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A new NF-κB inhibitor based on the amino-epoxyquinol core of DHMEQ

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ABSTRACT

The amino-epoxyquinols **6a** and **6b** were synthesized as soluble derivatives of an NF-κB inhibitor DHMEQ (**1**). In spite of the opposite configuration from **1**, **6b** rather than **6a** affected the deactivation of NF-κB, based on NO secretion and MALDI-TOF MS analysis. It was indicated that **6b** inhibited the activation by different manner from that of **1**.

The NF-κB signaling pathway plays a central role not only in inflammation but also in the development of cancer. Therefore, NF-κB inhibitors are expected to be novel candidates as chemotherapeutic agents for inflammatory and cancer diseases, as well as bioprobes for the characterization of intracellular biological responses and cell function.[1] Over the past decade, a number of structurally diverse small molecules that block the NF-κB signaling pathway, have been identified. Especially, the epoxyquinol class NF-κB inhibitors, such as DHMEQ (**1**),[2] cycloepoxydon (**2**),[3] and panepoxydon (**3**),[4] were found to exhibit remarkable inhibitory activity against NF-κB activation (Fig. 1). In our previous study, we synthesized parasitenone (**4**) carrying the same epoxyquinol moiety as DHMEQ (**1**), and the quinol moiety was shown to be crucial for its inhibitory activity against NF-κB activation.[5] Despite their structural similarity (Fig. 1), all of them showed completely different modes of action in the inhibition of NF-κB activation. Whereas DHMEQ (**1**) exhibits inhibitory activity by covalently binding to the NF-κB components, cycloepoxydon (**2**) and panepoxydon (**3**) show inhibition by interfering with the degradation of IκB-α and activation of IκB kinase (IKK).[3,4] In this study, we synthesized an epoxyquinol derivative carrying the same amide moiety as **1** to examine the structure–activity relationship.

Compound **5**, with a free amine instead of the salicylamide of DHMEQ (**1**), was considered the simplest analog of **1**. However, several attempts to obtain **5** in the pure state have been unsuccessful, probably due to the extremely unstable β-keto enamine structure. In fact, although isolation of MM14201 (Fig. 1), which is a regioisomer of **5**, has been reported, its biological properties have not been discussed due to its instability.[6] Therefore, we prepared the N-protected epoxyquinol **6** and studied their inhibitory activities.

Wipf et al. reported an efficient route for obtaining amino-substituted epoxyquinols in the synthesis of LL-C10037α (Scheme 1).[7] We used their intermediate **11** for synthesis of our desired epoxyquinol derivatives with several improvements. As shown in Scheme 1, the synthesis commenced with protection of 2,5-dimethoxyanilline (**7**) to afford the protected **8**. Oxidation by iodobenzene diacetate under neutral conditions afforded the acetal **9**, which was regioselectively hydrolyzed in AcOH–acetone, yielding the dimethoxyquinone **10** (80% in three steps). Epoxidation of **10** with H_2O_2 and

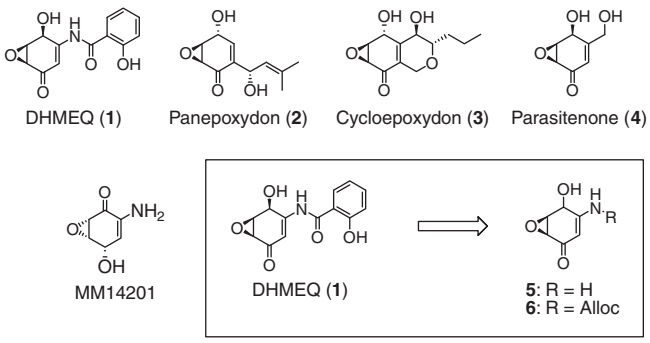

Figure 1. NF-κB inhibitors possessing epoxyquinol moiety.

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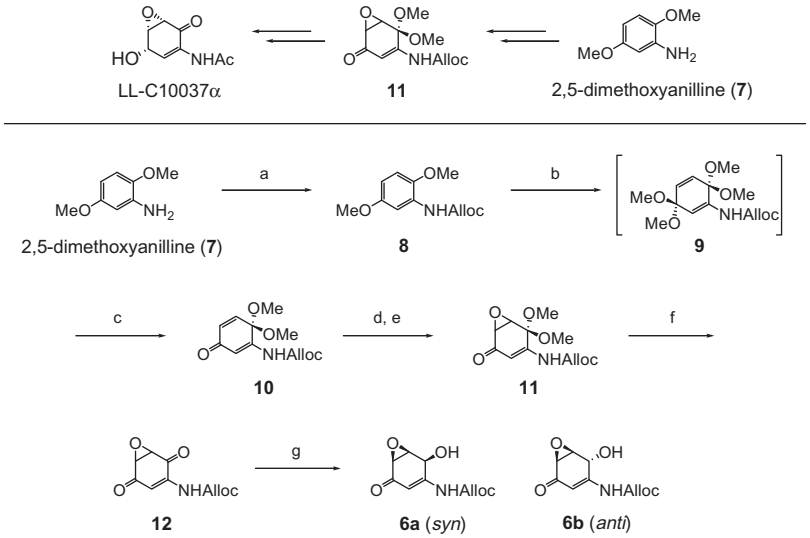
- Wipf's total synthesis of LL-C10037a



Scheme 1. Reagents and conditions: (a) AllocCl, TEA, THF, rt; (b) $PhI(OAc)_2$, $NaHCO_3$, MeOH, rt; (c) AcOH, acetone, rt, 80% in three steps; (d) 30% H_2O_2 aq, 1 M K_2CO_3 aq, THF; (e) $TsOH{\cdot}H_2O$, acetone, 60% in two steps; (f) TFA; (g) $NaBH(OAc)_3$, MeOH, 60% in two steps, **6a**:**6b** = 4:1.

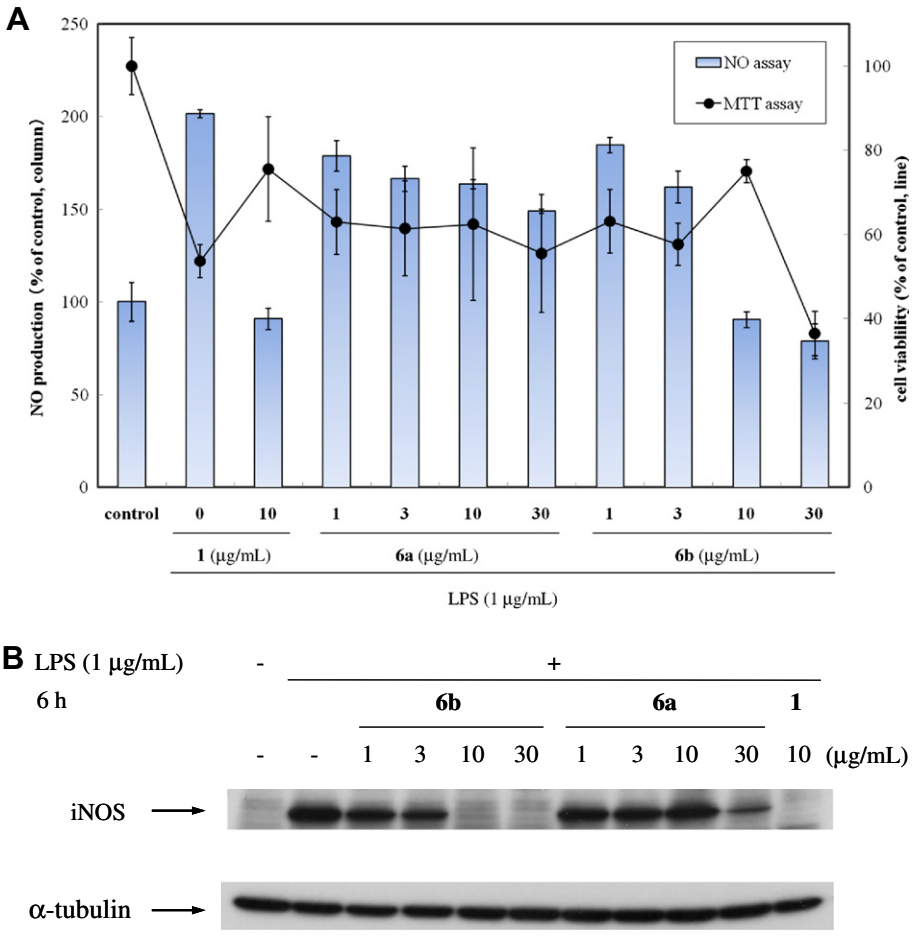


Figure 2. Inhibition of NO secretion and iNOS expression by **6a** and **6b**. (A) Effects on NO secretion in RAW264.7 cells and cell viability. Cells were treated with or without chemicals at indicated concentrations for 1 h, and stimulated with or without 1 μg/mL LPS for 24 h. NO secretion was assessed by Griess reaction. The cell viability was assessed by MTT exclusion. (B) Effects on iNOS expression. Cells were treated with or without **6a** and **6b** at indicated concentrations for 1 h, then stimulated with 1 μg/mL LPS for 6 h. Total cell lysates were subjected to SDS-PAGE and immunoblotted with anti-iNOS antibody. Tubulin was used as a control.

K_2CO_3 in THF gave a 3:2 mixture of **11** and **10**, which on treatment with $TsOH{\cdot}H_2O$ in acetone gave **11** (60% in two steps). During this reaction, the unreacted acetal **10** was smoothly deprotected into the corresponding benzoquinone, whereas **11** remained intact

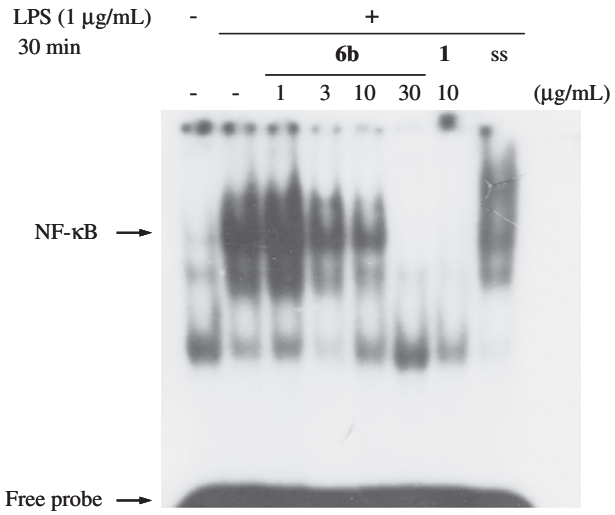


Figure 3. Effects of **6b** on NF-κB activation in RAW264.7 cells. RAW264.7 cells were treated with or without **6b**, then stimulated or not 1 μg/mL LPS for 30 min, and thereafter, total nuclear extracts containing equal amounts of protein (5 μg) were then analyzed by electrophoretic mobility shift assay (EMSA) for DNA binding activity of NF-κB using a ^{32}P-labeled oligonucleotide with the high affinity site 5′-AGTTGAGGGGACTTTCCCAGGC-3′. The inducible NF-κB complex is indicated. A section of the fluorogram from a native gel is shown.

under the reaction conditions. Finally, the dimethoxy acetal of **11** was removed by trifluoroacetic acid, followed by selective reduction of a ketone to afford a mixture of the epoxyquinols[8] (60% in two steps, **6a** (*syn*):**6b** (*anti*) = 4:1).

DHMEQ (**1**) suppressed the lipopolysaccharide (LPS)-induced expression of inflammatory mediators and cytokines, such as iNOS, COX-2, IL-6, and TNF-α, in the mouse macrophage cell line RAW264.7.[9] We evaluated the inhibitory activities of *syn*- and *anti*-derivatives (**6a** and **6b**) carrying the amino-substituted epoxyquinol moiety similar to **1**, against secretion of inflammatory mediators.

Compound **6a**, which has the same configuration as that of **1**, scarcely inhibited NO production at even 30 μg/mL. On the other hand, **6b** dose-dependently reduced NO production; at 30 μg/mL, and showed cellar toxicity (Fig. 2A). Inducible NO synthase (iNOS) is also a major inflammatory mediator contributing to the pathogenesis of cancer and inflammation. As shown in Fig. 2B, **6b** inhibited LPS-induced iNOS expression again more strongly than **6a**. Inhibition of NF-κB by **6b** was confirmed by electrophoretic mobility shift assay (EMSA), as shown in Figure 3. Since **1** is known to bind to NF-κB components, including p65,[10] the binding property of **6a** and **6b** with p65 was evaluated by MALDI-TOF MS analysis (Fig. 4). Compound **6a** showed a mass-shift at 4 equiv mol concentration (**1** effected the mass-shift by addition of 2 equiv mol), whereas no shift was observed in **6b**. It was suggested that **6a** binds weakly and nonspecifically to p65, and **6b** did not bind to p65. Therefore, the mechanism of inhibition should be different from that of **1**.

To examine the effects of chirality, optical resolution of **6b** was attempted. The optical resolution was accomplished with a chiral stationary phase column (Daicel Chiralpak AS) with MeOH as the

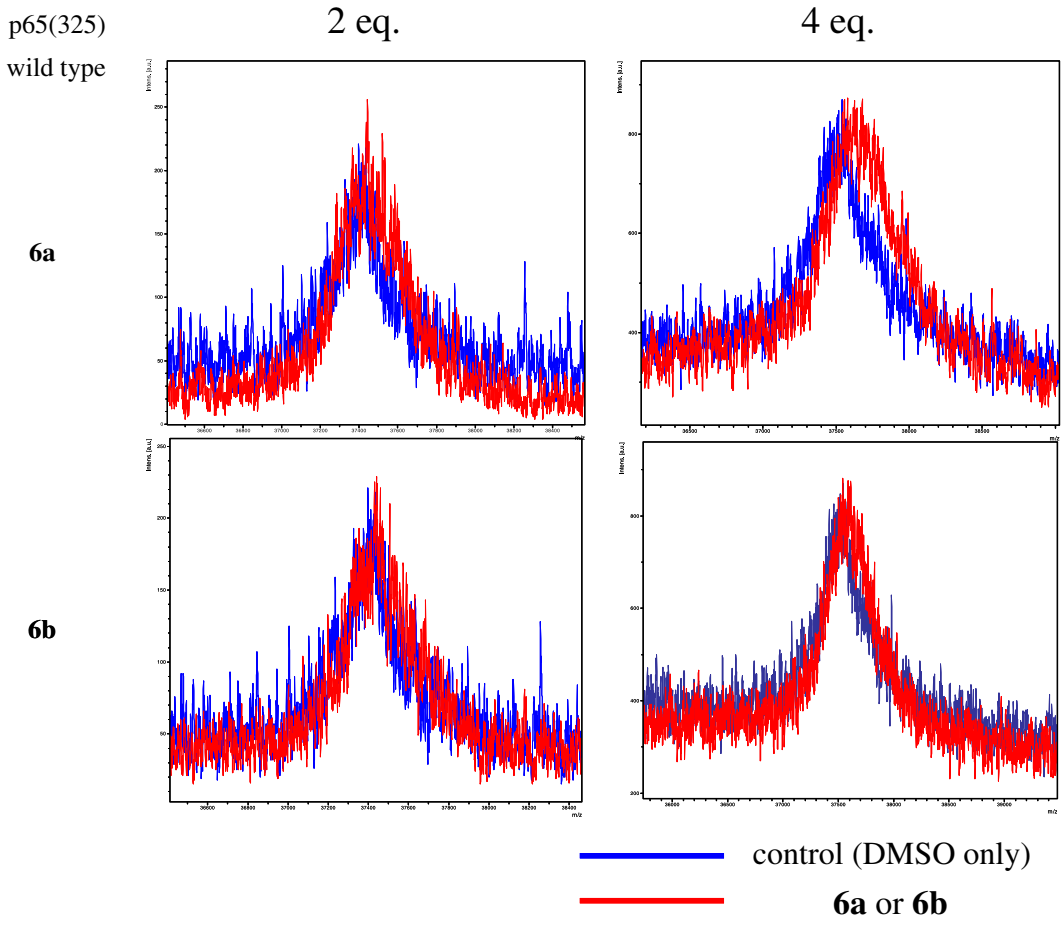


Figure 4. MALDI-TOF MS analysis of p65(1–325) with **6a** and **6b**. Recombinant p65(1–325) was treated with or without 2 or 4 equiv (±)-**6a** and **6b**. The p65(1–325) protein (20 μM) was treated with indicated equivalent of (±)-**6a** or **6b** for 1 h at 4 °C. After incubation, the proteins were used for MALDI-TOF MS analysis.

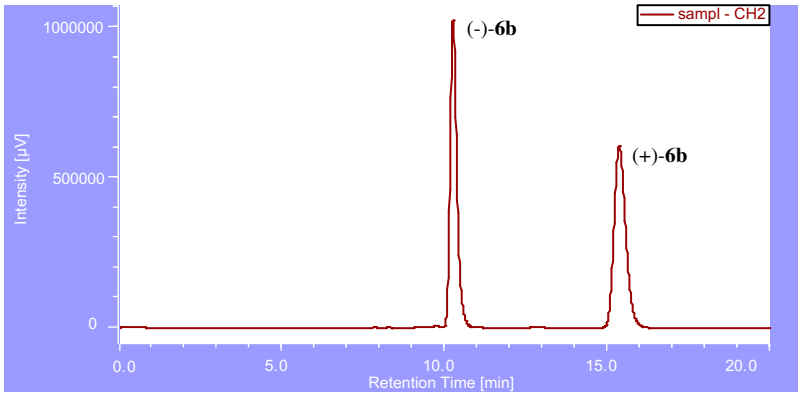


Figure 5. Chiral HPLC profile of **6b**. Column: Daicel Chiralpak AS, column size: 10 mm I.D. 250 mm; eluent: methanol; flow rate: 2.0 mL/min; detection: 274 mm.

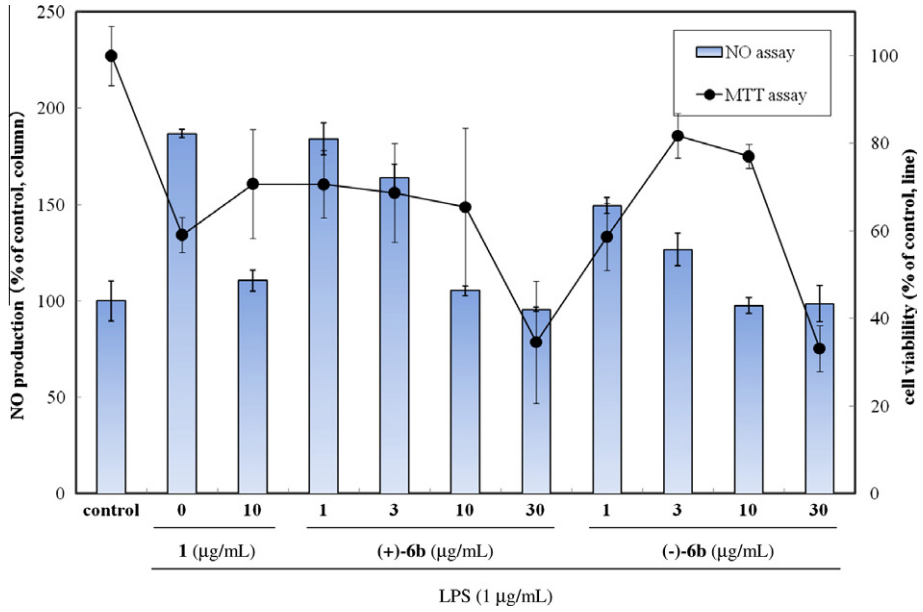


Figure 6. Inhibition of NO secretion by (+)-**6b** and (−)-**6b**. Effects on NO secretion in RAW264.7 cells and cell viability. Cells were treated with or without chemicals at indicated concentrations for 1 h, and stimulated with or without 1 μg/mL LPS for 24 h. NO secretion was assessed by Griess reaction. The cell viability was assessed by MTT exclusion.

eluent. Figure 5 shows the HPLC profile of **6b**. Aliquots of 30 mg of racemic **6b** were added to the chiral column to give 11.8 and 8.5 mg of the corresponding (−)- and (+)-enantiomers. The former showed optical rotation of $[\alpha]_D^{25}$ −103.3 (*c* 1.00, MeCN), while the latter showed optical rotation of $[\alpha]_D^{25}$ +96.5 (*c* 1.00, MeCN). Their enantiomeric purities were >98% ee based on the results of HPLC analyzes. In NO secretion assay of DHMEQ enantiomers, we observed that (−)-**1** was 10-times more effective than (+)-**1**.[11] However, as can be seen in Figure 6, approximately same activity was observed in the case of (+)-**6b** and (−)-**6b**.

In conclusion, we synthesized the N-protected amino-epoxyquinol derivatives **6a** and **6b**, and assessed their inhibitory activities against NF-κB activation. The results of the biological investigation of **6a**, which has the same configuration of the epoxyquinol moiety as that of **1**, indicated that the *syn* relationship of the epoxy-alcohol is crucial for binding to the cysteine residue of NF-κB. Compound **6b** inhibited the NF-κB activation by a different mode of action from **1**, which may inhibit the IKK activity. The *syn*- and *anti*-stereochemistry controlled binding to NF-κB. Furthermore, although DHMEQ (**1**) exhibited remarkable activity, **1** has been applied only in DMSO solution. To inspect biological activity, it is generally necessary to dissolve test samples in various solvents. The high solubility of **6b** in such solvents as MeOH, EtOH, and MeCN will provide further biological information.

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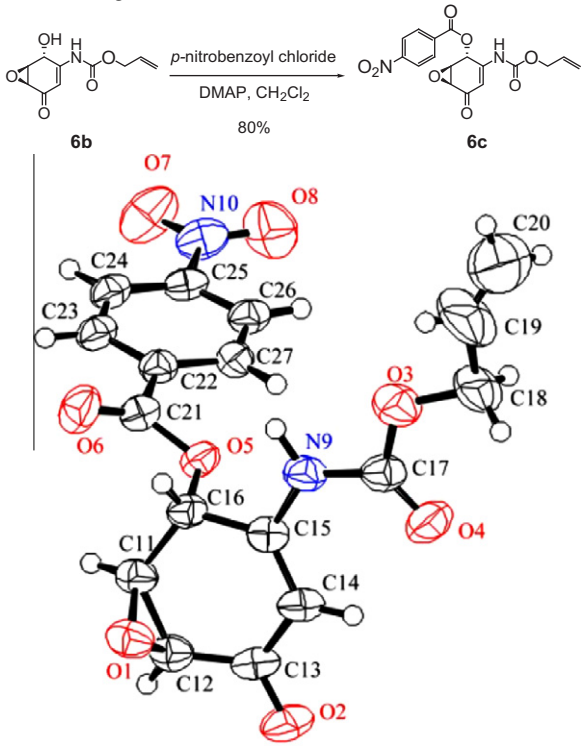
8. (a) *Selected data. Compound* **6a**: HRMS (ESI-MS) calcd for $C_{10}H_{12}NO_5$ $(M+H)^+$ 226.0715, obsd *m*/*z* 226.0708. ^1H NMR ($CDCl_3$): δ 3.18 (br s, 1H), 3.52 (m, 1H), 3.86 (t, 1H, *J* = 2.0 Hz), 4.58 (br s, 1H), 4.64 (d, 2H, *J* = 3.7 Hz), 5.30 (m, 2H), 5.91 (m, 1H), 6.58 (s, 1H), 7.33 (br s, 1H). ^{13}C NMR ($CDCl_3$): δ 53.5, 54.0, 65.4, 66.9, 105.1, 119.4, 131.5, 149.3, 152.2, 192.7. *Compound* **6b**: HRMS (ESI-MS) calcd for $C_{10}H_{12}NO_5$ $(M+H)^+$ 226.0715, obsd *m*/*z* 226.0703. ^1H NMR ($CDCl_3$): δ 3.46 (d, 1H, *J* = 2.3 Hz), 3.81 (dd, 1H, *J* = 0.9, 2.3 Hz), 4.41 (br s, 1H), 4.66 (d, 2H, *J* = 3.7 Hz), 4.99 (s, 1H), 5.30–5.38 (m, 2H), 5.74 (s, 1H), 5.91 (m, 1H), 7.09 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 52.3, 53.3, 63.1, 67.4, 108.9, 119.8, 131.1, 151.3, 152.8, 192.7.
   (b) Relative configuration of **6b**.



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Since the crystals of (±)-**6b** were very thin needles, the *p*-nitrobenzoate derivative **6c** has been prepared and the crystal structure determined by X-ray diffraction method to confirm the *anti* configuration. For **6c**, monoclinic, $P2_1$, a = 17.065(3), b = 14.071(3), c = 7.1425(14) Å, β = 100.352(16)°, V = 1687.2(6) $Å^3$, and Z = 4. The structure of **6c** has been deposited at the Cambridge Crystallographic Data Center and allocated the deposition number CCDC 782800.