Natural Products

DOI: 10.1002/anie.201100926

Synthesis of *ent*-Nanolobatolide**

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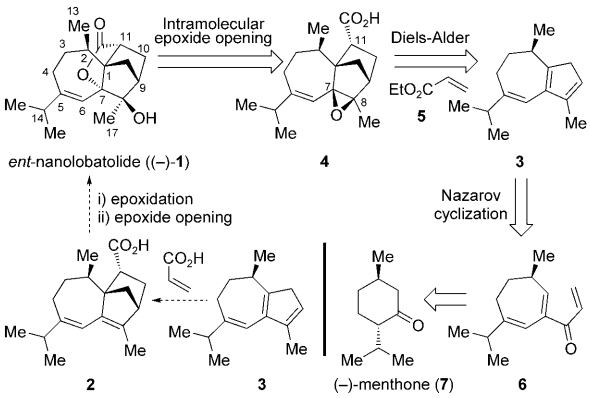
Neurodegeneration is a physiological phenomenon characterized by the progressive loss either of neurons or their function. The prevalence of neurodegenerative disorders, such as Alzheimer's disease and Parkinson's disease, has placed an ever-increasing burden on the healthcare system as a result of the debilitating social and economical impact of these diseases.[1] Indeed, the search for effective therapeutic intervention for these detrimental diseases continues to be of high priority on the global health agenda, and the identification of novel chemical entities that possess neuroprotective properties has an undisputed role in this context. In 2009, Sheu and co-workers reported the isolation and structural elucidation of nanolobatolide, a novel C18 terpenoid obtained from the extracts of Formosan soft coral *Sinularia nanolobata*.[2] Besides its structural intricacy, the biological effects of nanolobatolide on the neurological system were particularly fascinating. At a concentration of 10 μM, nanolobatolide not only displayed a cytotoxic effect against microglial cells, but was also found to exhibit anti-neuroinflammatory activity with a 45.5% reduction in the INF-γ stimulated expression of proinflammatory protein iNOS relative to the control cells, which were treated only with INF-γ.[2] Furthermore, nanolobatolide showed a neuroprotective effect in the 6-OHDA (6-hydroxydopamine) induced neurotoxicity of neuroblastoma SH-SY5Y, with neuroprotective activities ranging between 41.4% and 83.3% across the 0.01–10 μM concentration range.[2] The novel molecular architecture of nanolobatolide, coupled with its impressive biological properties, prompted us to undertake its synthesis. Herein we report the total synthesis of *ent*-nanolobatolide ((−)-**1**; Scheme 1), through a strategy that also provided evidence in support of its biogenetic origin.

Inspired by the speculated biosynthetic pathway that suggested a diene acid precursor (**2**) and a guaiane-type triene precursor (**3**),[2] a logical synthetic strategy was formulated, the retrosynthetic analysis of which is shown in Scheme 1. Thus, an intramolecular epoxide-opening reaction involving the C11 carboxylate and the C7–C8 epoxide was reserved as a late-stage synthetic maneuver to complete the oxo-bridged tetracyclic framework of the nanolobatolide structure. The thus obtained retrosynthetic intermediate, tetracyclic epoxide **4**, was expected to be derived from triene **3** through an intermolecular Diels–Alder reaction[3] with ethyl acrylate (**5**) and a subsequent chemo- and stereoselective epoxidation. By recognizing the positions of the olefinic functionalities within the [5,7]-bicyclic system of **3**, a Nazarov reaction[4] involving trienone **6** was envisaged for its construction. Finally, the asymmetric information, that was present throughout the synthesis and ultimately resulted in *ent*-nanolobatolide ((−)-**1**), originates from the readily available chiral building block, (−)-menthone (**7**).



Scheme 1. Molecular structure of *ent*-nanolobatolide ((−)-**1**) and proposed biosynthesis via diene acid **2** and triene **3**. Retrosynthetic analysis of *ent*-nanolobatolide ((−)-**1**) leading to epoxide **4**, triene **3**, trienone **6** and (−)-menthone (**7**).

As shown in Scheme 2, our initial foray towards the synthesis of the nanolobatolide structure featured an oxidative ring-expansion of (−)-menthone (**7**) followed by an intramolecular Dieckmann condensation to construct the [5,7]-bicyclic motif of enedione **11**. This first-generation strategy, as we shall see, enabled the validation of the proposed intermolecular Diels–Alder reaction and the intramolecular epoxide opening, which led to the preparation of the highly advanced tetracyclic intermediates **16** and **17**.

In this case, kinetic TMS silyl enol ether formation of (−)-menthone (**7**) followed by Simmons–Smith cyclopropanation[5] afforded cyclopropane **8** in 85% yield over the two steps. Oxidative ring-enlargement of cyclopropane **8** took place smoothly in the presence of CAN and NaI,[6a] and the initially formed β-iodo cycloheptanone underwent further elimination in the presence of NaOAc to furnish cycloheptenone **9** (86% yield over the two steps).[6b] Conversion of enone **9** into keto ester **10** required a three-carbon homologation reaction, which was readily accomplished through

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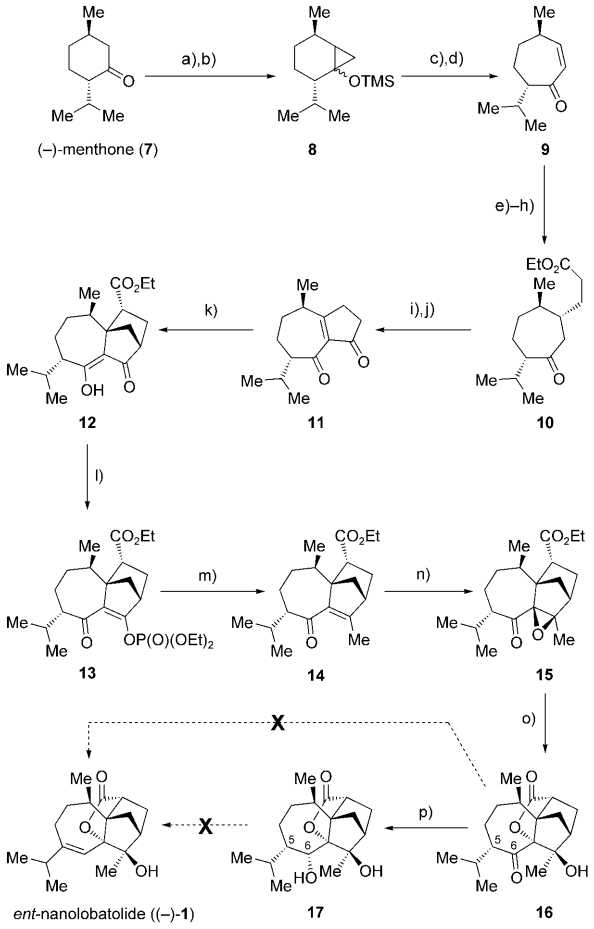
[**] We thank Doris Tan (ICES) for assistance with high-resolution mass spectrometry (HRMS). Financial support for this work was provided by A*STAR, Singapore.

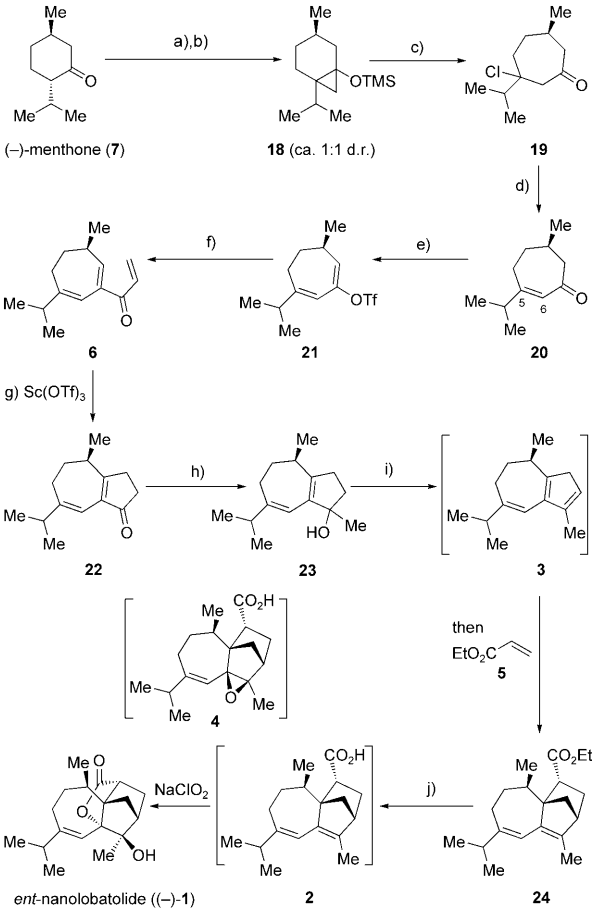
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201100926.



Scheme 2. Synthesis of advanced tetracyclic intermediates **16** and **17**. Reagents and conditions: a) LDA (0.5 M in THF, 1.3 equiv), THF, −78 → 0 °C, 1 h; then TMSCl (1.3 equiv), 0 °C, 1 h, 92%; b) CH_2I_2 (2.0 equiv), Et_2Zn (1.0 M in hexanes, 2.0 equiv), hexanes, 0→25 °C, 12 h, 92%; c) CAN (2.0 equiv), NaI (1.0 equiv), CH_3CN/H_2O (8:1), 0 °C, 1 h; d) NaOAc (6.0 equiv), MeOH, 80 °C, 12 h, 86% over two steps; e) vinylmagnesium bromide (1.4 M in THF, 1.7 equiv), CuI (0.1 equiv), THF, −78 °C, 1 h, 86%; f) O_3, CH_2Cl_2, −78 °C, 30 min; then PPh_3 (1.0 equiv), 25 °C, 16 h, 100%; g) Ph_3PCHCO_2Et (1.2 equiv), CH_2Cl_2, 40 °C, 12 h, 93%; h) H_2, Pd/C (10 wt.% , 0.1 equiv), EtOH, 25 °C, 12 h, 95%; i) KO*t*Bu (3.0 equiv), THF, 0 °C, 45 min, 89%; j) PhSeCl (1.05 equiv), py (1.1 equiv), CH_2Cl_2, 0 °C, 30 min; then H_2O_2 (70% aq., 3.8 equiv), CH_2Cl_2, 0 °C, 30 min, 60%; k) LDA (0.2 M in THF, 1.5 equiv), THF, −78 °C, 1 h; then ethyl acrylate (**5**, 2.0 equiv), −78 °C, 2 h, 86%; l) $ClPO(OEt)_2$ (1.5 equiv), HMPA (1.5 equiv), Et_3N (1.5 equiv), DMAP (0.15 equiv), Et_2O, 0 °C, 3 h, 78%; m) $[Fe(acac)_3]$ (5.0 equiv), MeMgBr (3.0 M in Et_2O, 20 equiv), THF/NMP (10:1), −30 °C, 15 min, 65%; n) *m*CPBA (1.0 equiv), CH_2Cl_2, 25 °C, 6 h, 92%; o) $TiCl_4$ (1.5 equiv), CH_2Cl_2, −78→25 °C, 12 h, 55%; p) $NaBH_4$ (1.5 equiv), MeOH, 0 °C, 3 h, 92%. acac = acetoacetonate, CAN = ceric ammonium nitrate, DMAP = 4-methylaminopyridine, HMPA = hexamethylphosphoryl amide, LDA = lithium diisopropylamide, *m*CPBA = *meta*-chloroperoxybenzoic acid, NMP = *N*-methylpyrrolidone, py = pyridine, THF = tetrahydrofuran, TMS = trimethylsilyl.

conjugate addition, oxidative olefin cleavage, Wittig olefination, and hydrogenation, with a 76% overall yield for this four-step transformation. An intramolecular Dieckmann condensation of keto ester **10** in the presence of K*t*OBu took place smoothly to afford the corresponding bicyclic diketone in 89% yield; this compound was treated with PhSeCl and then H_2O_2 to deliver enedione **11** in 60% yield. Gratifyingly, the proposed biomimetic Diels–Alder reaction[2] using the lithium enolate of enedione **11** (formed upon treatment of **11** with LDA) and ethyl acrylate (**5**) proceeded smoothly to give tricycle **12** in 86% yield as a single diastereoisomer, which exists in its enol form. Conversion of enol ketone **12** into enone **14** required extensive screening of reaction protocols, and ultimately a $[Fe(acac)_3]$-mediated[7] coupling between enol phosphate **13** (prepared from **12** upon its treatment with $ClP(O)OEt_2$, Et_3N, and DMAP; 78% yield) and MeMgBr was successful and gave **14** in 65% yield. In preparation for the intramolecular epoxide-opening reaction, for assembly of the tetracyclic core of the nanolobatolide structure, enone **14** was treated with *m*CPBA to afford epoxide **15** as a single diastereoisomer in 92% yield. In the presence of $TiCl_4$, epoxide **15** underwent lactone formation to construct the final ring required in the nanolobatolide structure in 55% yield. With the tetracyclic keto lactone **16** secured, its conversion into the nanolobatolide structure merely required the final introduction of the C5–C6 trisubstituted olefin. Much to our disappointment, this seemly simple transformation has remained elusive to date, primarily because of the inability of ketone **16** to undergo enolization under a variety of reaction conditions.[8] Reduction of the ketone moiety in **16** afforded the corresponding secondary alcohol **17** as a single stereoisomer, and this alcohol also resisted further elimination through transformations involving either E1 or E2 mechanistic pathways.[9] Attempts to form the C5–C6 trisubstituted olefin from tricyclic enone **14** or epoxide **15**, prior to the bridged lactone formation also failed. We rationalized that the severe steric congestion experienced at C6, which is shielded by the neighboring seven-membered ring that contains an isopropyl substituent, and the [2,2,1]-bicyclic system, is likely to be responsible for this late-stage obstacle. For this reason we opted for a revised strategy that involved an early introduction of the C5–C6 trisubstituted olefin.

Recognizing the shortfall in our initial attempts at the synthesis of the nanolobatolide structure, a revised strategy, which ultimately brought our synthetic route to fruition, is outlined in Scheme 3. Conveniently, (−)-menthone (**7**) once again served as the chiral starting material, and cycloheptenone **20**, which contains the target C5–C6 trisubstituted olefin, was prepared by a synthetic sequence analogous to that developed for its regioisomeric congener **9**. The only deviation in the synthesis was the initial TMS silyl enol ether formation, which was carried out under thermodynamic conditions. A Simmons–Smith cyclopropanation[5] of this TMS silyl enol ether afforded cyclopropane **18** (72% yield over the two steps from **7**), which was readily transformed to cycloheptenone **20** through the action of $FeCl_3$ and then NaOAc in 56% overall yield.[6b] In preparation for the proposed Nazarov cyclization to construct the [5,7]-bicyclic framework of dienone **22**, enone **20** was converted into the intermediate triflate **21**; the cross-coupling of **21** with tetravinyl tin in the presence of CO under palladium catalysis ($[Pd(PPh_3)_4]$) led to the trienone **6** (60% yield over the two



Scheme 3. Total synthesis of *ent*-nanolobatolide ((−)-**1**). Reagents and conditions: a) iPr$_2$NH (1.2 equiv), MeMgBr (3.0 M in Et$_2$O, 1.2 equiv), Et$_2$O, 25 °C, 12 h; then **7** (1.0 equiv), TMSCl (2.0 equiv), Et$_3$N (2.5 equiv), 25 °C, 8 h; b) CH_2I_2 (2.4 equiv), Et$_2$Zn (1.0 M in hexanes, 2.4 equiv), hexanes, 0→25 °C, 12 h, 72% over the two steps; c) $FeCl_3$ (3.0 equiv), DMF, 0 °C, 4 h; d) NaOAc (5.0 equiv), MeOH, 80 °C, 10 h, 56% over two steps; e) LHMDS (1.0 M in THF, 1.2 equiv), $PhNTf_2$ (1.2 equiv), THF, −78→0 °C, 2 h, 85%; f) [Pd(PPh$_3$)$_4$] (0.05 equiv), LiCl (3.0 equiv), tetravinyl tin (1.5 equiv), CO, DMF, 60 °C, 1.5 h, 70%; g) Sc(OTf)$_3$ (0.2 equiv), CH_2Cl_2, 0→25 °C, 3 h, 75%; h) MeLi (1.6 M in Et$_2$O, 5.0 equiv), THF, −78→0 °C, 1 h; i) $BF_3{\cdot}OEt_2$ (5.0 equiv), ethyl acrylate (**5**)/toluene (1:5), −20→0 °C, 3 h, 34% (38% brsm from **22**); j) DIBAL-H (1.0 M in toluene, 5.0 equiv), CH_2Cl_2, −78 °C, 1.5 h; then DMP (3.0 equiv), $NaHCO_3$ (10.0 equiv), CH_2Cl_2, 2 h; then $NaClO_2$ (3.0 equiv), 2-methyl-2-butene (30 equiv), tBuOH/pH 7 buffer (1:1), 18 h, 48% over three steps. brsm = based on recovered starting material, DIBAL-H = diisobutylaluminium hydride, DMF = *N*,*N*′-dimethylformamide DMP = Dess–Martin periodinane, LHMDS = lithium hexamethyldisilazide, Tf = trifluoromethanesulfonyl.

steps). Pleasingly, the Nazarov cyclization[4] of trienone **6** took place smoothly when using Sc(OTf)$_3$, to give dienone **22** in 75% yield. Unfortunately, our previously developed reaction conditions for the biomimetic intermolecular Diels–Alder reaction (i.e., LDA, ethyl acrylate, **11**→**12**) proved unsuccessful with this newly prepared dienone substrate **22**. Extensive screening of reaction conditions, which included (but were not limited to) base (KHMDS and LHMDS), and a route through the silyl enol ether derivative of dienone **22** (LDA/TMSCl or Et$_3$N/TMSI), failed to give the desired tricycle. We considered whether an alternative diene system that could be readily synthesized from dienone **22** would undergo a related intermolecular Diels–Alder reaction, thereby furnishing the carbocyclic backbone of nanolobatolide while retaining the C5–C6 olefin. At this point, prompted by biosynthetic considerations, triene system **3** became a natural and enticing option. As such, we envisaged a methyl addition/dehydration sequence to access the targeted triene system **3** (Scheme 3). In this context, while the intermediate tertiary alcohol **23** could be readily prepared from dienone **22** through a 1,2-addition (MeLi, ca. 1:1 d.r.), the subsequent dehydration of **23** proved capricious and generally afforded a complex mixture containing varying amounts (determined by ^{1}H NMR spectroscopy) of the desired triene **3** accompanied with regioisomeric triene systems and unidentified by-products. In view of the transiency and liability of the triene system **3**, the proposed Diels–Alder reaction was sucessfully achieved by intercepting the in situ generated triene **3** with ethyl acrylate (**5**), without isolation of **3**, in the presence of $BF_3{\cdot}OEt_2$ as the Lewis acid promoter (both for the generation of triene **3** from tertiary alcohol **23**, and for the intermolecular Diels–Alder reaction). This reaction gave tricyclic diene ester **24** in 38% overall yield from dienone **22**. In accordance with the biosynthetic pathway leading to the nanolobatolide structure (Scheme 1), the conversion of diene ethyl ester **24** into the diene acid **2** was required. The reluctance of ethyl ester **24** to undergo saponification necessitated a three-step reduction/oxidation sequence to afford diene acid **2**. Pleasingly, we serendipitously discovered that during the $NaClO_2$-mediated oxidation of the aldehyde precursor to the diene acid **2**, the nanolobatolide structure could be detected (by ^{1}H NMR spectroscopy) and was subsequently isolated in 48% yield (from **24**). This unexpected one-pot transformation suggested a facile oxidation of the initially formed diene acid **2** to epoxy acid **4** under the $NaClO_2$ conditions, followed by intramolecular epoxide opening/lactonization. Synthetic nanolobatolide structure exhibited identical ^{1}H and ^{13}C NMR spectra and mass spectra to those reported for the natural product,[2] and the optical rotation measurement of the synthetic material (synthetic: $[\alpha]_D^{25} = -10.8\ \mathrm{deg\,cm^3\,g^{-1}\,dm^{-1}}$ ($c = 0.13\ \mathrm{g\,cm^{-3}}$, $CHCl_3$) and Ref. [2] $[\alpha]_D^{25} = +11.4\ \mathrm{deg\,cm^3\,g^{-1}\,dm^{-1}}$ ($c = 0.72\ \mathrm{g\,cm^{-3}}$, $CHCl_3$)) implied that the naturally occurring substance (**1**) should be represented as the enantiomer of the structures shown in Scheme 1–3.[10]

In summary, the total synthesis of *ent*-nanolobatolide ((−)-**1**) has been accomplished by using an efficient strategy that obviates the use of protecting and blocking groups. The developed synthetic sequence from (−)-menthone (**7**) featured an oxidative ring-expansion, a Nazarov cyclization,[4] an intermolecular Diels–Alder reaction,[3] and an intramolecular epoxide-opening reaction. The successful execution of the two latter transformations also provided support to the speculated biosynthetic pathway of nanolobatolide.[2] In view of the neuroprotective properties of nanolobatolide, the work described herein (Scheme 2 and Scheme 3) should pave way for the synthesis of a diverse array of rationally designed nanolobatolide analogues for chemical and biolog-

ical investigations. These activities are currently underway in our laboratory.

Received: February 6, 2011
Revised: March 3, 2011
Published online: April 6, 2011

Keywords: biomimetic synthesis · cycloaddition · cyclization · natural products · neurological agents

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[8] Attempts to install the C5–C6 olefin from ketone **16** through the corresponding enol triflate, enol phosphate, vinyl halide, and hydrazone all proved unsuccessful, leading to either recovery or extensive decomposition of the starting material **16**.

[9] Attempts to eliminate alcohol **17** through its corresponding triflate, selenide, halide, xanthate, or under Mitsunobu, Martin's sulfurane, Burgess reagent, and $POCl_3$/$SOCl_2$–pyridine conditions all proved ineffective for this process.

[10] Notably, although (+)-menthone, which would lead to the naturally occurring form of (+)-nanolobatolide (**1**) in accordance to our synthetic strategy, is less readily available and more expensive than (−)-menthone, both enantiomeric forms of menthol are readily available, and this compound can be easily oxidized to give (+) and (−)-menthone.

