Synthesis of Optically Active Methyl 7β-Hydroxykaurenoate with Potent Neuroprotective Activity

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(−)-Methyl 7β-hydroxykaurenoate (3) and its 4-demethyl acetate (−)-4 were both synthesized *via* methods that contained radical cyclization and intramolecular Diels-Alder reactions as key steps. Both compounds displayed potent neuroprotective activity against *N*-methyl-D-aspartate toxicity in cultured cortical neurons.

Key words kaurene diterpenoid; neuroprotective activity; chiral synthesis; radical cyclization

It has been suggested by a number of previous studies[1—4] that excess activation of glutamate receptors causes severe and irreversible damage to the mammalian central nervous system (CNS). Akaike and coworkers have recently reported the isolation of atisane diterpenes, serofendic acids A (**1**) and B (**2**) (Fig. 1), from fetal calf serum and described their potent protective activity in cortical neurons against both nitric oxide donor and glutamate cytotoxicity.[5] These observations prompted us to examine the possible neuroprotective activity of our synthetic compounds that have a variety of bridged skeletons. Here we report that kaurene derivatives act as potential neuroprotective agents.

Among the many compounds that we tested, racemates of 7β-hydroxykaurenoate (**3**)[6] and its demethyl acetate **4**[6] display potent activity for lactate dehydrogenase (LDH)[7] induced by *N*-methyl D-aspartate (NMDA) in cortical cell cultures (Fig. 2). The activity was comparable to that of memantine, which received US FDA approval for the treatment of moderate to severe Alzheimer's disease.

To elucidate further the mechanisms of their biological activity, the corresponding optically active compounds were then synthesized. For the purposes of preparing chiral compounds that have the same absolute configuration as serofendic acids, a suitable chiral precursor was sought. The half ester **5**, which can be readily synthesized in large quantities with high optical purity from the corresponding diester using porcine liver esterase (PLE),[8] was eventually selected. Compound **5**, which has an optical purity of 95% ee, was then treated with isobutene in the presence of concentrated sulfuric acid, followed by reaction with sodium hydroxide in methanol (Chart 1). This generated the *t*-butyl ester **6** in 67% overall yield. A subsequent Grignard reaction and treatment with diluted sulfuric acid produced the lactone **7** in an overall yield of 45%. Optically active (−)-**7** was transformed into the kaurene-type diterpene **3** utilizing the same procedure previously described for the racemate synthesis.[6] The optical purity of product **7** was determined at a later stage.

Introduction of a propargyl group into lactone **7** produced **8** in 92% yield and this product was then subjected to radical cyclization. The desired tricyclic compound **9** was successfully obtained in 93% yield following treatment with silica gel. Reduction of the lactone **9** with lithium aluminum hydride generated the diol product **10** in 97% yield, which was then converted into the corresponding (*R*)-methoxy(trifluoromethyl)phenylacetate **11**, the ^{1}H-NMR spectrum of which indicated an optical purity of 94% ee. This therefore indicated that no racemization occurred during the transformation reactions.

Following acetylation of **10**, the resulting acetate **12**, obtained in 99% yield, was treated with phosphorous oxychloride in pyridine to produce the olefin **13** in 93% yield (Chart 2). This compound was further converted into aldehyde **15** in two steps. The subsequent addition to **15** of an anion[9] produced from 3-triethylsilyloxy-1,4-pentadiene, followed by acetylation, generated a 3 : 1 mixture of two epimeric acetates **16** in 81% overall yield. The *trans*-fused tetracyclic compound **17** was then obtained in an overall yield of 42% by the intramolecular Diels–Alder reaction of the epimeric mixture **16**, conducted in toluene in a sealed tube at 200 °C, followed by treatment with tetrabutylammonium fluoride. An optical purity of 95% ee was further confirmed by the conversion of **17** into **19** through **18**.

A Corey–Chaykovsky reaction[10] of **17** produced the epoxide **20** as a single stereoisomer in 60% yield, which was subsequently converted into the ester **21** in three steps in an

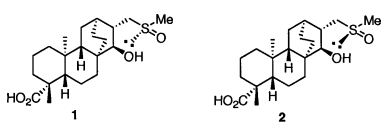


Fig. 1. Structures of Serofendic Acids A (**1**) and B (**2**)

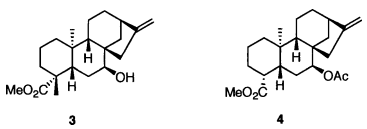
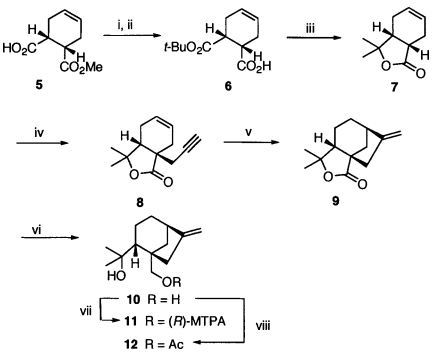


Fig. 2. Structures of Kaurene Derivatives with Potent Neuroprotective Activity



Reagents and conditions: (i) isobutene, concentrated H_2SO_4, Et_2O, room temperature; (ii) NaOH, MeOH, room temperature; (iii) MeMgBr, THF, then 10% H_2SO_4; (iv) LDA, propargyl bromide, HMPA, THF, room temperature; (v) Bu_3SnH, AIBN, benzene, reflux, then SiO_2; (vi) $LiAlH_4$, Et_2O; (vii) (*R*)-MTPACl, DMAP, Et_3N, CH_2Cl_2, room temperature; (viii) Ac_2O, pyridine, room temperature.

Chart 1

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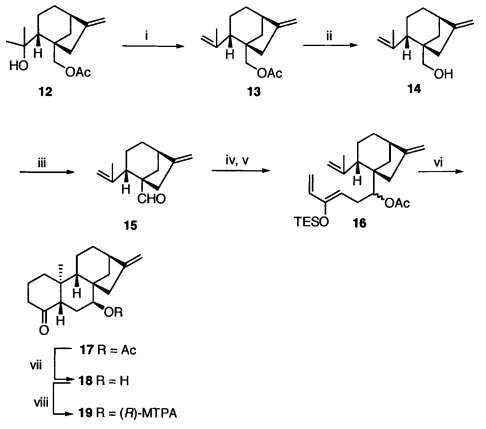
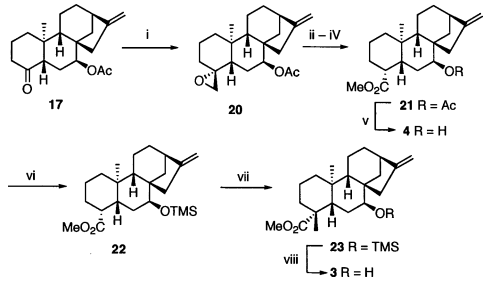


Reagents and conditions: (i) $POCl_3$, pyridine, room temperature; (ii) K_2CO_3, MeOH, room temperature; (iii) Et_3N, DMSO, $SO_3 \cdot Py$, CH_2Cl_2, room temperature; (iv) 3-triethylsilyloxy-1,4-pentadiene, *s*-BuLi, THF, −78 °C; (v) Ac_2O, DMAP, CH_2Cl_2, room temperature; (vi) 200 °C, toluene, then TBAF, THF, room temperature; (vii) K_2CO_3, MeOH, 50 °C; (viii) (*R*)-MTPACl, DMAP, Et_3N, $ClCH_2CH_2Cl$, room temperature.

Chart 2



Reagents and conditions: (i) $Me_3S^+OI^-$, NaH, DMSO, 45 °C; (ii) $BF \cdot OEt_2$, toluene; (iii) $NaClO_2$, KH_2PO_4, 2-methyl-2-butene, *t*-BuOH, H_2O, room temperature; (iv) MeI, DBU, MeCN, room temperature; (v) K_2CO_3, MeOH, 50 °C.; (vi) TMSOTf, 2,6-lutidine, CH_2Cl_2, room temperature; (vii) MeI, LDA, HMPA, THF, −78 °C; (viii) TBAF, THF, room temperature.

Chart 3

overall yield of 62% (Chart 3). Following the exchange of the protecting group for a hydroxyl group *via* (−)-**4**, the resulting silyl ether **22** was methylated to generate **23** in an overall yield of 89% with high diastereoselectivity. Removal of the silyl group of **23** synthesized the compound (−)-**3** in 93% yield.

Evaluation of the synthetic products **3** and **4** for possible neuroprotective activity was then carried out by examining the effects of these kaurene derivatives on NMDA toxicity in cultured cortical neurons. The neuronal cells were exposed to NMDA for 24 h, followed by the addition of the kaurene derivatives to the culture medium 24 h prior to the addition of NMDA. Racemates of both **3** and **4** at a concentration of 10 μM were found to inhibit NMDA toxicity in a dose-dependent manner to 56% and 51% of control levels, respectively, whereas the (−)-**3** and (−)-**4** derivatives inhibited this toxicity completely at the same dosages (Fig. 3). It is noteworthy that the optically active compound showed about twice the activity of the corresponding racemate.

An NMDA glutamate receptor subtype is thought to play a predominant role in triggering glutamate neurotoxicity, but kaurene derivatives did not block [^{3}H]-NMDA binding using rat brain synaptosomes (data not shown). It has been reported that serofendic acid does not block glutamate receptor-mediated currents in cortical neurons, despite its pronounced activity in preventing glutamate neurotoxicity.[11] Based upon these findings, it was speculated that the neuroprotective effects of these compounds do not involve the inhibition of glutamate receptor channel activities. The neuroprotective mechanisms of kaurene derivatives are still unknown. However, from our results showing that compounds (−)-**3** and (−)-**4** displayed more potent neuroprotective effects than the corresponding racemate products, we postulate that a receptor other than the NMDA receptor might be involved in the neuroprotective effects of these molecules.

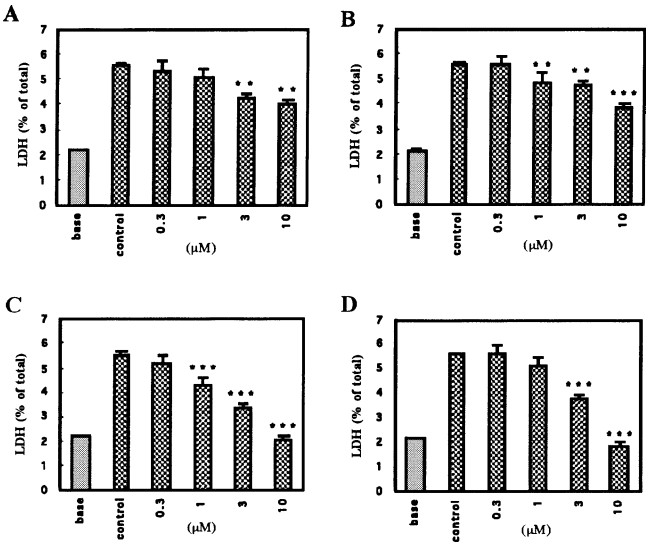


Fig. 3. Effects of Methyl 7β-Hydroxykaurenoate (**3**) and Its Demethyl Acetate **4** on NMDA Toxicity in Cortical Neurons

Lightly shaded columns (base) indicate NMDA-untreated cells and darkly shaded columns indicate NMDA-treated cells. A, racemate of methyl 7β-hydroxykaurenoate (±)-(**3**); B, (±)-**4**; C, (−)-methyl 7β-hydroxykaurenoate (−)-(**3**); D, (−)-**4**. Each value represents the mean±S.E.M. (n=3—6). $^{**}p<0.01$ and $^{***}p<0.001$ *vs.* NMDA-treated control cells. Data were analyzed using ANOVA and Dunnett's multiple comparison methods.

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