Cyclopropanation Reactions for the Synthesis of 2-Azabicyclo[4.1.0]heptane Derivatives with Nitric Oxide Synthase Inhibitory Activity

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Synthesis of new bicyclic structures containing cyclopropanes, related to selective iNOS inhibitor ONO-1714, is described. We have evaluated the effect of the compounds obtained on the production of nitric oxide in lipopolysaccharide and interferon-gamma stimulated mouse peritoneal macrophages and on in vitro iNOS activity assays.

Nitric oxide (NO) and the enzymes that produce it, *nitric oxide synthases* (NOS), regulate numerous physiological processes.[1] Among the NOS subclasses, the iNOS is induced by inflammatory stimuli, which may result in an excessive production of NO. It has been suggested that iNOS could be part of the physiopathology of a number of diseases such as septic shock, inflammation, and carcinogenesis.[2]

The search for more active and selective inhibitors of a particular NOS isoform is a challenge.[3] Recently, a new iNOS inhibitor, ONO-1714, has been described (Figure 1).[4]

This compound is a potent and selective inhibitor of iNOS and is currently undergoing evaluation in a Phase II clinical trial in Japan.[5] We have been engaged in new structural modifications of this compound. Thus, we have synthesized and evaluated new compounds in which a polar side chain has been attached to the cyclopropane ring.

Synthesis of the test compounds was planned using a cyclopropanation reaction of cyclic enamides **4** with ethyl diazoacetate (EDA). **4a** and **4b** were obtained by aminolysis of **1** following a procedure described by Kawanaka et al.[4] which formed the imides **3**. We carried out the reduction of **3** with DIBAL, and dehydrated the resulting intermediate using mesyl chloride, which formed **4** in 85–87% yield (Scheme 1).

The cyclopropanation reaction with EDA was first done with rhodium acetate (Table 1). In the reactions with compound **4a**, *trans*- and *cis*-**5a** were isolated jointly with unreacted starting material and insertion product **6a**. Major product was *trans*-**5a**. The conditions shown in Entry 1 were then used with chiral copper complexes, observing an improvement in yields keeping a high diastereomeric ratio. These catalysts were prepared in situ treating CuOTf with ligands **A** or **B**. As shown in Entries 2 and 3 these ee are not completely satisfactory although it is interesting that the major enantiomer is different in reactions of Entries 2 and 3, which will make it possible to have information on biological activity of both enantiomers.

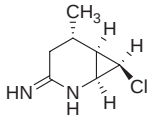
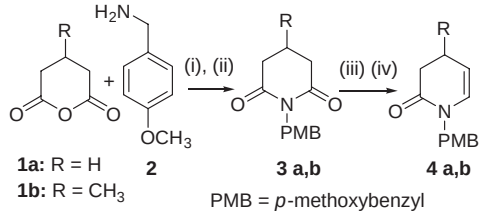
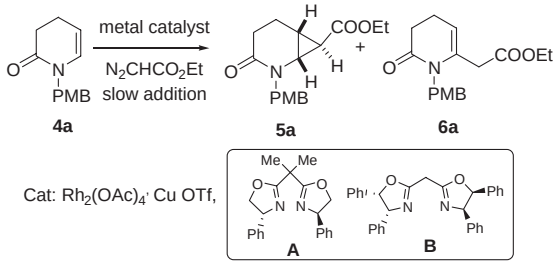


Figure 1. Structure of ONO-1714.



Scheme 1. Synthesis of olefins **4a** and **4b**. (i) THF, rt; (ii) Et_3N, Ac_2O (90–95% in two steps); (iii) DIBAL, CH_2Cl_2, −78 °C (80–85%); (iv) Et_3N, MsCl, CH_2Cl_2, 0 °C (85–87%).

Table 1. Cyclopropanation of **4a** with EDA[a]

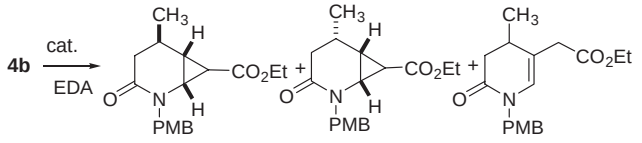


Entry	Catalyst	mol % cat.	Yield/% 4a	*trans*-5a	*cis*-5a	6a	% dr[b] trans:cis	ee % *trans*-5a[c]
1	$Rh_2(OAc)_4$	4	25	45	5	5	90:10	—
2	CuOTf:**A**[6]	1:1	28	52	4	7	87:13	75[d]
3	CuOTf:**B**[7]	1:1	18	62	10	5	78:22	55[d]

[a]All reactions in DCM, at rt, with 2 equivalents of EDA slowly added with a pump syringe over 4 h; relative stereochemistry of products determined by NOE experiments, see Supporting Information. Yields are given for pure products after chromatographic separation. [b]% dr was determined by ^{1}H NMR of the crude mixture. [c]% ee was determined by HPLC analysis. [d]Major enantiomer was the first eluted in reaction of Entry 2 and the second in Entry 3.

Our next target was the cyclopropanation of 4-methyl-substituted lactam **4b**.[4] The starting material was racemic but we decided to use chiral catalysts, in addition to rhodium acetate, as they gave better yields in the case of **4a**, and a kinetic resolution could be envisioned. Thus, **4b** was treated under the conditions indicated in Table 2, giving mixtures of the four possible diastereomers **5b**. In all cases the anti–trans isomer was the major product. A small amount of insertion compound **6b** was also obtained. This time, insertion of EDA occurred in position 5 of the ring. The best result was achieved with complex catalyst CuOTf:**A**, in terms of total yield and diastereoselectivity (Entry 3, Table 2). The major product, *anti–trans*-**5b** was isolated in 50% yield as a pure scalemic compound (18% ee). Overall yield of this reaction was 82%. All the isomers could be separated by

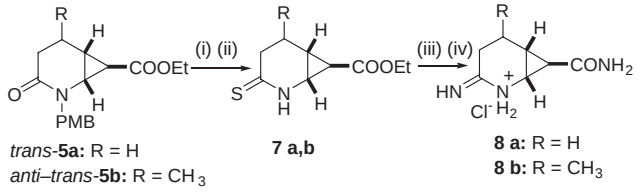
Table 2. Cyclopropanation of **4b** with EDA[a]



anti-**5b** *syn*-**5b** **6b**

Entry	Catalyst	Yield/% 4b	5b mix.	*anti–trans*-5b	*anti–cis*-5b	6b	% dr[b]	% ee *anti–trans*-5b[c]
1	$Rh_2(OAc)_4$	30	60	36	18	8	57:28:8:7	—
2	$Cu(OAc)_2$	22	72	41	16	6	16:43:25:16	—
3	CuOTf:**A**[6]	30	41	25	10	2	80:12:0:0	18
4	CuOTf:**B**[7]	18	72	50	10	7	57:25:12:6	14

[a]See Table 1, footnote a. [b]% dr was determined by ^{1}H NMR spectra of the crude reaction mixture. (*anti–trans*:*anti–cis*:*syn–trans*:*syn–cis*). [c]% ee was determined by HPLC analysis.



trans-**5a:** R = H
anti–trans-**5b:** R = CH_3

7 a,b

8 a: R = H
8 b: R = CH_3

Scheme 2. Synthesis of amidines **8**. (i) TFA, 80 °C (20–25%); (ii) Lawesson's reagent (87–90%); (iii) MeOH sat. NH_3; (iv) HCl–MeOH (80–85%).

column chromatography on silica gel and characterized.[8] Experiments to assign relative stereochemistry are described in the supporting information.

The racemic major compounds *trans*-**5a** and *anti–trans*-**5b**, were transformed into the corresponding imino derivatives following previously reported procedures.[9] After trying several deprotection methods, the only reaction that afforded the desired products was with TFA, although it gave a sluggish crude from which only 25% yield of pure material could be isolated. Then, reaction with Lawesson's reagent gave thioamides **7**. When (±)-**7a** and -**7b** were stirred with a saturated solution of NH_3 in methanol, concurrent transformation of the ester group into an amide group via ammonolysis was observed. Acidification of the crude product with HCl–CH_3OH gave the corresponding hydrochloride salts (±)-**8**. (Scheme 2).

Biological experiments were conducted to investigate the effects of the new compounds on NO production and enzymatic activity, using a lipopolysaccharide (LPS) and interferon gamma (INF-γ) stimulated mouse peritoneal macrophage model.[10] The results were compared with those obtained with ONO-1714, as a reference drug. As shown in Figure 2, the treatment with LPS (100 ng/mL) and INF-γ (100 ng/mL) markedly increased the production of NO from the basal level, 0.28 to 31.11 μM following 48 h incubation. When cells were simultaneously treated with various concentrations of ONO-1714 and LPS/INF-γ,

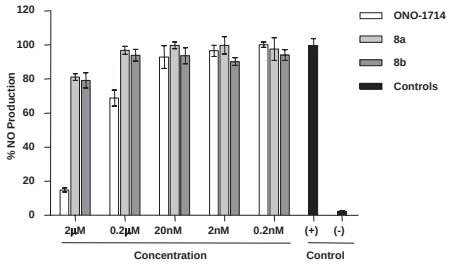


Figure 2. Effect of compounds **8a**, **8b**, and ONO-1714 on nitrite production in primary peritoneal macrophages measured 48 h after activation.

NO production was significantly inhibited in a dose-dependent manner. Over 95% inhibition of NO production was shown at 2 μM ONO-1714 with an apparent IC_{50} of 0.5 μM. But when the cells were cotreated with compounds **8a** and **8b** no significant inhibition was observed.

Finally, inhibitory activities of compounds **8a** and **8b** against iNOS were examined using the ^{3}H-citrulline assay in which the conversion of L-[^{14}H]arginine to L-[^{14}H]citrulline is measured. Compounds **8a** and **8b** were ineffective to inhibit iNOS activity ($IC_{50} > 100$ μM), whereas our results with ONO-1714 ($IC_{50} = 0.011$ μM) are in accordance with data by Kawanaka et al.[4]

In conclusion, new bicyclic [4.1.0] compounds related to iNOS selective inhibitor ONO-1714 have been synthetized and evaluated.[9,11]

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References and Notes

1 E. Culotta, D. E. Koshland, Jr., *Science* **1992**, *258*, 1862.
2 S. Ekmekcioglu, C.-H. Tang, E. A. Grimm, *Curr. Cancer Drug Targets* **2005**, *5*, 103.
3 S. Proskuryakov, A. Konoplyannikov, V. Skvortsov, A. Mandrugin, V. Fedoseev, *Biochemistry (Moscow)* **2005**, *70*, 8.
4 a) Y. Kawanaka, K. Kobayashi, S. Kusuda, T. Tatsumi, M. Murota, T. Nishiyama, K. Hisaichi, A. Fujii, K. Hirai, M. Naka, M. Komeno, Y. Odagaki, H. Nakai, M. Toda, *Bioorg. Med. Chem.* **2003**, *11*, 1723. b) Y. Kawanaka, K. Kobayashi, S. Kusuda, T. Tatsumi, M. Murota, T. Nishiyama, K. Hisaichi, A. Fujii, K. Hirai, M. Naka, M. Komeno, H. Nakai, M. Toda, *Eur. J. Med. Chem.* **2003**, *38*, 277.
5 B. J. Whittle, *IDrugs* **2002**, *5*, 590.
6 D. A. Evans, K. A. Woerpel, M. M. Hinman, M. M. Faul, *J. Am. Chem. Soc.* **1991**, *113*, 726.
7 R. E. Lowenthal, S. Masamune, *Tetrahedron Lett.* **1991**, *32*, 7373.
8 The absolute stereochemistry of the products could not be determined at this stage. The synthesis of enantiomerically pure **5b**, starting from a chiral precursor is underway.
9 D. S. Bredt, H. H. H. W. Schmidt, in *Methods in Nitric Oxide Research*, ed. by M. Feelisch, J. S. Stamler, Chichester, UK. John Wiley & Sons Ltd., **1996**, pp. 249–255.
10 F. Meng, C. A. Lowell, *J. Exp. Med.* **1997**, *185*, 1661.
11 Supporting Information is also available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

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