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Stereoselective semi-synthesis of the neuroprotective natural product, serofendic acid

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We have recently demonstrated a synthetic biology-enabled semi-synthesis of the potent neuroprotective compound, serofendic acid. An engineered bacterium produces *ent*-atis-16-en-19-oic acid, which has six of eight chiral carbons configured with the appropriate stereochemistry. Setting the configuration of the C15 hydroxyl group and C16 methylene is a critical step that occurs late in each published total or formal synthesis. Here we explore the use of alternative reducing reagents, stereochemical directing agents, reaction order, and product recycling to improve the diastereoselectivity of this step. We find that installing and oxidizing the C17 methylsulfide prior to reducing the C15 ketone provides the greatest yield of the desired C15,C16 diastereomer. This represents an improved total synthesis of serofendic acid.

Introduction

Chronic neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease and others affect over 100 million Americans and result in an annual economic burden of $800B.[1] Together with acute neurodegenerative diseases, such as ischemic stroke, these diseases were responsible for 9 million deaths globally in 2015[2,3] and are leading causes of disability in the USA and the world.[4] Each of these acute and chronic neurodegenerative diseases involve programmed cell death (PCD) as a key aspect of their pathology. One solution to limit neurodegeneration is the inhibition of signals or metabolic cascades that lead to PCD in neurons.

Recently, serofendic acid epimers **1A** and **1B** (**Fig. 1A**), diterpenoid natural products originally isolated from fetal calf serum[5] have both showed promising cell-protective activity at nanomolar concentrations. **1** has been successfully used to prevent PCD in *in vitro* models (cardiac cells[6,7], auditory hair cells[8] and neurons[9–11]), in animal models (stroke[12,13] and infarction[14]), and Parkinson's disease.[15]

Despite these encouraging results, the scarcity of **1** in nature (3 mg per 250 L of fetal calf serum)[5] and the complex chemical structure (nine chiral centers and four fused rings) have hindered drug development efforts. Efficient and sustainable production of the serofendic acid **1** scaffold will accelerate mechanism of action and structure-activity-relationship studies.

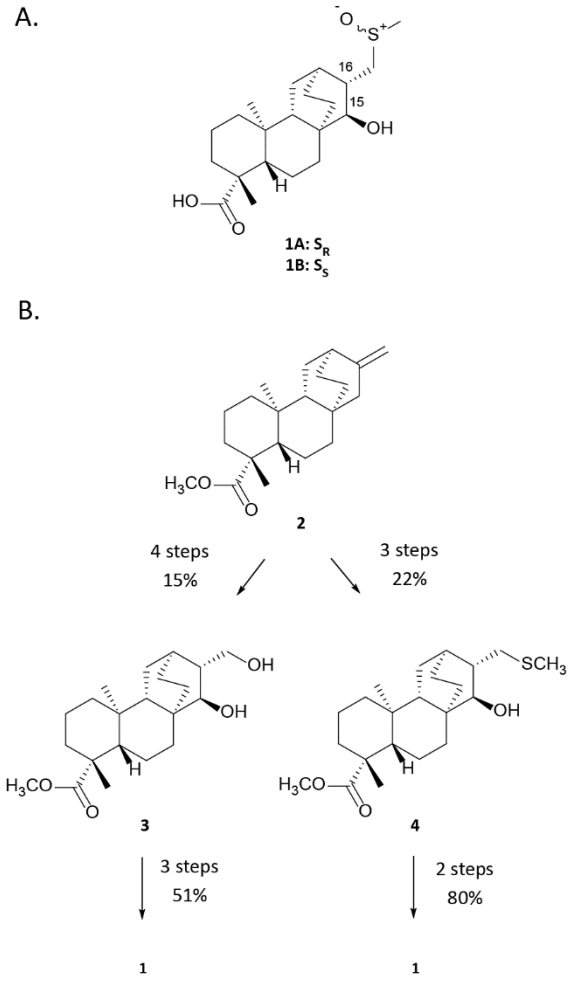

Fig. 1. Summary of previous approaches for setting C15/C16 configuration. (A) Chemical structure of serofendic

acids **1**. (B) Efficiency of reactions and key intermediates used to establish C15/C16 configuration by Terauchi *et al.*[16] (left) and Toyota *et al.*[17] (right).

The first two syntheses of **1** published in literature both involve the key intermediate, methyl *ent*-atis-16-en-19-oate **2**, which was transformed into **1** through different reactions (**Fig. 1B**).[5,16–18] In the synthesis reported by Terauchi and co-workers, (-)-isosteviol **S1** was converted to **2** in 8 steps with a combined yield of 13.4% (**Supplementary Fig. S1**). The stereochemistry of C15 and C16 was set through a four-step stereoselective oxidation-reduction cascade to convert **2** to methyl (15R,16S)-15,17-dihydroxy-*ent*-atisanoate **3** with an overall yield of 15% (**Fig. 1B**) (**Supplementary Fig. S1**).[16] Three more steps afforded **1** with a combined yield of 51%. The overall yield displayed for this 15-step synthesis was 1.01% (**Supplementary Fig. S1**).

The second total synthesis, reported by Toyota and co-workers, was similar in length and overall yield (**Fig. 1B**).[17,19] After producing **2** from commercial 2-allylcyclohexanone **S5**, oxidation to enone **S7** followed by thiomethylation led to a 1:1 epimeric mixture of α and β methyl 15-oxo-17-methylsulfenyl-*ent*-atisanoates **S8** and **S9** respectively (**Supplementary Fig. S2**). Using the complex borane in THF to reduce the ketone group of **S8** and **S9** produced all possible isomers of methyl 15-hydroxy-17-methylsulfenyl-*ent*-atisanoate with a slight selectivity of this reaction towards the *trans* isomers **4** and **S10** (27% each) over the *cis* ones **S11** and **S12** (18% each) (**Fig. 1B**) (**Supplementary Fig. S2**). Two more steps afforded **1** with a combined yield of 80%. The overall yield of this 17-step total synthesis was 0.90% (**Supplementary Fig. S2**).

Obtaining the *ent*-atisane scaffold and the desired (15R,16S) configuration of the moieties on **1** are the crux of these syntheses and improvement of both will substantially impact the overall yield. After having reported a sustainable way to produce the *ent*-atisane scaffold by bacterial fermentation (Hsu, et al., in revision), we describe our efforts to improve the conversion efficiency of the reduction step leading to **1**. We explored the use of different reducing agents, stereochemical directing agents, reaction order, and finally the recycling of undesired stereoisomers.

Results and Discussion

Direct reduction of ent-15-oxo-17-methylsulfenylatisanoic acid (7-8).

We have recently developed an engineered metabolic pathway to produce *ent*-atis-16-en-19-oic acid **5** through bacterial fermentation (Hsu, et al., in revision). **5** is then purified from the culture medium and converted to **1** via chemical synthesis. While six of the eight chiral carbons on **1** are configured during the bacterial production of **5**, the C15 hydroxyl group and C16 alkyl methylsulfinyl group need to be installed during the chemical synthesis (**Fig. 2**).

Following the Toyota, et al. approach,[17] which is described above and in **Supplementary Fig. S2**, we arrive at an epimeric mixture of 17-methylsulfenyl-15-oxo-*ent*-atisan-19-oic acid **7** and **8** via the reactions described previously with similar yields (**Fig. 2**).[17]

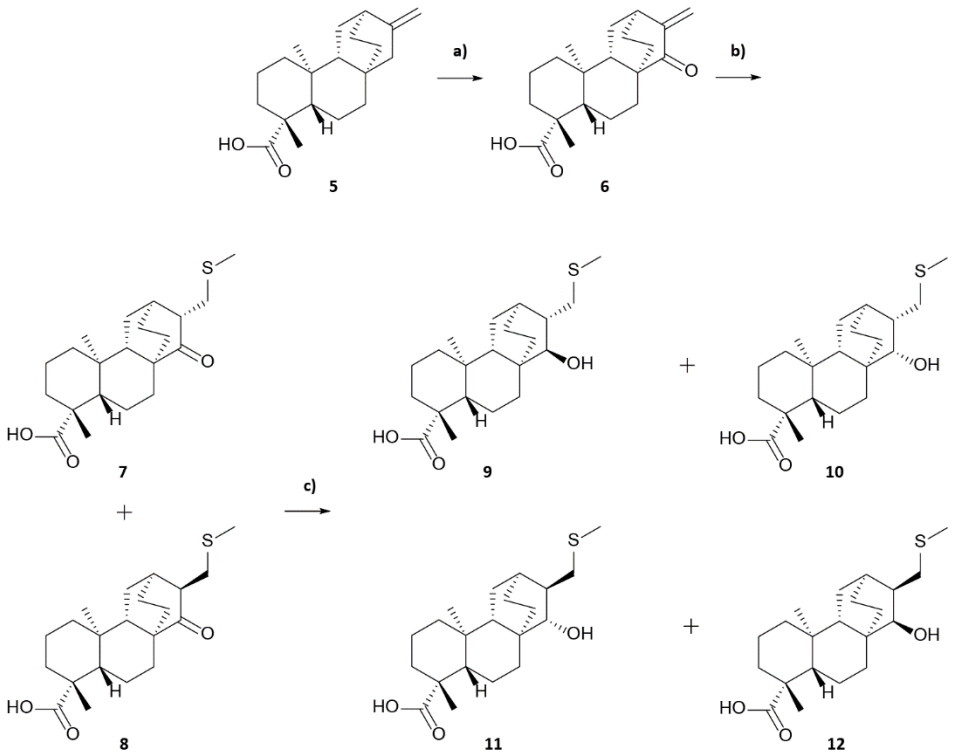

Fig. 2. Synthesis of 15-hydroxy-17-methylsulfenyl-*ent*-atisan-19-oic acids **9-12** from eAA **5** from Hsu, et al. Reagents and conditions: a) $(PhSeO)_2O$, benzene, reflux, 4 h, 75%; b) NaSMe aq., THF, r.t., 1 h; c) $NaBH_4$, EtOH, 0°C, 30 min, 10% (from **6**) **9**, 30% (from **6**) **10**, 11% (from **6**) **11**, 39% (from **6**) **12**. Overall yield: 7.5%.

The borane-THF complex, as reported previously for reducing the methyl ester-protected version of our substrate **7** and **8**,[17] was inefficient and led to degradation of the carboxylate group on the unprotected substrate. To overcome this issue, we substituted the borane-THF complex with sodium borohydride as a reducing agent. This substitution led to a clearer crude mixture in which only the four possible isomers **9-12** were observed. Unfortunately, diastereomer **9**, which is needed to complete the synthesis of **1**, was produced in the lowest yield (10% starting from **6**). Comparatively, the other diastereomers were produced at 30% (**10**), 11% (**11**), and 39% (**12**) starting from **6** (**Table 1**). Through this route, we had achieved at the time the synthesis of **5** with an overall yield of 7.5% starting from **1** (Hsu, et al., in revision).

As the limiting step of the chemical synthesis leading to **1** from **5** was the reduction step, we decided to seek for more selective reducing reagents towards the *trans* conformations. Small reducing agents such as borane-THF or sodium borohydride showed either low selectivity for *trans* compounds or undesired reactivity towards the carboxylic acid group on our products. We then tested several bulkier reducing reagents in an attempt to improve the stereoselectivity towards our desired product and prevent side reactions. None of the agents we tried (L-selectride, sodium triacetoxyborohydride,[18] and disiamylborane) produced the desired product **9**, as determined by LC/MS comparison with authentic standard (**Table 1**) (**Supplementary Fig. S3**).

Table 1: Experimental conditions tested to reduce 17-methylsulfenyl-15-oxo-*ent*-atisenoic acids 7 into 15-hydroxy-17-methylsulfenyl-*ent*-atisenoic acid 9.

Reducing reagent	Reaction Conditions	(15R,16S) 9
BH_3-THF¶	10 eq, 0°C, THF	-
$NaBH_4$¶	10 eq., 0 °C, EtOH	+
L-selectride	10 eq, 0°C, THF	-
$NaBH(OAc)_3$	5 eq, 0°C, AcOH* (10 eq), acetonitrile[18]	-
$NaBH(OAc)_3$	5 eq., 0°C, acetonitrile	-
$NaBH(OAc)_3$	10 eq, 0°C, acetonitrile	-
$(Sia)_2BH$	10 eq., 0°C, THF	-

¶Previous work
(+): Isomer **9** detected by LC/MS analysis (**Supplementary Fig. S3**)
(-): Isomer **9** not detected by LC/MS analysis (**Supplementary Fig. S3**)
*AcOH: glacial acetic acid

C15 reduction with stereochemical directing agents.

Since bulky reducing agents did not process C15 reduction efficiently, we next explored using chiral or achiral reagents to direct the sodium borohydride reduction. We first chose cerium (III) chloride due to its oxophile behavior. Cerium (III) chloride has been demonstrated to direct the selective 1,2 reduction of α,β unsaturated ketones.[20] As a bulky oxophile, we reasoned that the ketone group of methylsulfenyl-containing intermediates **7** and **8** would chelate the cerium on the sterically available face. This would allow the BH_4^- ion to attack on the same side of the methylsulfenyl group and favor formation of *trans* diastereomers (**Fig. 3A**). An analysis by ^1H NMR of the crude reaction mixture showed that the opposite occurred. The *cis* diastereomers **10** and **12** (36% and 48% conversion, respectively) were favored over the *trans* diastereomers **9** and **11** (8% conversion for both) (**Fig. 3B**). This result suggests that the cerium is chelated by both the ketone and sulfide groups in the substrate. The 6-membered ring resulting from such a chelation would hinder the side towards which was pointing the methylsulfenyl group (**Fig. 3C**).

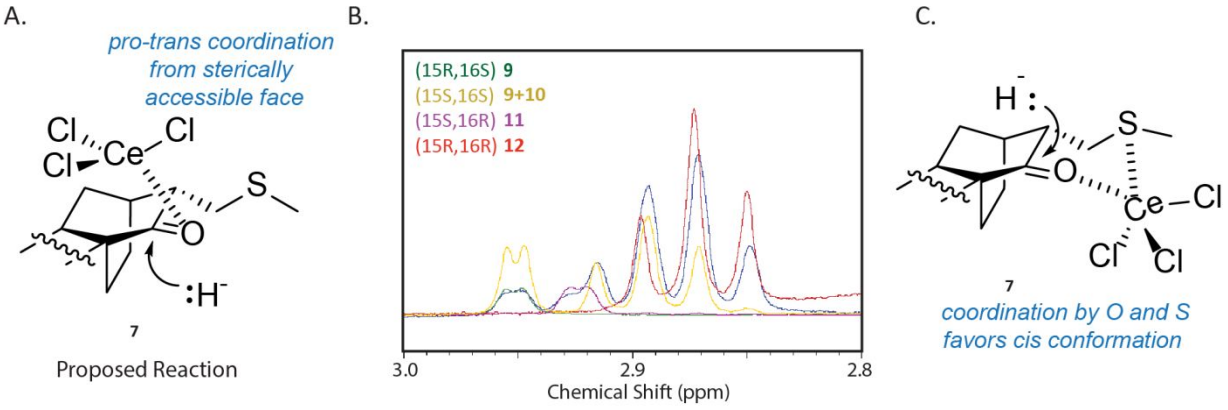

Fig. 3. Cerium (III) chloride directed reduction. (A) Proposed reaction mechanism relying on pro-*trans* coordination from less sterically-hindered face (epimer **7** used as an example). (B) ^{1}H NMR of reaction mixture showing enrichment of both *cis*-diastereomers. Reaction mixture is a blue trace, ^{1}H NMR shifts of isolated compounds are green (**9**), yellow (**9**+**10**), purple (**11**), and orange (**12**). (C) Possible reaction mechanism based on experimental results (epimer **7** used as an example).

Additional attempts to use directing groups that coordinate with the methylsulfenyl or ketone groups to control the stereochemistry of the ketoreduction were similarly ineffective. We tested *tert*-butoxide-activated pinacolborane (HBpin)[21] with and without with cerium (III) chloride, but each reaction favored the production of *cis* diastereomers **10** and **12** (**Supplementary Fig. S4**). Lastly, we tried using a Corey–Bakshi–Shibata (CBS) catalyst[22,23] for enantioselective ketone reduction. After 24 h at room temperature in THF, we saw partial reduction of the starting material (**Supplementary Fig. S5**), whereas the reductions using pinacolborane were total in the same period of time. However, most of the products observed at that time were *cis*-diastereomers **10** and **12** so we did not optimize the reaction further.

Oxidation of sulfide group to sulfoxide and then diastereoselective reduction of ketone

Since multiple attempts to stereoselectively reduce the C15 ketone of our γ-keto-sulfide group to the *trans* configuration were unfruitful, we decided to change the reaction sequence by first oxidizing the sulfide moiety to a sulfoxide prior to reducing the C15 ketone. Diisobutylaluminum hydride (DiBAl-H) has proven to be a reagent that can selectively reduce β and γ-keto sulfoxide groups.[24–26] In the reported cases, the sulfoxide stereochemistry in the presence or absence of Lewis acids could control the stereochemistry of the hydroxyl moiety in the final product. Unlike those previous reports, our substrates have a chiral α carbon in a single configuration and a sulfoxide with different configurations. Our first goal was to determine if the chiral α carbon or the sulfoxide group could guide selective *trans* reduction.

Oxidation of an epimeric mixture of **7** and **8** was achieved using Davis oxaziridine reagent with a combined yield of 86% (from **6**) for the collection of a mixture of γ-keto sulfoxide diastereoisomers **13A**, **13B**, **14A** and **14B** (**Fig. 4**). This mixture was reduced with DiBAl-H in the presence or absence of Lewis acid (**Fig. 4**). The results of this assay are summarized in **Table 2**. Unlike previous reports with achiral substrates at the α carbon, the presence or absence of a Lewis acid did not drastically affect the stereochemistry of ketone reduction (**Table 2, lines 1-3**). The *cis*-diastereoisomers were the only product

seen by ^{1}H NMR by using either DiBAl-H or DiBAl-H with cerium (III) chloride (**Table 2, lines 1-2**) and using zinc chloride as Lewis acid gave approximately equal yields of *cis* **15** and **16** and *trans* **1** and **17** diastereoisomers (**Table 2, line 3**) (**Supplementary Fig. S6-11**). This suggests that the chiral α carbon plays a larger role than the sulfoxide for this reduction reaction.

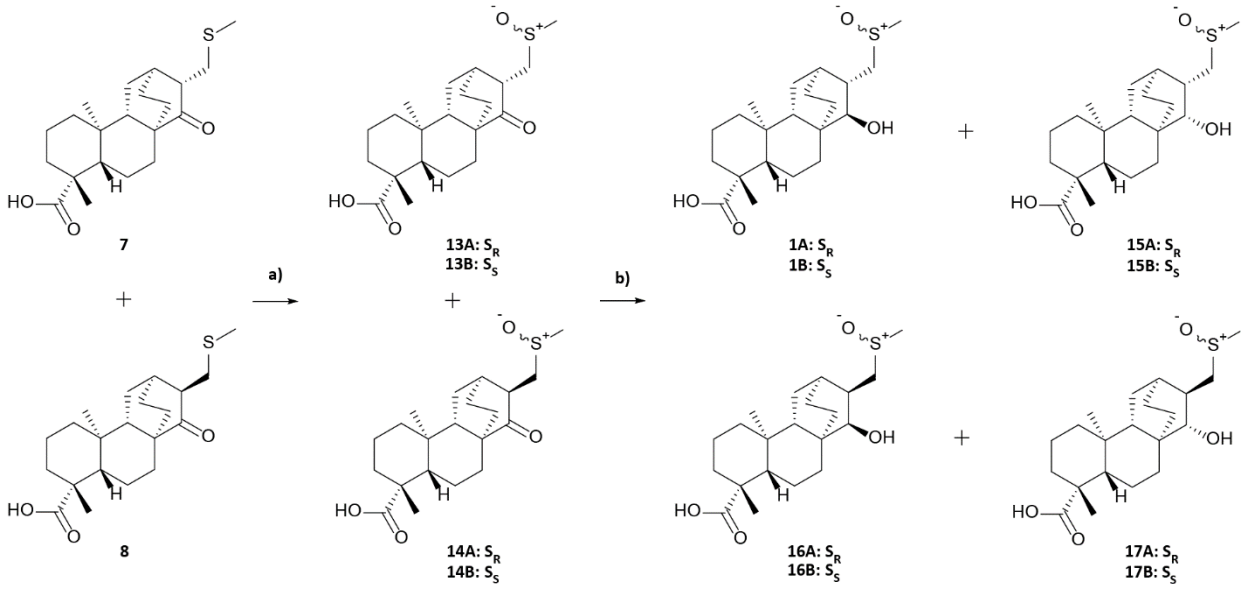

Fig. 4. Synthesis of 15-hydroxy-17-methylsulfinyl-*ent*-atisan-19-oic acids **1**, **15-17** from an epimeric mixture of **7** and **8**. Reagents and conditions: a) Davis oxaziridine (2-benzenesulfonyl-3-phenyloxaziridine), $CHCl_3$, r.t., 1 h, 86%; b) DiBAl-H, $ZnCl_2$, THF, 0°C, 1h, 47% **1** and **17**, 53% **15** and **16** (products **1**, **15-17** not isolated and conversion calculated by ^{1}H NMR).

Next, we tested several different reduction conditions (**Table 2, lines 3 and 7**) (**Supplementary Fig. S12-19**) and found that DiBAl-H plus zinc chloride was the best conditions to provide *trans* **1** and **17** diastereoisomers.

Table 2: Stereospecificity of 17-methylsulfinyl-15-oxo-*ent*-atisan-19-oic acids 13 and 14 reduction with different reducing reagents.

	Reducing reagent	Reaction Conditions	Reaction progress reactant[a]/product[b] (%/%)[c]	*cis*[d]/*trans*[e] (%/%)[c]
1	DiBAl-H	5 eq., THF, 0°C	19/81	100/0
2	DiBAl-H/$CeCl_3$	5 eq./1.1 eq., THF, 0°C	28/72	100/0
3	DiBAl-H/$ZnCl_2$	5 eq./1.1 eq., THF, 0°C	0/100	53/47
4	HBpin	5 eq., THF, 0°C	100/0	0/0
5	BH_3-THF	5 eq., THF, 0°C	ND[f]	ND
6	$NaBH_4$	10 eq., EtOH, 0°C	0/100	77/23

7	$NaBH_4/CeCl_3$	10 eq./1.1 eq, EtOH, 0°C	0/100	93/7
8	DiBAl-H/$ZnCl_2$	5 eq./1.1 eq., THF, 0°C	26[g]/74	68/32[h]

[a]Mixture of keto-sulfoxides **13** and **14**
[b]Mixture of hydroxy-sulfoxides **1** and **15-17**
[c]Conversion calculated by ^{1}H NMR (**Supplementary Fig. S7, S9, S11, S13, S15, S17 and S19**)
[d]Hydroxy-sulfoxides **15** and **16**
[e]Hydroxy-sulfoxides **1** and **17**
[f]ND: Not determined
[g]**13B** remaining only
[h]**15B**/**1B** ratio only

Inverting the sulfide oxidation with the ketone reduction step and using the DiBAl-H/$ZnCl_2$ couple to perform this reduction allowed an improvement in our overall yield to 15.2% for the conversion of **5** to **1** (**Fig. 6**). This doubles our previous overall yield from reduction of **7** and **8** with $NaBH_4$ followed by sulfide oxidation.

(16S,S)-17-methylsulfinyl-15-oxo-*ent*-atisan-19-oic acid recycling

Syntheses of **1** that generate the undesired epimer of the C15 hydroxyl group in **15A** and **15B** (Toyota, et al, Hsu, et al.) could be improved by recycling these via re-oxidation and reduction to produce more **1**. We started with (15S,16S)-15-hydroxy-17-methylsulfenyl-*ent*-atisan-19-oic acid **10**, that was produced as an undesired *cis* diastereomer from reduction reactions described above. We oxidized the sulfide group using Davis oxaziridine[16–18] to produce an epimeric mixture of (15S,16S)-15-hydroxy-17-methylsulfinyl-*ent*-atisan-19-oic acids **15A** and **15B** (46 % and 44% yield respectively), C15-*cis* analogs of **1A** and **1B**, respectively (**Fig. 5A**). Oxidation of the hydroxyl group of **15B** was achieved using Dess-Martin periodinane[27], leading to (16S,S)-17-methylsulfinyl-15-oxo-*ent*-atisan-19-oic acid **13B** and with 93 % yield (**Fig. 5B**).

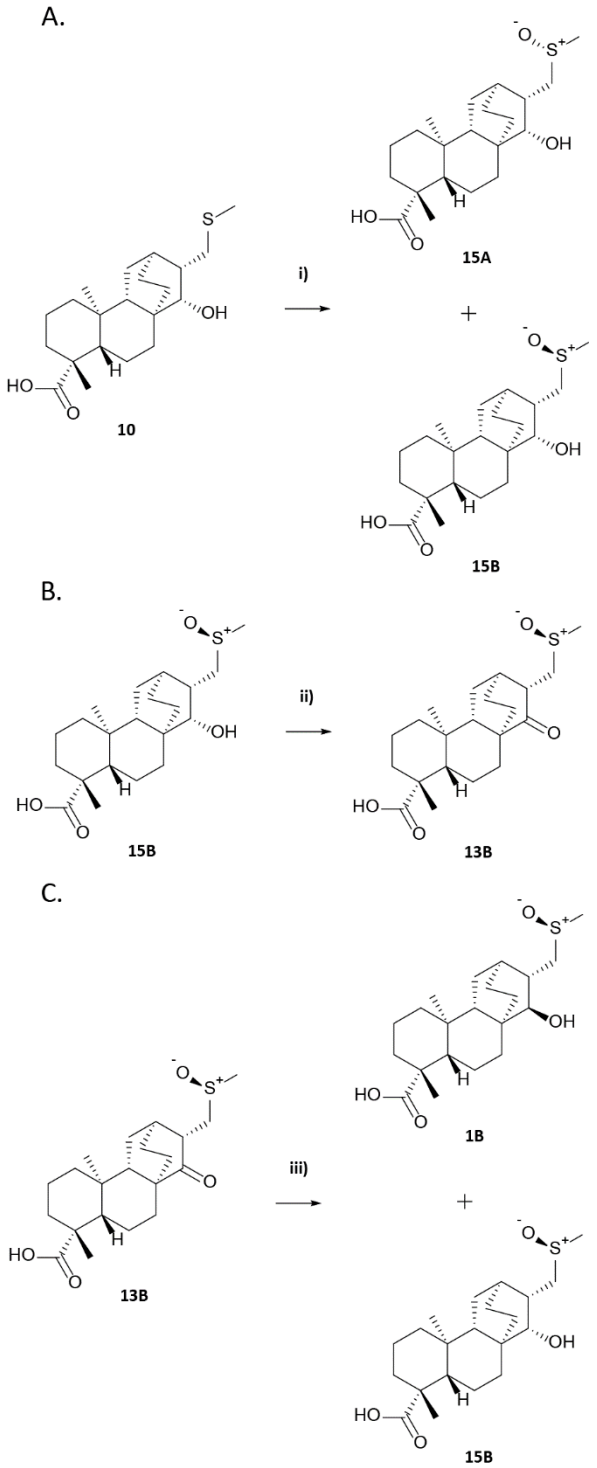

Fig. 5. Recycling undesired diastereomers to SA **1**. (A) Oxidation of methylsulfenyl group of undesired diastereomer **10** to 17-methylsulfinyl-15-oxo epimers **15A** and **15B**. Reagents and conditions: i) Davis oxaziridine (2-benzenesulfonyl-3-phenyloxaziridine), $CHCl_3$, r.t., 1 h, 46% **15A**, 44% **15B**. (B) Oxidation of 17-methylsulfinyl-15-oxo isomer **15B** to 15-keto-17-methylsulfinyl **13B**. Reagents and conditions: ii) Dess Martin periodinane, H_2O, $CHCl_3$, 0°C to r.t., 30 min, 93%. (C) Reduction of compound **13B** to produce SA **1B** and **15B**. Reagents and conditions: iii) DiBAl-H, $ZnCl_2$, 0°C, 30 min, 24% **1B**, 50% **15B**.

We reduced (16S,S)-17-methylsulfinyl-15-oxo-*ent*-atisan-19-oic acid **13B**, using the DiBAl-H/$ZnCl_2$ couple, which yielded 32% **1B** and 68% *cis* analog **15B** (**Table 2, line 8**) (**Supplementary Fig.20-21**). Comparing the diastereomeric purity of this reaction to the reaction using a mixture of reagents (**Table 2**, **line 3**), we hypothesize that the sulfinyl isomer of either **13A** or **14A** would produce more *trans* (**1A** or **17A** respectively) versus *cis* (**15A** or **16A** respectively) products. However, we have not tested this experimentally.

Including one round of product recycling to our semi-synthesis of **1** starting from **5** displayed in **Fig. 4** allowed us to increase our overall yield to 17.0% (**Fig. 6**).

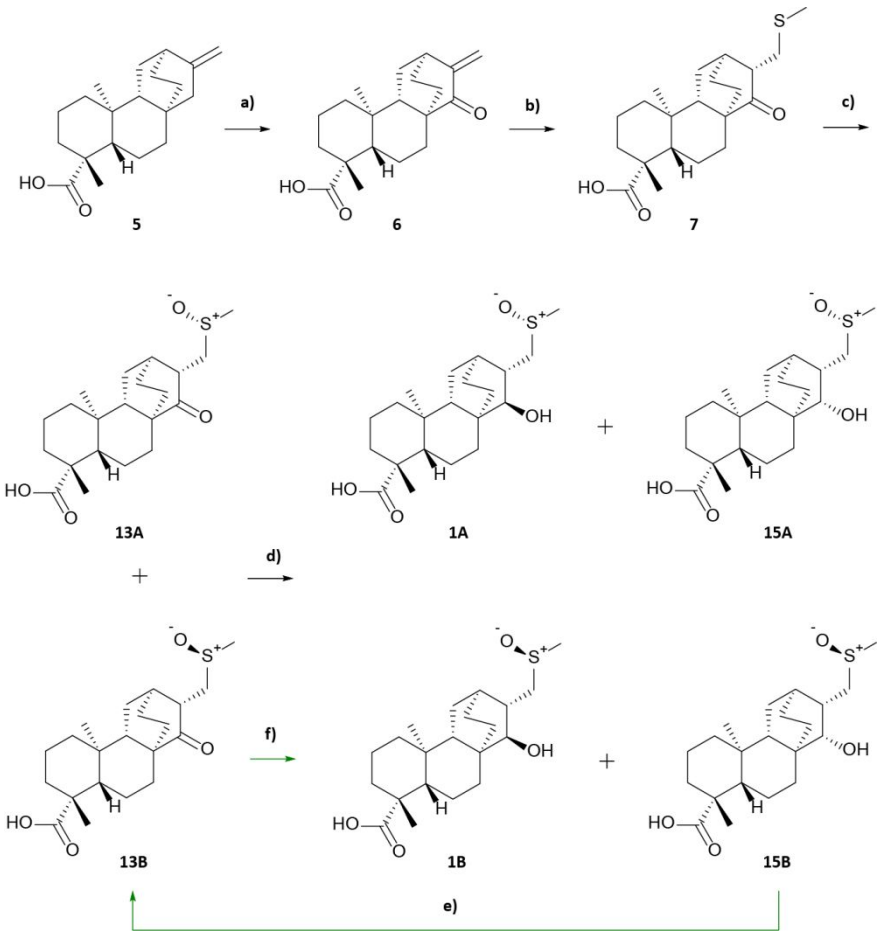

Fig. 6. Semi-synthesis of **1** from **5** (black arrows) including recycling of **15B** into **1B** (green arrows). Reagents and conditions: a) $(PhSeO)_2O$, benzene, reflux, 4 h, 75%; b) NaSMe aq., THF, r.t., 1 h; c) Davis' oxaziridine (2-benzenesulfonyl-3-phenyloxaziridine), $CHCl_3$, r.t., 1 h, 43% 2 steps; d) DiBAl-H, $ZnCl_2$, THF, 0°C, 30 min, 47% **1A**+**1B**, 53% **15A**+**15B**; e) Dess Martin periodinane, H_2O, $CHCl_3$, 0°C to r.t., 30 min, 93%; f) DiBAl-H, $ZnCl_2$, THF, 0°C, 30 min, 24% **1B**, 50% **15B**. Overall yield of **1** from **5**: 15.2% (without recycling), 17.0% (with one round of recycling).

Conclusions

The stereoselective reduction of **7** towards the desired (15R,16S) configuration proved to be challenging. Each strategy we attempted favored the *cis*-diastereoisomers over the *trans* ones. However, reversing the order of ketone reduction and sulfinyl oxidation proved to be a promising

strategy to improve the overall efficiency of the synthesis. Indeed, using the reducing system DiBAl-H/$ZnCl_2$ on γ-keto-sulfoxide groups instead of the γ-keto-sulfide ones allowed more steric hindrance of the pro-*cis* face of these systems.[24,25]

There are still opportunities to further improve the total synthesis of **1**. For instance, half of the material is still lost when C16 is configured during the sulfa-Michael addition step giving **7** and **8** from **6**. This lack of selectivity could be addressed by performing on **2** a thiol-ene reaction followed by a selective epoxidation. The epoxide could be then selectivity opened in the desired (15R,16S) configuration.

An alternative use of the undesired diastereoisomers that are produced during synthesis of **1** is to convert them into new SA isosteres. For example, the hydroxyl group on **15** could be transformed into a leaving group and used in S_N2 reactions to introduce halides at C15 in the desired (15R) configuration. The structure-activity-relationship studies of **1** that have been completed to date suggest that structural changes in this region of the molecule are tolerated without dramatic losses in bioactivity.[28]

Natural products are historically an excellent resource for new drug discovery.[29,30] The complex structures of many natural products, including fused ring systems and multiple chiral centers, makes complete chemical synthesis at scales required to support pre-clinical and clinical drug discovery challenging.[31] Previous total syntheses of **1** required more than a dozen steps with overall yields around 1%. Starting with an advanced intermediate, **2**, gave us an immediate boost in yield to 7.5% (Hsu, et al. in revision). By reversing the sulfinyl oxidation and ketone reduction steps and proceeding one round of recycling on one undesired stereoisomer, we have now further increased the overall yield to 17% (**Fig. 6**).

Experimental Methods

All chemicals and reagents were purchased from commercial sources and used directly without further purification. All non-aqueous reactions were performed under an atmosphere of argon in flame-dried glassware. Reaction progress was monitored by thin-layer chromatography (TLC) using silica gel plates (silica gel 60 F254). Eluted TLC plates were visualized with UV light (254 nm) and dyed using ethanol acidified with sulfuric acid (5% v/v). Compounds were purified either by flash silica gel (230-400 mesh) column chromatography or by Büchi C-700 chromatograph apparatus with 4 g or 25 g Büchi FlashPure EcoFlex cartridges. Diastereoisomers were purified by HPLC (Ultimate 3000 Rapid Separation (RS) system) using a semi-preparative column Betasil C18, 250 x 10 mm, 5µm particle size (Thermo Scientific) and the products were observed at 210 nm. Chromatography was performed at flow rate of 1.5 mL/min using 10 mM ammonium formate buffer (solvent A, pH 8.3) and 100% acetonitrile (solvent B). NMR experiments were performed on Bruker Advance III 500 MHz (Broadband Observe SmartProbe) or Bruker Advance 700 MHz (5-mm triple resonance cryoprobe) spectrometers. Chemical shifts were reported as ppm relative to either chloroform-d (7.26 ppm for ^1H, 77.16 ppm for ^{13}C) or methanol-d_4 (3.31 ppm for ^1H and 49.00 ppm for ^{13}C). ^1H constant coupling (*J*) are expressed in hertz (Hz), and multiplicity is described as follows: s = singlet, d = doublet, d_{app} = appearing doublet, t = triplet, br = broad, m = multiplet. Compounds were analyzed by HR-ESI-MS (ThermoScientific, Q Exactive, Quadrupole, Orbitrap, Heated-Electrospray Ionization probe source (HESI-II)).

Production of *ent*-atis-16-en-19-oic acid (5)

The production of **5** was performed as reported in literature (Hsu, et al., in revision). Briefly, fresh spores from a recombinant strain of *Streptomyces albus* J1704 were grown in 2 mL R2YE supplemented with apramycin (1/1000 (v/v)) (50 mg/L) in a 15 mL culture tube. The R2YE liquid culture was incubated in a 28°C shaker set up at 250 rpm for 48 hours. This preculture was used to inoculate (1/100 (v/v)) a 50 mL ISM3 supplemented with 50 µL of apramycin (50 mg/L). The ISM3 culture was incubated in a 28°C shaker set up at 250 rpm for 48 h. This 50 mL culture was used to inoculate six 500 mL ISM3 media supplemented with XAD-16 Amberlite resin (15% m/v) prepared in baffled Erlenmeyer flasks. The media were incubated in a shaker set up at 28°C for 5-7 days. Afterwards, the 500 mL fermentations were centrifuged at 4600 rpm for 30 min. The supernatants were discarded and the cell-resin pellets were washed with de-ionized water and centrifuged for 30 minutes at 4600 rpm for three times. The pellets were combined and extracted with methanol (250 mL and stirring for 15 min) 6 times. Liquid extracts were combined and evaporated under vacuum pressure and the residue was extracted with ethyl acetate (3 x 50 mL). The organic solution was then washed with brine (2 x 50 mL), dried over anhydrous magnesium sulfate, filtrated and evaporated under vacuum pressure. The powder obtained was washed with *n*-hexane and was collected over a frit to afford **5** as white powder (356.1 mg, 24%). For ^{1}H and ^{13}C NMR data, **Supplementary Fig. S22-24**. HRESIMS: *m/z* calculated for $C_{20}H_{29}O_2^-$ $[M\text{-}H^+]^-$ 301.2173, found 301.2173.

Synthesis of *ent*-15-oxoatis-16-en-19-oic acid (6)

The synthesis of **6** was performed as reported in literature (Hsu, et al., in revision). Briefly, under argon atmosphere, a solution of **5** (56.5 mg, 0.19 mmol) and benzeneseleninic anhydride (70.6 mg, 0.196 mmol) in benzene (36 mL) was refluxed for 4 h. The solution was then concentrated under vacuum pressure and the residue was purified by silica gel column chromatography using a *n*-hexane to *n*-hexane / ethyl acetate (4:1 v/v) gradient to afford **6** (44.6 mg, 75%) as a white solid. For ^{1}H and ^{13}C NMR data, see **Supplementary Fig. S25-27**. HRESIMS: *m/z* calculated for $C_{20}H_{27}O_3^-$ $[M\text{-}H]^-$ 315.1966, found 315.1966.

Synthesis of 17-methylsulfenyl-15-oxo-*ent*-atisan-19-oic acids (7-8)

The synthesis of **7** and **8** was performed as reported in literature (Hsu, et al., in revision). Briefly, to a solution of **6** (167.3mg, 0.53 mmol) in THF (30 mL) was added every 15 min an aqueous solution of sodium thiomethoxide (15% m/v, 512 µL, 1.06 mmol). After the fourth addition the medium was stirred for 15 min and brine (50 mL) was added to the solution. The mixture was extracted with ethyl acetate (2 x 50 mL) and the organic phase was washed with hydrochloric acid 1 M (2 x 20 mL). The organic solvent was then dried over anhydrous magnesium sulfate, evaporated under vacuum pressure. The solid collected was dried thoroughly under vacuum pressure overnight to afford a mixture of inseparable epimers of **7** and **8** (186.1 mg, 97%) as a white solid. HRESIMS: **7:** *m/z* calculated for $C_{21}H_{27}O_3S^-$ $[M\text{-}H]^-$ 363.1999 (100%), $[M+2\text{-}H]^-$ 365.1968 (4%), found 363.2013 (100%), 365.1966 (4%), **8:** *m/z* calculated for $C_{21}H_{27}O_3S^-$ $[M\text{-}H]^-$ 363.1999 (100%), $[M+2\text{-}H]^-$ 365.1968 (4%), found 363.2013 (100%), 365.1967 (4%).

Synthesis of 17-methylsulfinyl-15-oxo-*ent*-atisan-19-oic acids (13 and **14)**

To a solution of sulfides **7** and **8** (78.0 mg, 213.9 µmol) in chloroform (2 mL) was added a solution of the Davis oxaziridine (2-benzenesulfonyl-3-phenyloxaziridine) (58.7 mg, 224.6 µmol) in chloroform (500 µL). The mixture was then stirred at room temperature for 1 h and directly purified by column chromatography using a dichloromethane to dichloromethane/methanol (9:1 v/v) gradient. A fraction containing the 4 isomers **13A**, **13B**, **14A** and **14B** (69.4 mg, 86%) was collected. For ^{1}H and ^{13}C NMR data of **13B**, **14A** and **14B**, see **Supplementary Fig. S40-49**. ESIMS: *m/z* calculated for $C_{21}H_{31}O_4S^-$ [M-H]$^-$ 379.1949 (100%), [M+2-H]$^-$ 381.1906 (4%), found **13A**: 379.1951 (100%), 381.1908 (4%)**, 13B**: 379.1950 (100%), 381.1907 (4%)**, 14A:** 379.1952 (100%), 381.1909 (4%) and **14B**: 379.1949 (100%), 381.1906 (4%).

Synthesis of serofendic acids and 15-hydroxy-17-methylsulfinyl-*ent*-atisan-19-oic acids (1, **15-17)**

To a solution of **13** and **14** (4.0 mg, 10.5 µmol) in THF (300 µL) was added a solution of zinc chloride (1.6 mg, 11.6 µmol) in THF (300 µL) and stirred at room temperature for 1 h. After, the mixture was cooled down to 0°C and a solution of diisobutylaluminum hydride in THF 1 M (52.5 µL) was added. The reaction was stirred at cold temperature for 1h. The reaction was quenched by adding methanol (80 µL) and a solution of HCl 10% (40 µL). The mixture was concentrated and then diluted in a mixture of ethyl acetate and water. Once the layers separated, the organic phase was dried out. The residue was dissolved in methanol-d_4 for ^{1}H NMR analysis. The analysis showed total consumption of starting material and a conversion of **13** and **14** into *trans* diastereomers **1, 17**(47%) and *cis* diastereoisomers **15** and **16** (53%).

Synthesis of (15S,16S)-15-hydroxy-17-methylsulfinyl-*ent*-atisan-19-oic acids (15)

To a solution of sulfide **10** (53.0 mg, 144.6 µmol) in chloroform (1.3 mL) was added Davis oxaziridine (2-benzenesulfonyl-3-phenyloxaziridine) (39.7 mg, 151.8 µmol). After completion (1 h) the mixture was purified on silica gel column chromatography using a dichloromethane to dichloromethane/methanol (10:1 v/v) gradient to yield **15A** (24.4 mg, white solid, 46%) and **15B** (23.5 mg, white solid, 44%). For ^{1}H and ^{13}C NMR data of **15A** and **15B**, see **Supplementary Fig. S50-55.** HRESIMS: *m/z* calculated for $C_{21}H_{29}O_4S^-$ [M-H]$^-$ 381.2105 (100%), [M+2-H]$^-$ 383.2063 (4%), found **15A**: 381.2109 (100%), 383.2068 (4%), **15B**: 381.2110 (100%), 383.2068 (4%).

Synthesis of (16S,S)-17-methylsulfenyl-15-oxo-*ent*-atisan-19-oic acid (13B)

To a solution of **15B** (5.0 mg, 13.1 µmol) in chloroform (300 µL) was added a solution of Dess-Martin periodinane (7.8 mg, 18.3 µmol) in chloroform (150 µL). To that mixture was added H_2O (0.3 µL, 14.4 µL) and the solution was stirred at room temperature. After 30 min, the reaction was quenched with a saturated solution of sodium thiosulfate. The phases were separated and the aqueous solution was extracted twice with chloroform. The organic layer was dried with anhydrous magnesium sulfate, filtered and concentrated. The concentrate was purified on silica gel column chromatography using a dichloromethane to dichloromethane/methanol (30:1 v/v) gradient to yield **13B** (4.6 mg, 93%) as a clear film. For ^{1}H and ^{13}C NMR data, see **Supplementary Fig. S40-42**. HRESIMS: *m/z* calculated for $C_{21}H_{31}O_4S^-$ [M-H]$^-$ 379.1949 (100%), [M+2-H]$^-$ 381.1906 (4%), found 379.1950 (100%), 381.1907 (4%).

Synthesis of serofendic acid B and (15S,16S,S)-15-hydroxy-17-methylsulfinyl-*ent*-atisan-19-oic acid (1B and 15B)

To a solution of **13B** (4.6 mg, 12.1 µmol) in THF (400 µL) was added a suspension of zinc chloride (1.8 mg, 13.3 µmol) in THF (300 µL). The mixture was stirred at room temperature for 1 h and then was allowed to cool down to 0°C. After 5 min at cold temperature, diisobutylaluminum hydride in THF 1M (60.4 µL) was added and the mixture was stirred, still at cold temperature. After 30 min, the reaction was quenched with adding methanol and droplets of a solution of HCl 10%. The mixture was concentrated and the concentrate was diluted with a mixture of ethyl acetate and water. The layers were separated and the aqueous one was extracted once with ethyl acetate. The organic phase was washed with brine, dried with anhydrous magnesium sulfate, filtered and the solvent was evaporated *in vacuo*. The residue was analyzed by ^{1}H NMR, which showed a conversion of 74% of the starting material into **1B** (32%) and **15B** (68%). For ^{1}H and ^{13}C NMR data of **1B**, see **Supplementary Fig. S59-62**. HRESIMS: *m/z* calculated for $C_{21}H_{29}O_4S^-$ $[M-H]^-$ 381.2105 (100%), $[M+2-H]^-$ 383.2063 (4%), found **1B**: 381.2109 (100%), 383.2068 (4%) and **15B**: 381.2110 (100%), 383.2068 (4%).

Clarification of stereochemistry naming convention used herein.

Conventional approaches for compound nomenclature lead to confusing notation for the diastereomers produced in this study. For natural products, newly described compounds are given a common name, and enantiomers of these compounds are given the '*ent*-' designation. Our desired final product, serofendic acid, has a carbon scaffold with the same absolute configuration as the enantiomer of atisane (*i.e. ent*-atisane). IUPAC conventions for naming compounds are to use the '*ent*-' designation as a prefix in compound naming that applies to the whole molecule. This has the effect of inverting local annotations of stereochemistry. Thus, the configuration of the carbons 15 and 16 for *ent*-(15S,16R)-15-hydroxy-17-methylsulfenylatisan-19-oic acid **9** is identical to the desired final product (15R,16S)-serofendic acid.

In an attempt to clarify the orientation of C15/C16 throughout this semi-synthesis, we have adapted a non-conventional naming scheme for intermediates. We move the *ent*- designation internally and apply it only to the base carbon scaffold. In this way, *ent*-(15S,16R)-15-hydroxy-17-methylsulfenylatisan-19-oic acid **9** is written instead as (15R,16S)-15-hydroxy-17-methylsulfenyl-*ent*-atisan-19-oic acid **9**. In this way, the C15/C16 local stereochemistry designation does not need to be inverted. We have included an additional table in the Supplementary Information that gives the compound number, name used in this study, and IUPAC name.

Conflicts of Interest

We do not have any conflicts of interest to report.

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Stereoselective semi-synthesis of the neuroprotective natural product, serofendic acid

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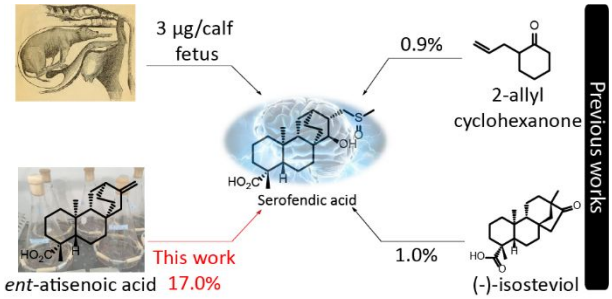

Our synthesis of neuroprotectant serofendic acid from biosourced *ent*-atis-16-en-19-oic acid represents the best route to date.