above squalene diols were prepared (Fig. 1) and separated by flash chromatography with silica gel. In addition, we present here the ^{1}H NMR data needed for the easy assignment of the absolute configuration of the derivatized secondary carbinols present in 2,3- and *erythro*-squalene diols and we compare these results with the more cumbersome assignation from data previously obtained for the above squalene diols derivatized with MTPA.[1]

2. Results and discussion

2.1. Preparation, derivatization and separation of the 2,3- and *erythro*-squalene diols

So far, there have been a great number of studies for the synthesis of chiral squalene epoxides. In most of these syntheses, 2,3-squalene diols were used as versatile key intermediates in the preparation of enantiomerally pure 2,3-squalene oxides. Other approaches were reported by using chiral synthons,[2] by enantioselective reactions[3] or by chemical resolution of the diols.[1,4] among others. Previously, we had performed the chemical resolution

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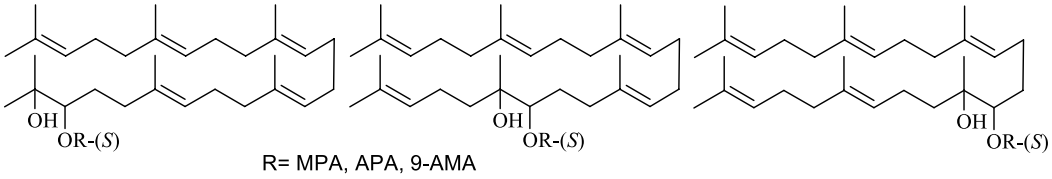


Figure 1. Arylacetic esters of 2,3- and *erythro*-6,7- and 10,11-squalene diols.

strategy involving the formation of the (*R*)-MTPA esters of the corresponding enantiomers of 2,3-, *erythro*-6,7- and *erythro*−10,11-squalene diols. The obtained esters were submitted to HPLC chromatographic resolution and used for further determination of the absolute configuration of the stereogenic centers present at the squalene skeleton for each diastereoisomer. But HPLC chromatographic resolution by reverse phase HPLC was very tedious and difficult.

Our ongoing interest in resolving secondary alcohols with lipases[5] has led us to undertake the search for a new enzymatic strategy. In this context, specific lipases could be able to remove selectively the arylacetic group from the derivatized diasteromeric (*S*)-(+)-esters of the corresponding squalene diols. As a result, we would be able to separate both enantiomers of the racemic mixture facilitating the stereochemical studies of the resolved diol intermediate and determining the absolute configuration of both diastereomeric esters. Consequently, we decided to prove this strategy for arylacetic derivatized compounds which could be potential substrates with acylases or lipases and had been utilized in determining the absolute configuration of secondary alcohols, such as (*S*)-(+)-MPA, (*S*)-(+)-APA, and (*S*)-(+)-9-AMA esters. In this context, some representative model secondary alcohols were derivatized and enzymatically hydrolyzed but results were not so good as anticipated and this approach was disregarded.

2,3-Squalene diol, *erythro*-6,7- and *erythro*−10,11-squalene diol were prepared according to procedures previously reported[1] and derivatization with (*S*)-(+)-MPA, (*S*)-(+)-APA, and (*S*)-(+)-9-AMA, was achieved by one of these two methods: (1) By previous preparation of the proper acid chloride and then reaction with the squalene diol in presence of DMAP and NEt_3[6] or (2) by using a carbodiimide in presence of DMAP.[5] Apparently, no important racemizations have been reported under the derivatization conditions described for these two methods and yields used to be higher than 90%.

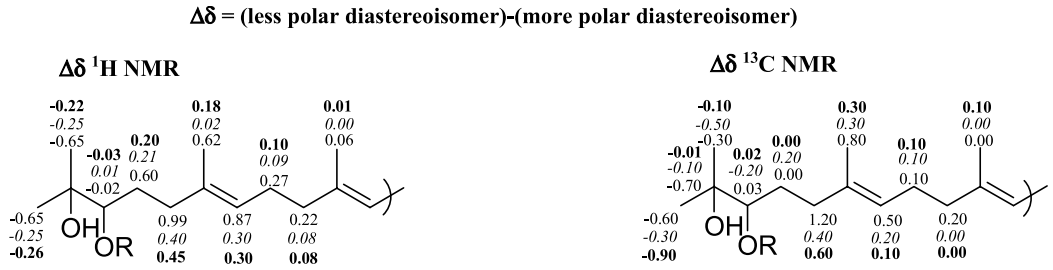
Surprisingly, when we analyzed the reaction mixture by TLC we observed high differences of R_f in the TLC plate for all the squalene arylacetic esters prepared. As an example, TLC separation of diasteromeric esters was better for the derivatizing acid in the order 9-AMA> APA≈MPA and for the derivatized diol in the order 10,11->6,7->2,3-squalene diol. As we expected, stability of products followed the order MPA >> APA > 9-AMA, since after 1 year of storage only 4% of MPA derivatized product was isomerized. ^{1}H, ^{13}C NMR and HPLC-MS-FIA data allowed us to determine that the two major products formed corresponded to the monoderivatized diastereoismers at the secondary carbinol of the squalene diol.

2.2. Absolute configuration determination of the 2,3-, *erythro*-6,7- and *erythro*-10,11-squalene diols

Since Mosher[7] proposed ^{1}H NMR to determine the absolute configuration of stereogenic centers of secondary alcohols derivatized with MTPA, many other researchers have reported similar methodologies and derivatizing agents. Most of these methodologies try to visualize important ^{1}H NMR chemical shifts changes due to the diamagnetic effect of the aromatic ring(s) on certain protons of the derivatized molecule. Obviously, the success to determine the absolute configuration of a derivatized alcohol by ^{1}H NMR depends on the $\Delta\delta$ value calculated from the different chemical shifts for the same hydrogen of both diastereoisomers. The greater is this anisotropy effect in both diastereomers, the easier is the proton chemical shifts differentiation and therefore the absolute configuration determination. When we applied this technique to determine the absolute configuration of the squalene diols derivatized with (*R*)-MTPA by NMR, the chemical shifts signals of the diasteromeric derivatives were too close with only small $\Delta\delta$ values among 0.15–0.05 ppm, complicating the assignment process. However, the recent availability of acids like MPA, APA and 9-AMA has facilitated the application of the corresponding stereochemical models for the assignment of absolute configurations. In order to extend the use of this methodology, we present here a comparative study of the $\Delta\delta$ values obtained for the assignment of the absolute configuration of the squalene diols and we compare these results with the previously studied derivatives obtained with Mosher's acid (MTPA).

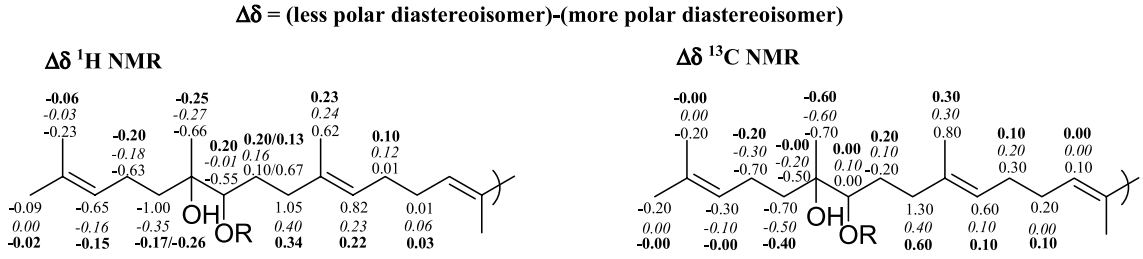
To this aim, ^{1}H, ^{13}C NMR, DQFCOSY and XH-CORFE spectra for the different arylacetic esters were registered, and interpretation of COSY and HETCOR data allowed the assignments of ^{1}H and ^{13}C NMR signals, which readily demonstrated that another difference between them was the asymmetric diamagnetic effect of the aromatic ring(s) on the ^{1}H NMR chemical shifts for the hydrogens with respect to each diastereoisomer.

The ^{1}H and ^{13}C NMR chemical shift increments ($\Delta\delta$) in each pair of diastereomeric esters are shown in Schemes 1–3. ^{1}H NMR $\Delta\delta$ calculations showed a similar sign behavior for the squalene diols substituents in all arylacetic esters. As we had checked previously for aliphatic chains, hydrogens at β position from the carbinol exhibited the highest shielding effect.[8] As expected, (*S*)-(+)-9-AMA showed the most important shielding values for both diastereomeric esters (i.e., 1 ppm for hydrogens at β position). However, results using APA were comparable to those obtained with MPA (i.e., 0.4 ppm for hydrogens at β position), but much better than those reported with MTPA (i.e, 0.15 ppm for hydrogens at β position). Likewise, ^{13}C NMR chemical shift assignments showed a ^{13}C NMR $\Delta\delta$ behavior similar to that



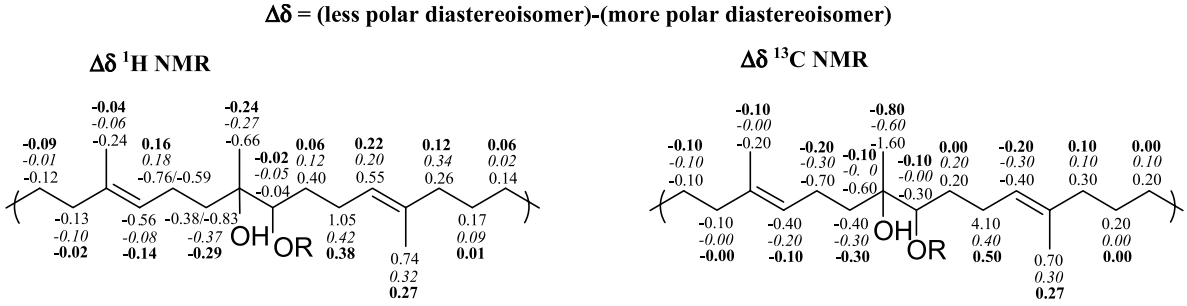
Plain R= (*S*)-(+)-9-AMA, *italic*=(*S*)-(+)-APA, **Bold**= (*S*)-(+)-MPA

Scheme 1. ^{1}H and ^{13}C NMR chemical shift differences (in ppm) for the positions closest to the substituted carbinolic center for the aryl acetic esters of the 2,3-squalene diol skeleton.



Plain R= (*S*)-(+)-9-AMA, *italic*=(*S*)-(+)-APA, **Bold**= (*S*)-(+)-MPA

Scheme 2. ^{1}H and ^{13}C NMR chemical shift differences (in ppm) for the positions closest to the substituted carbinolic center for the aryl acetic esters of the 6,7-*erythro*-squalene diol skeleton.



Plain R= (*S*)-(+)-9-AMA, *italic*=(*S*)-(+)-APA, **Bold**= (*S*)-(+)-MPA

Scheme 3. ^{1}H and ^{13}C NMR chemical shift differences (in ppm) for the positions closest to the substituted carbinolic center for the aryl acetic esters of the 10,11-*erythro*-squalene diol skeleton.

observed for ^{1}H NMR[9] and the highest anisotropy effect was observed for the carbons located at β position from the carbinol substitution. (cf Schemes 1–3).

Comparison with the corresponding stereochemical models for (*S*)-(+)-MPA,[10] (*S*)-(+)-APA,[11] and (*S*)-(+)-9-AMA[12] allowed us to determine the absolute configuration of the stereocenter of each diastereoisomer in the derivatized carbinol resulting in the "*S*" configuration for the less polar diastereoisomer (ca R_f 0.4, hexane–MTBE 7:3).

Reduction or saponification of individual isomers of Sdiols esters with $LiAlH_4$/Diethyl Ether or K_2CO_3 in MeOH, respectively, gave rise to the enantiomeric diols. As expected, sign of the optical rotation of the released squalene diol confirmed the absolute configuration assignment. Since resolution has been so good, we expected that the optical purity of the enantiomerically pure squalene diols were in agreement with the enantiomeric purity of the ester precursors. However, rederivatization with (*S*)-(+)-MPA of each one of the resulting squalene diols and TLC and HPLC analysis of the resulting esters showed that there was 6-8% of isomerized alcohol. This isomerization was much more important (up to 12%) for squalene diols previously derivatized with (*S*)-(+)-APA and by the acyl chloride method. These results are somewhat worse than those observed for the derivatized squalene diols obtained after rederivatization with MTPA that showed 5% of isomerization. In spite of the higher degree of isomerization, any of the recovered enantiomerically enriched squalene diols could be useful as squalene oxides intermediates to perform any biological experiment.

In conclusion, we have improved the methodology for

the separation and absolute configuration determination of the different squalene diol stereoisomers that afford the biologically active squalene oxides. In this context, MPA esters of squalene diols are the most appropriate and stable derivatized intermediates to separate and determine the absolute configuration of the enantiomers of the squalene diols.

3. Experimental

General methods. The liquid chromatography-mass spectrometry analyses (HPLC-MS-FIA) were performed with an apparatus with a chemical ionization interface at atmospheric pressure and diode array detector (CH_3CN/H_2O (80:20) at 1 mL/min; positive mode). All ^1H NMR spectra were acquired at 300 MHz, and ^{13}C NMR spectra, at 75 MHz in freshly neutralized $CDCl_3$ solutions, and chemical shifts are given in ppm using as internal standards $Si(CH_3)_4$ for ^1H, and $CDCl_3$ for ^{13}C. The standard ^1H DQFCOSY and XH-CORFE spectra for the determination of the absolute configuration of (*S*)-(+)-9-AMA, (*S*)-(+)-MPA and (*S*)-(+)-APA esters were recorded at 25 °C using the same concentration for both diastereomeric pairs as described elsewhere.[1] All IR spectra were run as film.

Unless otherwise stated, organic solutions obtained from workup of crude reaction mixtures were dried over $MgSO_4$. The purification procedures were carried out by flash chromatography on silica gel (230–400 mesh) and products were obtained as oils unless otherwise specified. Visualization of UV-inactive materials was accomplished by soaking the TLC plates in an ethanolic solution of anisaldehyde and sulfuric acid (v/v/v, 96:2:2). Optical rotations were determined at 25° in $CHCl_3$ solution at the specified concentration (g/100 mL). Enantiomeric and diastereomeric excesses (ee and de) values of the corresponding (*S*)-(+)-arylacetic diastereomeric esters were calculated by NMR or by HPLC analyses using Spherisorb ODS-2 (5 μm) columns (10×0.6 cm and 15×1 cm, respectively) and eluting with CH_3CN–H_2O mixtures at 1 mL/min. All TLC plates were eluted with hexanes–MTBE 7:3.

3.1. Preparation of esters from the acid chloride. General procedure

Oxalyl chloride (370 μL, 4.2 mmol) was added to a mixture of the corresponding acid [(S)-(+)-APA, -MPA, -9-AMA] (0.7 mmol) and DMF (10 μL, 0.12 mmol) in 30 mL of hexane at room temperature. After 2 days, solvent was evaporated to dryness at reduced pressure to afford the acid chloride. The freshly prepared acid chloride [APA-Cl, MPA-Cl, 9-AMA-Cl] was dissolved in 4 mL of CH_2Cl_2 and added to a solution of the squalene diol (250 mg, 0.56 mmol), Et_3N (240 μL, 1.7 mmol) and DMAP (60 mg, 0.55 mmol) in 20 mL of CH_2Cl_2. After 30 min, water was added (10 mL), decanted and the organic solution was dried, concentrated at reduced pressure to a residue which was purified by flash chromatography on silica gel using a gradient of 0–30% MTBE (methyl tert-butyl ether) in hexane (83–89% yield).

3.2. Preparation of esters by the carbodiimide methodology. General procedure

A mixture of DMAP (0.2 mmol), arylacetic acid (0.32 mmol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (0.3 mmol) and squalene diol (0.2 mmol) in 2 mL of CH_2Cl_2 was stirred for 1 h at RT, washed with $NaHCO_3$ solution, concentrated at reduced pressure and purified by flash chromatography on silica gel using a gradient of 0–30% MTBE in hexane (85–92% yield).

3.2.1. (*S*)-(+)-α-Methoxy-α-(9-anthryl)-acetate of (3*S*)-2,3-squalene diol (higher R_f in TLC). R_f=0.22; IR 3450, 2965, 2925, 2855, 1750 (CO), 1450, 1380, 1190, 1115, 730 cm^{-1}; ^1H NMR δ 8.61 (d, J=8 Hz, 2H), 8.47 (s, 1H), 8.02 (dd, J_1=8 Hz, J_2=1 Hz, 2H), 7.60–7.40 (4H), 6.33 (s, 1H), 5.20–5.00 (5H), 4.73 (dd, J_1=10 Hz, J_2=2.5 Hz, 1H), 3.46 (s, 3H), 2.20–1.92 (18H), 1.68 (s, 3H), 1.60 (bs, 12H), 1.58 (s, 3H), 1.60–1.20 (4H), 0.58 (s, 1H), 0.46 (s, 3H), 0.41 (s, 3H); ^{13}C NMR δ 170.7, 135.1, 135.0, 134.9, 133.9, 131.3, 131.2, 130.4, 129.3, 129.2, 127.1, 126.8, 125.1, 124.9, 124.4, 124.3, 124.2, 80.8, 77.2, 71.8, 57.4, 39.7, 39.7, 39.7, 36.0, 28.3, 27.9, 26.7, 26.6, 25.7, 25.3, 23.9, 17.7, 16.0, 16.0; HPLC-MS-FIA *m/z* 710 ($M^{\cdot+}$+18), 692 ($M^{\cdot+}$), 677 ($M^{\cdot+}$−18+3), 427 ($M^{\cdot+}$−265); $[\alpha]_D$=+43.4 (c=1, 98% de).

3.2.2. (*S*)-(+)-α-Methoxy-α-(9-anthryl)-acetate of (3*R*)-2,3-squalene diol (lower R_f in TLC). R_f=0.08; IR 3450, 2965, 2925, 2855, 1750 (CO), 1450, 1380, 1190, 1115, 730 cm^{-1}; ^1H NMR δ 8.61 (d, J=8 Hz, 2H), 8.47 (s, 1H), 8.02 (d, J=8 Hz, 2H), 7.60–7.40 (4H), 6.35 (s, 1H), 5.20–5.00 (4H), 4.73 (dd, J_1=10 Hz, J_2=2 Hz, 1H), 4.23 (m, 1H), 3.44 (s, 3H), 2.14–1.92 (12H), 1.79 (s, 1H), 1.78 (s, 3H), 1.68 (d, J=1 Hz, 3H), 1.60 (bs, 9H), 1.54 (d, J=1 Hz, 3H), 1.32 (m, 2H), 1.11 (s,3H), 1.06 (s,3H), 0.98 (d, J=1 Hz, 3H), 1.10–0.7 (2H); ^{13}C NMR δ 171.4, 135.1, 134.9, 133.3, 131.5, 131.2, 130.5, 129.2, 129.2, 127.1, 126.6, 125.0, 124.4, 124.4, 124.4, 124.2, 124.1, 80.6, 77.3, 72.5, 57.4, 39.7, 39.7, 39.5, 34.8, 28.2, 27.9, 26.7, 26.6, 26.6, 25.7, 24.2, 17.7, 16.0, 15.2; HPLC-MS-FIA *m/z* 710 ($M^{\cdot+}$+18), 691 ($M^{\cdot+}$−1), 677 ($M^{\cdot+}$−18+3), 427 ($M^{\cdot+}$−265); $[\alpha]_D$=+77.6 (c=1, 98% de).

3.2.3. (*S*)-(+)-α-Methoxy-α-(9-anthryl)-acetate of (6*R*,7*S*)-6,7-squalene diol (higher R_f in TLC). R_f=0.47; IR 3450, 2965, 2925, 2855, 1750 (CO), 1450, 1380, 1190, 1115, 730 cm^{-1}; ^1H NMR δ 8.59 (bb, 2H), 8.47 (s, 1H), 8.01 (d, J=8 Hz, 2H), 7.62–7.42 (4H), 6.32 (s, 1H), 5.22–5.02 (4H), 4.76 (dd, J_1=10 Hz, J_2=2.5 Hz, 1H), 4.23 (t, J=10 Hz, 1H), 3.46 (s, 3H), 2.16–1.90 (12H), 1.68 (d, J=1 Hz, 3H), 1.62 (d, J=1 Hz, 3H), 1.60 (bs, 12H), 1.59 (s, 3H), 1.56 (s, 3H), 1.60–1.10 (3H), 1.37 (s, 3H), 0.87 (m, 1H), 0.63 (m, 1H), 0.45 (m, 1H), 0.39 (s, 3H); ^{13}C NMR δ 170.6, 135.2, 134.8, 134.1, 131.3, 131.3, 131.2, 130.4, 129.3, 129.2, 127.1, 126.7, 125.1, 124.8, 124.3, 124.2, 124.1, 123.9, 80.1, 77.2, 73.5, 57.3, 39.7, 39.7, 36.5, 36.2, 28.3, 28.2, 27.5, 26.7, 26.6, 25.6, 25.5, 22.1, 21.2, 17.6, 17.4, 16.0, 16.0, 16.0; HPLC-MS-FIA *m/z* 710 ($M^{\cdot+}$+18), 692 ($M^{\cdot+}$), 675 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot+}$−265); $[\alpha]_D$=+43.4 (c=1, 98% de).

3.2.4. (*S*)-(+)-α-Methoxy-α-(9-anthryl)-acetate of (6*S*,7*R*)-6,7-squalene diol (lower R_f in TLC). R_f=0.15; IR 3450, 2965, 2925, 2855, 1750 (CO), 1450, 1375, 1190, 1115, 730 cm^{-1}; ^1H NMR δ 8.59 (bb, 2H), 8.47 (s, 1H), 8.01 (d, J=8 Hz, 2H), 7.60–7.40 (4H), 6.35 (s, 1H), 5.18–4.98 (4H), 4.78 (dd, J_1=10 Hz, J_2=2 Hz, 1H), 4.28 (t, J=6.5 Hz, 1H), 3.43 (s, 3H), 2.16–1.90 (10H), 1.90–1.64 (4H), 1.68 (s, 6H), 1.59 (s, 6H), 1.56 (s, 3H), 1.60–1.08 (6H), 1.05 (s, 3H), 0.98 (s, 3H), 0.84 (m, 2H); ^{13}C NMR δ 171.2, 134.9, 134.8, 133.6, 131.9, 131.4, 131.2, 130.5, 129.2, 129.1, 127.1, 126.5, 125.0, 124.3, 124.3, 124.2, 80.1, 77.3, 74.0, 57.4, 39.7 37.2, 34.9, 28.0, 28.0, 27.7, 26.7, 26.6, 25.7, 23.7, 21.9, 17.6, 17.6, 16.0, 15.2; HPLC-MS-FIA *m/z* 710 ($M^{\cdot+}$+18), 692 ($M^{\cdot+}$), 675 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot+}$−265); $[\alpha]_D$=+50.8 (c=1, 98% de).

3.2.5. (*S*)-(+)-α-Methoxy-α-(9-anthryl)-acetate of (10*R*,11*S*)-10,11-squalene diol (higher R_f in TLC). R_f=0.48; IR 3530, 2975, 2935, 2860, 1750 (CO), 1445, 1380, 1180, 1110, 725 cm^{-1}; ^1H NMR δ 8.62 (bb, 2H), 8.47 (s, 1H), 8.01 (d, J=8 Hz, 2H), 7.62–7.42 (4H), 6.32 (s, 1H), 5.20–5.00 (4H), 4.79 (dd, J_1=10 Hz, J_2=2 Hz, 1H), 4.48 (t, J=7 Hz, 1H), 3.46 (s, 3H), 2.16–1.90 (12H), 1.85 (m, 2H), 1.68 (s, 6H), 1.62 (s, 3H), 1.60 (bs, 9H), 1.70–1.10 (4H), 1.37 (s, 3H), 0.66 (s, 1H), 0.64 (m, 1H), 0.43 (m,1H), 0.39 (s, 3H); ^{13}C NMR δ 170.6, 136.0, 135.0, 131.3, 131.3, 131.2, 130.4, 129.3, 129.2, 127.1, 126.7, 125.1, 124.3, 124.2, 124.1, 123.8, 123.3, 80.0, 77.1, 73.5, 57.4, 39.7, 39.7, 39.6, 36.4, 29.4, 26.7, 26.6, 26.6, 25.7, 24.7, 22.2, 21.1, 17.7, 16.1, 16.0, 15.7; HPLC-MS-FIA *m/z* 710 ($M^{\cdot+}$+18), 692 ($M^{\cdot+}$), 675 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot+}$−265); $[\alpha]_D$=+41.1 (c=1, 98% de).

3.2.6. (*S*)-(+)-α-Methoxy-α-(9-anthryl)-acetate of (10*S*,11*R*)-10,11-squalene diol (lower R_f in TLC). R_f=0.20; IR 3530, 2975, 2935, 2855, 1745 (CO), 1445, 1380, 1180, 1110, 725 cm^{-1}; ^1H NMR δ 8.60 (bb, 2H), 8.46 (s, 1H), 8.00 (d, J=8 Hz, 2H), 7.60–7.40 (4H), 6.34 (s, 1H), 5.16–4.92 (4H), 4.85 (dd, J_1=10 Hz, J_2=2 Hz, 1H), 4.59 (t, J=7 Hz, 1H), 3.42 (s, 3H), 2.14–1.85 (12H), 1.74 (m, 2H), 1.68 (d, J=1 Hz, 3H), 1.66 (d, J=1 Hz, 3H), 1.61 (s, 3H), 1.58 (bs, 6H), 1.54 (d, J=1 Hz, 3H), 1.70–1.10 (6H), 1.06 (s, 3H), 0.88 (s, 3H), 1.10–0.80 (2H); ^{13}C NMR δ 171.3, 135.5, 135.5, 134.8, 131.4, 131.4, 130.8, 130.5, 129.2, 129.1, 127.1, 126.5, 125.0, 124.3, 124.2, 124.1, 124.0, 122.7, 80.3, 77.4, 74.1, 57.4, 39.7, 39.4, 37.1, 29.2, 26.7, 26.6, 26.4, 25.7, 23.8, 23.6, 21.8, 17.7, 17.6, 15.9, 15.9, 15.3; HPLC-MS-FIA *m/z* 710 ($M^{\cdot+}$+18), 692 ($M^{\cdot+}$), 675 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot+}$−265); $[\alpha]_D$=+57.4 (c=1, 98% de).

3.2.7. (*S*)-(+)-α-Methoxy-α-(phenyl)-acetate of (3*S*)-2,3-squalene diol (higher R_f). R_f=0.22; IR 3510, 2965, 2925, 2855, 1750 (CO), 1455, 1380, 1190, 1115, 730 cm^{-1}; ^1H NMR δ 7.52–7.42 (2H), 7.30–7.42 (3H), 5.22–5.00 (5H), 4.80 (s, 1H), 4.79 (dd, J_1=10 Hz, J_2=3 Hz, 1H), 3.44 (s, 3H), 2.14–1.86 (18H), 1.68 (d, J=1 Hz, 3H), 1.60 (bs, 12H), 1.56 (d, J=1 Hz, 3H), 1.60–1.20 (5H), 0.94 (s, 3H), 0.91 (s, 3H); ^{13}C NMR δ 170.4, 136.5, 135.1, 135.0, 134.9, 133.8, 131.2, 129.0, 128.7, 127.2, 125.0, 124.4, 124.3, 124.2, 82.6, 80.5, 72.3, 57.3, 39.7, 39.7, 39.6, 35.9, 28.3, 28.0, 26.7, 26.7, 26.6, 25.9, 25.7, 24.6, 17.7, 16.0, 16.0; HPLC-MS-FIA *m/z* 610 ($M^{\cdot+}$+18), 593 ($M^{\cdot+}$+1), 575 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot+}$−165); $[\alpha]_D$=+25.0 (c=1, 98% de).

3.2.8. (*S*)-(+)-α-Methoxy-α-(phenyl)-acetate of (3*R*)-2,3-squalene diol (lower R_f in TLC). R_f=0.15; IR 3510, 2965, 2925, 2855, 1750 (CO), 1455, 1380, 1190, 1115, 730 cm^{-1}; ^1H NMR δ 7.52–7.42 (2H), 7.30–7.42 (3H), 5.20–5.05 (4H), 4.81 (s, 1H), 4.78 (dd, J_1=10 Hz, J_2=2.5 Hz, 1H), 4.76 (t, J=6 Hz, 1H), 3.44 (s, 3H), 2.14–1.86 (18H), 1.68 (d, J=1 Hz, 3H), 1.60 (bs, 9H), 1.58 (d, J=1 Hz, 3H), 1.60–1.20 (5H), 1.38 (d, J=1 Hz, 3H), 1.16 (s, 3H), 1.16 (s, 3H); ^{13}C NMR δ 170.8, 136.3, 135.1, 135.0, 134.9, 133.5, 131.2, 128.8, 128.6, 127.1, 124.9, 124.4, 124.2, 82.7, 80.3, 72.4, 57.3, 39.7, 39.7, 39.6, 35.3, 28.2, 28.1, 26.7, 26.6, 25.7, 24.7, 17.6, 16.0, 16.0, 15.7; HPLC-MS-FIA *m/z* 610 ($M^{\cdot+}$+18), 593 ($M^{\cdot+}$+1), 575 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot+}$−165); $[\alpha]_D$=+28.8 (c=1, 98% de).

3.2.9. (*S*)-(+)-α-Methoxy-α-(phenyl)-acetate of (6*R*,7*S*)-6,7-squalene diol (higher R_f in TLC). R_f=0.30; IR 3525, 2965, 2925, 2855, 1750 (CO), 1455, 1375, 1200, 1175, 1115, 1000, 730, 695 cm^{-1}; ^1H NMR δ 7.52–7.42 (2H), 7.30–7.42 (3H), 5.20–5.02 (4H), 4.94 (m, 1H), 4.83 (dd, J_1=10 Hz, J_2=3 Hz, 1H), 4.78 (s, 3H), 3.43 (s, 3H), 2.14–1.80 (18H), 1.80–1.54 (4H), 1.68 (d, J=1 Hz, 3H), 1.66 (d, J=1 Hz, 3H), 1.60 (bs, 9H), 1.56 (bs, 6H), 1.26 (s, 1H), 1.18 (m, 2H), 0.87 (s, 3H); ^{13}C NMR δ 170.3, 136.5, 135.2, 134.9, 134.1, 131.9, 131.3, 128.9, 128.7, 127.2, 124.9, 124.4, 124.2, 124.2, 124.1, 82.6, 79.8, 74.0, 57.2, 39.7, 39.7, 37.1, 36.0, 28.3, 28.2, 27.6, 26.7, 26.7, 25.7, 22.9, 21.8, 17.7, 17.6, 16.0, 16.0; HPLC-MS-FIA *m/z* 610 ($M^{\cdot+}$+18), 593 ($M^{\cdot+}$+1), 575 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot+}$−165); $[\alpha]_D$=+21.2 (c=1, 98% de).

3.2.10. (*S*)-(+)-α-Methoxy-α-(phenyl)-acetate of (6*S*,7*R*)-6,7-squalene diol (lower R_f in TLC). R_f=0.25; IR 3505, 2965, 2925, 2855, 1750 (CO), 1455, 1375, 1200, 1175, 1115, 1000, 730, 695 cm^{-1}; ^1H NMR δ 7.52–7.42 (2H), 7.30–7.42 (3H), 5.20–5.02 (4H), 4.82 (dd, J_1=10 Hz, J_2=3 Hz, 1H), 4.80 (s, 3H), 4.81 (m, 1H), 3.43 (s, 3H), 2.14–1.80 (18H), 1.78–1.30 (5H), 1.68 (bs, 6H), 1.62 (d, J=1 Hz, 3H), 1.60 (bs, 9H), 1.37 (d, J=1 Hz, 3H), 1.12 (s, 3H); ^{13}C NMR δ 170.7, 136.3, 135.0, 134.9, 133.8, 132.0, 131.2, 128.8, 128.6, 127.1, 124.8, 124.4, 124.2, 124.2, 82.7, 79.8, 74.0, 57.3, 39.7, 39.7, 37.4, 35.4, 28.2, 28.1, 27.8, 26.7, 26.6, 25.7, 23.5, 22.0, 17.6, 16.0, 16.0, 15.7; HPLC-MS-FIA *m/z* 610 ($M^{\cdot+}$+18), 593 ($M^{\cdot+}$+1), 575 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot+}$−265); $[\alpha]_D$=+26.0 (c=1, 98% de).

3.2.11. (*S*)-(+)-α-Methoxy-α-(phenyl)-acetate of (10*R*,11*S*)-10,11-squalene diol (higher R_f in TLC). R_f=0.35; IR 3520, 2965, 2925, 2855, 1750 (CO), 1455, 1375, 1200, 1175, 1115, 1000, 730, 695 cm^{-1}; ^1H NMR δ 7.52–7.42 (2H), 7.42–7.28 (3H), 5.18–5.02 (4H), 4.95 (m, 1H), 4.86 (dd, J_1=9.5 Hz, J_2=3.5 Hz, 1H), 4.78 (s, 1H), 3.43 (s, 3H), 2.14–1.80 (18H), 1.80–1.60 (2H), 1.68 (bs, 6H), 1.60 (bs, 9H), 1.56 (bs, 6H), 1.27 (s, 1H), 1.18 (m, 2H), 0.87 (s, 3H); ^{13}C NMR δ 170.3, 136.5, 136.0, 135.5, 135.0, 131.4, 131.2, 128.9, 128.7, 127.3, 124.3, 124.2, 124.1, 124.0, 82.6, 79.8, 74.0, 57.3, 39.7, 39.7, 37.1, 29.3, 26.7, 26.6, 25.7, 24.6, 22.8, 21.7, 17.7, 16.0, 16.0, 15.9; HPLC-MS-FIA *m/z* 610 ($M^{\cdot+}$+18), 593 ($M^{\cdot+}$+1), 575 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot+}$−265); $[\alpha]_D$=+27.0 (c=1, 98% de).

3.2.12. (*S*)-(+)-α-Methoxy-α-(phenyl)-acetate of (10*S*, 11*R*)-10,11-squalene diol (lower R_f in TLC). R_f=0.27; IR 3520, 2965, 2925, 2855, 1750 (CO), 1455, 1375, 1200, 1175, 1115, 1000, 730, 695 cm^{-1}; ^1H NMR δ 7.52–7.42 (2H), 7.40–7.28 (3H), 5.18–5.02 (4H), 4.92 (m, 1H), 4.86 (ca, 1H), 4.81 (s, 1H), 3.44 (s, 3H), 2.16–1.80 (18H), 1.80–1.30 (5H), 1.68 (bs, 6H), 1.60 (bs, 9H), 1.58 (d, J=1 Hz, 3H), 1.33 (d, J=1 Hz, 3H), 1.26 (s, 1H), 1.11 (s, 3H); ^{13}C NMR δ 170.7, 136.3, 136.0, 135.7, 134.9, 131.4, 131.2, 128.8, 128.6, 127.0, 124.3, 124.2, 124.1, 124.0, 123.0, 82.8, 79.9, 74.1, 57.3, 39.7, 39.7, 39.6, 37.4, 29.3, 26.7, 26.6, 26.5, 25.7, 24.1, 23.6, 21.9, 17.7, 16.0, 16.0, 15.8; HPLC-MS-FIA *m/z* 610 ($M^{\cdot+}$+18), 593 ($M^{\cdot+}$+1), 575 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot}$ −265); $[\alpha]_D$=+26.6 (*c*=1, 98% de).

3.2.13. (*S*)-(+)-α-Acetoxy-α-(phenyl)-acetate of (3*S*)-2,3-squalene diol (higher R_f in TLC). R_f=0.30; IR 3510, 2970, 2925, 2855, 1750 (CO), 1450, 1380, 1200, 1175, 1115, 730, 695 cm^{-1}; ^1H NMR δ 7.58–7.46 (2H), 7.44–7.32 (3H), 5.93 (s, 1H), 5.24–5.02 (5H), 4.79 (dd, J_1=10 Hz, J_2=2.5 Hz, 1H), 2.21 (s, 3H), 2.14–1.84 (18H), 1.68 (d, J=1 Hz, 3H), 1.60 (bs, 12H), 1.57 (bs, 3H), 1.60–1.20 (5H), 0.95 (s, 6H); ^{13}C NMR δ 170.5, 168.6, 135.1, 135.1, 134.9, 133.9, 133.8, 131.2, 129.4, 128.8, 127.6, 125.0, 124.4, 124.3, 124.2, 81.2, 74.8, 72.2, 39.7, 39.7, 39.6, 35.6, 28.3, 28.2, 26.7, 26.6, 25.7, 24.6, 20.8, 17.7, 16.0, 16.0; HPLC-MS-FIA *m/z* 638 ($M^{\cdot+}$+18), 621 ($M^{\cdot+}$+1), 603 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot}$−193); $[\alpha]_D$=+26.0 (*c*=1, 98% de).

3.2.14. (*S*)-(+)-α-Acetoxy-α-(phenyl)-acetate of (3*R*)-2,3-squalene diol (lower R_f in TLC). R_f=0.16; IR 3510, 2970, 2925, 2855, 1750 (CO), 1450, 1380, 1200, 1175, 1115, 730, 695 cm^{-1}; ^1H NMR δ 7.58–7.46 (2H), 7.44–7.32 (3H), 5.86 (s, 1H), 5.22–5.02 (4H), 4.80 (dd, J_1=10 Hz, J_2=2 Hz, 1H), 4.74 (t, J=6 Hz, 1H), 2.20 (s, 3H), 2.14–1.85 (18H), 1.70–1.20 (5H), 1.68 (d, J=1 Hz, 3H), 1.60 (bs, 9H), 1.58 (d, J=1 Hz, 3H), 1.36 (d, J=1 Hz, 3H), 1.21 (s, 6H); ^{13}C NMR δ 170.7, 168.9, 135.1, 135.0, 134.9, 133.5, 133.4, 131.2, 129.4, 128.8, 128.8, 127.6, 124.8, 124.4, 124.2, 80.9, 75.0, 72.3, 39.7, 39.7, 39.6, 35.2, 28.2, 28.0, 26.7, 26.6, 26.0, 25.7, 25.1, 20.7, 17.6, 16.0, 16.0, 15.7; HPLC-MS-FIA *m/z* 638 ($M^{\cdot+}$+18), 621 ($M^{\cdot+}$+1), 603 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot}$−193); $[\alpha]_D$=+27.4 (*c*=1, 98% de).

3.2.15. (*S*)-(+)-α-Acetoxy-α-(phenyl)-acetate of (6*R*,7*S*)-6,7-squalene diol (higher R_f in TLC). R_f=0.40; IR 3520, 2965, 2925, 2855, 1750 (CO), 1455, 1375, 1230, 1175, 1055, 740, 695 cm^{-1}; ^1H NMR δ 7.56–7.44 (2H), 7.43–7.35 (3H), 5.91 (s, 1H), 5.20–5.05 (5H), 4.94 (m, 1H), 4.84 (dd, J_1=10 Hz, J_2=2.5 Hz, 1H), 2.21 (s, 3H), 2.14–1.80 (18H), 1.68 (d, J=1 Hz, 3H), 1.66 (d, J=1 Hz, 3H), 1.60 (bs, 9H), 1.60–1.20 (2H), 1.57 (bs, 6H), 1.40 (s, 1H), 1.28–1.13 (3H), 0.91 (s, 3H); ^{13}C NMR δ 170.5, 168.5, 135.1, 134.9, 134.1, 133.9, 131.9, 131.2, 129.4, 128.8, 127.6, 124.9, 124.4, 124.2, 124.1, 80.6, 74.8, 73.9, 39.7, 37.1, 35.7, 28.3, 28.2, 27.9, 26.7, 26.6, 25.7, 22.7, 21.7, 20.7, 17.6, 17.6, 16.0; HPLC-MS-FIA *m/z* 638 ($M^{\cdot+}$+18), 621 ($M^{\cdot+}$+1), 603 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot}$−193); $[\alpha]_D$=+38.6 (*c*=1, 98% de).

3.2.16. (*S*)-(+)-α-Acetoxy-α-(phenyl)-acetate of (6*S*,7*R*)-6,7-squalene diol (lower R_f in TLC). R_f=0.26; IR 3520, 2965, 2925, 2855, 1750 (CO), 1455, 1375, 1230, 1175, 1055, 740, 695 cm^{-1}; ^1H NMR δ 7.56–7.44 (2H), 7.43–7.35 (3H), 5.85 (s, 1H), 5.18–5.05 (5H), 4.85 (dd, J_1=10 Hz, J_2=2.5 Hz, 1H), 4.77 (m, 1H), 2.21 (s, 3H), 2.16–1.80 (18H), 1.68 (bs, 6H), 1.62 (d, J=1 Hz, 3H), 1.60 (bs, 9H), 1.70–1.20 (4H), 1.35 (s, 3H), 1.26 (s, 1H), 1.18 (s, 3H); ^{13}C NMR δ 170.6, 168.8, 135.1, 134.9, 133.8, 133.5, 132.1, 131.2, 129.4, 128.8, 127.6, 124.8, 124.4, 124.2, 124.2, 80.5, 75.0, 74.1, 39.7, 37.5, 35.3, 28.2, 28.1, 27.8, 26.7, 26.6, 25.7, 23.3, 22.0, 20.7, 17.7, 16.0, 15.7; HPLC-MS-FIA *m/z* 638 ($M^{\cdot+}$+18), 621 ($M^{\cdot+}$+1), 603 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot}$−193); $[\alpha]_D$=+29.0 (*c*=1, 98% de).

3.2.17. (*S*)-(+)-α-Acetoxy-α-(phenyl)-acetate of (10*R*,11*S*)-10,11-squalene diol (higher R_f in TLC). R_f=0.40; IR 3530, 2965, 2925, 2855, 1750 (CO), 1455, 1375, 1230, 1180, 1060, 740, 695 cm^{-1}; ^1H NMR δ 7.55–7.44 (2H), 7.43–7.34 (3H), 5.93 (s, 1H), 5.15–5.03 (4H), 4.95 (m, 1H), 4.84 (dd, J_1=9 Hz, J_2=3.5 Hz, 1H), 2.21 (s, 3H), 2.14–1.76 (18H), 1.68 (bs, 6H), 1.60 (bs, 9H), 1.56 (bs, 6H), 1.70–1.20 (2H), 1.37 (s, 1H), 1.28–1.13 (3H), 0.91 (s, 3H); ^{13}C NMR δ 170.5, 168.5, 136.1, 135.6, 134.9, 133.9, 131.5, 131.3, 129.4, 128.8, 127.6, 124.4, 124.2, 123.9, 123.2, 80.6, 74.8, 73.9, 39.7, 39.7, 37.1, 29.6, 26.7, 26.6, 26.6, 25.7, 24.4, 22.7, 21.6, 20.8, 17.7, 16.0; HPLC-MS-FIA *m/z* 638 ($M^{\cdot+}$+18), 621 ($M^{\cdot+}$+1), 603 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot}$−193); $[\alpha]_D$=+31.2 (*c*=1, 98% de).

3.2.18. (*S*)-(+)-α-Acetoxy-α-(phenyl)-acetate of (10*S*,11*R*)-10,11-squalene diol (lower R_f in TLC). R_f=0.30; IR 3520, 2965, 2925, 2855, 1750 (CO), 1455, 1375, 1230, 1180, 1060, 740, 695 cm^{-1}; ^1H NMR δ 7.56–7.44 (2H), 7.43–7.33 (3H), 5.86 (s, 1H), 5.17–5.01 (4H), 4.95–4.85 (2H), 2.20 (s, 3H), 2.18–1.82 (14H), 1.68 (bs, 6H), 1.62 (d, J=1 Hz, 3H), 1.60 (bs, 6H), 1.57 (d, J=1 Hz, 3H), 1.72–1.20 (2H), 1.27 (d, J=1 Hz, 3H), 1.18 (s, 3H); ^{13}C NMR δ 170.6, 168.9, 135.9, 135.7, 134.9, 133.4, 131.4, 131.2, 129.4, 128.8, 127.6, 124.3, 124.2, 124.1, 122.9, 80.6, 75.0, 74.1, 39.7, 39.6, 37.4, 29.3, 26.7, 26.6, 26.5, 25.7, 24.0, 23.3, 21.9, 20.7, 17.7, 16.0, 16.0, 15.8; HPLC-MS-FIA *m/z* 638 ($M^{\cdot+}$+18), 621 ($M^{\cdot+}$+1), 603 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot}$−193); $[\alpha]_D$=+36.0 (*c*=1, 98% de).

3.3. Preparation of squalene diols by reduction of arylacetic esters. General procedure

A solution of the corresponding arylacetic ester in Et_2O, maintained under nitrogen atmosphere and at room temperature, was treated with 4.5 molar equiv of $LiAlH_4$. The mixture was stirred until the reaction was completed. After the usual workup, the residue was purified by flash chromatography using as eluent hexanes–MTBE 85:15 to afford the corresponding enantiomeric diol (87–93% yield).

Squalene diols reobtained by reduction with $LiAlH_4$ of the corresponding (*R*)-(−)-MPA derivatives showed the following optical rotations: (3*S*)-2,3-Dihydroxy-2,3-dihydrosqualene (from higher R_f) $[\alpha]_D$=−11.8 (*c*=1, 97% ee); (3*R*)-2,3-Dihydroxy-2,3-dihydrosqualene (from lower R_f) $[\alpha]_D$=+11.4 (*c*=1, 97% ee); (6*R*,7*S*)-6,7-Dihydroxy-6,7-dihydrosqualene (from higher Rf) $[\alpha]_D$=−10.0 (*c*=1, 97% ee); (6*S*,7*R*)-6,7-Dihydroxy-6,7-dihydrosqualene (from lower R_f) $[\alpha]_D$=+10.2 (*c*=1, 97% ee); (10*R*,11*S*)-10,11-Dihydroxy-10,11-dihydrosqualene

(from higher R_f) $[\alpha]_D = -6.6$ ($c=1$, 97% ee); (10*S*,11*R*)-10,11-Dihydroxy-10,11-dihydrosqualene (from lower R_f) $[\alpha]_D = +6.5$ ($c=1$, 97% ee). These data are in agreement with the previously reported optical rotations for these compounds.[1]

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