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Synthetic neovibsanes and their ability to induce neuronal differentiation in PC12 cells

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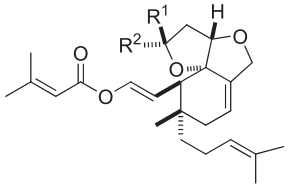
ABSTRACT

A series of neovibsanin A and B derivatives and lower homologues were synthesized to study their neurotrophic ability with PC12 cells. 4,5-Bis-*epi*-neovibsanin A displayed prominent ability to induce neurite outgrowth compared to control cultures. Herein we describe the total synthesis of 4,5-bis-*epi*-neovibsanin A and B as well as comparing the biological activity of several neovibsane derivatives.

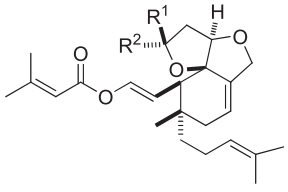
1. Introduction

Adult neurogenesis and the regenerative potential of neural stem cells offer a tantalizing possibility for restoring neurons and lost neural circuitry in neurodegenerative disorders such as Alzheimer's and Parkinson's disease. Neurotrophins, such as nerve growth factor (NGF), neurotrophin 3 (NT3), neurotrophin 4/5 (NT4/5) and brain-derived neurotrophic factor (BDNF), are a family of polypeptides, which are essential for the differentiation, growth, development, survival and functional maintenance of neurons and several other neuroectoderm-derived cellular populations.[1–4] Deregulation of neurotrophins or their receptors have been implicated in neurodegeneration, neuropathies, pain and cancer, thus providing a rationale for the synthetic development of neurotrophic factors for possible therapeutic applications in disease.[5,6] So far, peptidyl neurotrophic factors have been tested in neurodegenerative disorders but none, however, have been effective due to the drawbacks generally associated with using large polypeptides as drugs.[7,8] These drawbacks include short half-lives in vivo, poor pharmacokinetic profiles, proteolytic degradation and undesired pleiotropic effects.

The problems associated with protein-based therapies have encouraged the development of small molecule agents, which can mimic or enhance the neurotrophic activities of endogenous neurotrophins.[5–10] These compounds are often sourced from the reservoir of natural products, which are continuously being screened for compounds displaying neurotrophic activity. Two natural products isolated by Fukuyama,[11] neovibsanin A (**1**) and B (**2**), have been shown to induce neurite outgrowth in primary rat cortical neurons at 0.01 μM, whilst displaying very weak cytotoxicity [KB cells (IC_{50} 30 and 33 μM, respectively) Fig. 1].[12]



Neovibsanin A (**1**, R^1=OCH_3, R^2=CH_3)
Neovibsanin B (**2**, R^1=CH_3, R^2=OCH_3)



4,5-bis-*epi*-neovibsanin A (**3**, R^1=OCH_3, R^2=CH_3)
4,5-bis-*epi*-neovibsanin B (**4**, R^1=CH_3, R^2=OCH_3)

Figure 1. Structures of neovibsanin A (**1**) and B (**2**) and the natural product epimers 4,5-bis-*epi*-neovibsanin A (**3**) and B (**4**).

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Neovibsanin A (**1**) and B (**2**) belong to a family of rare vibsane compounds, many of which are of biological and synthetic interest.[13] Recently, Imagawa, Fukuyama and Nishizawa reported the first total synthesis of (±)-neovibsanin B (**2**). Tested for its neurotrophic activity using PC12 cells (derived from rat pheochromocytoma), a widely used model for studying NGF-induced neuronal differentiation,[14] (±)-neovibsanin B displayed comparable neurotrophic activities to the natural product (+)-neovibsanin B.[15] This suggests that both enantiomers are able to induce neuronal differentiation.

Our research efforts towards synthesizing neovibsanin A (**1**) and B (**2**) resulted in the total synthesis of (±)-4,5-bis-*epi*-neovibsanin A (**3**) and B (**4**). This paper describes the syntheses of these natural product diastereomers and an extensive evaluation of their activity compared to other structural analogues by quantifying the differentiation of PC12 cells.

2. Results and discussion

2.1. Chemistry

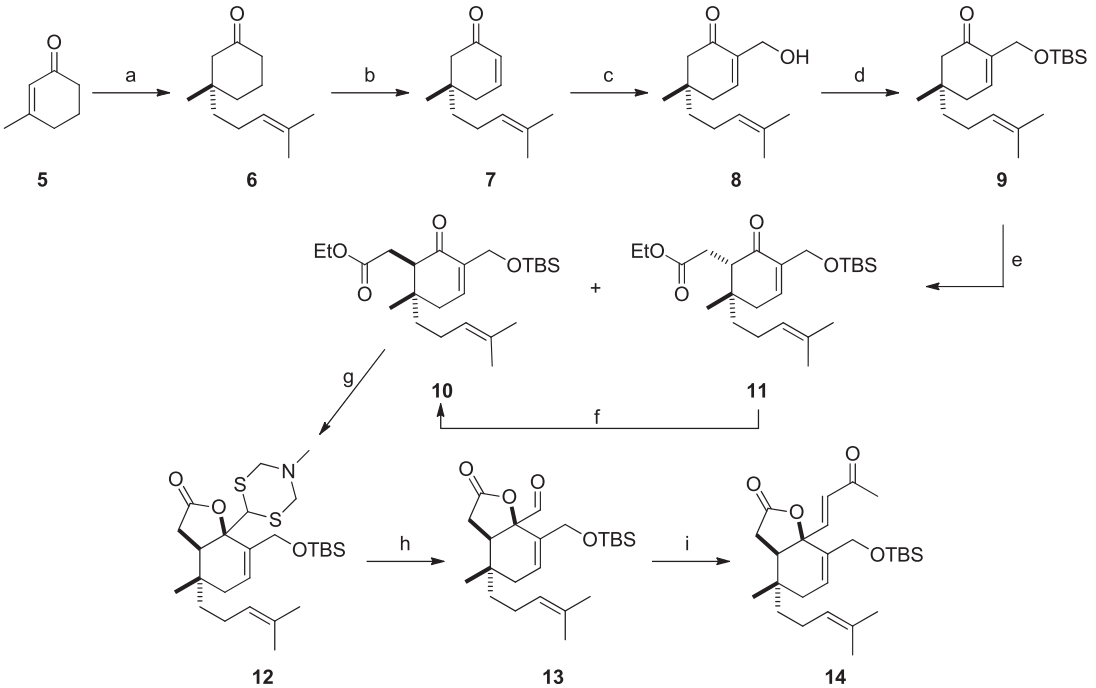
(±)-4,5-Bis-*epi*-neovibsanin A (**3**) and B (**4**) were synthesized in 12 steps via a modified biomimetic approach involving an acid-catalyzed, one-pot, five-step cascade reaction to access the tricyclic core. Based on previous work[16–24] and on the synthesis of (±)-2-*O*-methylneovibsanin H,[25] enone **14** was prepared from 3-methylcyclohexanone **5** (Scheme 1). Ketone **6** was prepared via a copper mediated 1,4-addition to cyclohexanone **5** in 83% yield, followed by dehydrogenation with IBX·NMO[26,27] to afford the α,β-unsaturated ketone **7** in 78% yield. The allylic alcohol **8** was obtained using a modified Baylis–Hillman reaction in water,[28,29] which was protected as the *tert*-butyldimethylsilyl ether **9** in 54% yield over two steps. Alkylation using lithium diisopropylamide with iodoacetate gave a 3:2 mixture of diastereomers with the major isomer **10** having the correct configuration (61% yield, 80% based on recovered starting material). Compound **11** could be epimerized with LDA to furnish further supplies of **10**. To obtain enone **14**, required an initial one-carbon unit integration using lithium dithiazide, providing compound **12** in 57% yield, followed by a mercury mediated deprotection to reveal the aldehyde unit **13** (73% yield). Aldehyde **13** was subjected to prolonged reflux in chloroform with ylide 1-(triphenylphosphoranylidene)propanone in an *E* selective olefination to obtain enone **14** in 94% yield.

Treatment of enone **14** with an excess of concentrated sulfuric acid in anhydrous methanol at 4 °C promoted a five-step cascade reaction to afford the tricyclic methyl esters **19** and **20** in 73% overall yield in a 5:1 ratio, respectively (Scheme 2). The cascading steps involve an initial deprotection of the TBS ether, leading to a Michael addition of the primary alcohol to give **16**, which underwent a lactone ring opening with the solvent to form ester **17**, followed by hemiacetal formation and ketalization with methanol to provide **19** and **20**. In parallel, the two epimeric esters were reduced using $LiAlH_4$ then oxidized using pyridine-buffered Dess–Martin periodinane in an overall yield of 54%. Finally the enol ester side chain was installed using microwave irradiation to give 4,5-bis-*epi*-neovibsanin A (**3**) and B (**4**) (30%, *E*/*Z* ratio 5:1; 14% *E*/*Z* ratio 3:2, respectively).

The ^1H and ^{13}C NMR spectroscopic data of the natural products and the diastereomers are quite similar (Table 1). Notable differences occur at position 5 where the stereoconfiguration differs, especially between neovibsanin B (**2**) and its diastereomer (**4**) with a difference of 0.54 ppm and 3.6 ppm in the ^1H and ^{13}C data, respectively. Position 9 is also worth noting with a proton chemical shift difference of about 0.7 ppm for the neovibsanin A comparison and 0.8 ppm for the neovibsanin B comparison.

2.2. Biological evaluation

In view of the prominent biological activity of natural neovibsanin A (**1**) and B (**2**), as well as the activity of the racemic mixture synthesized by Fukuyama, compounds **19**–**22** and the two natural product diastereomers **3** and **4** were examined for their ability to stimulate neurite outgrowth in PC12 cells.



Scheme 1. Reagents and conditions: (a) 5-bromo-2-methyl-2-pentene, Mg, CuI, THF, 83%; (b) IBX·NMO, DMSO, 45 °C, 78%; (c) DMAP, CH_2O, SDS, H_2O, 56%; (d) Imidazole, TBSCl, CH_2Cl_2, 97%; (e) LDA, ICH_2CO_2Et, THF, 50 °C, 61%; (f) LDA, 85%; (g) *N*-methyl dithiazine, *n*-BuLi, THF, −78 °C, 57%; (h) $Hg(ClO_4)_2$, $CaCO_3$, H_2O/THF, 73%; (i) $Ph_3PCHCOCH_3$, $CHCl_3$, reflux, 94%.