Organic LETTERS

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Double [3,3]-Sigmatropic Rearrangement in the Enzymatic Dioxygenation of Benzyl Azide: Preparation of Novel Synthetically Valuable Azido-diols

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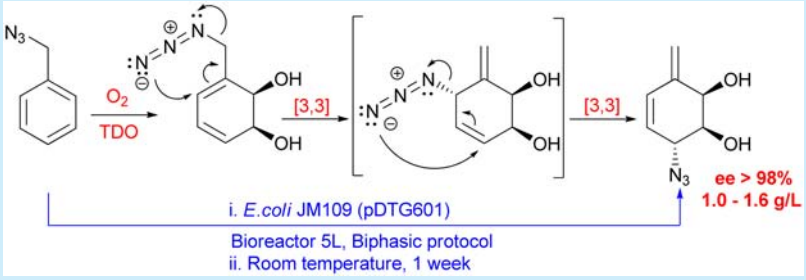
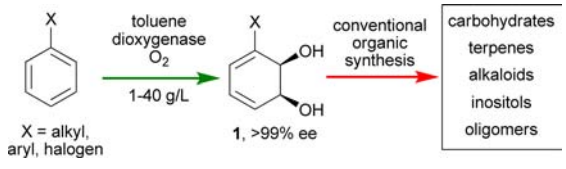
Supporting Information



ABSTRACT: Enzymatic dioxygenation of benzyl azide by toluene dioxygenase produces the expected enantiopure *cis*-cyclohexadienediol along with an exocyclic diene formed by a spontaneous sequence of two [3,3] sigmatropic shifts. This novel dienediol presents high synthetic potential for natural product synthesis. The sigmatropic rearrangements can be reversed by protection of the diol moiety. An optimized production protocol for either of these valuable diols is presented.

Enantiopure *cis*-cyclohexadienediols of the general type **1** have been widely used as starting materials for enantioselective organic syntheses of complex natural products (Scheme 1).[1] The useful array of functional groups present in these diols allows the use of regio- and stereocontrolled organic transformations resulting in short and efficient synthetic schemes.[1d]

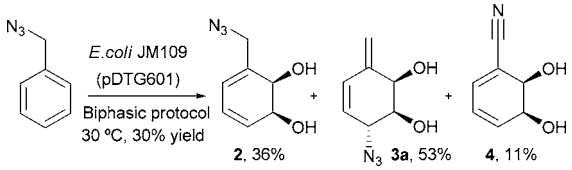
Scheme 1. *cis*-Cyclohexadienediols from Microbial Origin Have Been Extensively Used As Starting Materials for Enantioselective Synthesis of Diverse Natural Products



These compounds are prepared from the corresponding monosubstituted arenes by dihydroxylation via the toluene dioxygenase (TDO) enzymatic system. Currently, no other efficient chemical method for their production at a preparative scale is available. Recently our group published an optimized biphasic procedure for the biocatalytic production of *cis*-cyclohexadienediols in high yields using *E. coli* JM109 (pDTG601) in a 5 L bioreactor.[2]

In connection with our ongoing efforts to prepare complex alkaloids using these diols as starting materials, we started a program to screen for nitrogen containing substrates of the TDO system. The trials were performed in shake flasks using resting cells of *E. coli* JM109 (pDTG601) in a biphasic medium. After a series of unsuccessful attemps using protected anilines and benzylamines, we found that the biotransformation of benzyl azide produced the expected cyclic diene **2**, along with compounds **3** and **4** (Scheme 2).

Scheme 2. Dioxygenation of Benzyl Azide in Shake Flasks by *E. coli* JM109 (pDTG601) Give Three *cis*-Cyclohexadienediols



We immediately became aware of the synthetic potential of compound **3a** for the preparation of amino conduritols and cyclitols, so we decided to carry on an in-depth study of its formation and eventually optimize its production.

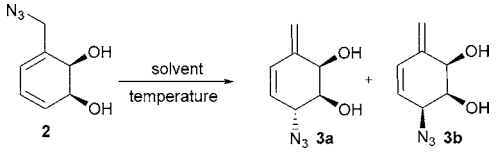
Since formation of **3a** could be explained considering a stereoselective transposition of the azide group from **2** we tried

Received: December 24, 2014
Published: January 28, 2015

DOI: 10.1021/ol503708v
Org. Lett. 2015, 17, 684–687

several conditions for its preparation, using the isolated *cis*-cyclohexadienediol **2** as starting material. Product **2** was quantitatively transformed to **3a** and its epimer **3b** by heating in different solvent systems (Table 1, entries 1−8). The

Table 1. Azide Rearrangement Optimization



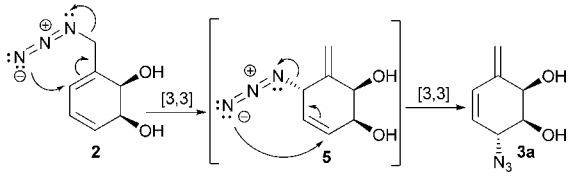
entry	solvent	temp[a] (°C)	3a:3b[b]	time (h)[c]
1	toluene	100	75:25	12
2	tetradecane	100	76:24	12
3	THF	100	80:20	6
5	DMSO	100	78:22	6
6	EtOH	100	80:20	6
7	EtOH/H_2O (1:1)	100	73:27	6
8	H_2O[d]	100	74:26	6
9	D_2O	37	96:4	96
10	D_2O	25	97:3	168
11	D_2O	4	98:2	672

[a]Reactions run in a sealed vial. [b]Ratio determined by NMR on the reaction crude. [c]Time for complete conversion. [d]Na_2CO_3 was added to reach pH = 8 to prevent aromatization.

stereochemical outcome of the reaction is not appreciably affected by the choice of solvent, and compound **3a** (where the azide group is anti to the diol functionality) is obtained as the major product in all the cases. The polarity of the solvent has only a kinetic impact in the system since the transformation seems to be accelerated in the most polar ones (entries 1−2 vs 3−8). Temperature affects the diastereoselectivity of the process (Table 1, entries 8−11), and the best conditions for a complete conversion are shown at room temperature in aqueous media to afford a 97:3 mixture of the exocyclic epimers in 1 week. The optical purity of the major diastereomer **3a** was confirmed by preparing the corresponding Mosher ester derivatives (see Supporting Information).

Regarding the mechanistic aspects of this transformation, we proposed a hypothesis based on two [3,3] sigmatropic rearrangements of the allylic azide functionality as it is shown in Scheme 3.

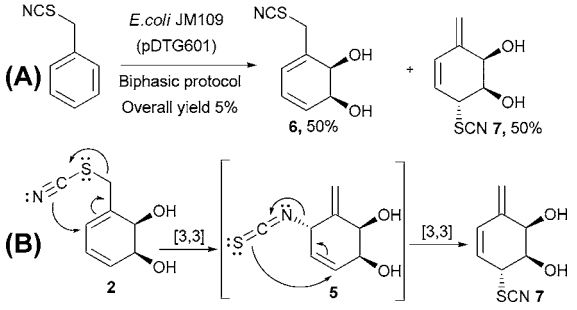
Scheme 3. Mechanistic Proposal for Formation of 3a



Allylic-azide sigmatropic shift is a well-known transformation,[3] which has been synthetically exploited in both acyclic[4] and cyclic[5] substrates in the past. In our system, intermediate **5** was not detected during the transformation, which could be expected considering the loss of diene conjugation in this structure, which is recovered in the final product. The stereochemical outcome of the overall transformation could be determined by the relative stability of the products **3** (see preliminary theoretical calculations).

In order to prove this mechanistic hypothesis we tried the corresponding biotransformation using benzyl thiocyanate as the substrate (Scheme 4A), since a similar rearrangement is

Scheme 4. (A) Enzymatic Dioxygenation of Benzyl Thiocyanate; (B) Mechanistic Proposal



described in the literature for the transformation of allylic thiocyanates to isothiocyanates.[6] Although the yield of the process was very low (possibly due to the high toxicity of the substrate) products **6** and **7** were isolated in a 1:1 ratio. The structure of diol **7** is in accordance with the two sigmatropic rearrangements proposed since the shifted group is attached as a thiocyanate and not as an isothiocyanate in this product (Scheme 4B).

To confirm that azide migration in **2** is not mediated by any carbocation intermediates, the reaction was carried out in EtOH/H_2O adding an excess of NaSCN. Only products **3a** and **3b** were detected, and no traces of **6** or **7** were found (which would be expected from the collapse of any carbocation intermediate and the SCN^- ion).

Preliminary theoretical calculations support the mechanistic proposal, showing a structural stabilization of products **3a** and **3b** due to hydrogen bonding between the azide and one of the hydroxyl groups. This stabilization cannot happen in **2** since the azide group is positioned far away from the diol function owing to electrostatic repulsion (Figure 1). Hydrogen bonds in azido diols have been proposed before as an important stabilization factor to promote allylic azide rearrangements.[4c,5c] In agreement with these results, the estimated $\Delta G°$ values at 25 °C (and also at 100 °C) indicate that the [3,3] sigmatropic shifts

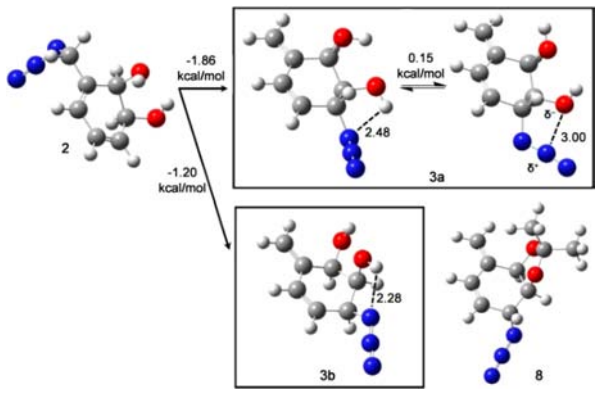


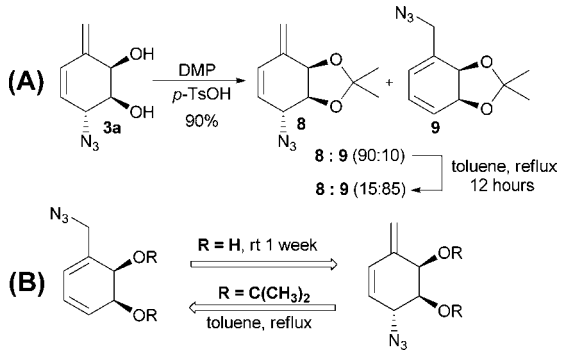
Figure 1. RB3LYP/6-31+G(d,p) optimized geometries for **2**, **3a**, **3b**, and **8**. The calculated $\Delta G°$ values at 25 °C are shown for each reaction. The intramolecular hydrogen bonds and electrostatic interactions involving the azide group are depicted as dashed lines, with the corresponding distances in Å. Color code: C (gray), H (white), O (red), N (blue).

DOI: 10.1021/ol503708v
Org. Lett. 2015, 17, 684−687

by both faces of the ring are spontaneous, although the product **3a** where the azide and diol groups are anti is thermodynamically more favored (Figure 1). According to the computational results, the latter phenomenon is partly explained by an additional stabilization of **3a** brought about by the rapid conformational change this compound undergoes in solution, allowing it to maximize the through-space electrostatic interactions between the azide group and the nearest hydroxyl group.

We wondered what would happen with the driving force of the sigmatropic shift if the diol moiety was protected to avoid hydrogen bonding. With this aim we protected compound **3a** with the isopropylidene group in standard conditions (Scheme 5A). The NMR of the crude reaction mixture showed a 90:10

Scheme 5. Isopropylidene Protection of 3a Produces a Mixture 90:10 of Compounds 8:9 Which Can Be Inverted to 15:85 by Refluxing in Toluene (A); in This Manner Protection of the Diol Function Allows to Manipulate the Direction of the Sigmatropic Rearragments into the Exo or Endo Diene (B)

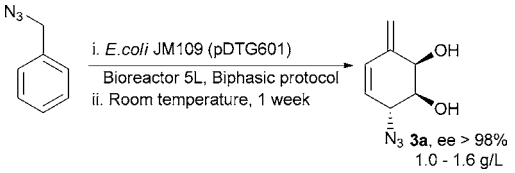


ratio of products **8**:**9**, which could be transformed to a 15:85 ratio after reflux in toluene for 12 h. This result indicates that in these conditions the thermodynamic product is diene **9** instead of product **8**. This observation is also supported by the theoretical calculations, in which no hydrogen-bond mediated stabilization is predicted for **8** (see Figure 1 and the Supporting Information). In this manner protection of the diol function allows the direction of the sigmatropic rearragments into the exo- or endodiene to be manipulated, which is relevant to exploit the synthetic potential of this system (Scheme 5B).

In order to use the described azido diols for synthetic purposes, we decided to optimize its production in a 5 L bioreactor using our previously described biphasic procedure with high cell density cultures of *E. coli* JM109 (pDTG601).[2] When the biotransformation is over, cells and liquid paraffin are separated by centrifugation and the clean culture media is left for 1 week at room temperature to ensure complete conversion of the sigmatropic rearrangement. After isolation and column purification (to separate it from nitrile **4**) the azido diol **3a** is obtained in 1.0−1.6 g/L of culture media (Scheme 6). Minor diastereomer **3b** is not detected in appreciable amounts after the whole procedure. As stated before, compound **3a** can be converted to **9** after diol protection and refluxing in toluene, allowing the endodiene system to be obtained for synthetic purposes.

Having completed the optimization of the production of these azido diols, we turned our attention to the third product obtained, the benzonitrile diol **4**. The generation of this

Scheme 6. Production of Diol 3a in a 5 L Bioreactor



product is intriguing, since it could arise from oxidation of either the primary benzyl azide in the starting compound or the allyl azide in diol **2**. Also, this dehydrogenation could be enzymatic or chemically mediated. In order to determine if this oxidation is enzymatically mediated by the TDO system we carried out the corresponding controls using benzyl azide in the reaction media without cells (to consider possible spontaneous oxidation under the conditions of the experiment) and during a biotransformation using *E. coli* JM109 (no TDO expression, to evaluate the role of other enzymes in the nitrile generation). In both cases benzonitrile was not detected in the reaction media. These preliminary results suggest that the TDO complex is involved in this oxidation. This is remarkable, since to our knowledge there is no report of this type of biotransformation. Further studies on this topic will be reported in due course by our research group.

In summary, we describe an optimized protocol for the preparation of an enantiopure azido diol **3a** that is generated from benzyl azide by enzymatic dioxygenation followed by a spontaneous double [3,3] sigmatropic rearrangement. The last process can be reversed by protection of the diol functionality and refluxing in toluene, obtaining the expected *cis*-cyclohexadienediol **2**. In this manner the preparation of two new synthetically valuable azido diols is presented. The use of these diols for the preparation of conduramines and aminocyclitols will be published by our group in due course.

■ ASSOCIATED CONTENT

Supporting Information

Experimental data for biotransformations and chemical reactions in addition to characterization of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the Agencia Nacional de Investigación e Innovacion (Project FCE 6045) for financial support and Prof. Sonia Rodríguez (Cátedra de Microbiología, Facultad de Química − UdelaR) for her assistance and continuous support with the microbiogical process. We also thank Prof. Alejandra Rodríguez and David Menchaca (Polo Tecnológico de Pando, Facultad de Química, UdelaR) for the high resolution mass spectroscopy analysis.

DOI: 10.1021/ol503708v
Org. Lett. 2015, 17, 684−687

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DOI: 10.1021/ol503708v
Org. Lett. 2015, 17, 684−687