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Chemoenzymatic synthesis of (2*S*,3*S*,4*S*)-form, the physiologically active stereoisomer of dehydroxymethylepoxyquinomicin (DHMEQ), a potent inhibitor on NF-κB

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ABSTRACT

A new route for (2*S*,3*S*,4*S*)-form, the physiologically active stereoisomer of dehydroxymethylepoxyquinomicin (DHMEQ), a potent NF-κB inhibitor, was established by chemoenzymatic approach. Elaboration on the asymmetric epoxidation of a *p*-benzoquinone monoketal with benzylcinchonidinium *tert*-butylhydroperoxide yielded an epoxyenone, in 79.8% ee and 57% yield in reproducible manner. By way of the transformation of this key intermediate to enantiomerically pure (2*S*,3*S*,4*S*)-DHMEQ, the contaminating undesired enantiomer could be effectively removed by applying *Burkholderia cepacia* lipase-catalyzed hydrolysis of diacylated precursor. The above integrated combination of chemical asymmetric synthesis and enzyme-catalyzed kinetic resolution enabled us to prepare active DHMEQ in a large-scale.

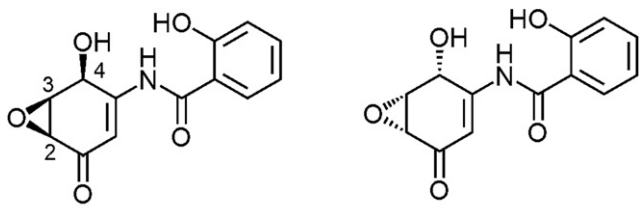
1. Introduction

DHMEQ (dehydroxymethylepoxyquinomicin, **1a**) is a newly designed and highly active NF-κB inhibitor,[1] and has shown potent anti-inflammatory and anticancer activities in animals.[2] Through extensive studies on structure-activity relationships,[3] it was revealed that (2*S*,3*S*,4*S*)-**1a** is more than ten times as physiologically active as its enantiomer (Fig. 1). So far, the chromatographic separation of racemate by means of a chiral stationary phase has only been the way to approach enantiomerically pure form of (2*S*,3*S*,4*S*)-**1a**.[4] Herein we report a new asymmetric synthesis of (2*S*,3*S*,4*S*)-**1a**.

2. Results and discussion

The synthetic plan of (2*S*,3*S*,4*S*)-**1a** is shown in Scheme 1. Enantiomerically enriched form of **2a** is a known building block developed by Taylor[5,6] by an asymmetric epoxidation of the precursor aminoquinine monoketal **3a** with the action of chiral quaternary ammonium *tert*-butylhydroperoxide.

First, according to the reported procedure,[7] amino group of commercially available 2,5-dimethoxyaniline **4a** was protected by Boc group and the resulting hydroquinone derivative **4b** was subsequently dehydrogenated by the action of $PhI(OAc)_2$ in methanol with a concomitant ketalization. The convergence of **5** to desired **3a** was important, because diketal **5**, the direct precursor for **3a**, behaves as the troublesome contaminant at the stage of purification. Then, the crude mixture was treated with 2 M hydrochloric acid according to the original procedure,[4] but such operation caused an unexpected decomposition of the materials to result in



(2*S*,3*S*,4*S*)-**1a**
active enantiomer

(2*R*,3*R*,4*R*)-**1a**

Figure 1. The enantiomers of dehydroxymethylepoxyquinomicin (DHMEQ, **1a**).

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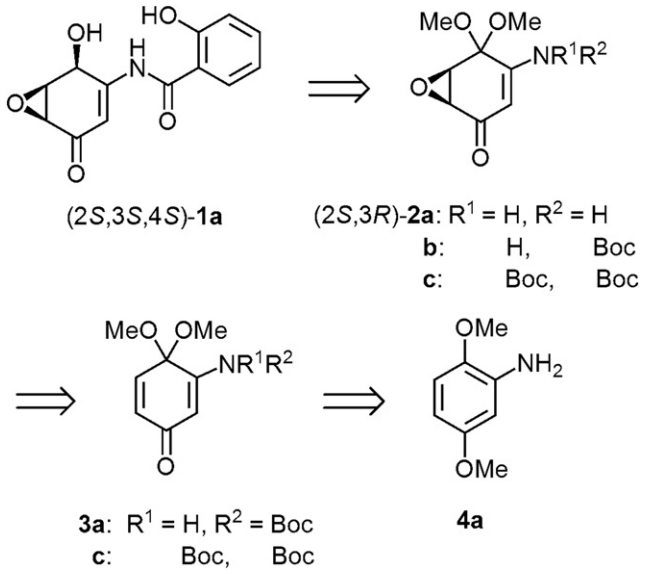
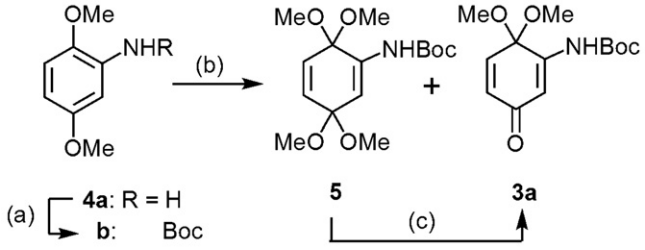


Scheme 1. Synthetic plan of (2*S*,3*S*,4*S*)-**1a**.

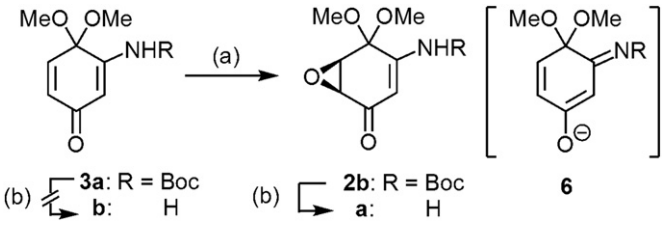
3a in as low as 21%. This observation promoted us to switch hydrochloric acid into weaker aqueous citric acid, and the deprotection proceeded in a reproducible manner to give **3a** in 48% yield. Moreover, careful examination in regard to oxidizing agent, the yield of the dehydrogenation of Boc derivative **4b** was improved as high as 80%, by applying $PhI(OPiv)_2$ (Scheme 2).



Scheme 2. (a) Boc_2O, THF, 99%; (b) $PhI(OPiv)_2$, MeOH; (c) citric acid aq soln, 80% over two steps.

In our hand, Taylor's asymmetric epoxidation of **3a** was not entirely reproducible,[5,6] in terms of either enantioselectivity or conversion. Even under the optimal conditions, the results were not satisfactory. For example, the yield and ee of the product fluctuated between 30 and 40%, and between 43.8 and 52.4%, respectively. This asymmetric epoxidation initiates with an enantiofacially preferential 1,4-attack of chiral *N*-benzylcinchonidinium salt of *tert*-butylhydroperoxide (TBHP) to prochiral enone **3a**. The epoxide **2b** was obtained only as an inseparable mixture with the starting material **3a**. Removal of Boc group in **2b** provided **2a** in pure form, however, the remaining enone **3a** decomposed via **3b** to an intractable waste. In this point of view, higher conversion in epoxidation is desirable. We improved the yield, by stepwise examination as follows: (1) the modification of the substrate structure; (2) careful adjustment of the equivalence of reagents; (3) additives, which suppress the undesired species, which would cause non-asymmetric epoxidation. The detail is shown below.

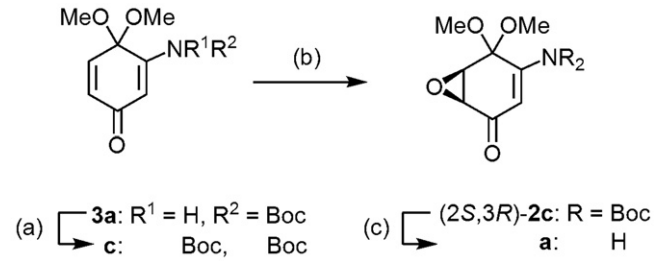
Under Taylor's procedure,[5] the epoxidation had been carried out in buffered conditions with a ratio of 4:1 between acidic hydrochloride and basic hydroxide of cinchonidine. An equilibration between **3a** and enolate **6** by the deprotonation of *tert*-butylcarbamate group under basic conditions (Scheme 3) can explain the necessity of neutral to slightly acidic environment, even sacrificing the nucleophilicity of *tert*-butylhydroperoxide.



Scheme 3. (a) *N*-Benzylcinchonidinium chloride, TBHP, 6 M NaOH aq soln, toluene, 40%, 52.4% ee; (b) TFA, CH_2Cl_2, quant.

Accordingly, a new substrate, bis-Boc derivative **3c**,[8] bearing no acidic proton on enamine nitrogen was designed. Under the conditions with cinchonidinium salt and free base in 1:1, the epoxidation proceeded smoothly at 25 °C in a conversion of 74%. The crude mixture was directly treated with trifluoroacetic acid, and the desired epoxyenamine (2*S*,3*R*)-**2a** was obtained in 30% yield over two steps. Since an unidentified byproduct was detected on TLC during the epoxidation, the reaction temperature was lowered to 0 °C, with increasing the equivalence of hydroperoxide from two to five, in order to compensate the low reactivity. After extensive optimization, we found that the equivalence of cinchonidinium salt and NaOH should be two and one, respectively, under carefully controlled reaction conditions.

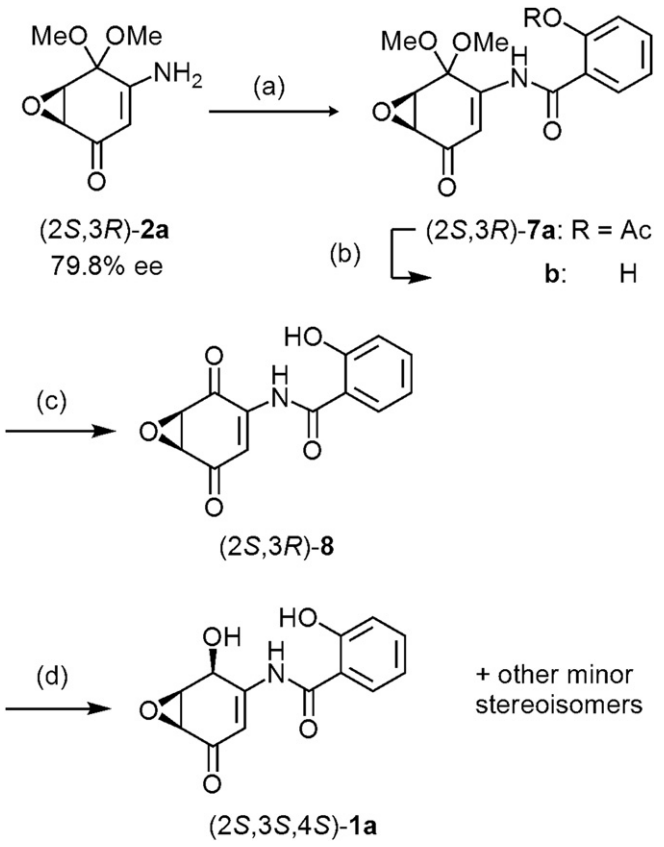
Finally, it was concluded that the absorption of any trace of water with MS 4 Å and trapping sodium cation with 15-crown-5 further were important. In this way, the yield involving epoxidation and deprotection was improved to be 43% (over two steps) from 29% in original procedure and ee of the product was constantly over 75% (Scheme 4).



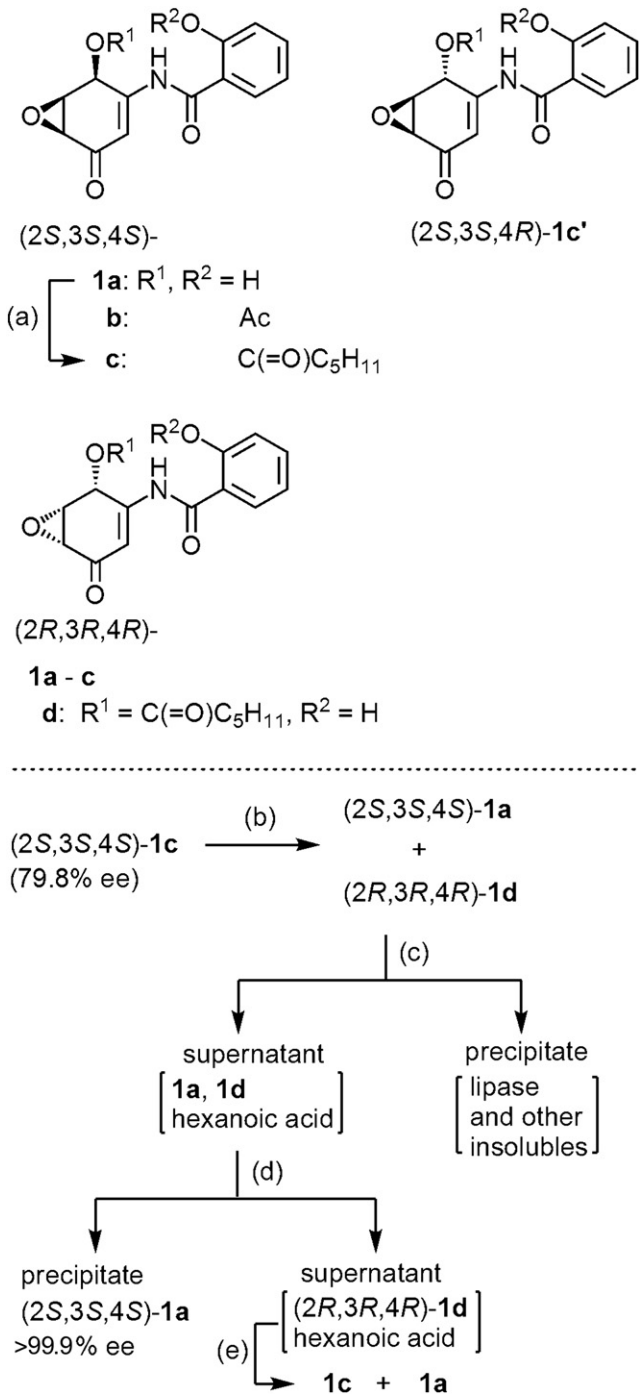
Scheme 4. (a) Boc_2O, DMAP, THF, 89%; (b) *N*-benzylcinchonidinium chloride, TBHP, NaOH, 15-crown-5, toluene, MS 4 Å, 57%; (c) TFA, anisole, CH_2Cl_2, 76%, 79.8% ee.

Although enantiomerically pure **2a** was obtained by recrystallization from ethyl acetate, the recovery was only as low as 8%. This situation prompted us to use complementary enzyme-catalyzed kinetic resolution to remove undesired enantiomer on DHMEQ itself or intermediates toward target molecule (Scheme 5), which had been established in the synthesis of racemate of DHMEQ.

After several trials, we turned our attention to the application of lipase-catalyzed hydrolysis on the diacylated form **1b** and **1c** of DHMEQ, whose acyl chains were introduced on both hydroxyl groups in secondary alcohol and phenol. Between two candidates, dihexanoate **1c** with two medium-length acyl chains was advantageous because of its stability on silica gel column chromatography, was much superior to that of diacetate **1b**. At this stage, diastereomerically pure **1c** free from (2*S*,3*S*,4*R*)- or (2*R*,3*R*,4*S*)-**1c**′ became in hand. Two commercially available lipases were compared, and *Burkholderia cepacia* lipase (Amano PS-IM) showed higher activity than that of *Candida antarctica*



Scheme 5. (a) Acetylsalicyloyl chloride, *t*-BuOLi, THF; (b) K_2CO_3, aq MeOH, 67% over two steps; (c) $BF_3{\cdot}OEt_2$, CH_2Cl_2, 42%; (d) $NaBH(OAc)_3$, MeOH.



Scheme 6. (a) $(C_5H_{11}CO)_2O$, DMAP, THF, 90%; (b) *B. cepacia* lipase (Amano PS-IM), acetone, water, 69%; (c) trituration in THF; (d) trituration in diisopropyl ether; (e) silica gel chromatography.

lipase B (Novozym 435). The hydrolysis of both acyl chain proceeded very smoothly in the case of (2*S*,3*S*,4*S*)-isomer. By virtue the unique properly of DHMEQ, the poor solubility in neither water nor organic solvent except for tetrahydrofuran, the purification protocol became very simple. First, the crude residue after evaporation of the solvent was triturated in tetrahydrofuran to recover all of organic materials. Then, the residue after concentration of the above extract was alternatively triturated in diisopropyl ether. Unreacted recovery and hexanoic acid resulted by the hydrolysis were removed, and pure DHMEQ **1a** was obtained as precipitated solid in 69% yield (Scheme 6). All of the spectral and physical properties were identical with those reported previously. The enantiomeric excess (>99.9% ee) was confirmed by HPLC analysis of **1c**, which was prepared by re-acylation of **1a**.

The lipase-catalyzed hydrolysis was really advantageous, as (2*S*,3*S*,4*S*)-isomer was readily transformed to **1a**, while the undesired (2*R*,3*R*,4*R*)-isomer stayed at the stage of monohexanoate **1d**. Any attempts for the isolation of (2*R*,3*R*,4*R*)-**1d** were unsuccessful, as the monohexanoate was labile on silica gel and is prone to isomerize into two components of **1a** and **1c** even on the occasion of TLC analysis. Thanks to the lipophilic property of **1d**, it remained in supernatant at the stage of trituration with diisopropyl ether, and silica gel chromatography could be avoided.

We refrain from discussing the enantioselectivity in a quantitative manner, for example, as *E* value, because precise evaluation of neither the conversion of the reaction nor the ee of the slow-reacting isomer (**1d**) was available. A very high enantioselectivity, however, is believed, as even from racemate of **1c**, enantiomerically pure (2*S*,3*S*,4*S*)-**1a** was obtained.

3. Conclusion

We established an efficient chemoenzymatic route to (2*S*,3*S*,4*S*)-DHMEQ **1a**. First, bis-Boc derivative **3c** was designed, and the desired epoxy ketone (79.8% ee) became available in a reproducible manner. In the final step, contaminated (10%) undesired enantiomer could be easily removed by lipase-catalyzed kinetic resolution. This achievement for large-scale and practical synthesis would promote the clinical trials of DHMEQ itself. Moreover, the stable, lipophilic acylated derivative is promising as the clue to design and synthesize pro-drugs.

4. Experimental

4.1. Material and methods

Merck silica gel 60 F_{254} thin-layer plates (1.05744, 0.5 mm thickness) and silica gel 60 (spherical and neutral; 100–210 μm, 37,560–79) from Kanto Chemical Co., were used for preparative thin-layer chromatography and column chromatography, respectively. Under anhydrous reaction conditions, molecular sieves were predried in vacuo for 1 h at 100 °C.

4.2. Analytical methods

All mps are uncorrected. IR spectra were measured as films for oils or KBr disks for solids on a Jasco FT/IR-410 spectrometer or ATR on a Jeol FT-IR SPX60 spectrometer. ^{1}H NMR spectra were measured in $CDCl_3$ at 270 MHz on a Jeol JNM EX-270 or 400 MHz on a VARIAN 400-MR spectrometer, and ^{13}C NMR spectra were measured in $CDCl_3$ at 100 MHz on a VARIAN 400-MR spectrometer. Optical rotation values were recorded on a Jasco P-1010 polarimeter. High resolution mass spectra were recorded on a Jeol JMS-700 MStation spectrometer. HPLC data were recorded on Jasco MD-2010 multichannel detectors and SHIMADZU SPD-M20A diode array detector.

4.3. *N*-*tert*-Butoxycarbonyl-2,5-dimethoxyaniline (4b)

A soln of **4a** (5.01 g, 32.6 mmol) and di-*tert*-butyl dicarbonate (8.55 g, 39.2 mmol) in THF (20 mL) was heated under reflux for 2 days. After removal of volatile materials in vacuo, the residue was purified by silica gel column chromatography (100 g) with hexane–EtOAc (10:1) to afford **4b** (8.16 g, 99%) as a colorless oil. Its ^{1}H NMR spectrum was identical with that reported previously.[8]

4.4. 3-*tert*-Butoxycarbonylamino-4,4-dimethoxycyclohexa-2,5-dien-1-one (3a)

To a soln of **4b** (3.20 g, 12.6 mmol) in anhydrous MeOH (45 mL) at 0 °C under N_2 were added MS 4 Å (1.2 g), and then portionwise $PhI(OPiv)_2$ (6.16 g, 15.2 mmol). The mixture was stirred for 24 h at 0 °C. The mixture was diluted with EtOAc (25 mL) and saturated citric acid aq soln (10 mL) was added to organic layer. After stirring for 30 min, the mixture was extracted with EtOAc, and the combined organic layer was washed with saturated $NaHCO_3$ aq soln, water, and brine. The organic phase was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by recrystallization from EtOAc–hexane to give **3a** (1.90 g, 60%) as colorless needles. The residue from mother liquor was further purified by silica gel column chromatography (90 g) with hexane–EtOAc (1:1) to afford **3a** (0.63 g, 20%). Mp 130–131 °C [lit.[7] mp 130–131 °C]. Its ^{1}H NMR spectrum was identical with that reported previously.[7]

4.5. (2*S*,3*R*)-5-*tert*-Butoxycarbonylamino-2,3-epoxy-4,4-dimethoxycyclohexa-5-en-1-one (2b)

According to the reported procedure,[6] **3a** (50.0 mg, 0.19 mmol) was treated with *N*-benzylcinchonidinium chloride (117 mg, 0.28 mmol), TBHP (32 μL, 0.30 mmol), and NaOH aq soln (6 M, 15 μL) in toluene (3 mL) for 1 h at −10 °C, and then allowed to warm to room temperature and stirred for further 48 h to afford **2b** (21.2 mg, 40%, 52.4% ee) as a colorless needle. Mp 118–119 °C [lit.[6] mp 116–117 °C]; $[\alpha]_D^{24}$ −103 (*c* 0.92, $CHCl_3$) [lit.[6] $[\alpha]_D$ −189.4 (*c* 1.0, $CHCl_3$)]. Its ^{1}H NMR spectrum was identical with that reported previously.[5] The product **2b** was analyzed by HPLC [column, Daicel CHIRALCEL® OD-H, 0.46 cm×25 cm; hexane–2-propanol (15:1); flow rate 0.5 mL/min; detected at 300 nm]: t_R (min)=18.8 (76.2%), 28.8 (23.8%).

4.6. Direct approach to (2*S*,3*R*)-5-amino-2,3-epoxy-4,4-dimethoxycyclohexa-5-en-1-one (2a)

In a similar manner as described above, to a soln of **3a** (2.50 g, 9.30 mmol), *N*-benzylcinchonidinium chloride (5.85 g, 13.9 mmol, 1.5 equiv) and TBHP (2.0 M in toluene, 1.6 mL, 15.0 mmol, 1.6 equiv) in toluene (150 mL) were added NaOH aq soln (6 M, 750 μL) at −10 °C. The similar workup as above yielded a mixture of (−)-**2b** and **3a** (total 2.71 g) as yellow oil.

Then, it was treated with TFA (25 mL, 27.0 mmol) according to the reported procedure[6] to give **2a** (528 mg, 30%, 43.8% ee) as dark brown oil. This was further purified by recrystallization from EtOAc to give (−)-**2a** (141 mg, 8.0% recovery, 99.0% ee) as colorless needles. Mp 162–163 °C [lit.[9] mp 161–162 °C]; $[\alpha]_D^{23}$ −106 (*c* 0.60, MeOH) [lit.[9] $[\alpha]_D$ −91.0 (*c* 1.0, MeOH)]. Its IR and ^{1}H NMR spectra were identical with those reported previously.[9] The product **2a** was analyzed by HPLC [CHIRALCEL® OD-H; hexane–2-propanol (2:1); flow rate 0.5 mL/min detected at 300 nm]: t_R (min)=17.1 (99.5%), 28.2 (0.5%).

4.7. 3-*N*,*N*-Bis-(*tert*-butoxycarbonyl)amino-4,4-dimethoxycyclohexa-2,5-dien-1-one (3c)

To a soln of **3a** (538 mg, 2.0 mmol) in anhydrous THF (6 mL) were added DMAP (122 mg, 1.0 mmol) and di-*tert*-butyl dicarbonate (874 mg, 4.0 mmol) at 25 °C. The mixture was stirred at 70 °C under argon atmosphere for 6 h. The reaction was quenched by the addition of water, and then extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (10 g). Elution with hexane–EtOAc (4:1) afforded **3c** (561 mg, 89%) as white solid. Mp 117–118 °C; IR ν_{max} 2981, 1753, 1718, 1674, 1340, 1234, 1122 cm^{-1}; ^{1}H NMR (400 MHz) δ: 1.47 (s, 18H), 3.29 (s, 6H), 6.28 (d, *J*=2.4 Hz, 1H), 6.36 (dd, *J*=2.4, 10.4 Hz, 1H), 6.80 (d, *J*=10.4 Hz, 1H); ^{13}C NMR (100 MHz) δ: 185.7, 150.8, 149.4, 142.1, 130.8, 130.6, 94.8, 83.3, 51.3, 27.8; MS (FAB): *m*/*e* (% relative intensity) 370.30 (33) $[M+1]^+$, 392.29 (22) $[M+23]^+$; HRMS (FAB): calcd for $C_{18}H_{27}NO_7$: $[M+1]^+$: 370.1866; found: *m*/*z*=370.1864.

4.8. Asymmetric epoxidation of 3c: (2*S*,3*R*)-5-*N*,*N*-bis-(*tert*-butoxycarbonyl)amino-2,3-epoxy-4,4-dimethoxycyclohexa-6-en-1-one (2c)

To a mixture of *N*-benzylcinchonidinium chloride (30.0 g, 70.5 mmol, 2.0 equiv) and MS 4 Å (20.0 g) in anhydrous toluene (130 mL) were added TBHP (2.0 M in toluene, 88 mL, 176 mmol, 5.0 equiv), NaOH (1.40 g, 35.2 mmol, 1.0 equiv), and 15-crown-5 (9.50 g, 8.50 mL, 39.0 mmol, 1.1 equiv) at 10 °C. The resulting suspension was stirred for 30 min, and then to the mixture was added **3c** (13.0 g, 35.2 mmol) at 10 °C under argon atmosphere. After stirring for 24 h, the mixture was quenched with saturated $Na_2S_2O_3$ aq soln MS 4 Å and other insoluble materials were removed by filtration, and the filtrate was extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo to give a mixture of **2c** and **3c** (total 10.3 g) as oil. As the product **2c** and the starting material **3c** showed same R_fs on silica gel, the yield was estimated to be 57%, based on ^{1}H NMR with an internal standard [ethyl benzoate, Tokyo Kasei Co., B0069, analytically pure grade, standard signal at δ=4.36 (dd, *J*=6.8, 7.2 Hz, 2H)].

Then, the crude product in CH_2Cl_2 (120 mL) was treated with TFA (30 mL, 32.4 mmol) and anisole (12 mL), to give (2*S*,3*R*)-**2a** (2.90 g, 46% for two steps, 79.8% ee) as solid. HPLC analysis was performed in the same manner as above: t_R (min)=17.1 (89.9%), 28.1 (10.1%).

4.9. (2*S*,3*R*)-5-*N*-[(2-Acetoxybenzoyl)amino]-2,3-epoxy-4,4-dimethoxycyclohexa-6-en-1-one (7a)

To a soln of above-mentioned **2a** (2.80 g, 15.1 mmol) in THF (140 ml) at −78 °C were added the lithium *tert*-butoxide (Aldrich, 398185, 16.6 mL, 16.6 mmol), and subsequently acetylsalicyloyl chloride (3.50 g, 18.1 mmol) in portionwise. After stirring for 1 h at −78 °C, the mixture was quenched with saturated NH_4Cl aq soln and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (240 g). Elution with hexane–EtOAc (2:1) afforded **7a** (3.50 g) as white solid. Mp 84–85 °C; IR ν_{max} 3367, 2920, 2848, 1772, 1670, 1597, 1495, 1456, 1363, 1273, 1234, 1178, 1109, 1045 cm^{-1}; ^1H NMR (400 MHz) δ: 2.37 (s, 3H), 3.29 (s, 3H), 3.55 (dd, *J*=2.0, 4.4 Hz, 1H), 3.67 (s, 3H), 3.83 (d, *J*=4.4 Hz, 1H), 7.15 (dd, *J*=1.2, 8.4 Hz, 1H), 7.31 (d, *J*=2.0 Hz, 1H), 7.33 (ddd, *J*=1.2, 7.6, 8.4 Hz, 1H), 7.54 (ddd, *J*=1.6, 7.6, 8.0 Hz, 1H), 7.85 (dd, *J*=1.6, 8.0 Hz, 1H), 8.73 (br s, 1H); ^{13}C NMR (100 MHz) δ: 192.7, 168.7, 164.1, 148.0, 145.1, 133.2, 130.5, 126.9, 126.6, 123.5, 109.7, 95.7, 52.1, 51.6, 51.5, 50.8, 20.9. This was employed for the next step without further purification.

4.10. (2*S*,3*R*)-2,3-Epoxy-5-*N*-[(2-hydroxybenzoyl)amino]-4,4-dimethoxycyclohexa-6-en-1-one (7b)

To a soln of **7a** (3.50 g) in MeOH (42 mL) and H_2O (14 mL) was added K_2CO_3 (1.40 g, 10.1 mmol). After stirring for 30 min at room temperature, the mixture was diluted with H_2O (20 mL), and then extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ aq soln and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to afford **7b** (3.10 g, 67% for two steps) as white solid. Mp 117–118 °C. Its IR and ^1H NMR spectra were identical with those reported previously.[4] This was employed for the next step without further purification.

4.11. (2*S*,3*R*)-2,3-Epoxy-5-*N*-[(2-hydroxybenzoyl)amino]-cyclohexa-6-en-1,4-dione (8)

According to the reported procedure,[4] ketal **7b** (2.90 g, 9.50 mmol) was treated with $BF_3\cdot OEt_2$ (3.74 mL, 29.0 mmol) in CH_2Cl_2 (15 mL) for 1 h at −20 °C, and then allowed to warm to room temperature and stirred for further 7 h to afford **8** (1.03 g, 42%) as yellow solid. Its ^1H NMR spectrum was identical with that reported previously.[4] This was employed for the next step without further purification.

4.12. (2*S*,3*S*,4*S*)-2,3-Epoxy-4-hexanoyloxy-5-*N*-[(2-hexanoyloxybenzoyl)amino]-cyclohexa-6-en-1-one (dihexanoyl DHMEQ, 1c)

According to the reported procedure,[4] diketone **8** (1.0 g, 3.90 mmol) was treated with $NaBH(OAc)_3$ (1.70 g, 7.80 mmol) in MeOH (100 mL) for 20 min at 0 °C, and then the mixture was allowed to warm to room temperature and stirred for further 4 h to afford crude mixture (2.7 g) containing **1a**.

The residue was suspended in THF (100 mL) and to that were added hexanoic anhydride (2.80 mL) and DMAP (16.0 mg). After stirring for 30 min at room temperature, the mixture was quenched with ice-water (20 mL), then stirred for 20 min at room temperature and extracted with EtOAc. The organic layer was washed with hydrochloric acid (0.5 M), saturated $NaHCO_3$ aq soln and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (190 g). Elution with hexane–EtOAc (4:1) to afford (2*S*,3*S*,4*S*)-**1c** (1.30 g, 72%, 79.8% ee) as yellow oil. $[\alpha]_D^{23}$ −82.0 (*c* 1.00, $CHCl_3$); ^1H NMR (270 MHz) δ: 0.85 (t, *J*=6.8 Hz, 3H), 0.91 (t, *J*=6.8 Hz, 3H), 1.30 (m, 4H), 1.34 (m, 4H), 1.70 (m, 4H), 2.53 (t, *J*=7.8 Hz, 2H), 2.56 (t, *J*=7.8 Hz, 2H), 3.50 (dd, *J*=2.0, 3.9 Hz, 1H), 3.91 (dd, *J*=2.9, 3.9 Hz, 1H), 5.85 (dd, *J*=1.5, 2.9 Hz, 1H), 7.02 (dd, *J*=1.5, 2.0 Hz, 1H), 7.10 (d, *J*=7.8 Hz, 1H), 7.35 (dd, *J*=7.8, 7.8 Hz, 1H), 7.54 (ddd, *J*=1.5, 7.8, 7.8 Hz, 1H), 7.67 (dd, *J*=1.5, 7.8 Hz, 1H), 7.99 (br s, 1H); ^{13}C NMR (100 MHz) δ: 192.2, 173.3, 172.3, 164.3, 147.7, 133.0, 129.6, 127.9, 126.6, 123.5, 109.9, 66.5, 52.8, 34.2, 34.0, 31.2, 24.5, 22.3, 13.9, 13.8; IR ν_{max} 3372, 3284, 3256, 3217, 3148, 3102, 3043, 2956, 2930, 2871, 1752, 1699, 1642, 1520, 1282, 1241, 1214, 1155, 1099, 1033, 905, 781 cm^{-1}. Anal. Calcd for $C_{25}H_{31}NO_7$: C, 65.6; H, 6.83; N, 3.06. Found: C, 65.6; H, 6.73; N, 3.10. The product **1c** was analyzed by HPLC [CHIRALCEL® AD-H, 0.46×25 cm; hexane–2-propanol (7:1); flow rate 0.5 mL/min; detected at 290 nm]: t_R (min)=28.3 (10.1%), 30.9 (89.9%).

4.13. *B. cepacia* lipase-catalyzed hydrolysis of (2*S*,3*S*,4*S*)-1c

To a soln of (2*S*,3*S*,4*S*)-**1c** (46.0 mg, 79.8% ee, 0.10 mmol) in acetone (0.7 mL) and water (0.7 mL) was added *B. cepacia* lipase (Amano PS-IM, 70 mg). After stirring for 24 h at room temperature, the mixture was concentrated in vacuo for 1 h to dryness and then mixed with THF (10 mL). Ultrasonic vibration (200 W) was applied for the resulted suspension for 10 min, and the mixture was filtered through a pad of Celite to remove insoluble materials. The filtrate was concentrated in vacuo and the residue was mixed with *i*-Pr_2O (10 mL). To the mixture was again applied the ultrasonic vibration (200 W) for 10 min, to wash out monohexanoyl DHMEQ **1d**. After filtration, the residue on the filter paper was charged on a silica gel column (300 mg), and the elution with THF afforded (2*S*,3*S*,4*S*)-**1a** (18.0 mg, 69%) as white solid. Mp 187 °C [lit.[4] mp 185 °C]. As the solubility of DHMEQ was very low in methanol, the comparison of the sign of rotations between that of present sample and authentic datum seemed not to be reliable enough, then the ee as well as the absolute configuration of the present DHMEQ was confirmed by derivation to the corresponding dihexanoate (2*S*,3*S*,4*S*)-**1c** again. Colorless oil; $[\alpha]_D^{23}$ −126 (*c* 0.66, $CHCl_3$). HPLC analysis was performed in the same manner as above: t_R (min)=30.9 (single peak). This retention time was in good accordance with that of an authentic specimen of (2*S*,3*S*,4*S*)-**1c**, and there was no peak at 28.3 min ascribable to (2*R*,3*R*,4*R*)-**1c**.

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2010.07.013.

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