Tetrahedron 66 (2010) 7083–7087

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

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

Manabu Hamada [a], Yukihiro Niitsu [b], Chihiro Hiraoka [c], Ikuko Kozawa [a], Toshinori Higashi [b], Mitsuru Shoji [b], Kazuo Umezawa [a,*], Takeshi Sugai [b,*]

[a] Department of Applied Chemistry, Keio University, 3-14-1 Hiyoshi, Yokohama 223-8522, Japan
[b] Faculty of Pharmacy, Keio University, 1-5-30 Shibakoen, Minato-ku, Tokyo 105-8512, Japan
[c] Department of Chemistry, Keio University, 3-14-1 Hiyoshi, Yokohama 223-8522, Japan

ARTICLE INFO

Article history:
Received 2 June 2010
Received in revised form 6 July 2010
Accepted 7 July 2010
Available online 24 July 2010

Keywords:
DHMEQ
Asymmetric epoxidation
Lipase
Hydrolysis
Kinetic resolution

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.

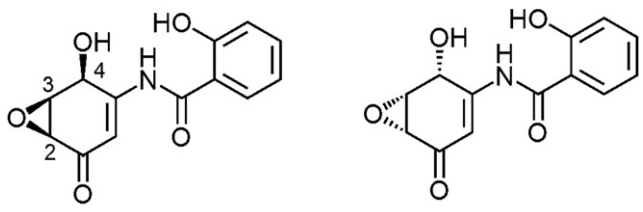
1. Introduction

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



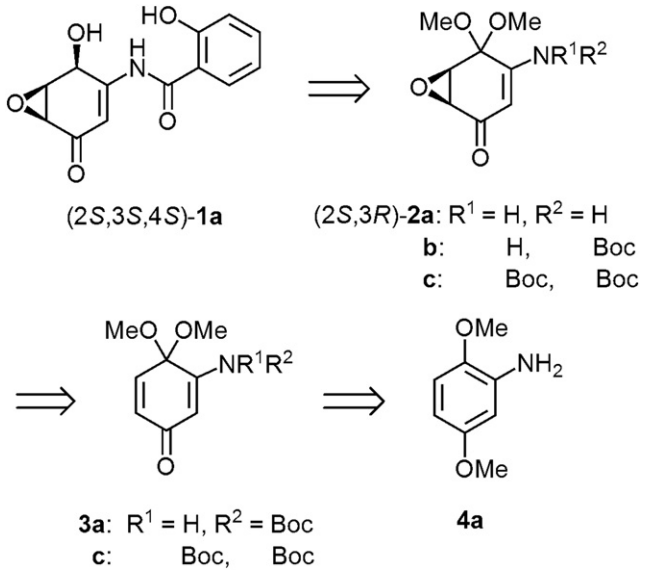
(2*S*,3*S*,4*S*)-**1a**
active enantiomer

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

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

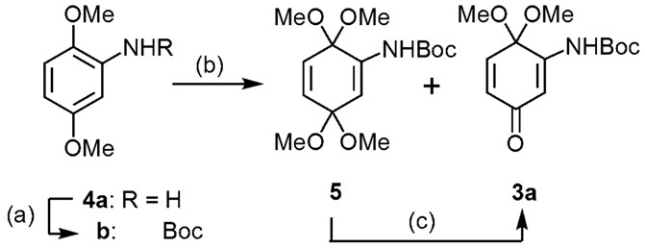
* Corresponding authors. Tel./fax: +81 3 5400 2665; e-mail address: sugai-tk@pha.keio.ac.jp (T. Sugai).

0040-4020/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2010.07.013



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

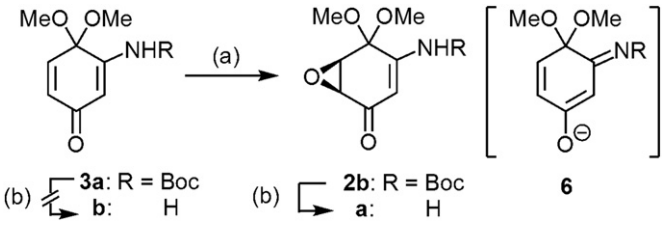
3a in as low as 21%. This observation promoted us to switch hydrochloric acid into weaker aqueous citric acid, and the deprotection proceeded in a reproducible manner to give **3a** in 48% yield. Moreover, careful examination in regard to oxidizing agent, the yield of the dehydrogenation of Boc derivative **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.

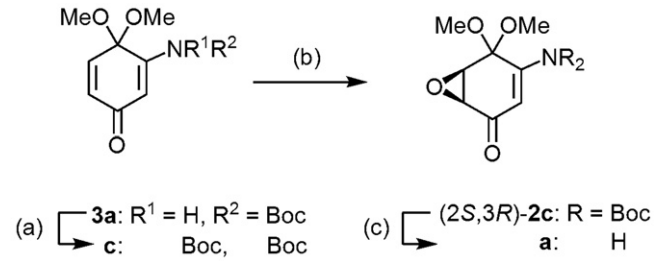
Under Taylor's procedure,[5] the epoxidation had been carried out in buffered conditions with a ratio of 4:1 between acidic hydrochloride and basic hydroxide of cinchonidine. An equilibration between **3a** and enolate **6** by the deprotonation of *tert*-butylcarbamate group under basic conditions (Scheme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