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Synthesis and pharmacological profile of serofendic acids A and B

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Abstract—We present efficient syntheses of serofendic acids A and B (SA-A and SA-B), novel neuroprotective substances isolated from fetal calf serum. Biological and pharmacological evaluation showed that SA-A and SA-B have potent protective action against glutamate-induced neurotoxicity, but do not interact directly with glutamate receptors. A pharmacokinetic study showed that they have good oral bioavailability in rats. The results indicate that SA-A and SA-B are potential lead compounds for candidate drugs to treat various neurological disorders.

1. Introduction

L-Glutamate (Glu) is a major excitatory neurotransmitter in the central nervous system (CNS) and plays an important role in neurological processes, including cognition, learning, and memory.[1] Excessive stimulation of glutamate receptors, under pathophysiological conditions, leads to neuronal damage and death. This Glu neurotoxicity is associated with various neurological disorders, including hypoxic–ischemic brain injury,[2] Alzheimer's disease,[3] Huntington's disease, and Parkinson's disease.[4] MK-801 (Dizocilpine) and phencyclidine, typical *N*-methyl-D-aspartate receptor (NMDAR) antagonists, showed potent neuroprotective effects in various neurodegenerative models,[5] but in clinical trials they induced schizophrenia-like symptoms, which were ascribed to the NMDAR-antagonistic activity itself.[6] Therefore, substances which can prevent Glu neurotoxicity without acting directly on NMDARs would be candidate drugs for the treatment of various neurological and neurodegenerative diseases.

Recently, many drug discovery programs have been based on high-throughput screening of huge chemical libraries, including natural products derived from marine sources or plants.[7] We have sought to discover novel neuroprotective substances from animal sources, based on the hypothesis that animals may have intrinsic anti-neurodegenerative mechanisms.

We previously reported serofendic acids A and B (SA-A **1** and SA-B **2**, respectively) (Fig. 1), which were found in a lipophilic fraction of fetal calf serum, as novel neuroprotective substances.[8] These compounds have a unique structure, that is, an atisane-type diterpenoid (15-hydroxy-17-methylsulfinylatisane-19-oic acid) with a sulfoxide group, the epimers of which correspond to SA-A and SA-B. In order to clarify the pharmacological profile of the serofendic acids and their derivatives, we required a synthetic route to provide substantial amounts of the compounds. Here, we report the synthesis and pharmacokinetic profile of SA-A and SA-B and their derivatives.

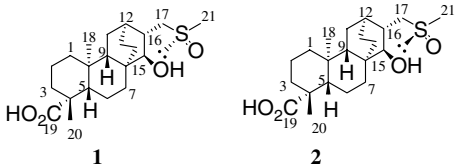

Figure 1. Structure of serofendic acids A (**1**) and B (**2**).

Keywords: Serofendic acid; Neuroprotective; Neurodegenerative disease; Neurotoxicity; Glutamate; Atisane.
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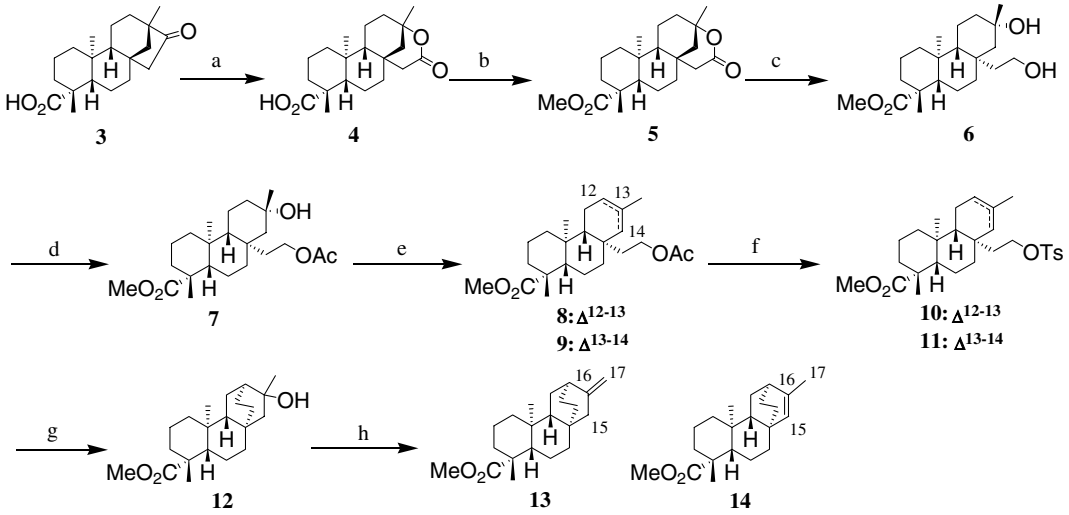
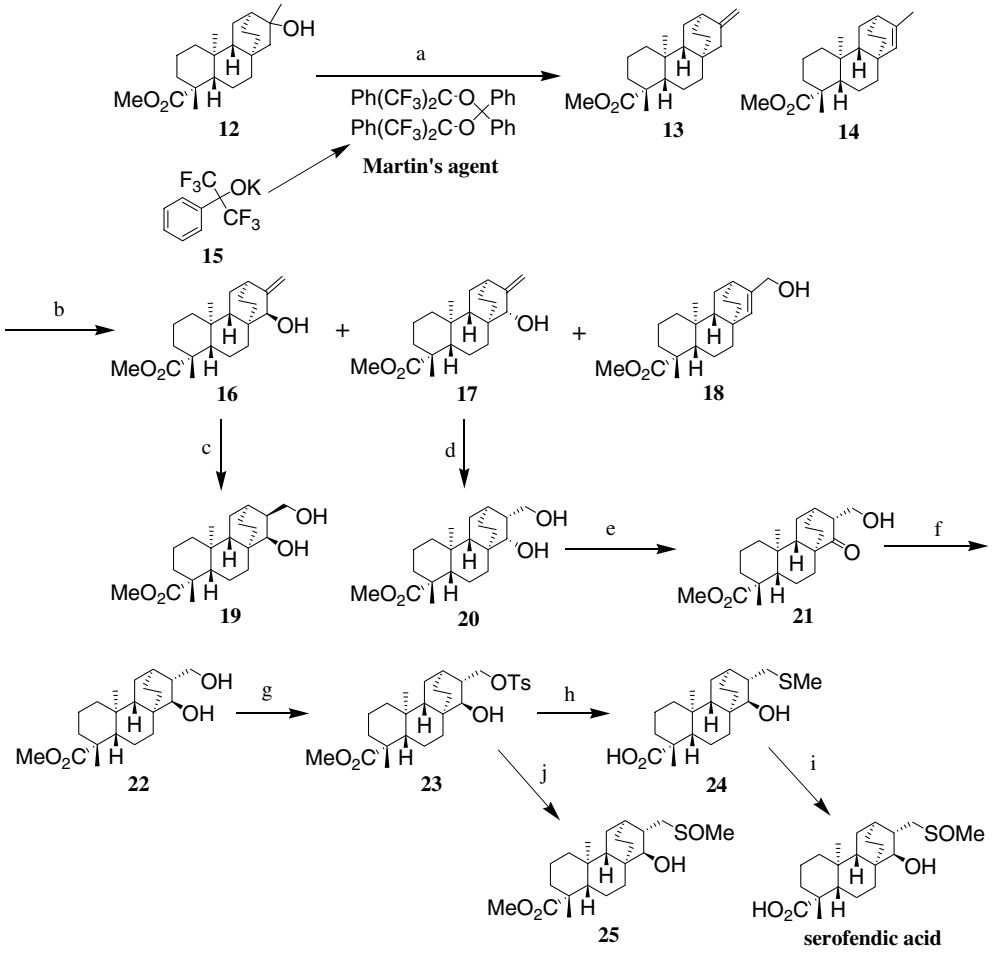
2. Results and discussion

2.1. Chemistry

Our synthetic route is depicted in Schemes 1 and 2.[9] The diterpene skeleton of SA-A and SA-B was derived from commercially available isosteviol (**3**) according to Coates and Bertram's method with some modifications.[10] Isosteviol (**3**) was converted to **4** by Baeyer–Villiger oxidation, followed by esterification and reduction with lithium borohydride to provide the diol **6**. Acetylation of the primary hydroxyl group of **6** gave **7**. Coates reported that dehydration with thionyl chloride–collidine/CH_2Cl_2 afforded the desired olefin **8** and undesired olefin **9** in 2:1 ratio. To increase the selectivity between **8** and **9**, we tried to use only acid. Use of methanesulfonic acid gave a complex reaction mixture that did not include **8** or **9**, but use of TFA gave **8** in a 6:1 ratio over **9**. Hydrolysis of the mixture of **8** and **9** with NaOH/EtOH provided a primary alcohol. The C19 carbomethoxy group remained intact, presumably because of high steric hindrance. Tosylation then gave a mixture of **10** and **11**. Solvolysis of the tosylate mixture, followed by alkaline hydrolysis, gave the cyclized **12** and unchanged **11**. Dehydration of the tertiary alcohol **12** with thionyl chloride/pyridine–CH_2Cl_2 afforded the exo-olefin **13** and endo-olefin **14** in 1:1 ratio (76% yield). Although regioselectivity was not obtained, both isomers might lead to the desired product. Thus, we tried to introduce the functional groups at the C15 and C16 positions of **13** and **14** by means of SeO_2 oxidation followed by hydroboration. Oxidation of **13** gave the corresponding oxidized products, while the reaction of **14** did not proceed at all. This result presumably reflects steric hindrance at C15, which is the primary reaction site for SeO_2. Next, chemoselective dehydration of **12** to **13** was investigated. Satisfactory results were not achieved with TFA or MsCl–collidine (Table 1). It has been reported that 2-methylbicyclo[2.2.1]heptan-2-ol was predominantly dehydrated on the exocyclic double bond with the $(COCl)_2$–DMSO–TEA reagent system.[11] When this condition was examined, we obtained **13** with a modestly improved selectivity (**13**:**14** = 4.5:1, 95% yield). Regarding E2 elimination, it is known that Hofmann elimination tends to be favored over Saytzeff elimination when the leaving group is large or cationic. Based on this insight, dehydration of **12** with Martin's dehydrating agent was conducted.[12] Finally, we found that preparation of the reagent and the dehydration reaction could be conducted in one pot to obtain the olefins with high selectivity (**13**:**14** = 11.5:1) and high yield (93%). Next, treatment of the olefins **13** and **14** with SeO_2 afforded **16**, **17**, and **18**, together with unchanged **14**. Each alcohol was isolated and the stereochemistries of **16** or **17** were determined by means of NOESY experiments (Fig. 2). Although we tried to get the diol **22** by straightforward oxidation, hydroboration of **16** with the same stereochemistry at the C15 position as those of the serefendic acids gave a *syn*-diastereomer **19** as the only product. Similarly, hydroboration of **17** afforded the corresponding *syn*-diastereomer **20**. The relative configuration at C15 and C16 of **19** or **20** was determined based on the coupling constants for H15 and H16 in the ^1H NMR spectra. The results indicated that the borane reagent selectively approached from the opposite side to the secondary alcohol at the C15 position. Since the product obtained by the oxidation reaction did not exhibit the natural configuration, inversion of the secondary alcohol on **20** by the Mitsunobu method (HCO_2H, DEAD, PPh_3, and THF)[13] was investigated. However, the reaction did not proceed at all. Then, we tried to invert the secondary alcohol of **20** by means of an oxidation followed by reduction process. Selective oxidation of the secondary alcohol of the diol **20** was attempted. Use of $NaBrO_3$–$NaHSO_3$[14] gave the keto alcohol **21** in high yield (80%), and this was reduced with $NaB(OAc)_3H$ to provide the diol **22** in 73% yield. Selective tosylation of the resulting diol **22** followed by thiomethylation and simultaneous hydrolysis of the methylester with NaSMe gave the sulfide **24** in 51%



Scheme 1. Reagents and conditions: (a) H_2O_2 aq, H_2SO_4, AcOH, rt, 71 h, 93%; (b) i—$(COCl)_2$, DMF, CH_2Cl_2, rt, 2.5 h; ii—TEA, MeOH, rt, 1 h, 92%; (c) $LiBH_4$, THF, rt, 61 h, 72%; (d) Ac_2O, pyridine, rt, 9 h, 97%; (e) TFA, CH_2Cl_2, rt, 18 h; (f) i—NaOH, EtOH, rt; ii—TsCl, pyridine, rt, 16 h; (g) i—Na_2CO_3, HCO_2H, rt, 16 h; ii—NaOH, EtOH, rt, 3 h, 61% from **7**; (h) dehydrating condition.



Scheme 2. Reagents and conditions: (a) Ph–C$(CF_3)_2$–OK, $(Ph)_2S$, Br_2, CCl_4, rt, 3 h then **12**, $CHCl_3$, rt, 3 h, 93%, **13**:**14** = 11.5:1; (b) SeO_2, *t*-BuOOH, CH_2Cl_2, rt, 15 h, 26% **16**, 28% **17**, 2.9% **18**; (c) BH_3–THF, THF, rt, 4 h then NaOH aq, H_2O_2 aq, rt, 2 h, 80%; (d) BH_3–THF, THF, rt, 4 h then NaOH aq, H_2O_2 aq, rt, 3 h, 91%; (e) $NaBrO_3$, $NaHSO_3$, CH_3CN, H_2O, rt, 3 h, 80%; (f) $NaB(OAc)_3H$, AcOH, CH_3CN, rt, 4 h, 73%; (g) TsCl, DMAP, pyridine, rt, 17 h, 67%; (h) NaSMe, HMPA, 80 °C, 43 h, 76%; i—Davis's oxaziridine (2-benzenesulfonyl-3-phenyloxazirine), $CHCl_3$, 0 °C, 2 h, quant; (j) i—NaSMe, DMF, rt, 2 h; ii—Davis's oxaziridine (2-benzenesulfonyl-3-phenyloxazirine), $CHCl_3$, 0 °C, 1 h, 73%, 2 step.

Table 1. Dehydration of **12**

12 → **13** **14** Condition (equiv.)	**13**:**14**	**Yield (%)**
1 $SOCl_2$ (4.5), pyridine–CH_2Cl_2, rt, 5 min	1:1	76
2 TFA (10), CH_2Cl_2, rt, 3 h	—	0
3 MsCl (3), collidine (10), CH_2Cl_2, −60 °C → rt, 14 h	1.4:1	—[a]
4 DMSO (8), $(COCl)_2$ (4), TEA (12) CH_2Cl_2, −70 °C → rt, 2 h	4.5:1	95
5 Martin's agent (2), CH_2Cl_2, rt, 3 h	11.5:1	93

[a] Yield was not confirmed.

overall yield. Finally, oxidation of the sulfide group in **24** by using Davis's oxaziridine[15] quantitatively yielded SA-A (**1**) and SA-B (**2**) as a 1:2 mixture. After separation of the diastereomers by HPLC, it was confirmed that each synthetic isomer was consistent with the corresponding natural isomer in physico-chemical properties and biological activity.[8] The absolute stereochemistry was estimated by chiral derivatization and HPLC-MS analysis.[9a] X-ray structure determination of SA-A (**1**) or SA-B (**2**) (Fig. 3) confirmed the structures.

The corresponding ester derivatives of the serofendic acids were also prepared.[16] Since the ester group at C4 is resistant to hydrolysis at room temperature, the methylester analogues of serefendic acid (**25**) were obtained by thiomethylation at room temperature, followed by oxidation of the sulfide group in the same way as described for the synthesis of serofendic acid. HPLC separation afforded **25A** and **25B**.

2.2. Neuroprotective activity

Neuroprotective effect of SA-A (**1**) or SA-B (**2**) was evaluated on primary neuronal cultures obtained from the cerebral cortex of fetal rats (17–19 days of gestation).[17] Neurotoxicity was induced by incubating the cells with 500 μM Glu for 10 min. Under this condition, MK-801 (1 μM) completely blocked the Glu-induced neurotoxicity (data not shown). As shown in Figure 4, incubation of the cells with SA-A (**1**) or SA-B (**2**) for 1 h before, 10 min during, and 24 h after Glu exposure had a protective effect. In order to identify the mechanism of action, we employed a panel assay with 76 receptors and ion channels, including NMDAR and

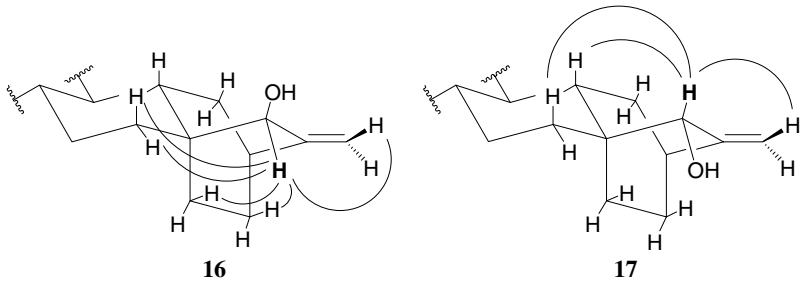


Figure 2. Protons of **16** and **17** were assigned with COSY spectra, NOESY spectra, TOCSY spectra, HMQC spectra, and HMBC spectrum. The key NOEs were observed in the NOESY spectra which indicate the C15 stereochemistry as shown.

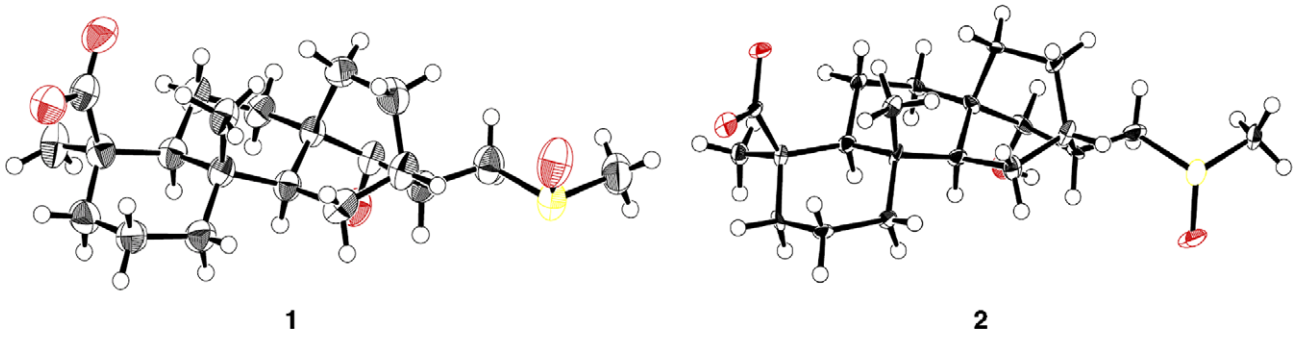


Figure 3. Single crystal X-ray structure determination of serofendic acids A (**1**) and B (**2**).

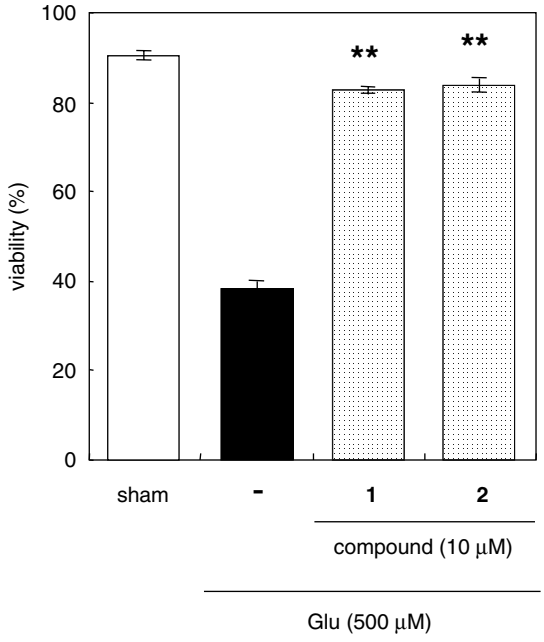


Figure 4. Protective effect of synthetic serofendic acids against Glu neurotoxicity in cultured cortical neurons. Cultures were exposed to Glu (500 μM) for 10 min and then incubated in glutamate-free medium for 24 h. Cultures were treated with serofendic acid for 1 h before, 10 min during, and 24 h after Glu exposure. $^{**}P < 0.01$, compared with Glu alone.

α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor (AMPAR). The serofendic acids had no effect on any of them up to 10 μM. Next, the effect of the serofendic acids on neurotoxicity induced by nitric oxide or oxidative stress was investigated. [8,18] The neuroprotective effect was found to be as potent as that against Glu neurotoxicity. Further, it has been reported that the serofendic acids protect cardiac myocytes against oxidant-induced cell death,[19] and prevent the loss of mitochondrial membrane potential and the activation of caspase-3 induced by Glu exposure in rat cultured cortical cells.[20] Since nitric oxide or oxidative stress activates caspase-3[21] and activated caspase-3 induces over-release of Glu in synapses,[22] the serofendic acids might act at some point prior to over-activation of glutamate receptors, for example, by protecting the functional integrity of mitochondria. Detailed investigation of the mechanism of action is ongoing.

2.3. Pharmacokinetics

Since the serofendic acids showed attractive in vitro biological activity, an in vivo study was conducted. Table 2 shows the pharmacokinetic parameters of SA-A (**1**), SA-B (**2**), and the methylester analogue **25** after intravenous administration at a dose of 1 mg/kg in rats. The AUC (area under the concentration–time curve) value of **2** was 1.4 times higher than that of **1**. The stereochemistry of the sulfoxide moiety thus influenced the pharmacokinetic profile. Although biological conversion of sulfoxide isomers might occur via a reduction–oxidation process, in fact, interconversion between **1** and **2** was not observed when either isomer was administered. The methylester analogue **25**, a mixture of isomers, exhibited a relatively high clearance value and low AUC value in a preliminary study, presumably reflecting its greater lipophilicity. In addition, the serofendic acids were detected as metabolites (AUC value: 0.08 μg h/ml).

Table 2. Pharmacokinetic result in rats for intravenous administration of serofendic acids (**1**, **2**) and methylester analogue (**25**) (1 mg/kg each)

Compound	$T_{1/2}$ (h)	V_{dss} (l/kg)	Cl total (l/h/kg)	AUC (μg h/ml)	B/P 0.5 h	B/P 2 h
1 (1 mg/kg iv)	0.48	0.33	0.52	1.95	N.T	N.T
2 (1 mg/kg iv)	0.65	0.33	0.36	2.78	0.021	N.D
25 (1 mg/kg iv)	0.55	0.57	0.88	1.44	2.20	2.21

N.T, not tested; N.D, no detected.

Table 3. Pharmacokinetic result in rats for oral administration of serofendic acid B (**2**) (5 mg/kg)

Compound	T_{max} (h)	$T_{1/2}$ (h)	C_{max} (μg/ml)	AUC (μg h/ml)	B.A. (%)
2 (5 mg/kg po)	0.33	3.90	2.05	5.64	41

Brain concentrations of the compounds were measured at 0.5 h and 2 h after administration. While the methylester analogue showed a high brain-to-plasma (B/P) concentration ratio, serofendic acid (only isomer B was measured) had a very low B/P value. We speculate that the carboxylic group of serofendic acid might be the main cause of the low permeability into the brain. A pharmacokinetic study of serofendic acid B (**2**) in rats after oral administration at a dose of 5 mg/kg (Table 3) showed relatively good oral bioavailability (B.A.). On the other hand, compound **25** was not detected in plasma after oral administration at a dose of 5 mg/kg (data not shown). These data indicate that the serofendic acids and **25** may be ineffective in the clinical context. Work to improve the brain penetration or oral bioavailability by modification of the serofendic acids or **25** is ongoing.

3. Conclusion

We have developed an efficient synthesis of serofendic acids A and B, novel neuroprotective substances isolated from fetal calf serum. Biological and pharmacological evaluation indicated that the serofendic acids exhibited a potent protective action against neurotoxicity induced by glutamate, but this did not involve direct interaction with glutamate receptors. A pharmacokinetic study showed good oral bioavailability, but low concentration in the brain. In contrast, the methylester analogues exhibited high concentrations in the brain. Single crystal X-ray diffraction studies of the serofendic acids supported the stereochemistry of the sulfoxide group indicated by NMR spectroscopic analysis. SA-A and SA-B appear to be potential lead compounds for candidate drugs to treat various neurological disorders.

4. Experimental

4.1. Chemistry

Melting points were taken with a Yanaco MP-S3. The infrared absorption spectra were obtained on an FT/IR-620 spectrometer (Jasco, Tokyo). The following NMR spectra were recorded on a Varian Unity 400, a Varian Unity INOVA 500, or a JEOL JNM-α600 spectrometer: ^{1}H NMR, ^{13}C NMR, COSY, NOESY, TOCSY, HMQC, and HMBC. Chemical shifts are given in ppm (δ) from tetramethylsilane as an internal standard and coupling constants are given in Hz (J). When CD_3OD was used as the NMR solvent, chemical shifts were referred to the solvent peaks: δ_H 3.35 for CD_2HOD and δ_C 49.0 for CD_3OD. Column chromatography was performed on Fuji Silysia BW silica gel (200–400 mesh). TLC was carried out on silica gel Merck $60F_{254}$. Reagents and solvents were purchased and used without further purification. HPLC separation was performed with a Biotage Parallex Flex or a MDA-0054 (Waters) apparatus. Electrospray ionization (ESI) MS were recorded on a Thermo Fisher Scientific SSQ7000 mass spectrometer. ESI-HRMS spectra were recorded with a Q-TOF Ultima Global Alliance 2695 (Waters) and API-III plus mass spectrometer (Applied Biosystems). Specific rotations were measured on a JASCO DIP-1000 digital polarimeter.

4.1.1. (1*R*,4*S*,5*R*,9*S*,10*R*,13*S*)-5,9,13-Trimethyl-15-oxo-14-oxatetracyclo[11.3.1.0^{1,10}.0^{4,9}]heptadecane-5-carboxylic acid (4). To a solution of isosteviol (30 g, 94.1 mmol) in AcOH (315 ml) and H_2SO_4 (5.25 ml) was added dropwise a 30% hydrogen peroxide solution (105 ml) over 30 min at 0 °C. The reaction mixture was then stirred for 71 h at room temperature and cooled with a dry-ice ethanol bath (ca. −40 °C). Saturated aq $Na_2S_2O_3$ was slowly added, while the temperature was kept below 20 °C. The disappearance of peroxide was confirmed with potassium iodide-starch paper, then the mixture was diluted with water (2000 ml) and extracted with Et_2O (700 ml ×2). The organic layer was washed with brine (300 ml), dried over $MgSO_4$, and evaporated under reduced pressure. The resulting solid was washed with *n*-heptane/Et_2O (4:1, v/v, 400 ml) and collected by filtration to afford **4** (29.1 g, 93%) as a white solid. mp 268–270 °C. $[\alpha]_D^{26}$ −50.90 (*c* 1.007, $CHCl_3$). ESI-MS *m*/*z* 357 $(M+Na)^+$. ^{1}H NMR ($CDCl_3$): δ 0.83–1.52 (m, 9H), 0.87 (s, 3H), 1.25 (s, 3H), 1.35 (s, 3H), 1.53–1.62 (m, 2H), 1.66–1.75 (m, 1H), 1.76–2.02 (m, 5H), 2.05 (d, J = 18.4 Hz, 1H), 2.18 (broad d, J = 13.6 Hz, 1H), 3.12 (dd, J = 18.8, 2.8 Hz, 1H). Anal. Calcd for $C_{20}H_{30}O_4$ (334.2144): C, 71.82; H, 9.04. Found: C, 71.62; H, 8.90.

4.1.2. Methyl (1*R*,4*S*,5*R*,9*S*,10*R*,13*S*)-5,9,13-Trimethyl-15-oxo-14-oxatetracyclo [11.3.1.0^{1,10}.0^{4,9}] heptadecane-5-carboxylate (5). Oxalyl chloride (22.4 ml, 261 mmol) was added to a solution of **4** (29.1 g, 87 mmol) in CH_2Cl_2

(426 ml) and DMF (2.8 ml) at 0 °C. The mixture was at room temperature for 2.5 h, then cooled to 0 °C again, and MeOH (71 ml) was added, followed by triethylamine (60.6 ml, 436 mmol). The reaction mixture was stirred for 1 h at room temperature, solvent was evaporated under reduced pressure, and water (500 ml) was added. The mixture was extracted with EtOAc (400 ml ×2), and the organic layer was washed with brine (250 ml), dried over $MgSO_4$, and evaporated under reduced pressure. The resulting solid was washed with MeOH (300 ml) and collected by filtration to afford **5** (27.8 g, 92%) as a white solid. mp 206–207 °C. $[\alpha]_D^{26}$ −40.90 (*c* 1.392, $CHCl_3$). ESI-MS *m/z* 371 $(M+Na)^+$. ^1H NMR ($CDCl_3$): δ 0.76 (s, 3H), 0.81–0.91 (m, 1H), 0.94 (dd, $J = 12.4$, 3.0 Hz, 1H), 1.01 (td, $J = 13.6$, 4.4 Hz, 1H), 1.08 (dd, $J = 11.8$, 2.8 Hz, 1H), 1.18 (s, 3H), 1.19–1.32 (m, 2H), 1.35 (s, 3H), 1.35–1.50 (m, 3H), 1.52–1.63 (m, 2H), 1.65–1.94 (m, 5H), 1.95–2.03 (m, 1H), 2.04 (d, $J = 18.8$ Hz, 1H), 2.18 (broad d, $J = 13.6$ Hz, 1H), 3.08 (dd, $J = 18.8$, 2.6 Hz, 1H), 3.63 (s, 3H). Anal. Calcd for $C_{21}H_{32}O_4$ (348.2300): C, 72.38; H, 9.26. Found: C, 72.32; H, 9.17.

4.1.3. Methyl (5β,8α,9β,10α,13α)-13-Hydroxy-8-(2-hydroxyethyl)-13-methylpodocarpan-15-oate (6). $LiBH_4$ (6.52 g, 299 mmol) was added to a solution of **5** (17.4 g, 49.9 mmol) in THF (250 ml) at 0 °C. The reaction mixture was stirred for 61 h at room temperature, and then water (300 ml) was added at 0 °C. The mixture was stirred for 3 h at room temperature, then extracted with EtOAc (300 ml ×2), and the organic layer was washed with brine (200 ml), dried over $MgSO_4$, and evaporated under reduced pressure. The resulting solid was washed with *n*-heptane/Et_2O (1:2, v/v, 200 ml) to afford **6** (12.7 g, 72%) as a white solid. mp 213–214 °C. $[\alpha]_D^{26}$ −26.63 (*c* 0.815, $CHCl_3$). ESI-MS *m/z* 375 $(M+Na)^+$. ^1H NMR ($CDCl_3$): δ 0.68 (s, 3H), 0.74–0.94 (m, 4H), 1.01 (td, $J = 13.6$, 4.4 Hz, 1H), 1.09 (dd, $J = 12.0$, 3.2 Hz, 1H), 1.16 (s, 3H), 1.17 (s, 3H), 1.36 (td, $J = 13.2$, 4.4 Hz, 1H), 1.40–1.52 (m, 2H), 1.53–1.94 (m, 8H), 1.98 (dd, $J = 14.8$, 2.4, 1H), 2.10–2.20 (m, 2H), 3.63 (s, 3H), 3.69–3.79 (m, 2H). Anal. Calcd for $C_{21}H_{36}O_4$ (352.2613): C, 71.56; H, 10.29. Found: C, 71.27; H, 10.18.

4.1.4. Methyl (5β,8α,9β,10α,13α)-8-(2-Acetoxyethyl)-13-hydroxy-13-methylpodocarpan-15-oate (7). According to the procedure reported by Coates, **6** (27.3 g, 77.4 mmol) was converted to **7** (29.5 g, 97%, white solid). mp 145–147 °C. $[\alpha]_D^{26}$ −37.21 (*c* 0.088, $CHCl_3$). ESI-MS *m/z* 417 $(M+Na)^+$. ^1H NMR ($CDCl_3$): δ 0.71 (s, 3H), 0.74–0.95 (m, 4H), 1.01 (td, $J = 13.6$, 4.4 Hz, 1H), 1.09 (dd, $J = 12.2$, 2.6 Hz, 1H), 1.16 (s, 3H), 1.17 (s, 3H), 1.33 (td, $J = 13.6$, 4.4 Hz, 1H), 1.40–1.65 (m, 3H), 1.69–1.98 (m, 8H), 2.05 (s, 3H), 2.15 (broad d, $J = 13.6$ Hz, 1H), 2.26–2.36 (m, 1H), 3.65 (s, 3H), 4.03 (td, $J = 10.0$, 5.6 Hz, 1H), 4.34 (td, $J = 10.0$, 5.6 Hz, 1H). Anal. Calcd for $C_{23}H_{38}O_5$ (394.2719): C, 70.02; H, 9.71. Found: C, 69.97; H, 9.66.

4.1.5. Methyl (5β,8α,9β,10α)-8-(2-Acetoxyethyl)-13-methylpodocarp-12-en-15-oate (8) and methyl (5β,8α,9β,10α)-8-(2-acetoxyethyl)-13-methylpodocarp-13-en-15-oate (9). TFA (58 ml) was added dropwise to a solution of **7** (29.5 g, 74.8 mmol) in CH_2Cl_2 (300 ml) over 30 min at 0 °C. The reaction mixture was stirred for 18 h at room temperature and then evaporated to half the initial volume under reduced pressure. Water (500 ml) was added, and the mixture was extracted with Et_2O (300 ml ×2). The organic layer was washed with water (200 ml), saturated aq $NaHCO_3$ (200 ml), and brine (200 ml), dried over $MgSO_4$, and evaporated under reduced pressure to afford a mixture of **8** and **9** as a crude light yellow oil (31.8 g, 100%). The ratio of **8** and **9** was determined from the olefin proton NMR peak integration value, and was found to be 6:1. HRESI(+)MS calcd for $C_{23}H_{36}NaO_4$ $(M+Na)^+$ 399.2511; found: 399.2538. ^1H NMR ($CDCl_3$): δ 0.68 (s, 3H, for **9**), 0.72 (s 3H, for **8**), 0.78–2.24 (m, 23H), 1.17 (s, 3H, for **9**), 1.18 (s 3H, for **8**), 2.04 (s, 3H), 3.65 (s, 3H), 3.86–3.98 (m, 1H), 4.0–4.14 (m, 1H), 5.08 (broad s, 1H, for **9**), 5.35 (broad s, 1H, for **8**).

4.1.6. Methyl (5β,8α,9β,10α,12α)-16-Hydroxyatisan-19-oate (12). According to the procedure reported by Coates, the crude mixture of **8** and **9** (34.3 g) was converted to diastereomeric **12** (15.9 g, 61% from **7**, white solid), from which the diastereomers **12-A** and **12-B** were slightly separated by column chromatography on silica gel with *n*-heptane/EtOAc (100:0 to 6:1, v/v) as an eluent. **12-A**: mp 139–141 °C. $[\alpha]_D^{26}$ −51.76 (*c* 0.701, $CHCl_3$). ESI-MS *m/z* 357 $(M+Na)^+$ ^1H NMR ($CDCl_3$): δ 0.77 (s, 3H), 0.78–1.90 (m, 19H), 1.17 (s, 3H), 1.28 (s, 3H), 1.92–2.04 (m, 1H), 2.16 (broad d, $J = 12.4$ Hz, 1H), 3.64 (s, 3H). Anal. Calcd for $C_{21}H_{34}O_3$ (334.2507): C, 75.41; H, 10.25. Found: C, 75.30; H, 10.14. **12-B**: mp 148–150 °C. $[\alpha]_D^{26}$ −36.73 (*c* 0.802, $CHCl_3$). ESI-MS *m/z* 357 $(M+Na)^+$. ^1H NMR ($CDCl_3$): δ 0.78 (s, 3H), 0.79–0.89 (m, 1H), 0.90–1.09 (m, 5H), 1.17 (s, 3H), 1.17–1.69 (m, 9H), 1.28 (s, 3H), 1.69–1.76 (m, 2H), 1.76–1.91 (m, 2H), 1.96–2.07 (m, 1H), 2.16 (broad d, $J = 13.2$ Hz, 1H), 3.64 (s, 3H). Anal. Calcd for $C_{21}H_{34}O_3$ (334.2507): C, 75.41; H, 10.25. Found: C, 75.35; H, 10.14.

4.1.7. Methyl (5β,8α,9β,10α,12α)-Atis-16-en-19-oate (13). Br_2 (2.63 ml, 51.4 mmol) was added dropwise to a solution of potassium 1,1,1,3,3,3-hexafluoro-2-phenyl-2-propoxide (29 g, 103 mmol)[12a] and diphenyl sulfide (8.56 ml, 51.4 mmol) in CCl_4 (200 ml) over 10 min at room temperature. The reaction mixture was stirred for 3 h at rt, then cooled with an ice-NaCl bath (ca. −15 °C), and a solution of diastereomeric **12** (8 g, 23.9 mmol) in $CHCl_3$ (120 ml) was added dropwise for 15 min. The reaction mixture was stirred for 3 h at room temperature and then evaporated under reduced pressure. The mixture was diluted with Et_2O (300 ml), washed with 5 N aq NaOH (100 ml ×2), water (100 ml), saturated aq NH_4Cl (100 ml), and brine (100 ml), dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with *n*-heptane/Et_2O (100:0 to 25:1, v/v) as an eluent to afford **13** (7.05 g, 93%) as a white solid. The resulting **13** contained **14** in ca. 11.5:1 ratio, as determined from the olefin proton NMR peak integration values. HRESI(+)MS calcd for $C_{21}H_{32}NaO_2$ $(M+Na)^+$ 339.2300; found: 339.2343. ^1H NMR ($CDCl_3$): δ 0.78 (s, 3H), 0.84 (td, $J = 13.6$,

4.0 Hz, 1H), 0.93–1.16 (m, 5H), 1.18 (s, 3H), 1.34–1.65 (m, 7H), 1.67–2.00 (m, 5H), 2.04 (dt, J = 16.8, 2.0 Hz, 1H), 2.13–2.20 (m, 1H), 2.22 (quintet, J = 3.0 Hz, 1H), 3.65 (s, 3H), 4.57 (q, J = 2.0 Hz, 1H), 4.73 (q, J = 2.0 Hz, 1H), 5.58 (broad t, J = 1.8 Hz, 1 H for **14**).

4.1.8. Methyl (5β,8α,9β,10α,12α,15β)-15-Hydroxyatis-16-en-19-oate (16) and methyl (5β,8α,9β,10α,12α,15α)-15-hydroxyatis-16-en-19-oate (17) and methyl (5β,8α,9β,10α,12α)-17-hydroxyatis-15-en-19-oate (18). To a suspension of SeO_2 (246 mg, 2.22 mmol) in CH_2Cl_2 was added 70% aq *tert*-butyl hydroperoxide (9.21 ml, 66.6 mmol) at room temperature. The mixture was stirred for 15 min at room temperature, then cooled to 0 °C, and **13** (7.01 g, 22.2 mmol) was added. Stirring was continued for 15 h at room temperature, then the mixture was cooled to 0 °C, and saturated aq $Na_2S_2O_3$ (200 ml) was added. The whole was extracted with EtOAc (200 ml ×2), and the organic layer was washed with brine (200 ml), dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with *n*-heptane/EtOAc (20:1 to 8:1, v/v) to afford **16** (1.9 g, 26%, white solid), **17** (2.04 g, 28%, white solid), and **18** (217 mg, 3%, white solid). Compound **16**: mp 194–195 °C. $[\alpha]_D^{26}$ −43.54 (c 0.790, $CHCl_3$). ESI-MS m/z 355 $(M+Na)^+$. ^1H NMR ($CDCl_3$): δ 0.81 (s, 3H), 0.81–1.09 (m, 4H), 1.14–1.21 (m, 1H), 1.18 (s, 3H), 1.32–1.45 (m, 3H), 1.45–1.60 (m, 4H), 1.64–1.89 (m, 4H), 1.97–2.07 (m, 1H), 2.12–2.20 (m, 1H), 2.27–2.33 (m, 1H), 3.59 (dt, J = 5.6, 2.0 Hz, 1H), 3.65 (s, 3H), 5.00 (broad t, J = 1.6 Hz, 1H), 5.06 (broad t, J = 1.6 Hz, 1H). Anal. Calcd for $C_{21}H_{32}O_3$ (332.2351): C, 75.86; H, 9.70. Found: C, 75.74; H, 9.66. Compound **17**: mp 134–135 °C. $[\alpha]_D^{26}$ −57.39 (c 0.905, $CHCl_3$). ESI-MS m/z 355 $(M+Na)^+$. ^1H NMR ($CDCl_3$): δ 0.80–0.91 (m, 1H), 0.83 (s, 3H), 0.93–1.15 (m, 4H), 1.19 (s, 3H), 1.33–1.66 (m, 7H), 1.66–1.90 (m, 5H), 2.14–2.21 (m, 1H), 2.26–2.31 (m, 1H), 3.58–3.62 (m, 1H), 3.65 (s, 3H), 4.98 (broad t, J = 1.6 Hz, 1H), 5.05 (broad t, J = 1.6 Hz, 1H). Anal. Calcd for $C_{21}H_{32}O_3$ (332.2351): C, 75.86; H, 9.70. Found: C, 75.89; H, 9.66. Compound **18**: mp 97–99 °C. $[\alpha]_D^{26}$ −61.62 (c 0.472, $CHCl_3$). ESI-MS m/z 355 $(M+Na)^+$. HRESI(+)MS calcd for $C_{21}H_{32}NaO_3$ $(M+Na)^+$ 355.2249; found: 355.2202. ^1H NMR ($CDCl_3$): δ 0.74–0.91 (m, 2H), 0.80 (s, 3H), 0.95–1.11 (m, 3H), 1.15–1.64 (m, 7H), 1.19 (s, 3H), 1.71–1.93 (m, 4H), 2.00 (dt, J = 9.6, 3.2 Hz, 1H), 2.11–2.20 (m, 1H), 2.42–2.48 (m, 1H), 3.65 (s, 3H), 4.13 (d, J = 1.6 Hz, 2H), 5.83 (s, 1H).

4.1.9. Methyl (5β,8α,9β,10α,12α,15β,16β)-15,17-Dihydroxyatisan-19-oate (19). Diborane (1 M in THF, 18.1 ml, 18.1 mmol) was added to a solution of **16** (2 g, 6.02 mmol) in THF (60 ml) at 0 °C. The reaction mixture was stirred for 4 h at room temperature and then cooled to 0 °C again. Water (9 ml), 2 N aq NaOH (20 ml), and 30% aq H_2O_2 (18 ml) were added, and the mixture was stirred for 2 h at room temperature. Saturated aq $Na_2S_2O_3$ (100 ml) was added, the mixture was extracted with EtOAc (100 ml ×2), and the organic layer was washed with brine (100 ml), dried over $MgSO_4$, and evaporated under reduced pressure.The resulting solid was washed with *n*-heptane/Et_2O (2:1, v/v, 100 ml) and collected by filtration to afford **19** (1.68 g, 80%) as a white solid. mp 185–187 °C. $[\alpha]_D^{26}$ −3.86 (c 0.609, $CHCl_3$). ESI-MS m/z 373 $(M+Na)^+$. HRESI(+)MS calcd for $C_{21}H_{34}NaO_4$ $(M+Na)^+$ 373.2355; found: 373.2355. ^1H NMR (CD_3OD): δ 0.83 (s, 3H), 0.84–0.99 (m, 2H), 0.99–1.15 (m, 4H), 1.17 (s, 3H), 1.34–1.44 (m, 1H), 1.44–1.55 (m, 2H), 1.55–1.71 (m, 5H), 1.73–2.04 (m, 5H), 2.14 (broad d, J = 12.8 Hz, 1H), 3.40 (d, J = 9.2 Hz, 1H), 3.57 (dd, J = 10.4, 7.8 Hz, 1H), 3.63 (s, 3H), 3.88 (dd, J = 10.4, 7.6 Hz, 1H).

4.1.10. Methyl (5β,8α,9β,10α,12α,15α)-15,17-Dihydroxyatisan-19-oate (20). Diborane (1.1 M in THF, 16.7 ml, 18.4 mmol) was added to a solution of **17** (2.04 g, 6.14 mmol) in THF (60 ml) at 0 °C. The reaction mixture was stirred for 4 h at room temperature and then cooled to 0 °C again. Water (10 ml), 5 N aq NaOH (15 ml), and 30% aq H_2O_2 (20 ml) were added, and the mixture was stirred for 3 h at room temperature. Saturated aq $Na_2S_2O_3$ (100 ml) was added, the mixture was extracted with EtOAc (100 ml ×2), and the organic layer was washed with brine (100 ml), dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with *n*-heptane/EtOAc (8:1 to 3:2, v/v) as an eluent to afford **20** (1.95 g, 91%) as a white solid. mp 155–156 °C. $[\alpha]_D^{26}$ − 92.67 (c 0.319, $CHCl_3$). ESI-MS m/z 373 $(M+Na)^+$. HRESI(+)MS calcd for $C_{21}H_{34}NaO_4$ $(M+Na)^+$ 373.2355; found: 373.2358. ^1H NMR (CD_3OD): δ 0.85 (s, 3H), 0.93 (td, J = 13.6, 4.0 Hz, 1H), 0.98–1.11 (m, 4H), 1.17 (s, 3H), 1.21–1.33 (m, 1H), 1.35–1.54 (m, 4H), 1.54–1.93 (m, 8H), 1.94–2.04 (m, 1H), 2.14 (broad d, J = 12.8 Hz, 1H), 3.42 (dd, J = 9.6, 1.6 Hz, 1H), 3.56 (dd, J = 11.2, 7.6 Hz, 1H), 3.63 (s, 3H), 3.90 (dd, J = 10.8, 7.6 Hz, 1H).

4.1.11. Methyl (5β,8α,9β,10α,12α)-17-Hydroxy-15-oxoatisan-19-oate (21). A solution of $NaHSO_3$ (1.39 g, 13.3 mmol) in H_2O (13.4 ml) was added dropwise to a solution of **20** (1.95 g, 5.56 mmol) and $NaBrO_3$ (2.01 g, 13.3 mmol) in CH_3CN (22.4 ml)–H_2O (6.7 ml) for 10 min at 0 °C. The reaction mixture was stirred for 3 h at room temperature, and saturated aq $Na_2S_2O_3$ (30 ml) was added. The mixture was extracted with EtOAc (80 ml ×2), and the organic layer was washed with brine (80 ml), dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with *n*-heptane/EtOAc (10:1 to 2:1, v/v) as an eluent to afford **21** (1.55 g, 80%) as a white solid. mp 151 °C. $[\alpha]_D^{29}$ −22.13 (c 0.592, CH_3OH). ESI-MS m/z 371 $(M+Na)^+$. ^1H NMR (CD_3OD): δ 0.87–0.99 (m, 1H), 0.93 (s, 3H), 1.00–1.16 (m, 3H), 1.18 (s, 3H), 1.27–1.46 (m, 3H), 1.51–1.96 (m, 9H), 2.10–2.31 (m, 3H), 2.31–2.38 (m, 1H), 3.59 (dd, J = 10.8, 9.6 Hz, 1H), 3.65 (s, 3H), 3.87 (dd, J = 10.8, 5.0 Hz, 1H). Anal. Calcd for $C_{21}H_{32}O_4$ (348.2300): C, 72.38; H, 9.26. Found: C, 72.29; H, 9.23.

4.1.12. Methyl (5β,8α,9β,10α,12α,15β)-15,17-Dihydroxyatisan-19-oate (22). AcOH (2.55 ml, 44.5 mmol) was added to a solution of $NaB(OAc)_3H$ (4.72 g,

22.3 mmol) in CH_3CN (45 ml) at 0 °C. Then, a solution of **21** (1.55 g, 4.45 mmol) in CH_3CN (10 ml) was added, and the mixture was stirred for 4 h at 0 °C. H_2O (150 ml) was added, the mixture was extracted with EtOAc (2× 80 ml), and the organic layer was washed with brine (50 ml), dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with *n*-heptane/EtOAc (5:1 to 1:2, v/v) to afford **22** (1.14 g, 73%) as a white solid. mp 163–165 °C. $[\alpha]_D^{26}$ −74.56 (*c* 0.542, $CHCl_3$). ESI-MS *m/z* 373 $(M+Na)^+$. ^1H NMR (CD_3OD): δ 0.64–0.76 (m, 1H), 0.83 (s, 3H), 0.90–1.12 (m, 4H), 1.17 (s, 3H), 1.23–1.34 (m, 1H), 1.34–1.45 (m, 2H), 1.46–1.89 (m, 10H), 1.94 (ddd, *J* = 14.8, 11.2, 3.6 Hz, 1H), 2.16 (broad d, *J* = 13.4 Hz, 1H), 2.63 (d, *J* = 4.4 Hz, 1H), 3.42 (t, *J* = 10.6 Hz, 1H), 3.60–3.67 (m, 1H), 3.63 (s, 3H). Anal. Calcd for $C_{21}H_{34}O_4$ (350.2457): C, 71.96; H, 9.78. Found: C, 71.68; H, 9.69.

4.1.13. Methyl (5β,8α,9β,10α,12α,15β)-15-Hydroxy-17-{[(4-methylphenyl)sulfonyl]oxy}atisan-19-oate (23). TsCl (1.09 g, 5.71 mmol) and 4-dimethylaminopyridine (417 mg, 3.43 mmol) were added to a solution of **22** (400 mg, 1.14 mmol) in pyridine (11 ml) at 0 °C. This mixture was stirred for 17 h at room temperature, then H_2O (80 ml) was added. The mixture was extracted with EtOAc (50 ml ×2), and the organic layer was washed with brine (50 ml), dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with *n*-heptane/EtOAc (8:1 to 3:1, v/v) as an eluent to yield **23** (386 mg, 67%) as a white solid. mp 155 °C. $[\alpha]_D^{29}$ −42.57 (*c* 0.237, CH_3OH). ESI-MS *m/z* 527 $(M+Na)^+$. ^1H NMR ($CDCl_3$): δ 0.59–0.69 (m, 1H), 0.77 (s, 3H), 0.92 (td, *J* = 13.2, 4.0 Hz, 1H), 0.96–1.10 (m, 3H), 1.16 (s, 3H), 1.20–1.45 (m, 4H), 1.45–1.87 (m, 9H), 1.72 (d, *J* = 4.4 Hz, 1H) 1.93 (ddd, *J* = 14.4, 11.2, 3.2 Hz, 1H), 2.15 (broad d, *J* = 13.6 Hz, 1H), 2.45 (s, 3H), 2.80 (t, *J* = 4.2 Hz, 1H), 3.63 (s, 3H), 3.95 (t, *J* = 9.4 Hz, 1H), 4.05 (dd, *J* = 9.4, 7.4 Hz, 1H), 7.34 (d, *J* = 8.4 Hz, 2H), 7.79 (d, *J* = 8.4 Hz, 2H). Anal. Calcd for $C_{28}H_{40}O_6S$ (504.2545): C, 66.64; H, 7.99. Found: C, 66.50; H, 7.93.

4.1.14. Methyl (5β,8α,9β,10α,12α,15β)-15-Hydroxy-17-(methylsulfanyl)atisan-19-oate (24). NaSMe (1.16 g, 16.6 mmol) was added to a solution of **23** (1.66 g, 3.29 mmol) in HMPA (16.5 ml) at room temperature. The mixture was stirred for 43 h at 80 °C, then cooled to room temperature, and saturated aq NH_4Cl (100 ml) was added. The mixture was extracted with EtOAc (100 ml ×2), and the organic layer was washed with brine (100 ml), dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with *n*-heptane/EtOAc (5:1 to 2:1, v/v) as an eluent to afford **23** (923 mg, 76%) as a colorless solid. mp 145–147 °C. $[\alpha]_D^{26}$ −125.3 (*c* 0.255, $CHCl_3$). ESI-MS *m/z* 389 $(M+Na)^+$. ^1H NMR ($CDCl_3$): δ 0.69–0.81 (m, 1H), 0.92 (s, 3H), 0.92–1.18 (m, 4H), 1.25 (s, 3H), 1.25–1.46 (m, 4H), 1.46–1.75 (m, 6H), 1.76–1.92 (m, 3H), 1.99 (td, *J* = 11.4, 3.0 Hz, 1H), 2.11–2.20 (m, 1H), 2.14 (s, 3H), 2.53 (d, *J* = 7.6 Hz), 2.95 (d, *J* = 4.0 Hz, 1H). Anal. Calcd for $C_{21}H_{34}O_3S$ (366.22287): C, 68.81; H, 9.35. Found: C, 68.52; H, 9.32.

4.1.15. Serofendic acid A (1) and serofendic acid B (2). Davis's oxaziridine (2-benzenesulfonyl-3-phenyloxaziridine) (856 mg, 3.28 mmol) was added to a solution of **24** (923 mg, 2.52 mmol) in $CHCl_3$ (25.2 ml) at 0 °C. The reaction mixture was stirred for 2 h at 0 °C, Me_2S (1 ml) was added, and the whole was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with *n*-heptane/EtOAc (2:1, v/v) and CH_2Cl_2/MeOH (15:1 to 10:1, v/v) as eluents to yield serofendic acids A and B (1.01 g, 105%) as a white solid. The resulting isomer mixture (1 g) was purified by HPLC with a Daicel Chiralpak AD-H column and an *n*-hexane/EtOH (30%) isocratic solvent system to yield serofendic acid A (**1**) (393 mg, front peak, white solid) and serofendic acid B (**2**) (544 mg, back peak, white solid). Single crystals of the two compounds were obtained by crystallization from *n*-hexane/EtOAc. *Serofendic acid A*: mp 214–216 °C. $[\alpha]_D^{29}$ −122.9 (*c* 1.280, CH_3OH). HRESI(+)MS: $(M+H)^+$ *m/z* 383.2256 ($C_{21}H_{34}O_4S$, Δ 0.0 mmu). ESI(+)MS/MS: *m/z* 383, 365, 347, 319, 301, 283, 255. IR (KBr, cm^{-1}): 3432 (ν_{O-H}), 2927 (ν_{C-H}), 1698 ($\nu_{C=O}$). ^1H NMR (600 MHz, CD_3OD): δ 2.99 (1H, dd, *J* = 13.0, 9.3 Hz, H17a), 2.97 (1H, d, *J* = 3.7 Hz, H15), 2.87 (1H, dd, *J* = 13.0, 6.1 Hz, H17b), 2.72 (3H, s, H21), 2.17 (1H, brd, *J* = 14.2 Hz, H3α), 2.06 (1H, ddd, *J* = 14.2, 11.7, 2.9 Hz, H14α), 1.96 (1H, m, H16), 1.93 (1H, m, H2β), 1.90 (1H, m, H6α), 1.83 (1H, m, H6β), 1.74 (1H, ddd, *J* = 13.2, 13.2, 4.5 Hz, H7β), 1.71 (1H, m, H12), 1.68 (1H, m, H13α), 1.66 (1H, m, H1α), 1.63 (1H, m, H11β), 1.61 (1H, m, H9), 1.45 (1H, m, H11α), 1.42 (1H, m, H2α), 1.42 (1H, m, H13β), 1.24 (3H, s, H18), 1.11 (1H, *J* = ddd, 13.2, 2.9, 2.9 Hz, H7α), 1.07 (1H, m, H3β), 1.07 (1H, m, H5), 1.00 (1H, m, H1β), 0.99 (3H, s, H20), 0.87 (1H, ddd, *J* = 14.2, 12.0, 6.6 Hz, H14β). ^{13}C NMR (150 MHz, CD_3OD): δ 181.7 (C19), 81.3 (C15), 60.9 (C17), 57.8 (C5), 44.7 (C4), 44.5 (C16), 42.8 (C9), 41.2(C1), 39.3 (C3), 39.2 (C10), 38.8 (C21), 37.7 (C8), 34.3 (C7), 32.2 (C12), 30.0 (C11), 29.5 (C18), 28.2 (C14), 22.2 (C13), 20.9 (C6), 20.0 (C2), 13.4 (C20). Anal. Calcd for $C_{21}H_{34}O_4S$ (382.2177): C, 65.93; H, 8.96; O, 16.73; S, 8.38. Found: C, 65.64; H, 8.99; O, 16.81; S, 8.34. *Serofendic acid B*: mp 229–230 °C. $[\alpha]_D^{29}$ −13.73 (*c* 1.408, CH_3OH). HRESI(+)MS: $(M+H)^+$ *m/z* 383.2261 ($C_{21}H_{34}O_4S$, Δ +0.5 mmu). ESI(+)MS/MS: *m/z* 383, 365, 347, 319, 301, 283, 255. IR (KBr, cm^{-1}): 3420(ν_{O-H}), 2927 (ν_{C-H}), 1699 ($\nu_{C=O}$). ^1H NMR (500 MHz, CD_3OD): δ 3.04 (1H, dd, *J* = 13.2, 6.8, H17a), 2.95 (1H, d, *J* = 4.4 Hz, H15), 2.91 (1H, dd, *J* = 13.2, 9.0 Hz, H17b), 2.73 (3H, s, H21), 2.18 (brd, *J* = 13.2 Hz, H3α), 2.08 (1H, ddd, *J* = 14.0, 11.7, 3.0 Hz, H14α), 1.96 (1H, m, H2β), 1.92 (1H, m, H16), 1.90 (1H, m, H6α), 1.83 (1H, m, H6β), 1.79 (1H, m, H12), 1.73 (1H, ddd, *J* = 13.2, 13.2, 4.4 Hz, H7β), 1.66 (1H, m, H1α), 1.66 (1H, m, H13α), 1.63 (1H, m, H11β), 1.62 (1H, m, H9), 1.46 (1H, m, H11α), 1.44 (1H, m, H13β), 1.42 (1H, m, H2α), 1.23 (3H, s, H18), 1.10 (1H, ddd, *J* = 13.2, 2.9, 2.9 Hz, H7α), 1.06 (1H, m, H3β), 1.06 (1H, m, H5), 1.00 (1H, m, H1β), 0.99 (3H, s, H_2O), 0.86 (1H, ddd,

J = 14.0, 12.2, 6.5 Hz, H14β). ^{13}C NMR (125 MHz, CD_3OD): δ 181.0 (C19), 81.2 (C15), 59.8 (C17), 57.9 (C5), 44.8 (C4), 44.3 (C16), 42.9 (C9), 41.3 (C1), 39.4 (C3), 39.2 (C10), 38.3 (C21), 37.7 (C8), 34.3 (C7), 30.8 (C12), 29.9 (C11), 29.6 (C18), 28.4 (C14), 21.9 (C13), 21.0 (C6), 20.1 (C2), 13.5 (C20). Anal. Calcd for $C_{21}H_{34}O_4S$: C, 65.93; H, 8.96; O, 16.73; S, 8.38. Found: C, 65.73; H, 8.99; O, 16.85; S, 8.28.

4.1.16. Serofendic acid methylester (25). NaSMe (55.5 mg, 0.792 mmol) was added to a solution of **23** (200 mg, 0.396 mmol) in DMF (4 ml) at room temperature. The reaction mixture was stirred for 2 h at room temperature, then H_2O (40 ml) was added. The mixture was extracted with EtOAc (30 ml ×2), and the organic layer was washed with brine (30 ml), dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with *n*-heptane/EtOAc (10:1 to 5:1, v/v) to afford methyl (5β,8α,9β,10α,12α, 15β)-15-hydroxy-17-(methylsulfanyl)atisan-18-oate (121 mg, 80%) as a white solid. mp 107–108 °C. $[\alpha]_D^{26}$ −101.7 (*c* 0.200, $CHCl_3$). ESI-MS *m*/*z* 403 $(M+Na)^+$. ^{1}H NMR ($CDCl_3$): δ 0.69–0.80 (m, 1H), 0.80 (s, 3H), 0.85–1.14 (m, 4H), 1.18 (s, 3H), 1.23–1.46 (m, 4H), 1.46–1.88 (m, 9H), 1.91 (d, J = 4.0 Hz, 1H), 1.92–2.01 (m, 1H), 2.12–2.19 (m, 1H), 2.14 (s, 3H), 2.52 (s, 1H), 2.53 (d, J = 1.2 Hz, 1H), 2.94 (t, J = 4.0 Hz, 1H), 3.64 (s, 3H). Anal. Calcd for $C_{22}H_{36}O_3S$ (380.2385): C, 69.43; H, 9.53. Found: C, 69.31; H, 9.39.

Davis's oxaziridine (2-benzenesulfonyl-3-phenyloxaziridine) (91.6 mg, 0.35 mmol) was added to a solution of methyl (5β,8α,9β,10α,12α,15β)-15-hydroxy-17-(methylsulfanyl)atisan-18-oate (111 mg, 0.292 mmol) in $CHCl_3$ (2 ml) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C, Me_2S (0.1 ml) was added, and the mixture was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with *n*-heptane/EtOAc (2:1, v/v) and EtOAc/MeOH (15/1, v/v) to yield serofendic acid methylester (**25**) (105 mg, 91%) as a white solid.

The resulting isomeric mixture of serofendic acid methylester (60 mg) was purified by HPLC with a YMC pack ProC18 column and an H_2O/CH_3CN (37%) isocratic solvent system to afford serofendic acid methylester A (**25-A**) (15.1 mg, front peak, white solid) and serofendic acid methylester B (**25-B**) (24.3 mg, back peak, white solid). The stereochemistry of the sulfoxide group of **25-A** and **25-B** has not been determined. Compound **25-A**: mp 164–166 °C. $[\alpha]_D^{26}$ −132.0 (*c* 0.283, $CHCl_3$). ESI-MS *m*/*z* 419 $(M+Na)^+$. ^{1}H NMR (CD_3OD): δ 0.76–0.88 (m, 1H), 0.83 (s, 3H), 0.89–1.11 (m, 4H), 1.17 (s, 3H), 1.25–1.44 (m, 3H), 1.48–1.88 (m, 9H), 1.88–1.94 (m, 1H), 1.94–2.04 (m, 1H), 2.14 (broad d, J = 13.6 Hz, 1H), 2.67 (s, 3H), 2.81 (dd, J = 13.2, 6.0 Hz, 1H), 2.89–2.98 (m, 2H), 3.63 (s, 3H). Anal. Calcd for $C_{22}H_{36}O_4S$ (396.2334): C, 66.63; H, 9.15. Found: C, 66.48; H, 9.19. Compound **25-B**: mp 197–199 °C. $[\alpha]_D^{26}$ −24.98 (*c* 0.304, $CHCl_3$). ESI-MS *m*/*z* 419 $(M+Na)^+$.^{1}H NMR (CD_3OD): δ 0.76–0.88 (m, 1H), 0.83 (s, 3H), 0.90–1.01 (m, 1H), 1.01–1.11 (m, 3H), 1.17 (s, 3H), 1.27–1.45 (m, 3H), 1.48–1.91 (m, 10H), 1.94–2.04 (m, 1H), 2.14 (broad d, J = 13.6 Hz, 1H), 2.68 (s, 3H), 2.87 (dd, J = 13.2, 9.4 Hz, 1H), 2.91 (d, J = 4.4 Hz, 1H), 2.99 (dd, J = 13.2, 6.8 Hz, 1H), 3.63 (s, 3H). Anal. Calcd for $C_{22}H_{36}O_4S$ (396.2334): C, 66.63; H, 9.15. Found: C, 66.38; H, 9.02.

4.2. Single crystal X-ray structure determination

Single crystal X-ray diffraction data were collected with a R-AXIS RAPID II (Rigaku), using Cu Kα radiation (λ = 1.54187 Å). Crystallographic data, excluding structure factors, were deposited with the Cambridge Crystallographic Data Center as supplementary Publication numbers CCDC 647840 and 647841 for compounds **1** and **2**. These data can be obtained free of charge from the Cambridge Crystallographic Data Center via www.ccdc.cam..ac.uk/data_request/cif.

4.3. Neuroprotective activity

Eagle's minimal essential salt medium (Eagle's MEM) was purchased from Nissui Pharmaceutical (Tokyo, Japan). Fetal bovine serum and horse serum were purchased from JRH Biosciences (Lenexa, KS, USA). Poly(ethyleneimine) solution and cytosine β-D-arabinofuranoside hydrochloride were from Sigma–Aldrich Corp. (St. Louis, MO, USA). Trypan blue was from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Sodium pentobarbital was from Dainippon Pharmaceutical, Co, Ltd (Osaka, Japan). All other drugs were obtained from Nacalai Tesque (Kyoto, Japan).

Primary neuronal cultures were obtained from the cerebral cortex of fetal rats (17–19 days of gestation) according to the procedures described previously.[17] Briefly, pregnant Wistar rats (Nihon SLC, Shizuoka, Japan) were anesthetized with sodium pentobarbital, and the cerebral cortex was rapidly removed bilaterally and placed in ice-cold Hanks' solution (137 mM NaCl, 5.4 mM KCl, 3.4 mM Na_2HPO_4, 0.5 mM KH_2PO_4, 5.6 mM D-(+)-glucose, and 2.4 mM Hepes, pH 7.2). The cerebral cortex was mechanically dissociated with a scalpel and by trituration with a Pasteur pipette. Dissociated cells were filtered through a stainless steel mesh with a pore size of 150 μm and centrifuged at 200*g* for 3 min. The supernatant was removed, and cells were resuspended in complete medium (Eagle's MEM supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 11 mM D-(+)-glucose, 24 mM $NaHCO_3$, and 10 mM Hepes). Finally, single cell suspensions were plated onto 0.1% polyethyleneimine-coated glass coverslips, which were placed in 60 mm dishes (5.1×10^6 cells per dish). Cultures were maintained in the above-mentioned complete medium (1–8 days after plating) or Eagle's MEM supplemented with 10% heat-inactivated horse serum, 2 mM L-glutamine, 11 mM D-(+)-glucose, 24 mM $NaHCO_3$, and 10 mM Hepes (9–15 days after plating). Cultures were incubated at 37 °C in a humidified 5% CO_2 atmosphere. To prevent proliferation of non-neuronal cells, 10 μM cytosine β-D-arabinofuranoside hydrochloride was added after 6 days of plating. The culture medium was changed every

two days. Mature cells after 11–15 days in vitro were used in all experiments. Animals were treated in accordance with the guidelines of the Kyoto University animal experimentation committee and the guidelines of the Japanese Pharmacological Society.

Cell viability was assessed by means of trypan blue exclusion assay using Hoffman modulation contrast microscopy, as described in our previous report.[17a] On the day of the experiment, culture medium was replaced with drug-containing medium at 37 °C. After incubation, cultures were immediately stained with 1.5% trypan blue for 10 min at room temperature, fixed with isotonic formalin, and then rinsed with physiological saline. Cells stained with trypan blue were regarded as non-viable. The viability of the cultures was calculated as the percentage of the number of unstained cells (viable cells) relative to the total number of cells counted (viable cells plus non-viable cells). Over 200 cells per coverslip were counted. In each experiment, cells on five coverslips were counted to obtain means ± SEM of the cell viability.

Data were expressed as means ± SEM. The statistical significance of differences between groups was determined by one-way analysis of variance (ANOVA) followed by Dunnett's two-tailed test using the InStat program (Graph Pad Software, San Diego, CA, USA). Statistical significance was defined as a probability value of less than 5%.

4.4. Pharmacokinetic study

Fasted SD rats ($n = 3$, 290–260 g, obtained from Nihon SLC) were used for the study. The dosing solution containing the test compound (1 mg/ml) was administered (dosing solution: 30% DMSO/5% glucose for iv; 0.5% methyl cellulose for po). Blood samples were collected at designated time points: 0.25, 0.5, 1, 2, 4, 6, and 8 h. Brain samples were collected at designated time points: 0.5 and 2 h. MeOH/$HClO_4$ (500:1, 0.2 ml) solution was added to plasma (0.1 ml) or brain homogenate (20% saline) (0.1 ml), centrifuged (12,000 rpm, 5 min), and filtered. Then the samples were examined by using LC-MS methodology (MS: SIM positive 406; column: Develosil C30 2×150; column temperature: 40 °C; mobile phase: $CH_3CN/H_2O = 30/70$ containing 0.02% HCO_2H).

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