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Toosendanin: Synthesis of the AB-ring and investigations of its anti-botulinum properties (Part II)

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ABSTRACT

Botulinum neurotoxins (BoNTs) are the etiological agents responsible for botulism, a disease characterized by peripheral neuromuscular blockade and a characteristic flaccid paralysis of humans. The natural product toosendanin, a limonoid, is a traditional Chinese medicine that has reported anti-botulinum properties in animal models. Toosendanin effectively inhibits the biological activity of BoNT/A in neuronal cells at concentrations of 200 nM, and partial inhibition can be observed with concentrations as low as 8 nM. Mechanistically, toosendanin's inhibition is due to prevention of transduction of the BoNT LC through the HC channel. Intriguing questions as to the molecular architecture of toosendanin as related to its anti-botulinum properties have focused our attention on a synthesis of toosendanin's unusual AB-ring, containing a unique bridged hemi-acetal. Within the current work, a synthetic strategy allowing access to the AB-fragment of toosendanin was achieved from a *trans*-decalin system. In addition, this fragment was examined for its modulation of BoNT/A intoxication in a rat spinal cord cellular assay.

1. Introduction

Botulinum neurotoxins (BoNTs) are the most toxic poisons known to humans, with a lethal dose (LD_{50}) of approximately 1 ng per kg of body weight.[1] There are seven serologically distinct BoNTs (A–G); serotype A (BoNT/A) is the most potent, possessing a toxicity 10^6-fold higher than cobra toxin and 10^{11}-fold greater than cyanide.[2] BoNT intoxication is characterized by flaccid paralysis caused by the proteolytic cleavage of specific SNARE proteins critical for the release of the neurotransmitter acetylcholine from nerve cells.[3]

Limonoids (**1** and **2**, Fig. 1) are tetranortriterpenoids with a 4,4,8-trimethylfuranylsteroid skeleton derived from euphane or tirucallane triterpenoids.[4–6] A major limonoid constituent found in *Melia toosendan* is the compound toosendanin (**2**, Fig. 1) which appears to have multiple modes of action in insects including damage to midgut tissues, inhibition of esterases, cytochrome P450-aldrin epoxidase and proteinase activities.[7] Interest in the application of liminoid natural products, and in particular toosendanin, in pest management remains high, and while this area of toosendanin research remains fertile, we became intrigued by a series of reports over the past two decades, mainly originating from China detailing the special activity of toosendanin as it relates to BoNT. Most interesting were two reports both published in the early 1980s detailing toosendanin's in vivo activity.[8] Indeed, these cryptic reports identified toosendanin's anti-botulinum properties in both monkey and mouse models spanning these serotypes. Unfortunately, these reports proved to be difficult to substantiate either due to a lack of pure material (i.e., toosendanin) or inconsistencies with toosendanin in animal assays.

To resolve this dilemma, we reported the effects of well characterized toosendanin, using a sensitive and specific spinal cord cell-based assay, which validated toosendanin's activity in both BoNT serotypes A and E.[9] Thus, exposure of neurons to BoNT/A in presence of increasing concentrations of toosendanin resulted in the gradual preservation of intact, uncleaved synaptosomal associated protein of 25 kD (SNAP-25), the intracellular BoNT/A and BoNT/E substrate, becoming practically complete above 200 nM; excitingly, partial inhibition can be observed with concentrations as low as 8 nM for BoNT/A and 40 nM for BoNT/E, respectively. To further solidify toosendanin's BoNT inhibitory activity, we utilized

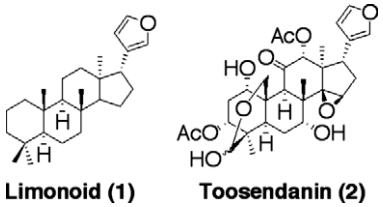

Figure 1. General structures of limonoid (**1**) and toosendanin (**2**).

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single molecule channel forming experiments that clearly demonstrated toosendanin's inhibition mode is due to prevention of transduction of the BoNT light chain (LC) through the heavy chain (HC) channel.[9,10] Thus, toosendanin selectively arrests the LC translocation step of intoxication with subnanomolar potency, and increases the unoccluded HC channel propensity to open with micromolar efficacy. Interestingly, these studies also provided strong evidence that toosendanin has an unprecedented dual mode of action within the protein-conducting channel acting both as a cargo-dependent inhibitor of translocation and as cargo-free channel activator. To further validate toosendanin's anti-botulinum properties, we have also investigated toosendanin's protective effects in a mouse lethality model. Positive findings have allowed us to begin to assign which chemical functionalities are important for the anti-botulinum properties found within toosendanin.[9] As a starting point we initially turned to semi-synthesis to generate a set of rationally designed toosendanin analogues (**3**–**6**, Fig. 2) that were prepared so as to probe the most salient functionalities embedded within toosendanin without perturbing its gross chemical structure. These analogues were examined in the mouse lethality assay wherein only **4** proved to have equivalent activity to toosendanin.

To both potentially improve and ultimately decipher toosendanin's anti-botulinum properties, the next tactic we envisioned embraced function-oriented synthesis (FOS) as advocated by Wender et al.[11] The underpinnings of FOS are that the function of a biologically active lead structure can be emulated, tuned, or possibly improved by replacement with simpler scaffolds designed to encompass the key activity-determining structural features of the natural product. As stated (vide supra), through our semi-synthetic efforts, the epoxide and acetoxy moieties were found to be important, while the furan ring was not. To further define toosendanin, we dissected the molecule into two fragments consisting of AB- and CD-rings. As such, we reported our initial research exploring the synthesis of the CD-ring of toosendanin and its potential biological function.[12] Our synthetic approach to the 4-acetoxy CD-ring of toosendanin was achieved starting from mesityl oxide and acetylacetone in 14 steps. This work represented the first semi-synthesis of the CD-ring of toosendanin correctly displaying all heteroatom substituents found within the natural product. In continuing with our general plan, we disclose our successful synthesis of toosendanin's AB-ring and testing of its biological activity (Fig. 3).

2. Results and discussion

In the search for a suitable *trans*-decalin system as a starting material with an angular substituent and oxygen functionalities

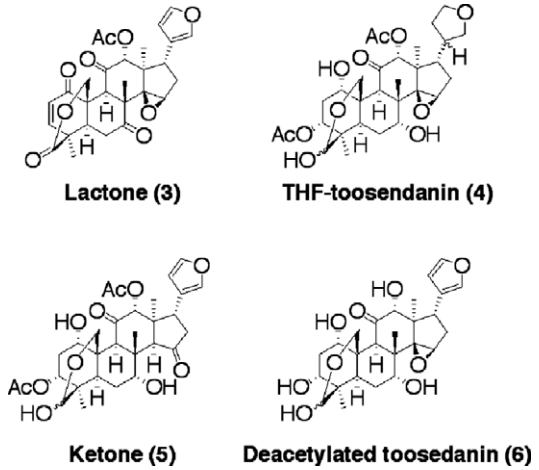


Figure 2. Structures of toosendanin's analogues (**3**–**6**).

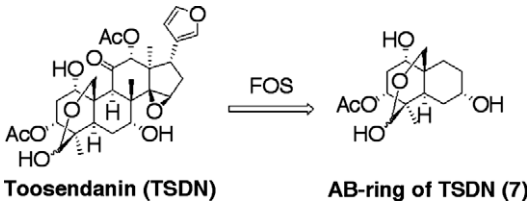


Figure 3. AB-ring of toosendanin (**7**).

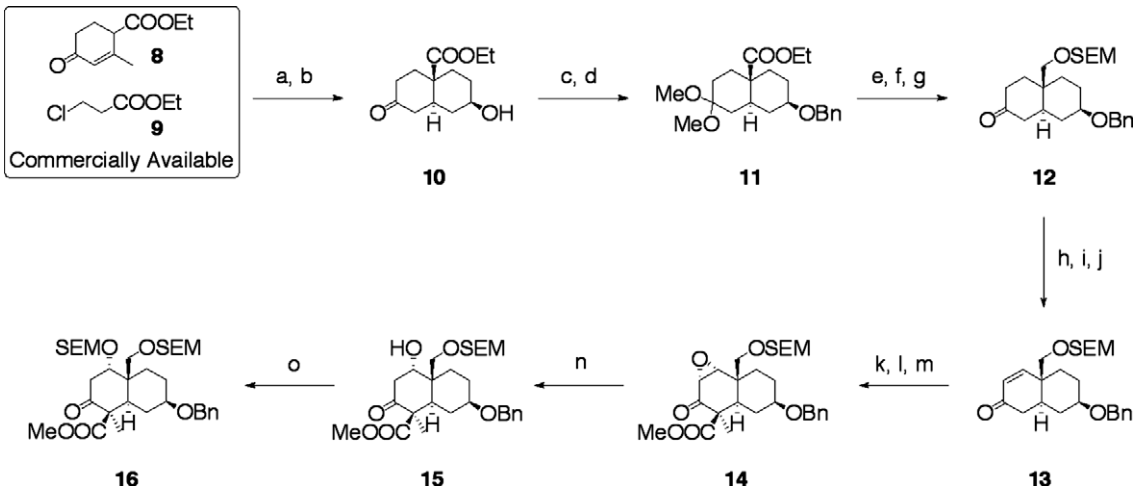
on both rings, we found ester (**10**) to be an ideal candidate for this study (Scheme 1). This compound is readily available in large scale, from commercially available starting materials (**8** and **9**) by a two-step modification as detailed by Jones and Dodds.[13] Thus, upon benzylation of the hydroxy group found within **10** under acidic conditions,[14] the resulting ketone was protected as the ketal (**11**) in 51% yield for the two steps. Reduction of **11** with LAH followed by acid-induced hydrolysis of the dimethyl ketal furnished a hydroxy ketone, which could then be utilized for protection of the primary alcohol with SEMCl affording **12** in 93% overall yield from **11**. The preparation of the desired enone (**13**) was accomplished by KHMDS-promoted phenyselenylation at −78 °C followed by oxidation and elimination of the resulting selenide using hydrogen peroxide in 83% yield.[15] Subsequent regioselective carboxymethylation with Mander's reagent[16] in the presence of LHMDS at −78 °C afforded a β-ketomethylester; this was followed by methylation, thus methyl iodide in the presence of NaH granted the corresponding α-methyl-β-ketomethylester.[17] Epoxidation of the enone moiety by TBHP in the presence of benzyltrimethylammonium hydroxide (triton B)[18] furnished stereoselectively epoxide (**14**), in 65% yield for three steps from **13**.[19] The regioselective opening of the epoxide moiety[20] found within **14** was established by PhSeNa (generated in situ from diphenyl diselenide and $NaBH_4$) in 86% yield followed by silylation of **15** with SEMCl, which led to disilyl ether (**16**) in 85% yield from **14** (Scheme 1).

The relative stereochemistry of **15** was ascertained through analysis of its 2D-ROESY NMR. Thus, for **15**, a ROESY correlation was observed between Me-1 (equatorial) and H-8a (axial); H-7 (axial) and H-8a; no long range interactions were observed between H-4 (equatorial) and H-8a protons (Fig. 4).

2.1. Synthesis of 7-epi AB-ring of toosendanin (TSDN)

To construct the diol directly, we attempted the stereoselective reduction of the ketone and methyl ester moiety of **16** with LAH or DIBAL at −78 °C, but unfortunately the undesired stereochemistry at C-2 position was obtained as a major isomer; the lone reaction that succeeded was $NaBH_4$ in CH_2Cl_2/MeOH (15:1). Again, however, the desired compound **17** was obtained as a minor isomer yet, the total yield was quantitative, hence, we invoked a recycling method via oxidation of 2β-form (**epi-17**) with Dess–Martin reagent that granted **16** in 96% yield. Reduction of ester (**17**) to the primary alcohol using LAH proceeded in an excellent yield. The resulting diol was selectively oxidized with 2,2,6,6-tetramethyl-1-piperidinyloxy free radical (TEMPO) under biphasic conditions to furnish an aldehyde followed by acetylation of the free secondary alcohol granted **18** in 85% yield for three steps from **17**.[21] Hydrogenation of **18** with palladium hydroxide on carbon provided **19** as a hemi-acetal moiety in 94% yield; as expected **20** was not observed. While, removal of SEM group with TFA furnished 7-epi AB-ring of toosendanin (**epi-7**) in 80% yield (Scheme 2).

With the first hurdle in the synthesis in place, that is, construction of the AB-ring, we were now faced with the challenge of inverting the stereochemistry at the C-7 position. Our initial attempt engaged the Mitsunobu reaction. Thus, **16**'s benzyl ether



Scheme 1. Synthesis of **16**. Reagents and conditions: (a) NaOEt, EtOH; (b) H_2, Pd/C, ether; (c) benzyl 2,2,2-trichloroacetimidate, cat. TfOH, cyclohexane/CH_2Cl_2 (2:1, v/v); (d) cat. H_2SO_4, MeOH, 51% for two steps; (e) LAH, THF, reflux; (f) 5 N HCl, THF; (g) SEMCl, DIPEA, CH_2Cl_2 93% for three steps; (h) KHMDS, THF, −78 °C, then TESCl, −78 °C; (i) PhSeCl, CH_2Cl_2, −78 °C; (j) 30% H_2O_2, THF, 83% for three steps; (k) LHMDS, THF, −78 °C, then $NCCO_2Me$, −78 °C; (l) NaH, MeI, THF; (m) TBHP, triton B, THF, 65% for three steps; (n) $(PhSe)_2$, $NaBH_4$, EtOH, 86%; (o) SEMCl, DIPEA, CH_2Cl_2, 99%.

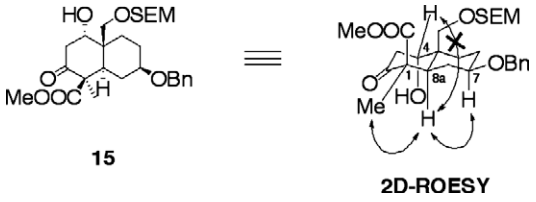
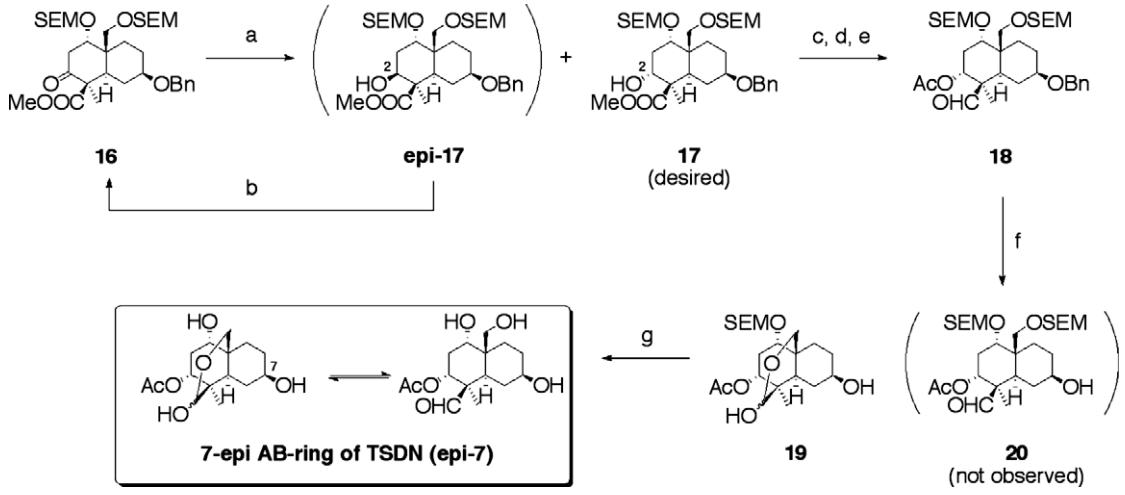


Figure 4. The relative stereochemistry of compound (**15**) as determined by 2D-ROESY experiment.

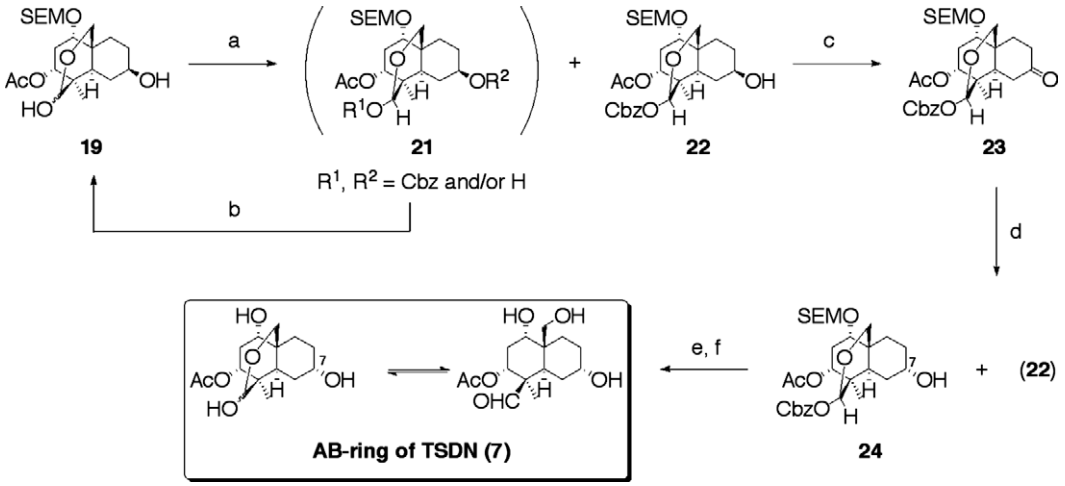
was removed by hydrogenation with palladium on carbon, and subsequent Mitsunobu reaction with 4-nitrobenzoic acid afforded 4-nitrobenzylester with the desired stereochemistry at C-7. Unfortunately, all attempts to hydrolyze this ester failed; we surmise that either the ester was resistant to hydrolysis or when hydrolysis was seen it coincided with elimination of SEM group resulting in the α,β-unsaturated ketone.

2.2. Synthesis of AB-ring of toosendanin (TSDN)

Since the necessary inversion of stereochemistry at C-7 was found untenable using a Mitsunobu sequence we were forced to rethink our strategy. We sought an advanced intermediate, and **19** appeared well-suited to meet this challenge. Thus, **19**'s hemiacetal was protected by treatment with CbzCl, however, to improve yields due to over reaction, (**21**), was recycled providing the desired alcohol (**22**). Oxidation of **22** using Dess–Martin reagent afforded ketone (**23**) in 96% yield. Stereoselective reduction was attempted using a plethora of reagents, however, none were satisfactory. Hence, we simply relied on reduction using $NaBH_4$ resulting in a mixture of **22** and the desired compound **24**. These compounds were readily separated and again recycling was used on **22** to obtain the correct stereochemistry at C-7. The corresponding AB-ring (**7**), as embedded within toosendanin was finally secured by hydrogenation of **24**, followed by SEM removal with TFA (Scheme 3).



Scheme 2. Synthesis of 7-epi AB-ring of toosendanin (**epi-7**). Reagents and conditions: (a) $NaBH_4$, CH_2Cl_2/MeOH (15:1, v/v), 99% (ratio of **epi-17**/**17** = 2/1); (b) Dess–Martin periodinane, CH_2Cl_2, 96%; (c) LAH, ether; (d) NaOCl, TEMPO, KBr, CH_2Cl_2/satd $NaHCO_3$ aq (2:1, v/v); (e) Ac_2O, pyridine, cat. DMAP, 85% for three steps; (f) H_2, $Pd(OH)_2$/C, CH_2Cl_2/MeOH (1:1, v/v), 94%; (g) TFA, CH_2Cl_2, 0 °C, 80%.



Scheme 3. Synthesis of AB-ring of toosendanin (**7**). Reagents and conditions: (a) CbzCl, TMEDA, CH_2Cl_2, −78 °C, 49% of **22**; (b) H_2, Pd/C, EtOH, 49% for two steps; (c) Dess–Martin periodinane, CH_2Cl_2, 96%; (d) $NaBH_4$, CH_2Cl_2/MeOH (15:1, v/v), 95% (ratio of **22**/**24** = 2.6/1); (e) H_2, Pd/C, EtOH; (f) TFA, CH_2Cl_2, 0 °C, 82% for two steps.

2.3. Testing toosendanin analogues 7 and epi-7 in an RSC assay

To examine the biological potency of toosendanin analogues **7** and **epi-7**, a primary rat spinal cord cellular assay (RSC assay) was engaged.[22] The value of this assay is it avoids the use of animals and lethality as an end-point. Here western blot analysis can be employed where visualization of intact SNAP-25 is a measure of a compounds efficacy against botulinum neurotoxin A. In these regards and based on our previous findings in terms of data accrued from our semi-synthetic and CD-ring analogues, we anticipated little or no biological potency. Yet, we felt it incumbent to examine these structures in this cellular assay both to contrast, and validate our previous results/hypotheses as well as provide a solid grounding for future FOS studies focused upon utilizing the AB-ring nucleus. Thus, primary rat spinal cord cells were exposed to 5.6 pM BoNT/A1 combined with 200 μM toosendanin (**2**), 1 mM AB-ring (**7**), or 1 mM 7-epi AB-ring (**epi-7**). The controls contain toxin only, or no toxin. Cell lysates were analyzed by Western blot using a monoclonal anti-SNAP-25 antibody (Synaptic Systems). As expected, addition of toosendanin at 200 μM resulted in complete inhibition of BoNT/A1 activity, as evidenced by the appearance of uncleaved SNAP-25 only (Fig. 5). Addition of **7** or **epi-7** at 1 mM concentrations consistently resulted in the same extent of SNAP-25 cleavage as the positive (toxin only) control. Thus, as expected, **7** and **epi-7** did not result in any detectable inhibition of BoNT/A1 activity in primary neuronal cells.

3. Conclusion

We have achieved the synthesis of the AB-ring (**7**) of toosendanin (5.8% overall yield in 23 steps from **10**) and the 7-epi AB-ring (**epi-7**) of toosendanin (10% overall yield in 19 steps from **10**). In accord with our previous findings neither structure afforded protection from BoNT/A intoxication within the RSC assay. However, noteworthy is that the merging of these studies with both our semi-synthetic and CD-ring analogues provides a unified picture emphasizing the importance of toosendanin's ABCD nucleus in the blocking of BoNT/A intoxication. Taken together a logical point for future studies, we will focus on the furan moiety found within toosendanin as its requirements appear more adaptable for analogues preparation and biological testing.

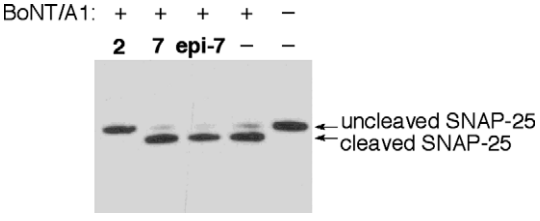


Figure 5. Western blot of RSC assay using toosendanin (**2**) and analogues **7**/**epi-7**.

4. Experimental

4.1. Chemistry

In general, CH_2Cl_2 and MeOH were distilled from CaH_2. Tetrahydrofuran (THF) was distilled from Sodium. Reagents were purchased from commercial sources and used without further purification. Synthetic reactions were monitored by analytical thin layer chromatography (TLC) carried out on 0.25 mm silica gel plates (60F-254). All flash column chromatography was performed using Silica Gel 60 (230–400 mesh). Preparative TLC was also performed using Merck Kieselgel 60F-254 silica gel plates (0.5 or 1 mm). ^{1}H NMR spectra were recorded on Bruker DRX-600 (600 MHz) or DRX-500 (500 MHz) spectrometers, and ^{13}C NMR spectra was recorded on Bruker DRX-600 (150 MHz) or DRX-500 (125 MHz) spectrometers. Chemical shifts were reported in parts per million (ppm) on the δ scale from an internal standard (NMR descriptions: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet). Electrospray ionization (ESI) time-of-flight reflectron experiments were performed at The Scripps Research Institute on an Agilent ESI-TOF mass spectrometer. Samples were electrosprayed into the TOF reflectron analyzer at a ESI voltage of 4000 V and a flow rate of 200 μL/min. Toosendanin was purchased from AvaChem Scientific. Subsequently, the purity of the toosendanin was confirmed by both TLC and HPLC. Toosendanin by TLC with EtOAc–hexane (3:1, v/v) runs as two spots with R_f values of 0.38 and 0.56 representing a mixture of *endo* and *exo* isomers. Finally, we note that reported NMR data displays compound **7**, **epi-7**, and **19** as a mixture of both *endo* and *exo* isomers.

4.1.1. (4a*RS*,7*RS*,8a*SR*)-Ethyl 7-(benzyloxy)-2,2-dimethoxydecahydronaphthalene-4a-carboxylate (11)

Catalytic amount of trifluoromethanesulfonic acid was added to a solution of (2*RS*,4a*RS*,8a*SR*)-ethyl 2-hydroxy-7-oxodecahydronaphthalene-4a-carboxylate (**10**)[13] (1.37 g, 5.7 mmol) and benzyl

2,2,2-trichloroacetimidate (1.2 mL, 6.3 mmol) in cyclohexane (20 mL) and CH_2Cl_2 (10 mL) at room temperature, and the mixture was stirred at room temperature for 9 h. The precipitate was filtered off, and the filtrate was washed with satd $NaHCO_3$ aq and brine, dried over Na_2SO_4. The solvent was evaporated under reduced pressure to leave an oil. The crude was dissolved in dry MeOH (40 mL), then addition of catalytic amount of concentrated H_2SO_4 at room temperature. The mixture was stirred at room temperature for 14 h (over night). The reaction mixture was poured into satd K_2CO_3 aq under ice-cooling. The mixture was extracted with $CHCl_3$, washed with brine, dried over Na_2SO_4. The solvent was evaporated under reduced pressure to leave an oil, which was purified by flash chromatography on SiO_2 with EtOAc–hexane (1:4, v/v) to give **11** (1.10 g, 51%, for two steps from **10**) as a yellow oil: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.33–7.30 (m, 4H), 7.27–7.23 (m, 1H), 4.54 (q, J = 12.4 Hz, 2H), 4.17–4.12 (m, 2H), 3.39 (sept, J = 5.1 Hz, 1H), 3.18 (s, 3H), 3.12 (s, 3H), 2.17–2.14 (m, 1H), 2.06 (t, J = 13.2 Hz, 1H), 1.98–1.79 (m, 5H), 1.75–1.71 (m, 1H), 1.48–1.43 (m, 1H), 1.30–1.12 (m, 7H, including 1.25 (t, J = 7.2 Hz, 3H)); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 174.6, 138.9, 128.3, 127.5, 127.4, 100.1, 77.1, 69.8, 60.0, 47.6, 47.5, 47.3, 38.9, 36.2, 35.5, 34.8, 33.6, 29.6, 29.4, 14.3; ESI-TOF MS (m/z): $[MNa]^+$ calcd for $C_{22}H_{32}O_5Na$ 399.2142, found 399.2144.

4.1.2. (4a*RS*,7*RS*,8a*SR*)-7-(Benzyloxy)-4a-(((2-(trimethylsilyl)-ethoxy)methoxy)methyl)octahydronaphthalen-2(1*H*)-one (12)

$LiAlH_4$ (2.0 M in THF, 2.1 mL, 4.1 mmol) was added to a solution of **11** (779 mg, 2.1 mmol) in dry THF (8 mL) at 0 °C, and the mixture was stirred at 100 °C (reflux condition) for 4 h. After addition of EtOAc, 1 N HCl, and Rochelle salt, the precipitate was filtered off, then the whole was washed with 1 N HCl, satd $NaHCO_3$ aq, and brine, dried over Na_2SO_4. The solvent was evaporated under reduced pressure to leave an oil. The crude was dissolved in THF (16 mL), then addition of 5 N HCl (4 mL) at room temperature. The mixture was stirred at room temperature for 1 h. After addition of solid $NaHCO_3$, water was added to the mixture until the sediment dissolved (pH 9). The whole was extracted with CH_2Cl_2, washed with brine, dried over Na_2SO_4. The solvent was evaporated under reduced pressure to leave an oil. The crude was dissolved in dry CH_2Cl_2 (8 mL), then DIPEA (0.75 mL, 4.6 mmol) and SEMCl (0.73 mL, 4.1 mmol) were added at 0 °C. The mixture was stirred at room temperature for 15 h (over night). After addition of EtOAc, the whole was washed with 1 N HCl, satd $NaHCO_3$ aq, and brine, then dried over Na_2SO_4. The solvent was evaporated under reduced pressure to leave an oil, which was purified by flash chromatography on SiO_2 with EtOAc–hexane (1:5, v/v) to give **12** (809 mg, 93%, for three steps from **11**) as a pale yellow oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.34–7.32 (m, 4H), 7.29–7.26 (m, 1H), 4.69 (s, 2H), 4.56 (s, 2H), 3.80 (s, 2H), 3.62–3.59 (m, 2H), 3.41 (sept, J = 5.3 Hz, 1H), 2.52–2.46 (m, 1H), 2.34–2.29 (m, 2H), 2.22–2.14 (m, 2H), 2.06–1.99 (m, 2H), 1.80–1.78 (m, 1H), 1.73–1.62 (m, 1H), 1.61–1.56 (m, 1H), 1.40 (q, J = 12.2 Hz, 1H), 1.27–1.21 (m, 1H), 0.98–0.91 (m, 3H), 0.02 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 210.8, 138.7, 128.4, 127.5, 95.1, 76.9, 70.1, 65.2, 63.6, 44.2, 42.4, 37.9, 36.1, 34.6, 34.5, 32.7, 27.6, 18.1, −1.5; ESI-TOF MS (m/z): $[MNa]^+$ calcd for $C_{24}H_{38}O_4SiNa$ 441.2431, found 441.2448.

4.1.3. (4a*SR*,7*RS*,8a*SR*)-7-(Benzyloxy)-4a-(((2-(trimethylsilyl)-ethoxy)methoxy)methyl)-4a,5,6,7,8,8a-hexahydronaphthalen-2-(1*H*)-one (13)

KHMDS (0.5 M in toluene, 26.4 mL, 13.2 mmol) was added (dropwise over the course of 10 min) to a solution of **12** (3.67 g, 8.8 mmol) in dry THF (75 mL) at −78 °C. After 40 min of stirring at −78 °C, TESCl (2.2 mL, 13.2 mmol) was added, and the mixture was stirred at −78 °C for 40 additional min. After addition of satd $NaHCO_3$ aq, the whole was extracted with ether, washed with brine, dried over Na_2SO_4. The solvent was evaporated under reduced pressure to leave an oil. The crude was dissolved in dry CH_2Cl_2 (75 mL), cooled to −78 °C, and addition of a solution of PhSeCl (98%, 2.1 g, 10.5 mmol) in dry CH_2Cl_2 (15 mL). The mixture was stirred at −78 °C for 30 min. After 30 min, the mixture was washed with satd $NaHCO_3$ aq and brine, dried over Na_2SO_4. The solvent was evaporated under reduced pressure to leave an oil. The crude was dissolved in THF (75 mL), cooled at 0 °C, then H_2O_2 (30 wt % in water, 2.7 mL, 26.3 mmol) was added. The mixture was stirred at room temperature for 1 h. After addition of EtOAc, the mixture was washed with 5% Na_2CO_3 aq and water, the aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4. The solvent was evaporated under reduced pressure to leave an oil, which was purified by flash chromatography on SiO_2 with EtOAc–hexane (1:5, v/v) to give **13** (3.02 g, 83%, for three steps from **12**) as a pale yellow oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.34–7.33 (m, 4H), 7.29–7.24 (m, 1H), 6.83 (d, J = 10.0 Hz, 1H), 5.97 (d, J = 10.0 Hz, 1H), 4.60–4.54 (m, 4H), 3.81 (d, J = 9.4 Hz, 1H), 3.77 (d, J = 9.4 Hz, 1H), 3.56–3.53 (m, 2H), 3.45 (sept, J = 5.1 Hz, 1H), 2.54 (dd, J = 17.7, 14.6 Hz, 1H), 2.24 (dd, J = 17.7, 4.3 Hz, 1H), 2.11–1.97 (m, 3H), 1.92–1.89 (m, 1H), 1.67–1.61 (m, 1H), 1.51 (q, J = 12.3 Hz, 1H), 1.30–1.24 (m, 1H), 0.97–0.90 (m, 2H), 0.01 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 199.2, 157.7, 138.5, 128.8, 128.3, 127.5, 127.4, 94.8, 76.5, 70.1, 67.9, 65.1, 40.7, 40.1, 39.2, 33.4, 31.2, 27.7, 17.9, −1.5; ESI-TOF MS (m/z): $[MNa]^+$ calcd for $C_{24}H_{36}O_4SiNa$ 439.2275, found 439.2285.

4.1.4. (1a*RS*,3*RS*,3a*RS*,5*RS*,7a*RS*,7b*RS*)-Methyl 5-(Benzyloxy)-3-methyl-2-oxo-7a-(((2-(trimethylsilyl)ethoxy)methoxy)methyl)decahydronaphtho[2,1-*b*]oxirene-3-carboxylate (14)

LiHMDS (1.0 M in THF, 10.5 mL, 10.5 mmol) was added in a single portion to a solution of **13** (2.18 g, 5.2 mmol) in dry THF (80 mL) at −78 °C. After 40 min of stirring at −78 °C, methyl cyanoformate (0.63 mL, 7.7 mmol) was added, and the mixture was stirred at −78 °C for 4 h. After addition of water and EtOAc, the whole was extracted with EtOAc, washed with brine, dried over Na_2SO_4. The solvent was evaporated under reduced pressure to leave an oil, which was purified by flash chromatography on SiO_2 with EtOAc–hexane (1:4, v/v) to give β-**ketomethylester** (2.33 g, 94%) as a pale yellow oil. The β-**ketomethylester** (2.33 g, 4.9 mmol) was dissolved in dry THF (80 mL), cooled at 0 °C, and addition of NaH (60%, 0.24 g, 5.9 mmol). After 5 min of stirring at 0 °C, methyl iodide (1.5 mL, 24.6 mmol) was added, and the mixture was stirred at room temperature for 14 h (over night). After addition of satd NH_4Cl aq, the whole was extracted with EtOAc, washed with brine, dried over Na_2SO_4. The solvent was evaporated under reduced pressure to leave an oil. The crude was dissolved in THF (80 mL), cooled at 0 °C, and addition of triton B (40 wt % in water, 3.9 mL, 9.8 mmol) and TBHP (70 wt % in water, 1.4 mL, 9.8 mmol). The mixture was stirred at room temperature for 3 h. After addition of EtOAc, the whole was washed with 10% $Na_2S_2O_3$ aq, then the aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4. The solvent was evaporated under reduced pressure to leave an oil, which was purified by flash chromatography on SiO_2 with EtOAc–hexane (1:5, v/v) to give **14** (1.71 g, 65%, for three steps from **13**) as a very pale yellow oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.34–7.24 (m, 5H), 4.63 (s, 2H), 4.56–4.51 (m, 2H), 3.85 (d, J = 10.4 Hz, 1H), 3.66–3.58 (m, 6H, including 3.64 (s, 3H)), 3.55–3.51 (m, 1H), 3.42 (d, J = 4.4 Hz, 1H), 3.34 (sept, J = 5.2 Hz, 1H), 2.32–2.29 (m, 1H), 2.06–2.03 (m, 2H), 1.84–1.82 (m, 1H), 1.56–1.49 (m, 1H), 1.41–1.36 (m, 4H, including 1.40 (s, 3H)), 1.24–1.18 (m, 1H), 0.95–0.89 (m, 2H), 0.01 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 205.3, 170.9, 138.4, 128.4, 127.6, 127.5, 95.0, 77.0, 70.3, 66.9, 65.2, 64.2, 57.4, 55.9, 52.2, 42.6, 38.8, 29.4,

28.9, 27.1, 23.8, 18.1, −1.4; ESI-TOF MS (*m/z*): $[MNa]^+$ calcd for $C_{27}H_{40}O_7SiNa$ 527.2435, found 527.2441.

4.1.5. (1*R*,4*S*,4a*R*,7*R*,8a*R*)-methyl 7-(benzyloxy)-4-hydroxy-1-methyl-2-oxo-4a-(((2-(trimethylsilyl)ethoxy)methoxy)-methyl)decahydronaphthalene-1-carboxylate (15)

Acetic acid (7.1 μL, 0.12 mmol) was added to an ethanolic solution of PhSeNa which prepared by the reduction of $(PhSe)_2$ (98%, 119 mg, 0.37 mmol) with $NaBH_4$ (28 mg, 0.75 mmol) in EtOH (2 mL), and the mixture was stirred at room temperature for few minutes until the bright yellow solution turned colorless. The resulting solution was added to a solution of **14** (125 mg, 0.25 mmol) in EtOH (3 mL), and stirred at room temperature for 30 min. After addition of EtOAc, the whole was washed with satd NH_4Cl aq and brine, dried over Na_2SO_4. The solvent was evaporated under reduced pressure to leave an oil, which was purified by flash chromatography on SiO_2 with EtOAc–hexane (1:3, v/v) to give **15** (108 mg, 86%) as a yellow oil. ^1H NMR ($CDCl_3$, 600 MHz) δ 7.37–7.33 (m, 4H), 7.29–7.26 (m, 1H), 4.63 (s, 2H), 4.59 (s, 2H), 4.26 (br s, 1H), 3.85 (d, J = 10.4 Hz, 1H), 3.68 (s, 3H), 3.62 (d, J = 10.4 Hz, 1H), 3.58–3.55 (m, 2H), 3.34–3.31 (m, 1H), 3.27 (dd, J = 15.1, 2.9 Hz, 1H), 2.55 (dd, J = 15.1, 3.3 Hz, 1H), 2.22–2.20 (m, 1H), 2.05–2.02 (m, 2H), 1.89–1.80 (m, 2H), 1.71 (br s, 1H), 1.59–1.54 (m, 2H), 1.36 (s, 3H), 0.98–0.89 (m, 2H), 0.02 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 206.6, 173.5, 138.6, 128.4, 127.6, 127.5, 94.9, 77.8, 71.7, 70.2, 65.2, 64.7, 57.3, 52.3, 45.2, 44.4, 42.0, 29.4, 27.2, 26.7, 20.8, 18.1, −1.5; ESI-TOF MS (*m/z*): $[MNa]^+$ calcd for $C_{27}H_{42}O_7SiNa$ 529.2592, found 529.2596.

4.1.6. (1*RS*,4*SR*,4a*RS*,7*RS*,8a*RS*)-Methyl 7-(benzyloxy)-1-methyl-2-oxo-4-((2-(trimethylsilyl)ethoxy)methoxy)-4a-(((2-(trimethylsilyl)ethoxy)methoxy)methyl)decahydronaphthalene-1-carboxylate (16)

The **15** (108 mg, 0.21 mmol) was dissolved in dry CH_2Cl_2 (3 mL), then DIPEA (0.19 mL, 1.2 mmol) and SEMCl (0.19 mL, 1.1 mmol) were added at 0 °C. The mixture was stirred at room temperature for 39 h. After addition of EtOAc, the whole was washed with 1 N HCl, satd $NaHCO_3$ aq, and brine, then dried over Na_2SO_4. The solvent was evaporated under reduced pressure to leave an oil, which was purified by flash chromatography on SiO_2 with EtOAc–hexane (1:5, v/v) to give **16** (134 mg, 99%) as a colorless oil. ^1H NMR ($CDCl_3$, 600 MHz) δ 7.37–7.33 (m, 4H), 7.29–7.27 (m, 1H), 4.70 (d, J = 7.2 Hz, 1H), 4.62 (s, 2H), 4.60 (d, J = 7.2 Hz, 1H), 4.59 (s, 2H), 4.09 (br t, J = 2.9 Hz, 1H), 3.86 (d, J = 10.4 Hz, 1H), 3.69–3.58 (m, 5H, including 3.67 (s, 3H)), 3.57–3.53 (m, 3H), 3.35–3.29 (m, 1H), 3.11 (dd, J = 15.3, 2.8 Hz, 1H), 2.72 (dd, J = 15.3, 3.3 Hz, 1H), 2.20–2.18 (m, 1H), 2.01–1.96 (m, 2H), 1.85–1.75 (m, 2H), 1.60–1.54 (m, 2H), 1.35 (s, 3H), 0.98–0.89 (m, 4H), 0.03 (s, 9H), 0.03 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 205.9, 173.5, 138.7, 128.3, 127.5, 127.5, 94.9, 94.2, 77.7, 77.4, 70.1, 65.4, 65.2, 64.3, 57.1, 52.2, 45.1, 42.2, 42.0, 29.5, 27.2, 26.6, 20.9, 18.1, 18.0, −1.5; ESI-TOF MS (*m/z*): $[MNa]^+$ calcd for $C_{33}H_{56}O_8Si_2Na$ 659.3406, found 659.3406.

4.1.7. (1*RS*,2*RS*,4*SR*,4a*RS*,7*RS*,8a*RS*)-Methyl 7-(Benzyloxy)-2-hydroxy-1-methyl-4-((2-(trimethylsilyl)ethoxy)methoxy)-4a-(((2-(trimethylsilyl)ethoxy)methoxy)methyl)decahydronaphthalene-1-carboxylate (17)

$NaBH_4$ (226 mg, 5.8 mmol) was added to a solution of **16** (929 mg, 1.5 mmol) in CH_2Cl_2 (15 mL) and MeOH (1 mL) at 0 °C, the mixture was stirred at room temperature for 24 h. After addition of CH_2Cl_2, the whole was washed with satd NH_4Cl aq and brine, then dried over Na_2SO_4. The solvent was evaporated under reduced pressure to leave an oil, which was purified by flash chromatography on SiO_2 with EtOAc–hexane (1:5, v/v) to give **17** (286 mg, 31%) as a colorless oil and **epi-17** (644 mg, 68%) as a colorless oil in the order of elution. Compound **17**: ^1H NMR ($CDCl_3$, 600 MHz) δ 7.38–7.34 (m, 4H), 7.29–7.26 (m, 1H), 4.75–4.74 (m, 1H), 4.62–4.53 (m, 5H), 4.10 (br s, 1H), 4.07 (br s, 1H), 3.94 (br s, 1H), 3.76–3.71 (m, 1H), 3.67–3.54 (m, 5H, including 3.65 (s, 3H)), 3.53–3.48 (m, 2H), 3.36–3.32 (m, 1H), 3.08 (d, J = 10.2 Hz, 1H), 2.25–2.18 (m, 2H), 2.09–2.06 (m, 1H), 2.03–1.84 (m, 3H), 1.79–1.77 (m, 1H), 1.53–1.44 (m, 2H), 1.29 (s, 3H), 0.96–0.90 (m, 4H), 0.03–0.01 (m, 18H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 177.2, 138.9, 128.4, 127.6, 127.5, 95.2, 94.8, 78.5, 77.7, 72.0, 70.1, 66.0, 65.0, 64.0, 51.5, 48.2, 42.5, 38.5, 29.3, 29.1, 27.4, 26.9, 23.6, 18.1, 18.0, −1.4; ESI-TOF MS (*m/z*): $[MNa]^+$ calcd for $C_{33}H_{58}O_8Si_2Na$ 661.3562, found 661.3562. Compound **epi-17**: ^1H NMR ($CDCl_3$, 600 MHz) δ 7.37–7.33 (m, 4H), 7.29–7.26 (m, 1H), 4.73 (d, J = 7.0 Hz, 1H), 4.62–4.54 (m, 5H), 3.89 (br s, 1H), 3.68 (s, 3H), 3.65–3.60 (m, 3H), 3.57–3.50 (m, 3H), 3.29 (br sept, J = 5.0 Hz, 1H), 3.22 (d, J = 9.9 Hz, 1H), 2.23–2.15 (m, 2H), 2.07–1.95 (m, 2H), 1.78–1.64 (m, 4H), 1.54–1.37 (m, 6H, including 1.40 (s, 3H)), 0.94–0.92 (m, 4H), 0.03–0.01 (m, 18H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 178.2, 138.8, 128.4, 127.6, 127.5, 94.8, 94.2, 78.2, 75.5, 73.1, 70.1, 65.4, 64.9, 63.9, 51.6, 49.2, 44.6, 42.2, 32.7, 29.7, 27.1, 26.7, 23.2, 18.1, 18.0, −1.4; ESI-TOF MS (*m/z*): $[MNa]^+$ calcd for $C_{33}H_{58}O_8Si_2Na$ 661.3562, found 661.3576. The **epi-17** (826 mg, 1.3 mmol) was dissolved in dry CH_2Cl_2 (10 mL), then Dess–Martin periodinane (97%, 679 mg, 1.6 mmol) was added at 0 °C. The mixture was stirred at room temperature for 1 h. After addition of sat. $NaHCO_3$ aq, the whole was extracted with CH_2Cl_2, washed with brine, dried over Na_2SO_4. The solvent was evaporated under reduced pressure to leave an oil, which was purified by flash chromatography on SiO_2 with EtOAc–hexane (1:5, v/v) to give **16** (790 mg, 96%) as a colorless oil.

4.1.8. (1*RS*,2*RS*,4*SR*,4a*RS*,7*RS*,8a*RS*)-7-(Benzyloxy)-1-formyl-1-methyl-4-((2-(trimethylsilyl)ethoxy)methoxy)-4a-(((2-(trimethylsilyl)ethoxy)methoxy)methyl)decahydronaphthalen-2-yl acetate (18)

$LiAlH_4$ (1.0 M in Et_2O, 3.6 mL, 3.6 mmol) was added to a solution of **17** (1.16 g, 1.8 mmol) in dry Et_2O (12 mL) at 0 °C, and the mixture was stirred at room temperature for 1.5 h. After careful addition of 1 mL of water, 3 mL of 15% NaOH aq, and 1 mL of water, the white precipitate was filtrated off. The aqueous layer was extracted with ether, washed with brine, dried over Na_2SO_4. The solvent was evaporated under reduced pressure to leave an oil, which was purified by flash chromatography on SiO_2 with EtOAc–hexane (1:2, v/v) to give **diol** (1.02 g, 91%) as a colorless oil. ^1H NMR ($CDCl_3$, 600 MHz) δ 7.35–7.33 (m, 4H), 7.29–7.26 (m, 1H), 4.75 (d, J = 7.0 Hz, 1H), 4.62–4.54 (m, 5H), 3.95 (br s, 1H), 3.83 (br s, 1H), 3.76–3.71 (m, 2H), 3.64–3.56 (m, 3H), 3.50 (d, J = 11.0 Hz, 1H), 3.46–3.43 (m, 2H), 3.41–3.37 (m, 1H), 2.06–1.97 (m, 4H), 1.89–1.82 (m, 2H), 1.51–1.42 (m, 2H), 1.34 (br q, J = 12.3 Hz, 1H), 1.25 (s, 1H), 1.12 (s, 3H), 0.96–0.92 (m, 4H), 0.03–0.02 (m, 18H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 138.8, 128.4, 127.6, 127.5, 95.0, 95.0, 78.4, 77.4, 71.1, 70.1, 66.0, 65.9, 65.8, 65.2, 43.1, 42.5, 39.7, 28.4, 28.0, 27.7, 27.4, 22.2, 18.2, 18.1, −1.4; ESI-TOF MS (*m/z*): $[MH]^+$ calcd for $C_{32}H_{59}O_7Si_2$ 611.3794, found 611.3795. The **diol** (1.02 g, 1.7 mmol) was dissolved in CH_2Cl_2 (40 mL), then TEMPO (21.0 mg, 0.13 mmol), KBr (31.7 mg, 0.27 mmol), and satd $NaHCO_3$ aq (20 mL) ware added at room temperature. The resulting biphasic mixture was cooled at 0 °C, and then treated with NaOCl (4% in water, 3.0 mL, 1.8 mmol), the mixture was stirred at room temperature for 20 min. After addition of brine and satd $NaHCO_3$ aq the whole was extracted with CH_2Cl_2, washed with brine, dried over Na_2SO_4. The solvent was evaporated under reduced pressure to leave an oil. The crude was dissolved in pyridine (6 mL), and then Ac_2O (3 mL) and catalytic amount of DMAP were added at room temperature. The mixture was stirred at room temperature for 17 h (over night). The solvent was removed under reduced

pressure to leave an oil, which was purified by flash chromatography on SiO_2 with EtOAc–hexane (1:4, v/v) to give **18** (1.01 g, 85% for three steps from **17**) as a colorless oil. ^1H NMR ($CDCl_3$, 600 MHz) δ 9.65 (s, 1H), 7.37–7.32 (m, 4H), 7.31–7.28 (m, 1H), 5.36 (br s, 1H), 4.65–4.63 (m, 2H), 4.59–4.52 (m, 4H), 3.82 (br s, 1H), 3.68 (d, J = 9.8 Hz, 1H), 3.62–3.48 (m, 4H), 3.44 (br sept, J = 5.1 Hz, 1H), 3.18 (d, J = 9.8 Hz, 1H), 2.23–2.16 (m, 3H), 2.08–2.02 (m, 4H, including 2.07 (s, 3H)), 1.95–1.91 (m, 1H), 1.76–1.66 (m, 3H), 1.54–1.47 (m, 1H), 1.04 (s, 3H), 0.94–0.88 (m, 4H), 0.02 (s, 9H), 0.01 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 201.7, 170.4, 138.6, 128.4, 127.6, 127.6, 94.7, 93.6, 77.9, 73.1, 71.2, 70.2, 65.3, 65.2, 64.8, 51.0, 42.0, 39.3, 27.2, 27.1, 27.1, 26.2, 21.3, 19.3, 18.1, 18.0, −1.4, −1.4; ESI-TOF MS (m/z): $[MNa]^+$ calcd for $C_{34}H_{58}O_8Si_2Na$ 673.3562, found 673.3562.

4.1.9. 4-((2-Trimethylsilyl)ethoxy)methoxy-7-epi AB-ring of TSDN (19)

A solution of **18** (135 mg, 0.21 mmol) in dry CH_2Cl_2 (2 mL) and dry MeOH (2 mL) was hydrogenated in the presence of $Pd(OH)_2$ on carbon (20 wt %, 60 mg) at room temperature, and 1 atom for 1 h. $Pd(OH)_2$ on carbon was filtered off, and the filtrate was evaporated under reduced pressure to leave an oil, which was purified by flash chromatography on SiO_2 with EtOAc–hexane (2:1, v/v) to give **19** (84.0 mg, 94%) as a colorless oil. Compound **19** is an inseparable mixture of diastereomers (dr = 5:2). ^1H NMR ($CDCl_3$, 500 MHz) δ 5.22 (d, J = 3.7 Hz, 1H), 4.95 (s, 1H), 4.74 (s, 1H), 4.72 (d, J = 3.7 Hz, 1H), 4.70 (d, J = 7.0 Hz, 1H), 4.69 (d, J = 7.0 Hz, 1H), 4.53 (d, J = 7.0 Hz, 1H), 4.53 (d, J = 7.0 Hz, 1H), 4.28 (d, J = 12.0 Hz, 1H), 4.12 (d, J = 12.0 Hz, 1H), 3.85 (br s, 1H), 3.66–3.44 (m, 8H), 3.41 (br s, 1H), 3.30 (d, J = 12.0 Hz, 1H), 3.07 (d, J = 12.0 Hz, 1H), 2.56–2.51 (m, 2H), 2.36–1.24 (m, 20H, including 2.04 (s, 3H) and 2.03 (s, 3H)), 1.06–1.03 (m, 2H), 0.95–0.83 (m, 10H, including 0.91 (s, 3H) and 0.86 (s, 3H)), 0.00 (s, 18H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.7, 96.3, 95.8, 93.3, 93.2, 78.1, 78.0, 76.0, 72.7, 71.1, 70.9, 66.7, 65.4, 61.8, 40.1, 39.6, 36.5, 36.5, 33.9, 32.4, 31.8, 31.7, 31.5, 31.1, 30.8, 30.1, 27.8, 27.0, 21.3, 21.3, 18.9, 18.2, 18.0, −1.5; ESI-TOF MS (m/z): $[MNa]^+$ calcd for $C_{21}H_{38}O_7SiNa$ 453.2279, found 453.2283.

4.1.10. 7-Epi AB-ring of TSDN (epi-7)

TFA (2 mL) was added to a solution of **19** (45.4 mg, 0.11 mmol) in CH_2Cl_2 (2 mL) at 0 °C, the mixture was stirred at 0 °C for 1.5 h. The solvent was removed under reduced pressure to leave an oil, which was purified by flash chromatography on SiO_2 with EtOAc–MeOH (95:5, v/v) to give **epi-7** (25.2 mg, 80%) as a colorless oil. Compound **epi-7** is an inseparable mixture of diastereomers (dr = 7:1). ^1H NMR (CD_3OD, 600 MHz) δ 5.17 (d, J = 3.6 Hz, 1H), 4.87 (s, 1H), 4.67 (d, J = 3.6 Hz, 1H), 4.62 (s, 1H), 4.27 (d, J = 12.0 Hz, 1H), 4.11 (d, J = 12.0 Hz, 1H), 3.63–3.62 (m, 1H), 3.49–3.43 (m, 3H), 3.24 (d, J = 12.0 Hz, 1H), 2.98 (d, J = 12.0 Hz, 1H), 2.69–2.63 (m, 2H), 2.33 (dd, J = 13.5, 3.8 Hz, 1H), 2.23–1.72 (m, 15H, including 2.03 (s, 3H) and 2.02 (s, 3H)), 1.52–1.46 (m, 1H), 1.37–1.24 (m, 3H), 1.05–1.01 (m, 2H), 0.87 (s, 3H), 0.81 (s, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 172.8, 172.7, 97.4, 96.8, 78.2, 74.8, 74.2, 74.2, 72.2, 72.0, 67.8, 62.7, 41.7, 41.2, 38.0, 37.9, 36.9, 36.7, 34.7, 33.4, 32.5, 32.0, 31.7, 31.2, 29.2, 28.4, 21.4, 21.3, 19.6, 19.0; ESI-TOF MS (m/z): $[MNa]^+$ calcd for $C_{15}H_{24}O_6Na$ 323.1465, found 323.1452.

4.1.11. Hemi-acetal-protected 4-((2-trimethylsilyl)ethoxy)methoxy-7-epi AB-ring of TSDN (22)

CbzCl (95%, 0.10 mL, 0.70 mmol) was added to a solution of **19** (177 mg, 0.41 mmol) in dry CH_2Cl_2 (5 mL) and TMEDA (55.5 μL, 0.37 mmol) at −78 °C, and the mixture was stirred at −78 °C for 2 h. After addition of CH_2Cl_2, The whole was washed with water, 1 N HCl aq, and brine, dried over Na_2SO_4. The solvent was evaporated under reduced pressure to leave an oil, which was purified by flash chromatography on SiO_2 with EtOAc–hexane (1:1, v/v) to give **21** (165 mg, including di-Cbz and/or undesired mono-Cbz products) as a colorless oil, **22** (115 mg, 49%) as a colorless oil, and **incompletely purified** recovery of **19** (14.3 mg) as a colorless oil in the order of elution. Compound **22**: ^1H NMR ($CDCl_3$, 600 MHz) δ 7.38–7.33 (m, 5H), 5.71 (s, 1H), 5.19–5.15 (m, 3H), 4.71 (d, J = 7.0 Hz, 1H), 4.54 (d, J = 7.0 Hz, 1H), 4.22 (d, J = 12.4 Hz, 1H), 3.68–3.54 (m, 3H), 3.49 (d, J = 3.6 Hz, 1H), 3.39 (d, J = 12.4 Hz, 1H), 2.54–2.43 (m, 2H), 2.10–1.83 (m, 7H, including 2.04 (s, 3H)), 1.60–1.54 (m, 1H), 1.39–1.32 (m, 1H), 1.10–1.08 (m, 1H), 0.96–0.84 (m, 5H, including 0.86 (s, 3H)), 0.01 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.3, 154.2, 134.7, 128.6, 128.6, 128.4, 97.9, 93.2, 77.7, 72.8, 70.8, 70.1, 67.4, 65.5, 38.9, 36.4, 34.1, 31.2, 30.9, 30.3, 26.8, 21.3, 18.2, 18.1, −1.4; ESI-TOF MS (m/z): $[MNa]^+$ calcd for $C_{29}H_{44}O_9SiNa$ 587.2647, found 587.2648. A solution of **21** (165 mg) in dry EtOH (5 mL) was hydrogenated in the presence of Pd on carbon (10 wt %, 80 mg) at room temperature, and 1 atom for 1.5 h. Pd on carbon was filtered off, and the filtrate was combined with **incompletely purified** recovery of **19** (14.3 mg), then evaporated under reduced pressure to leave an oil, which was purified by flash chromatography on SiO_2 with EtOAc–hexane (2:1, v/v) to give **19** (86.1 mg, 49% for two steps from **19** via **21**) as a colorless oil.

4.1.12. Hemi-acetal-protected 4-((2-trimethylsilyl)ethoxy)methoxy-7-keto AB-ring of TSDN (23)

The **22** (134 mg, 0.24 mmol) was dissolved in dry CH_2Cl_2 (5 mL), then Dess–Martin periodinane (97%, 311 mg, 0.71 mmol) was added at 0 °C. The mixture was stirred at room temperature for 17 h (over night). After addition of 10% $Na_2S_2O_3$ aq, the whole was extracted with CH_2Cl_2, washed with sat. $NaHCO_3$ aq and brine, dried over Na_2SO_4. The solvent was evaporated under reduced pressure to leave an oil, which was purified by flash chromatography on SiO_2 with EtOAc–hexane (1:2, v/v) to give **23** (127 mg, 96%) as a colorless oil. ^1H NMR ($CDCl_3$, 600 MHz) δ 7.39–7.33 (m, 5H), 5.70 (s, 1H), 5.20–5.16 (m, 3H), 4.70 (d, J = 7.1 Hz, 1H), 4.54 (d, J = 7.1 Hz, 1H), 4.27 (d, J = 12.6 Hz, 1H), 3.64–3.59 (m, 3H), 3.55–3.50 (m, 1H), 2.90 (dd, J = 14.2, 4.7 Hz, 1H), 2.55–2.51 (m, 2H), 2.45–2.33 (m, 3H), 2.25–2.20 (m, 1H), 2.16–2.04 (m, 1H), 2.02 (s, 3H), 1.37–1.33 (m, 1H), 0.95–0.83 (m, 5H, including 0.84 (s, 3H)), 0.00 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 210.3, 170.1, 154.1, 134.5, 128.7, 128.6, 128.4, 97.6, 92.9, 77.7, 72.3, 70.2, 67.4, 65.7, 39.0, 37.4, 36.5, 36.4, 35.5, 31.0, 28.3, 21.2, 18.1, 18.0, −1.5; ESI-TOF MS (m/z): $[MNa]^+$ calcd for $C_{29}H_{42}O_9SiNa$ 585.2490, found 585.2501.

4.1.13. Hemi-acetal-protected 4-((2-trimethylsilyl)ethoxy)methoxy AB-ring of TSDN (24)

$NaBH_4$ (17.3 mg, 0.45 mmol) was added to a solution of **23** (127 mg, 0.23 mmol) in CH_2Cl_2 (5.6 mL) and MeOH (0.4 mL) at 0 °C, the mixture was stirred at room temperature for 16 h (over night). After addition of CH_2Cl_2, the whole was washed with satd NH_4Cl aq and brine, then dried over Na_2SO_4. The solvent was evaporated under reduced pressure to leave an oil, which was purified by flash chromatography on SiO_2 with CH_2Cl_2–MeOH (93:7, v/v) to give mixture of **22** and **24** (122 mg, 95%, **22**/**24** = 2.6/1 by analytical HPLC) as a colorless oil. The mixture was completely separated by p-TLC and HPLC technique. Compound **24**: ^1H NMR ($CDCl_3$, 600 MHz) δ 7.38–7.33 (m, 5H), 5.68 (s, 1H), 5.19–5.15 (m, 3H), 4.74 (d, J = 7.1 Hz, 1H), 4.58 (d, J = 7.1 Hz, 1H), 4.21–4.19 (m, 2H), 3.67–3.63 (m, 1H), 3.60–3.55 (m, 1H), 3.44 (d, J = 3.7 Hz, 1H), 3.40 (d, J = 12.2 Hz, 1H), 2.80–2.77 (m, 1H), 2.54–2.50 (m, 1H), 2.34–2.28 (m, 1H), 2.11–2.01 (m, 5H, including 2.04 (s, 3H)), 1.80–1.64 (m, 3H), 0.95–0.79 (m, 6H, including 0.83 (s, 3H)), 0.01 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.5, 154.3, 134.7, 128.6,

128.6, 128.3, 98.1, 93.2, 78.1, 73.1, 70.0, 67.1, 66.0, 65.3, 38.5, 36.8, 31.2, 29.0, 27.9, 27.2, 21.9, 21.4, 18.1, −1.4; ESI-TOF MS (*m/z*): $[MNa]^+$ calcd for $C_{29}H_{44}O_9SiNa$ 587.2647, found 587.2646.

4.1.14. AB-ring of TSDN (7)

A solution of **24** (12.0 mg) in dry EtOH (2 mL) was hydrogenated in the presence of Pd on carbon (10 wt %, 5 mg) at room temperature, and 1 atom for 1 h. Pd on carbon was filtered off, then evaporated under reduced pressure to leave an oil. The crude was dissolved in CH_2Cl_2 (2 mL), cooled at 0 °C, then TFA (2 mL) was added. The mixture was stirred at 0 °C for 1.5 h. The solvent was removed under reduced pressure to leave an oil, which was purified by flash chromatography on SiO_2 with EtOAc–MeOH (95:5, v/v) to give **7** (5.2 mg, 82% for two steps from 24) as a colorless oil. Compound **7** is an inseparable mixture of diastereomers (dr = 2:1). ^1H NMR ($CDCl_3$, 600 MHz) δ 5.44 (d, J = 3.3 Hz, 1H), 4.96 (d, J = 2.5 Hz, 1H), 4.94 (d, J = 3.3 Hz, 1H), 4.79 (d, J = 2.5 Hz, 1H), 4.29 (d, J = 12.1 Hz, 1H), 4.21 (br s, 1H), 4.17 (br s, 1H), 4.12 (d, J = 12.1 Hz, 1H), 3.67 (br s, 2H), 3.47 (br s, 2H), 3.32 (d, J = 12.1 Hz, 1H), 3.09 (d, J = 12.1 Hz, 1H), 2.85–2.79 (m, 2H), 2.69 (d, J = 3.5 Hz, 1H), 2.59 (dd, J = 13.3, 4.2 Hz, 1H), 2.47 (dd, J = 13.3, 4.2 Hz, 1H), 2.41–2.33 (m, 3H), 2.12 (s, 3H), 2.11 (s, 3H), 1.93–1.62 (m, 10H), 0.92–0.84 (m, 8H, including 0.91 (s, 3H) and 0.87 (s, 3H)); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 169.9, 169.7, 96.5, 95.9, 74.6, 74.5, 74.1, 66.3, 66.1, 66.1, 61.4, 60.4, 40.3, 39.6, 37.5, 35.7, 35.6, 29.7, 29.1, 28.2, 28.1, 28.1, 26.9, 25.8, 22.9, 22.2, 21.5, 21.4, 18.6, 18.0; ESI-TOF MS (*m/z*): $[MNa]^+$ calcd for $C_{15}H_{24}O_6Na$ 323.1465, found 323.1463.

4.2. Biological evaluation

4.2.1. Details of assay procedures

Pure botulinum neurotoxin type A1 was purified from *Clostridium botulinum* strains Hall A hyper as described.[23] The specific activity was determined via the mouse bioassay[24] to be 7.8 *pg*/mouse LD_{50}. The toxins were stored in 40% glycerol, 15 mM sodium phosphate, 90 mM NaCl at −20 °C.

4.2.2. RSC assay

Primary rat spinal cord cells were prepared as described previously,[22] and seeded into collagen coated 96-well plates (BD Biosciences) at a density of 75,000 cells/well. The RSC assay was essentially performed as described,[9,22] after the cells had matured for at least three weeks. In short, the cells were exposed to a mixture of the indicated concentrations of toosendanin or analogues and 5.6 *p*M BoNT/A1 (42 *p*g or about 5.5 mouse LD_{50} Units) in 50 μL of culture medium containing 1% DMSO. The positive control contained toxin and 1% DMSO, and the negative control contained 1% DMSO only in culture medium. All samples were tested in replicates of three. After 24 h of incubation at 37 °C, 5% CO_2, the cells were harvested by lysis in 1 × LDS sample buffer (Invitrogen), and analyzed by Western blot as described.[9,22]

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