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Total Synthesis of (±)-4,5-Bis-*epi*-Neovibsanin A and B: A Neurite Outgrowth Comparison Study

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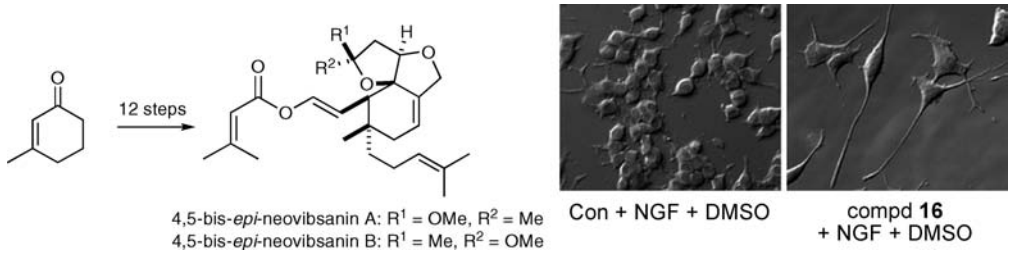
ABSTRACT



(±)-4,5-Bis-*epi*-neovibsanin A and B were synthesized in 12 steps. The acid-catalyzed, one-pot, five-step cascade reaction was central toward the formation of the tricyclic core. The two diastereomers of natural neovibsanin A and B acted as desirable derivatives for structure−activity relationship studies to probe neurotrophic activity. Both (±)-4,5-bis-*epi*-neovibsanin A and B strongly potentiate neurite outgrowth in NGF-stimulated PC12 cells. Furthermore, the tricyclic core appears to be largely responsible for promoting a biological response.

Neurotrophic factors (neurotrophins) are proteins secreted by neurons, or neuronal targets, that regulate a vast range of neuronal activities, including cell death, differentiation, neurite outgrowth, remyelination, collateral sprouting, synaptogenesis, maintenance of original and regenerated fiber networks, neurotransmitter release, and training-induced plasticity.[1] Understanding and identifying the biological mode of action is pivotal for progress toward treatment of neurodegenerative disorders such as Alzheimer's and Parkinson's disease. Controlling neurite outgrowth, for example, could potentially promote nerve cell regeneration by reconstructing cell networks. A host of neurotrophic factors are well understood [i.e., nerve growth factor (NGF)], but these are naturally occurring polypeptides or proteins which, although proven to prevent neuronal death, are not effective due to poor pharmacokinetic profiles (i.e., unable to cross the blood−brain barrier).[2] In recent years, however, a limited collection of natural products displaying neurotrophic activity have been discovered, many of which are terpene based and have succumbed to elegant total synthesis by the Danishefsky group.[3] The two natural products, neovibsanin A (**1**) and B

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(2) Pardridge, W. M. *Bioconjugate Chem.* **2008**, *19*, 1327.

(3) (a) Wilson, R. M.; Danishefsky, S. J. *Acc. Chem. Res.* **2006**, *39*, 539. (b) Wilson, R. M.; Danishefsky, S. J. *J. Org. Chem.* **2006**, *71*, 8329. (c) Pettus, T. R. R.; Chen, X.-T.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1998**, *120*, 12684. (d) Birman, V.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2002**, *124*, 2080. (e) Meng, Z. Y.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2005**, *44*, 1511. (f) Waters, S.; Tian, Y.; Li, Y.-M.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2005**, *127*, 13514. (g) Hua, Z. H.; Carcache, D. A.; Tian, Y.; Li, Y.-M.; Danishefsky, S. J. *J. Org. Chem.* **2005**, *70*, 9849. (h) Cho, Y. S.; Carcache, D. A.; Tian, Y.; Li, Y.-M.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 14358. (i) Carcache, D. A.; Cho, Y. S.; Hua, Z. H.; Tian, Y.; Li, Y.-M.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2006**, *128*, 1016. (j) Cook, S. P.; Polara, A.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2006**, *128*, 16440. (k) Siegel, D. R.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2006**, *128*, 1048. (l) Min, S. J.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2007**, *46*, 2199. (m) Dai, M. J.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2007**, *129*, 3498.

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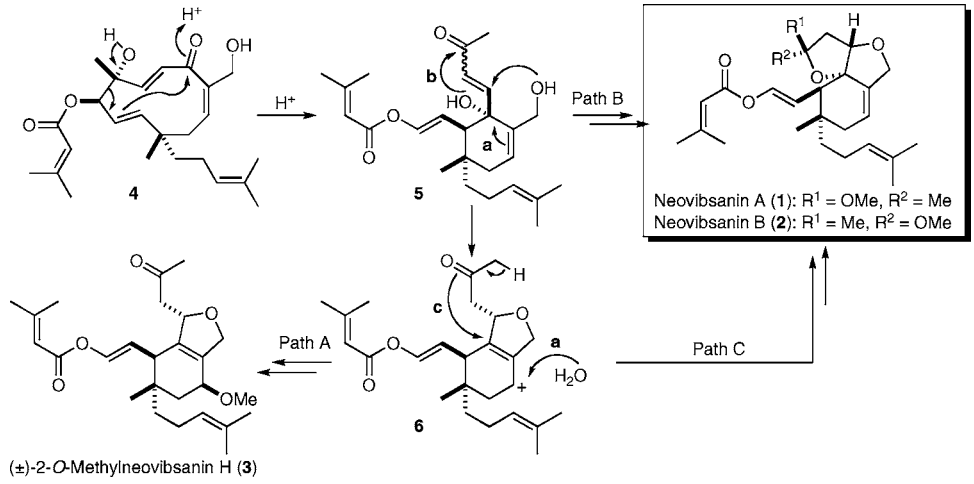
Scheme 1



(**2**) (Scheme 1), isolated by Fukuyama,[4] flaunt notable neurotrophic activity in the form of promoting neurite outgrowth in rat cortical neurons at 0.01 μM.[5,6] Considering, in addition to the above, that both neovibsanin A (**1**) and B (**2**) display very weak cytotoxicity [KB cells (IC_{50} 30 and 33 μM, respectively)],[5] **1** and **2** are of considerable biological interest, not to mention other vibsanin family members.[7] In fact, Imagawa, Fukuyama, and Nishizawa reported the first total synthesis of (±)-neovibsanin B (**2**) and demonstrated that the racemic material induced similar morphological changes in NGF-stimulated PC12 cells compared with the natural material [(+)-neovibsanin B].[8,9]

Our group has been very active in both synthetic studies[10] and total syntheses[11] of vibsane natural products; however, we recently became interested in neovibsanin A (**1**) and B (**2**) due to success with an expedient total synthesis of the closely related family member, (±)-2-*O*-methylneovibsanin H (**3**).[12] The synthetic approach taken for **3** was based on Fukuyama's proposed biosynthetic pathway arising from vibsanin B (**4**),[5] where the key proposed intermediates **5** and **6** can divert through a number of pathways leading to the various neovibsane natural products. For example, conjugate addition followed by solvolysis [i.e., path A (a)] and carbocation capture by water gives rise to (±)-2-*O*-methylneovibsanin H (**3**), whereas conjugate addition followed by hemiketalization [i.e., path B, (b)], or possibly even enol capture of the carbocation [i.e., path C, (c)] gives rise to neovibsanin A (**1**) and B (**2**) (Scheme 1).

Having successfully devised a synthesis of (±)-2-*O*-methylneovibsanin H (**3**) modeled on pathway A, we extended our study in this area by attempting to capitalize on synthetic routes modeled on either pathway B or C (assuming these pathways are valid) so to arrive at a key intermediate to access neovibsanin A (**1**) and B (**2**) (Scheme 1). To have any chance of achieving this goal, in our system, it would be crucial to promote and control path deviation in the acid-catalyzed cascade leading to (±)-2-*O*-methylneovibsanin H (**3**) (Scheme 2). After considerable investigation of reaction conditions, it was discovered that treatment of enone **7** with an excess of concentrated sulfuric acid in anhydrous methanol at 4 °C afforded the tricyclic methyl esters **15** and **16** in 73% yield in a 5:1 ratio, respectively, after 24 h (Scheme 2).

This surprising observation that the neovibsanin A (**1**) or B (**2**) core had been formed albeit epimeric at positions 4 and 5 (i.e., **15** and **16**) confirmed that the desired pathway C [i.e., **14** (**c** arrows)] was not viable and that pathway B shown in Scheme 1 is highly likely as a real biosynthetic pathway. With pathway C not in play, pathway B, as shown in Scheme 2, proceeds but is compromised by the β-stereochemistry of the hydroxyl group seen in **12** as initially incorporated from **7** (Scheme 2). The inversion of stereochemistry at position 4 clearly does not impede the five cascading step sequence, which includes the following: (1) TBS deprotection to give

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(6) Fukuyama, Y.; Esumi, T. *Org. Synth. Chem. Jpn* **2007**, *65*, 585.

(7) Wang, L.-Q.; Chen, Y.-G.; Xu, J.-J.; Liu, Y.; Li, X.-M.; Zhao, Y. *Chem. Biodiversity* **2008**, *5*, 1879.

(8) Early synthetic studies towards the total synthesis of neovibsanin A (**1**) and B (**2**) have been reported; see: (a) Esumi, T.; Zhao, M.; Kawakami, T.; Fukumoto, M.; Toyota, M.; Fukuyama, Y. *Tetrahedron Lett.* **2008**, *49*, 2692. (b) Mehta, G.; Bhat, B. A. *Tetrahedron Lett.* **2009**, *50*, 2474.

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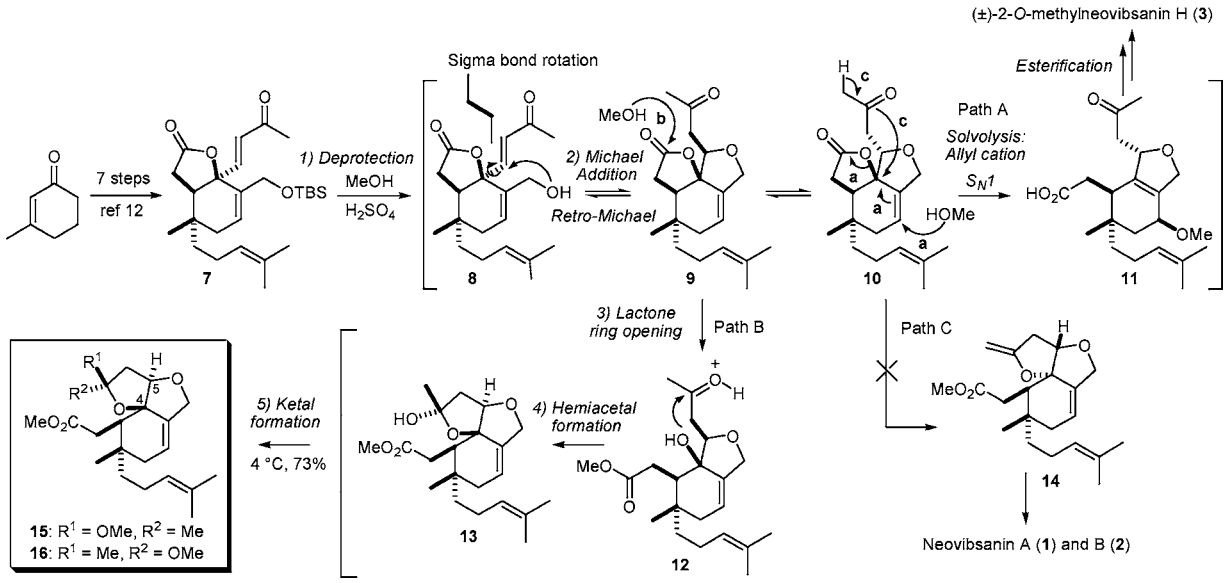
(12) Chen, A. P.-J.; Williams, C. M. *Org. Lett.* **2008**, *10*, 3441.

Scheme 2



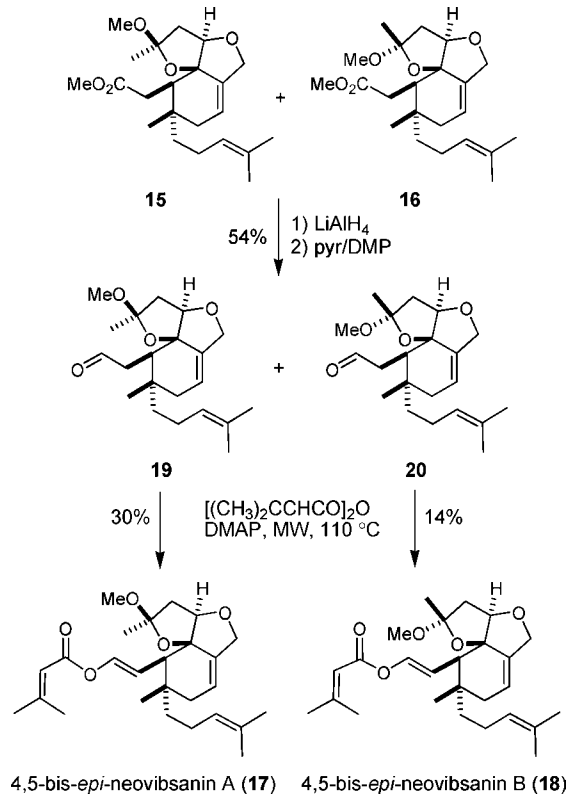
8, (2) Michael addition of the primary alcohol function to give **9**, (3) lactone **9** (**b** arrow) ring-opening to ester **12**, (4) hemiacetal formation of **13**, and (5) ketalization with methanol affording **15** and **16** (Scheme 2). The stereochemical outcome at position 5, however, is probably best explained by the existence of an equilibrium mixture of retro-Michael/Michael adducts (i.e., **8**, **9**, and **10**) arising from σ-bond rotation as indicated on structure **8**. Hence, at room temperature, **10** (**a** arrows) predominates and is carried through the cascade to **11**, whereas at cooler temperatures (e.g., 4 °C) adduct **9** is the major component (Scheme 2).

With these unexpected diastereomers, **15** and **16**, in hand it was decided to complete a total synthesis of (±)-4,5-bis-*epi*-neovibsanin A (**17**) and B (**18**) to probe the more intriguing question of neurite outgrowth promotion capability.

Global reduction of **15** and **16** with lithium aluminum hydride was easily accomplished. Global oxidation to access **19** and **20**, however, required pyridine-buffered Dess−Martin periodinane; otherwise, decomposition was observed. Finally, the 3,3-dimethylacroyl enol ester side chain was installed using the microwave irradiation modified Davies[10a,12] protocol giving 4,5-bis-*epi*-neovibsanin A (**17**) and B (**18**) [30%, *E*/*Z* ratio 5:1; 14%, *E*/*Z* ratio 3:2, respectively] (Scheme 3).

Compounds **15**−**20** were examined for their potential to induce neurite outgrowth in PC12 cells. As dimethyl sulfoxide (DMSO, 1.33%) inhibited neurite outgrowth to a small but observable extent, compound-induced outgrowth was compared to control cultures containing DMSO (1.33%). Figure 1 demonstrates that all compounds at 40 μM induced a marked increase in neurite outgrowth in the presence of NGF after 72 h when compared to cells cultured in NGF (+ DMSO) alone. In contrast, no compounds were capable of inducing neurite outgrowth in the absence of NGF. To further assess the extent of outgrowth, neurite length was scored using an arbitrary scale where 0 indicates no outgrowth and 7 indicates maximal outgrowth (Table 1). The ability of all compounds to stimulate neurite out-

Scheme 3



growth was already apparent after 24 h exposure (relative outgrowth rating of 2−3), and processes continued to extend over the following 2 days in all cases (relative

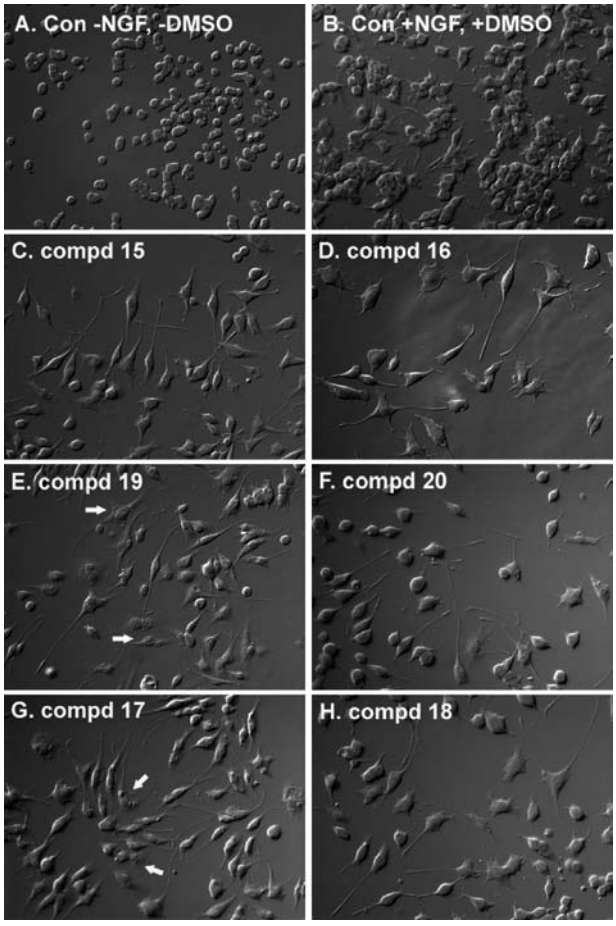


Figure 1. Comparison of morphological changes in PC12 cells induced by exposure to compounds **15**−**20** in the presence of NGF (C−H). Control (Con) panels indicate no neurite outgrowth in the absence of NGF (A) and the extent of outgrowth in the presence of NGF and DMSO (B). Arrows indicate cells exhibiting abnormal morphology.

outgrowth rating of 4−5 after 48 h, and 5−7 after 72 h). However, no outgrowth was observed in the presence of the compounds alone, indicating that they are unable to trigger the early signaling events that initiate neuronal differentiation.

Compounds **17** and **19** at a concentration of 40 μM appeared to be cytotoxic over the culture period as a substantial number of cells exhibited abnormal morphology (Figure 1E, G; arrows). Furthermore, a higher concentration (60 μM) of all compounds produced cytotoxic effects with the majority of cells undergoing cell death (data not shown).

Table 1. Neurite Outgrowth of PC12 Cells: Relative Outgrowth Rating

compd	24 h[a]	48 h	72 h
− NGF − DMSO	0[b]	0	0
+ NGF − DMSO	2	4	6
+ NGF + DMSO	1	2	3
15[c]	2−3	5	6−7
16	2−3	4−5	6
17	3	4	5−6
18	3	4−5	6
19	2−3	4	6
20	3	4−5	6

[a] Cells were fixed 24, 48, or 72 h after addition of compounds. [b] Relative outgrowth rating: 0 = no outgrowth, 7 = maximal outgrowth. [c] Cells were cultured in the presence of compounds (40 μM) (+ DMSO) + 20 ng/mL NFG.

In conclusion, synthetic efforts toward the total synthesis of neovibsanin A (**1**) and B (**2**) have resulted in an expedient total synthesis of (±)-4,5-bis-*epi*-neovibsanin A (**17**) and B (**18**) suggesting that the postulated biosynthetic pathway B (Scheme 1) proposed by Fukuyama is highly probable. Neurite outgrowth evaluation indicated that compounds **15**-**20** are capable of potentiating NGF-dependent neurite outgrowth in PC12 cells. This body of work represents the first structure activity relationship associated with the neovibsanin A and B structural motif and demonstrates that the core of the natural product is largely responsible for promoting a biological response.

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Supporting Information Available: Experimental procedures and copies of ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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