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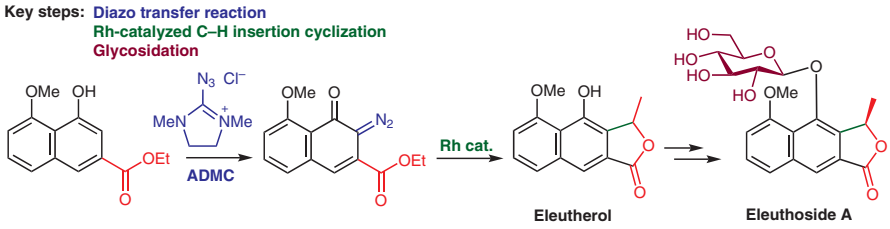
Total Synthesis of Eleuthoside A; Application of Rh-Catalyzed Intramolecular Cyclization of Diazonaphthoquinone

Dina I. A. Othman[a,b]
Kota Otsuka[a]
Shuhei Takahashi[a]
Khalid B. Selim[b]
Magda A. El-Sayed[b]
Atif S. Tantawy[b]
Tatsuo Okauchi[a]
Mitsuru Kitamura*[a]

[a] Department of Applied Chemistry, Kyushu Institute of Technology, 1-1 Sensuicho, Tobata, Kitakyushu, 804-8550, Japan
kita@che.kyutech.ac.jp
[b] Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt



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Abstract The first total synthesis of (±)-eleutherol and eleuthoside A, the natural cytotoxic substances extracted from medicinal Indonesian plant, is described. First, the synthesis of (±)-eleutherol has been accomplished in nine steps starting from bromo methoxy aldehyde with the aid of diazo-transfer chemistry approach. Second, a metal-catalyzed intramolecular cyclization reaction of the corresponding diazonaphthoquinone led to the desired eleuotherol, which served as a precursor to eleuthoside A. Then, several glycosidation routes, using different glucosyl donors, were experimented to reach effective O-glycosidation of eleutherol. The only successful strategy involved Koenigs–Knorr glycosidation using peracetyl glycosyl bromide in the presence of Ag_2O and quinoline. This strategy furnished our desired acetylated glycoside of β-configuration, regioselectively. Finally, deacetylation and successive separation of diastereomers were conducted to give eleuthoside A.

Key words diazonapthoquinone, rhodium, eleutherol, eleuthoside A, glycosidation, intramolecular cyclization, 1 Introduction, 2 Results and Discussion, 2.1 Synthetic Strategy, 2.2 Synthesis of Aglycone, Eleutherol, 2.3 Glycosidation and Synthesis of Eleuthoside A, 3 Conclusions

1 Introduction

In 1997, Hirotaka Shibuya and co-workers disclosed the isolation and structure of three new aromatic glycosides named eleuthosides A (**1**), B, and C.[1,2] They have been found in the bulbs of *Eleutherine palmifolia* (Iridaceae). This plant is an Indonesian medicinal one that is used traditionally as anticancer agent. In more detailed accounts in 2013, Young Ho et al. revealed further information about the anti-inflammatory properties of the same plant.[3]

In this paper, we made use of the efficient and short directed synthetic method of diazonaphthoquinone from naphthol by adopting our approach of diazo-transfer chemistry using 2-azido-1,3-dimethylimidazolinium chloride (ADMC).[4] Recently, this method is more likely to find widespread uses in the field of total synthesis of natural compounds with the aid of metal-catalyzed reactions.[5,6] To our knowledge, this developed diazo-transfer methodology has never been used so far to synthesize eleuthoside A (**1**). Moreover, the interesting biological activities of this substance **1** have inspired us toward these attempts (adopting this methodology). Hence, the first total synthesis of the cytotoxic natural eleuthoside A (**1**) was investigated via metal-catalyzed intramolecular C–H insertion cyclization reaction of the appropriate diazonaphthoquinone, followed by selective β-glycosidation and resolution of the final product. This developed strategy takes the advantage of both diazo-transfer reaction and metal-catalyzed reaction. In this investigation, we wish to describe the full details of our total synthesis of eleuthoside A (**1**) and eleutherol (**2**, Figure 1).

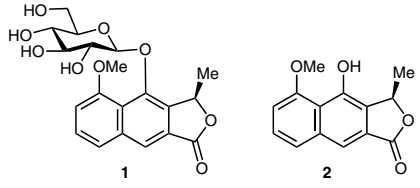
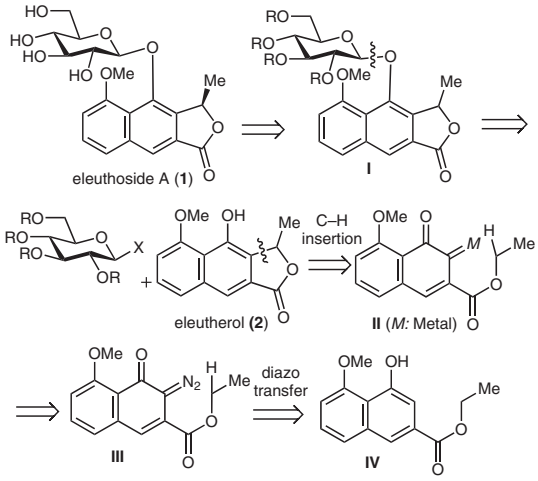


Figure 1 Structures of eleuthoside A (**1**) and eleutherol (**2**)

2 Results and Discussion

2.1 Synthetic Strategy

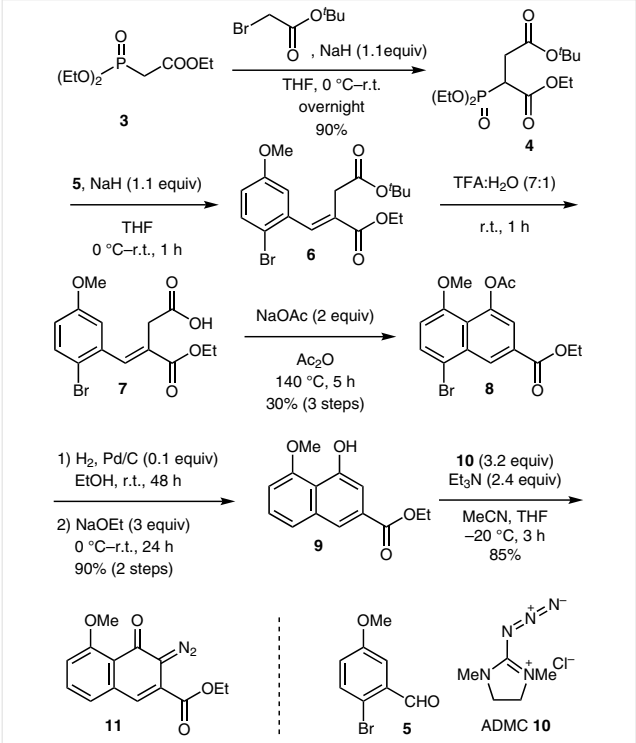
As obviously shown in Figure 1, the structure of eleuthoside A (**1**) is composed mainly of two domains: eleutherol (**2**) and glucosyl residue which are bonded together through β-glycosidic linkage.[2] The retrosynthetic analysis for eleuthoside A (**1**) is outlined in Scheme 1 which starts with the fragmentation of the target into two moieties, a sugar (glucose) and aglycone part, eleutherol (**2**). The lactone part in compound **2** could be constructed by C–H insertion reaction of metal carbene **II** formed from diazonaphthoquinone **III**,[7] which in turn would be synthesized from naphthol **IV** by diazo-transfer reaction with ADMC as mentioned previously (Scheme 1). An interesting feature is that the ethyl ester employed during the construction of naphthol **IV** is incorporated into our final product. During our work on the synthetic plan, two problems have emerged. At first, undesired intermolecular OH or benzyl insertion side products were observed with our desired compound **2** during C–H insertion cyclization reaction. Also, some difficulty was experienced when trying to attach the sugar ring in the glycosidation step, as will be explained later.



Scheme 1 Retrosynthetic analysis of eleuthoside A (**1**)

2.2 Synthesis of Aglycone, Eleutherol

As shown in Scheme 2, we began our investigation by the preparation of phosphonate **4** from the commercially available phosphonoacetate **3**.[8] Then, we followed the modified Wittig–Horner reaction of aldehyde **5** smoothly to afford *tert*-butyl diester **6**. The latter compound was selectively hydrolyzed under acidic conditions to give unsaturated acid **7**.[8b] The treatment of **7** with acetic anhydride in the presence of NaOAc led to naphthalene **8**. Both bromo and acetyl groups were removed from compound **8** by treatment with H_2/Pd(C) and NaOEt, respectively, to afford the corresponding naphthol **9** (90% yield over two steps). Then, naphthol **9** was anticipated to form eleutherol (**2**) via metal-catalyzed intramolecular cyclization of diazonaphthoquinone **11**. Compound **11** was prepared by the efficient one-step diazo-transfer reaction of naphthol **9** using ADMC (**10**).[4] It was observed that, using 3.2 equivalents of ADMC and 3.8 equivalents of Et_3N, efficiently afforded the diazonaphthoquinone **11** in 85% yield.

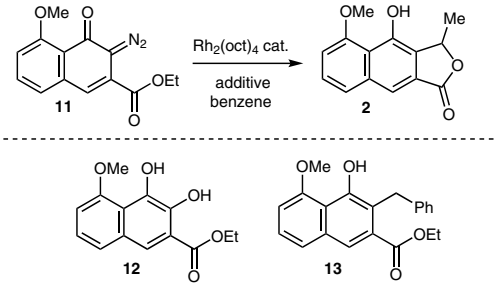


Scheme 2 Synthesis of ethyl 3-diazo-3, 4-dihydro-4-oxo-5-methoxy-2-naphthalenecarboxylate (**11**)

Finally, the resultant diazonapthoquinone **11** was subjected to intramolecular cyclization reaction to get the desired eleutherol (**2**, Table 1). When **11** was exposed to catalytic amount of rhodium(II) octanoate, dimer ($Rh_2(oct)_4$) in refluxing benzene, the desired compound **2** was not formed, whereas naphthalene diol **12** was mainly formed.[9] This might result from the O–H insertion reaction of diazonapthoquinone **11** with the contaminated water (Table 1, entry 1). As a result, the reaction was repeated in the presence of MS 4 Å (pellets; Table 1, entry 2). In this case, eleutherol (**2**) was difficultly isolated in 20% yield along with diol **12** (45% yield). To a great extent, this problem could be overcome by using 3 mol% $Rh_2(oct)_4$ in the presence of pre-activated powdered-type MS 4Å under completely anhydrous conditions to give compound **2** as a sole product in 63% yield (Table 1, entry 3).[10] Moreover, $Rh_2(oct)_4$-catalyzed reaction of **11** proceeded more rapidly

in refluxing toluene, but the yield of **2** was decreased and the benzyl insertion derivative **13** was observed as byproduct (Table 1, entry 4).

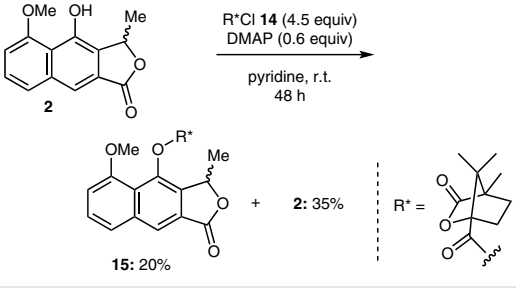
Table 1 Toward the Total Synthesis of Eleutherol (**2**): Studies for the Rh-Catalyzed Intramolecular Cyclization of Diazonaphthoquinone **11**



Entry	Rh cat. (mol%)	Additive	Temp (°C)	Time (min)	Product (%)
1	1.5	–	90	30	**12** 84
2	1.5	MS 4Å[a]	90	45	**2** 20, **12** 45
3	3	MS 4Å[b]	90	15	**2** 63
4[c]	3	MS 4Å[b]	110	10	**2** 51, **13** 12

[a] Pellets MS 4Å was used.
[b] Powdered MS 4Å was used.
[c] Reaction was carried out in toluene.

It is also worth noting that we tried to obtain eleutherol (**2**) as enantiomerically pure form through the optical resolution. (–)-(1*S*)-Camphanoyl chloride (**14**) has been proved to be an efficient and powerful chiral derivatization reagent for stereoisomeric separation used for stereoisomers containing hydroxy functional groups.[11] As a result, its utilities for chiral derivatization of **2** was taken into consideration, hoping to separate the enantiomeric pure compound prior to the glycosidation step, as shown in Scheme 3. However, no separation was detected for these diastereomers **15**. Therefore, we decided to go forward using the racemic mixture form **2**.



Scheme 3 Unsuccessful optical resolution protocol of eleutherol (**2**)

2.3 Glycosidation and Synthesis of Eleuthoside A

There are several well-known glycosyl donors for aromatic compounds, such as acetates, halides, and trichloroacetimidates.[12,13] As shown in Figure 2, a number of different donors, flurobenzylated glucose **16**,[14] trichloroacetimidate-acetylated glucose **17**,[15] and peracetyl glucosyl bromide **18**[15,16] have been synthesized, according to previously reported literatures, in order to be used for several glycosidation routes as will be illustrated.

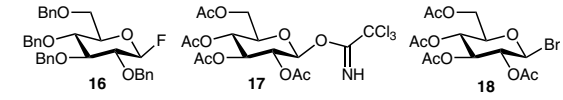
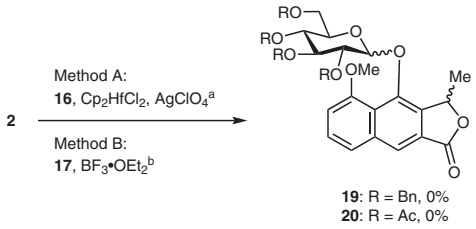


Figure 2 Glucosyl donors

The glycosidation step was so challenging. With the key eleutherol (**2**) and glucosyl donor fragments in hand, several trials have been made to join them into our final desired glycoside **1** (Scheme 4). Thus, a number of benzylated or acetylated glucosyl donors were treated with acceptor **2** under several glycosidation conditions (using different promoter systems). Throughout our attempts of O-glycosidation, we started with Mukaiyama–Suzuki conditions using 2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl fluoride (**16**) and the most powerful promotor system Cp_2HfCl_2·$AgClO_4$ (method A).[17] However, no glycosidation was observed. Thus, we increased the time of the reaction but many undesired side products were obtained.

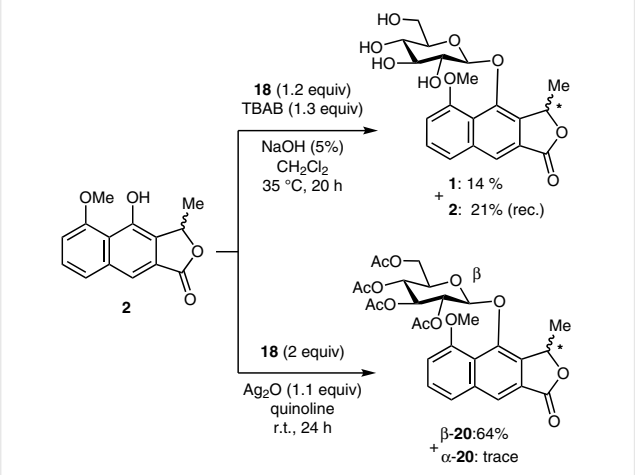


Scheme 4 Attempted O-glycosidation reaction toward the total synthesis of eleuthoside A (**1**); comparative studies for the attachment of the floride and imidate sugar donors. [a] Method A: **16** (0.5 equiv), Cp_2HfCl_2 (0.5 equiv), $AgClO_4$ (1 equiv), MS 4Å, CH_2Cl_2, –70 °C to r.t., 1 or 24 h. [b] Method B: **17** (2 equiv), BF_3·Et_2O (0.2 equiv), MS 4Å, CH_2Cl_2, –10 °C to r.t., 24 h.

Then, hoping for further improvement, less bulky glucosyl donor and another different promotor system have been examined. BF_3·OEt_2-catalyzed Schmidt glycosidation reaction which has used the acetyl-protected trichloroacetimidate derivative **17** as a glucosyl donor was adopted.[15b,18] This reaction was reported as the most common efficient method for the synthesis of such sensitive or complicated targets (method B). Unfortunately, this attempt gave back

the starting material **2**. These observed results suggested that the hydroxyl group in compound **2** was less suitable for such glycosidation conditions.

Due to the lack of success in O-glycosidation under both Mukaiyama–Suzuki's and Schmidt's methodology, bromides were taken into our consideration as one of the most frequently glycosyl donors described in similar prior work.[13] Therefore, direct O-glycosidation of **2** was performed with peracetyl glycosyl bromide **18** under Koenigs–Knorr conditions in a biphasic media (CH_2Cl_2/H_2O) using tetrabutylammonium bromide (TBAB) as phase-transfer catalyst and NaOH as a base (Scheme 5).[19] Again, this stage was troublesome owing to the aquatic basic conditions, which caused deacetylation of the sugar moiety and the product couldn't be confirmed clearly. At this juncture, we decided to avoid this aquatic environment by using quinoline and silver oxide as insoluble halophile promotor.[20]

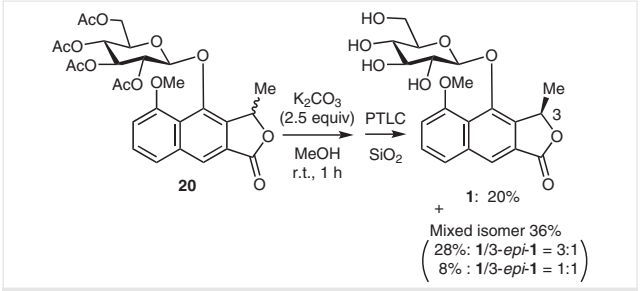


Scheme 5 Acetylated bromide donor for successful O-glycosidation of eleutherol (**2**)

As depicted in Scheme 5, we were excited to find that the coupling of peracetyl glycosyl bromide **18** and precursor **2** was successfully implemented affording our desired product **20**.

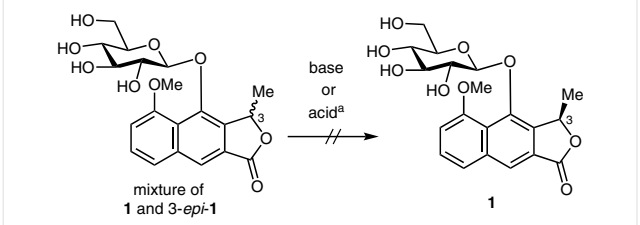
Judged by the ^{1}H NMR spectrum, **20** was exhibited as diastereisomeric mixture (1:1) in 64% yield due to the presence of stereocenter in the lactone ring. In addition, the configuration at the anomeric position was clarified as β-anomer as a major one along with traces of α-anomer, which was easily removed by chromatography. The exclusive formation of the major β-glycoside **20** was confirmed by the coupling constant of the anomeric proton (J = 7.8 Hz), while in case of α-anomer, the coupling constant (J) was 3.0 Hz. Furthermore, the spectrum of peracetate of eleuthoside A **20** was characterized by the disappearance of the hydroxyl signal of eleutherol (**2**) and the appearance of four acetoxymethyl groups.

At this point, our strategy had converged on this late-stage intermediate **20** as nonseparable diastereomers. Toward the completion of the total synthesis of eleuthoside A (**1**), careful deprotection of the four acetyl groups in the glucose unit was implemented smoothly using K_2CO_3 in methanol.[21] A mixture of eleuthoside A (**1**) and its α-epimer were obtained as white solid in 56% yield (Scheme 6).[22] All spectroscopic and analytical data of the synthesized material were in a close agreement with those reported for the naturally occurring compound published earlier by Shibuya et al.[2] It is also worth noting to mention that purification of this sensitive glycoside **1** could be simply achieved by PTLC silica gel chromatography. Running the plate twice using ($CHCl_3$/MeOH, 6:1) was useful to separate the single isomer in 20%, which was found to be identical to the natural glycoside. Also, 28% and 8% mixed isomers were obtained with a ratio of natural isomer to unnatural isomer 3:1 and 1:1, respectively, as illustrated in Scheme 6.



Scheme 6 The final step toward total synthesis of eleuthoide A (**1**)

For further enhancement of the yield, we expected that epimerization[23] may play a role in converting one isomer into another. As a result, epimerization was attempted under several conditions as illustrated in Scheme 7. However, we were not able to obtain one dominant isomer from the mixture of **1** and 3-*epi*-**1** either under basic epimerization conditions using DBU, *t*-BuOK or KOH or under acidic epimerization using $BF_3{\cdot}OEt_2$.[23]



Scheme 7 Unsuccessful epimerization attempts of eleuthoside A (**1**). [a] Examined conditions: DBU, THF, r.t.; *t*-BuOK, THF, r.t.; KOH, MeOH, r.t.; $BF_3{\cdot}OEt_2$, acetone, MS 4Å.

Despite these unfruitful epimerization trials, the protocol previously shown in Scheme 6 allowed us to successfully achieve our goal, and obtain eleuthoside A (**1**) in 20% single isomer.

3 Conclusions

The first total synthesis of the natural eleutherol (**2**) and eleuthoside A (**1**) has been accomplished, involving simple and readily accessible starting materials such as glucose and bromomethoxy aldehyde. Depending on our modest observations, the main challenge was that finding proper reaction conditions for both intramolecular cyclization reaction and glycosidation reaction. In brief, the presence of 3 mol% of Rh catalyst, pre-activated powdered molecular sieve, and anhydrous benzene were essential to avoid OH- or benzyl-insertion reactions. Furthermore, the best conditions found for smooth O-glycosidation involved the treatment of eleutherol (**2**) with two equivalents of acetobromoglucose in quinoline in the presence of Ag_2O at room temperature. Finally, subsequent deacetylation was done to furnish 20% eleuthoside A (**1**) in β-configuration. The spectral data of both eleutherol (**2**) and eleuthoside A (**1**) matched strongly with those previously reported for the natural one.[2] This reported chemistry allows not only access to the rare naturally occurring substances but also to attractive designed analogues as a new class of potential anticancer agents for future investigation.

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Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0036-1589118.

References and Notes

(1) There have been reported two kinds of eleuthoside A. One is isolated by Kashman Y. et al. and the other is (3*R*)-4-(β-d-glucopyranosyloxy)-5-methoxy-3-methyl-naphtho[2,3-*c*]furan-1(3*H*)-one, isolated by Shibuya H. et al.[2] In this paper, synthetic study of latter eleuthoside A and related compounds are described. For the isolation of eleuthoside A, see: Ketzinel, S.; Rudi, A.; Schleyer, M.; Benayahu, Y.; Kashman, Y. J. *Nat. Prod.* **1996**, *59*, 873.

(2) Shibuya, H.; Fukushima, T.; Ohashi, K.; Nakamura, A.; Riswan, S.; Kitagawa, I. *Chem. Pharm. Bull.* **1997**, *45*, 1130.

(3) Minh, Ha. L.; Huyen, D. T. T.; Kiem, P. V.; Minh, C. V.; Van N, T. H.; Nhiem, N. X.; Tai, B. H.; Long, P. Q.; Anh, B. K.; Hyun, K. S.; Hye-Jin, H.; Sohyun, K.; Young-Sang, K.; Young, Ho. K. *Bull. Korean Chem. Soc.* **2013**, *34*, 633.

(4) (a) Kitamura, M.; Sakata, R.; Tashiro, N.; Ikegami, A.; Okauchi, T. *Bull. Chem. Soc. Jpn.* **2015**, *88*, 824. (b) Kitamura, M.; Tashiro, N.; Sakata, R.; Okauchi, T. *Synlett* **2010**, 2503.

(5) For a review, see: Othman, D.; Kitamura, M. *Heterocycles* **2016**, *92*, 1761.

(6) (a) Kitamura, M.; Takahashi, S.; Okauchi, T. J. *Org. Chem.* **2015**, *80*, 8406. (b) Kitamura, M.; Kubo, K.; Yoshinaga, S.; Matsuzaki, H.; Ezaki, K.; Matsuura, T.; Matsuura, D.; Fukuzumi, N.; Araki, K.; Narasaki, M. *Tetrahedron Lett.* **2014**, *55*, 1653.

(7) For reviews, see: (a) Zhang, Z.; Wang, J. *Tetrahedron* **2008**, *64*, 6577. (b) Doyle, M. P.; Ye, T.; McKervey, M. A. *Modern Catalytic Methods for Organic Synthesis with Diazo Compounds*; John Wiley and Sons: New York, **1998**. (c) Ye, T.; McKervey, M. A. *Chem. Rev.* **1994**, *94*, 1091. (d) Padwa, A.; Austin, D. J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1797. (e) Doyle, M. P. *Chem. Rev.* **1986**, *86*, 919.

(8) (a) Owton, W. M.; Gallagher, P. T.; Juan-Montesinos, A. *Synth. Commun.* **1993**, *23*, 2119. (b) Snyder, S. A.; Sherwood, T. C.; Ross, A. G. *Angew. Chem. Int. Ed.* **2010**, *49*, 5146.

(9) Related reactions, see: Kitamura, M.; Otsuka, K.; Takahashi, S.; Okauchi, T. *Tetrahedron Lett.* **2017**, *58*, 3508.

(10) **Experimental Procedure and Physical Data of Eleutherol (2)**
To a solution of diazonaphthoquione **11** (100 mg, 0.37 mmol) in benzene (4 mL) in the presence of 0.2 g preactivated powdered MS 4Å, $Rh_2(oct)_4$ (8.6 mg, 0.011 mmol) was added at 90 °C as the bath temperature. The mixture was stirred for 15 min at the same temperature. After cooling, the mixture was filtered through Celite pad and concentrated in vacuo to afford the crude compound, which was purified by PTLC (silica gel, R_f = 0.7; toluene/acetone, 9:1) to give **2** (60 mg, 63%) as a yellow solid; mp 190 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.65 (s, 1 H), 7.90 (s, 1 H), 7.60 (d, 1 H, J = 8.0 Hz), 7.43 (dd, 1 H, J = 7.7, 8.0 Hz), 6.93 (d, 1 H, J = 7.7 Hz), 5.75 (q, 1 H, J = 6.6 Hz), 4.19 (s, 3 H), 1.75 (d, 3 H, J = 6.6 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.6, 156.5, 149.1, 137.2, 127.9, 126.5, 125.9, 123.6, 117.5, 116.5, 106.2, 77.4, 56.3, 19.1. IR (ATR): 3358, 2920, 1753, 1595, 1458 cm^{-1}. HRMS (FAB$^+$): *m/z* [M + H]$^+$ calcd for $C_{14}H_{13}O_4$: 245.0736; found: 245.0817.

(11) (a) Licea-Perez, H.; Wang, S.; Rodgers, C.; Bowen, C. L.; Fang, K.; Szapacs, M.; Evans, C. A. *Bioanalysis* **2015**, *7*, 3005. (b) Kitamura, M.; Ohmori, K.; Kawase, T.; Suzuki, K. *Angew. Chem. Int. Ed.* **1999**, *38*, 1229.

(12) (a) Garc, P. A.; Braga de Oliveira, A.; Batista, R. *Molecules* **2007**, *12*, 455. (b) Schmidt, R. R.; Castro-Palomino, J. C.; Retz, O. *Pure Appl. Chem.* **1999**, *71*, 729. (c) Jensen, K. J. J. *Chem. Soc., Perkin Trans. 1* **2002**, 2219.

(13) Jacobson, M.; Malmberg, J.; Ellervik, U. *Carbohydr. Res.* **2006**, *341*, 1266.

(14) (a) Brenstrum, T. J.; Brimble, M. A. *Arkivoc* **2001**, *(vii)*, 37. (b) Schmidt, O. T.; Auer, T.; Schmadel, H. *Chem. Ber.* **1960**, *93*, 556.

(15) (a) Mancini, R. S.; McClary, C. A.; Anthonipillai, S.; Taylor, M. S. J. *Org. Chem.* **2015**, *80*, 8501. (b) Aitken, H. R. M.; Johannes, M.; Loomes, K. M.; Brimble, M. A. *Tetrahedron Lett.* **2013**, *54*, 6916.

(16) (a) Yong-lin, J.; Bin, L.; Bai-chun, B. *Huaxue Yu Nianhe* **2007**, *29*, 189. (b) Shuhan, Z.; Kejun, S. CN 1803818 A, **2006**.

(17) (a) Matsumoto, T.; Katsuki, M.; Jona, H.; Suzuki, K. *J. Am. Chem. Soc.* **1991**, *113*, 6982. (b) Mukaiyama, T.; Murai, Y.; Shoda, S. *Chem. Lett.* **1981**, *10*, 431. (c) Oyama, K.; Kondo, T. *J. Org. Chem.* **2004**, *69*, 5240. (d) Matsumoto, T.; Katsuki, M.; Suzuki, K. *Chem. Lett.* **1989**, *18*, 437.

(18) (a) Zhao, Y.; Lu, Y.; Ma, J.; Zhu, L. *Chem. Biol. Drug Des.* **2015**, *86*, 691. (b) Tietze, L. F.; Gericke, K. M.; Güntner, C. *Eur. J. Org. Chem.* **2006**, 4910. (c) Kumazawa, T.; Ohki, K.; Ishida, M.; Sato, S.; Onodera, J.-I.; Matsuba, S. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 1379. (d) Toshima, K. *Carbohydr. Res.* **2000**, *327*, 15. (e) Yamaguchi, M.; Horiguchi, A.; Fukuda, A.; Minami, T. *J. Chem. Soc., Perkin Trans. 1* **1990**, 1079. (f) Oyama, K.; Kondo, T. *Synlett* **1999**, 1627. (g) Wozney, Y. V.; Kalicheva, I. S.; Galoyan, A. A. *Bioorg. Khim.* **1982**, *8*, 1388. (h) Giam, C. S.; Goldschmid, H. R.; Perlin, A. S. *Can. J. Chem.* **1961**, *39*, 2025.

(19) Chen, C.-Y.; Sun, J.-G.; Liu, F.-Y.; Fung, K.-P.; Wu, P.; Huang, Z.-Z. *Tetrahedron* **2012**, *68*, 2598.

(20) (a) Frackowiak, A.; Skibinski, P.; Gawel, W.; Zaczynska, E.; Czarny, A.; Gancarz, R. *Eur. J. Med. Chem.* **2010**, *45*, 1001. (b) Belyanin, M. L.; Stepanova, E. V.; Ogorodnikov, V. D. *Carbohydr. Res.* **2012**, *363*, 66.

(21) Zhao, Y.; Ni, C.; Zhang, Y.; Zhu, L. *Arch. Pharm. Chem. Life Sci.* **2012**, *345*, 622.

(22) **Experimental Procedure and Physical Data of Eleuthoside A (1)**

The solid of peracetyl glycoside **20** (8.0 mg, 0.1 mmol) was dissolved in MeOH (2 mL), and then, K_2CO_3 (4.8 mg, 0.25 mmol) was added. The reaction mixture was stirred at room temperature until TLC showed complete conversion of starting material (1 h). Then, the reaction mixture was concentrated under reduced pressure and purified by PTLC eluting with ($CHCl_3$/MeOH, 6:1) to afford (1.5 mg, 20%) eleuthoside A (**1**) as a single isomer along with 28% mixed isomer with the ratio **1**/3-*epi*-**1** (3:1) and 8% mixed isomer with the ratio 1:1. The spectral data of the white crystals of the natural isomer will be detailed below

Mp 210 °C. R_f = 0.42 ($CHCl_3$/MeOH, 6:1), $[\alpha]_D$ –58.6 (*c* 0.0015, in MeOH at 25 °C). ^{1}H NMR (500 MHz, CD_3OD): δ = 8.22 (s, 1 H, H-9), 7.68 (d, 1 H, J = 8.0 Hz, H-8), 7.54 (dd, 1 H, J = 7.9, 8.0 Hz, H-7), 7.15 (d, 1 H J = 7.9 Hz, H-6), 6.07 (q, 1 H, J = 6.6 Hz, H-3), 5.01 (d, 1 H, J = 7.6 Hz, H-1′), 4.02 (s, 3 H, OCH_3), 3.72 (dd, 1 H, J = 2.1, 11.6 Hz, H-6B′), 3.61 (dd, 2 H, H-2′, H-6A′), 3.49 (dd, 1 H, J = 9.2, 9.5 Hz, H-3′), 3.42 (dd, 1 H, J = 9.4, 9.5 Hz, H-4′), 3.12 (ddd, 1 H, J = 2.1, 5.8, 9.4 Hz, H-5′), 1.74 (d, 3 H, J = 6.6 Hz, 3-CH_3). ^{1} H NMR (500 MHz, $CDCl_3$): δ = 8.27 (s, 1 H, H-9), 7.68 (d, 1 H, J = 8.0 Hz, H-8), 7.53 (dd, 1 H, J = 7.9, 8.0 Hz, H-7), 7.10 (d, H J = 7.9 Hz, H-6), 5.91 (q, 1 H, J = 6.6 Hz, H-3), 4.95 (d, 1 H, J = 7.6 Hz, H-1′), 4.02 (s, 3 H, OCH_3), 3.82 (s, 2 H, br OH), 3.78–3.72 (m, 2 H, J = 7.4, 9.5 Hz, H-6B′, H-6A′), 3.68 (d, 2 H, H-2, H-4′, J = 8.8, 9.5 Hz), 3.49 (dd, 1 H, J = 9.2, 9.5 Hz, H-3′), 3.12 (ddd, 1 H, J = 4.5, 8.8, 9.0 Hz, H-5′), 2.84 (s, 1 H, br-OH), 2.73 (s, 1 H, br-OH), 1.73 (d, 3 H, J = 6.5 Hz, 3-CH_3). ^{13}C NMR (125 MHz, CD3OD): δ = 170.9, 156.0, 146.4, 139.3, 138.0, 127.06, 124.5, 122.6, 122.6, 122.3, 108.3, 104.7, 79.5, 76.9, 76.4, 74.6, 70.1, 61.0, 54.7, 17.9. IR (ATR): 3404 (OH), 2971, 1750 (C=O), 1586, 1033 cm^{-1}. HRMS (ESI^+): *m/z* = 429 [M + Na+]; calcd for $C_{20}H_{22}NaO_9$: 429.1162; found: 429.1154.

(23) Cheng, K.; Liang, G.; Hu, C. *Molecules* **2008**, *13*, 938.