



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 neovibsanin derivatives.

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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 μ M, whilst displaying very weak cytotoxicity [KB cells (IC₅₀ 30 and 33 μ M, respectively) Fig. 1].¹²

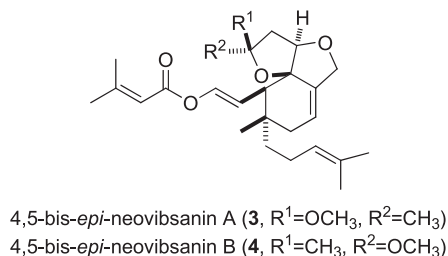
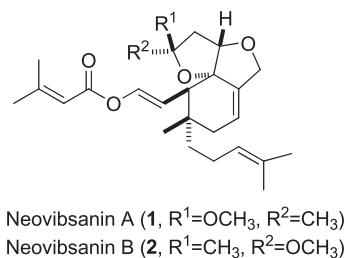


Figure 1. Structures of neovibsanin A (**1**) and B (**2**) and the natural product epimers 4,5-bis-*epi*-neovibsanin A (**3**) and B (**4**).

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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

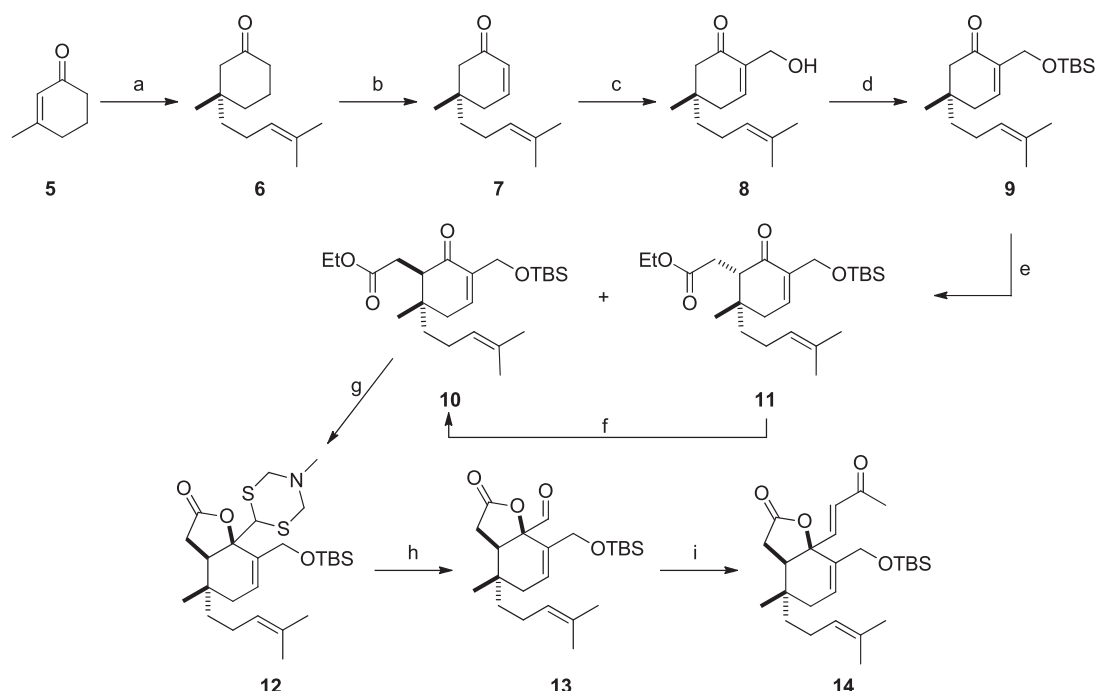
one-carbon unit integration using lithium dithiazide, providing compound **12** in 57% yield, followed by a mercury mediated deprotection to reveal the aldehyde unit **13** (73% yield). Aldehyde **13** was subjected to prolonged reflux in chloroform with ylide 1-(triphenylphosphoranylidene)propanone 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 LiAlH₄ 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).

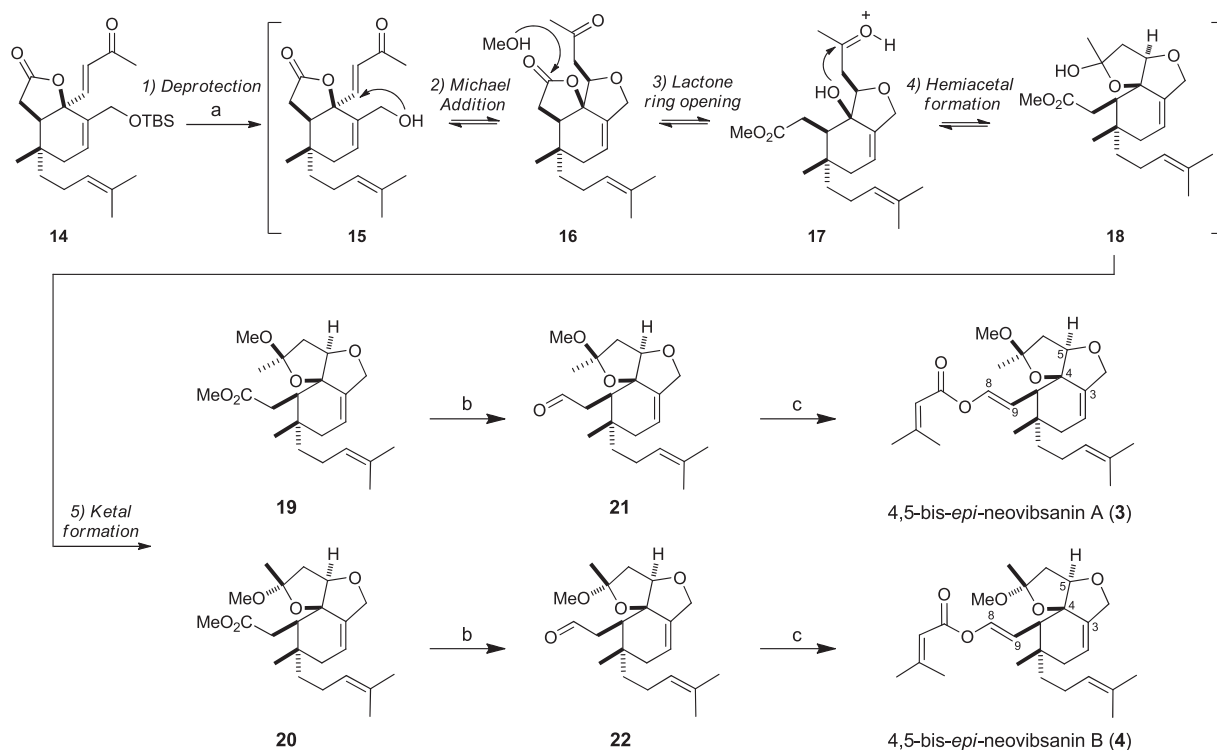
The ¹H and ¹³C NMR spectroscopic data of the natural products and the diastereomers are quite similar (Table 1). Notable differences occur at position 5 where the stereoconfiguration differs, especially between neovibsanin B (**2**) and its diastereomer (**4**) with a difference of 0.54 ppm and 3.6 ppm in the ¹H and ¹³C 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, CH₂O, SDS, H₂O, 56%; (d) Imidazole, TBSCl, CH₂Cl₂, 97%; (e) LDA, ICH₂CO₂Et, THF, 50 °C, 61%; (f) LDA, 85%; (g) *N*-methyl dithiazine, *n*-BuLi, THF, –78 °C, 57%; (h) Hg(ClO₄)₂, CaCO₃, H₂O/THF, 73%; (i) Ph₃PCHCOCH₃, CHCl₃, reflux, 94%.



Scheme 2. Reagents and conditions: (a) H_2SO_4 , MeOH, 4 °C, 73%; (b) LiAlH_4 , Et_2O , DMP, $\text{CH}_2\text{Cl}_2/\text{pyr}$, 55%; (c) $[(\text{CH}_3)_2\text{CCHCO}]_2\text{O}$, DMAP, Toluene, MW, 110 °C, 30% and 14% for **3** and **4**, respectively.

Table 1
Comparison of selected ^1H and ^{13}C NMR spectroscopic data between compounds **1–4**

Position	Neovibsanin A (1)		4,5-bis-epi-neovibsanin A (3)		Neovibsanin B (2)		4,5-bis-epi-neovibsanin B (4)	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}
3	—	137.8	—	139.0	—	137.9	—	139.2
4	—	90.7	—	90.9	—	91.6	—	90.6
5	4.56	87.7	4.31	89.2	4.62	86.4	4.08	90.0
8	7.50	137.4	7.36	137.1	7.5	137.8	7.42	136.8
9	5.00	113.1	5.7	111.5	5.22	113.0	6.02	112.2
OCH₃	3.32	50	3.14	48.7	3.32	48.8	3.28	49.5

Cells were exposed to compounds at a concentration of 40 μM in combination with NGF (20 ng/mL) for 48 to 72 h. As shown in Figure 2 and 3, a significant increase of neuronal differentiation determined by the outgrowth of neurites could be observed after 72 h for all compounds (Fig. 2a/b).

In particular, compound **3**, the diastereomer of natural product neovibsanin A, showed remarkable neuritogenic activity with an 11.5-fold increase in the percentages of neurite bearing cells compared to the control cultures containing NGF and DMSO (1.33%), the compound solvent. A 5-fold increase, however, was observed when compound **3** was compared to experiments controlling for the effect of DMSO (+NGF–DMSO). This suggests that 1.33% of DMSO is slightly cytotoxic to PC12 cells. A comparison between the 48 h and 72 h incubation time (Fig. 4), indicated that diastereomer **3** appears to reach its maximum effect after 48 h as there is no significant increase in neuronal differentiation after a further 24 h (timepoint 72 h). The other compounds, however, appear to show a continuous increase in outgrowth from 48 h to 72 h. In addition, compound **3** promotes a biological response faster as the number of cells with neurites is already higher after 48 h compared to the values of other compounds at 72 h.

Although higher concentrations (60 μM) induced neurite outgrowth, they also appeared to have cytotoxic effects with the

majority of cells dying, which may be due to the higher dose of DMSO. No compounds, however, were capable of inducing neurite outgrowth in the absence of NGF, suggesting a synergistic effect between the compounds and NGF (data not shown).

In terms of total outgrowth, all compounds significantly promoted neurite outgrowth compared to the control (+NGF+DMSO) (Fig. 2b). Compounds **19**, **22**, **3** and **4** appeared to promote a higher activity (***) $p < 0.001$) than compounds **20** and **21** (*) $p < 0.05$). Interestingly, all compounds induced less overall outgrowth compared to control cultures without DMSO (+NGF–DMSO). However, the reverse is observed for individual primary process lengths (Fig. 5a). This apparent contradiction in behaviour may be due to DMSO, restricting the ability of the cells to branch out (as observed in the highly branched nature of cells cultured without DMSO (Fig. 5b)).

Comparing the biological activity with the structures of individual compounds, it is hard to determine a definite structural feature that can help to explain the different neurogenic activities. With regards to the three different side chains, both compounds with an enol ester unit (i.e., **3** and **4**) appear to promote greater overall outgrowth (Fig. 2b). In comparison, only one of two compounds with the ester (i.e., **19**) or aldehyde (i.e., **22**) side chain showed comparable levels of activity (based on p values) to those of

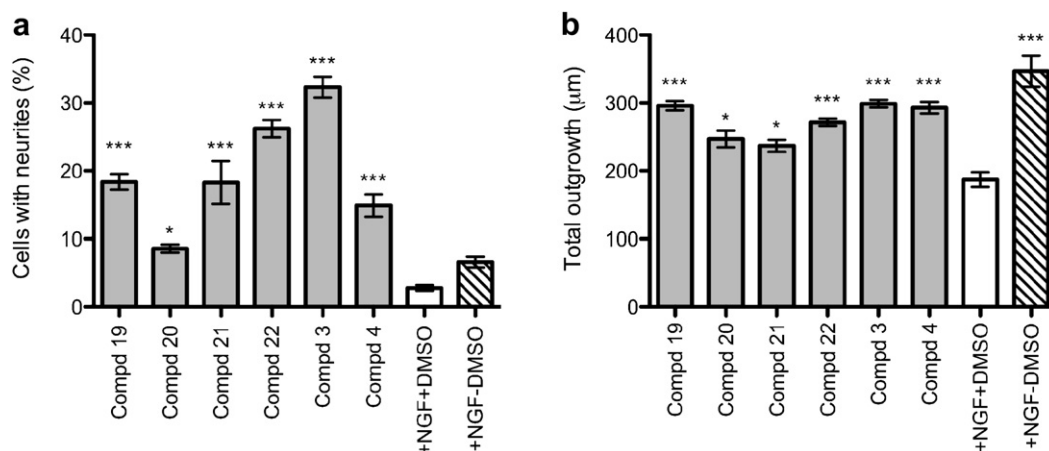


Figure 2. Quantitative analysis of neuronal differentiation of PC12 cells with compounds **19–22**, **3**, and **4** at 40 μM concentrations in the presence of NGF (20 ng/mL) for 72 h. (a) The percentages of cells with longer processes than one and a half cell body lengths and (b) the total neurite outgrowth were plotted as an index of neuronal differentiation. Data are presented as mean±SE (n=3). Statistical significance was tested against control+NGF+DMSO using a one-way ANOVA followed by Bonferroni post hoc means comparison with ** $p<0.01$ and *** $p<0.001$.

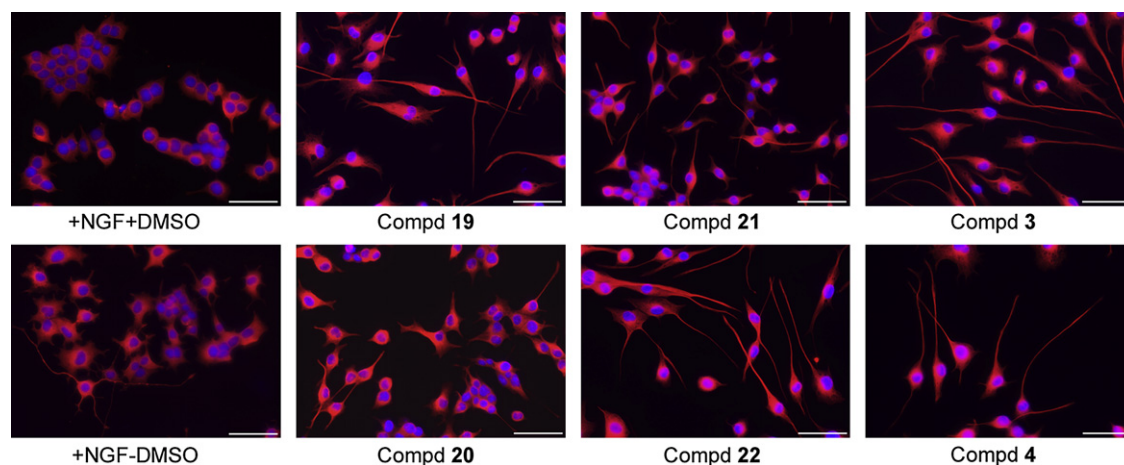


Figure 3. Morphological appearance of PC12 cells after treatment with compounds for 72 h. Neurite outgrowth was assessed by the presence, length and number of processes on cells and compared to controls (+NGF+DMSO, +NGF-DMSO). Cells were stained for β III-tubulin with Tuj1 (red) and the cell nuclei (DAPI; blue) (Bar=50 μm).

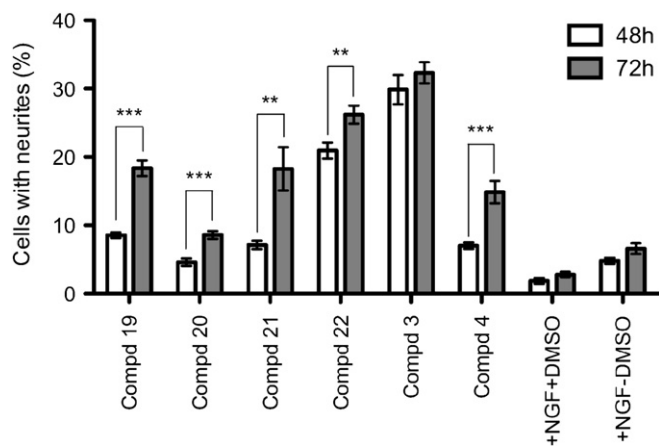


Figure 4. Neuronal differentiation of PC12 cells at 48 h and 72 h. Data are presented as mean±SE (n=3). Statistical analysis was performed between 48 h and 72 h using a *t*-test with ** $p<0.01$ and *** $p<0.001$.

3 and **4**. Of the compounds **19**, **22**, **3** and **4**, both stereo-configurations of the methoxy group are present in equal numbers.

In terms of the percentages of cells with neurites (Fig. 2a), compound **3** with the enol ester side chain showed higher levels of activity than compounds with the same stereoconfiguration of the methoxy group but with the ester (**19**) or aldehyde (**21**) unit. For the other methoxy group stereoconfiguration, however, it is the aldehyde (**22**), which induced higher biological activity than compounds with ester (**20**) or enol ester (**4**) side chains. Therefore, minor differences in the side chain functional groups, perhaps in combination with the different stereoconfiguration of the methoxy group appear to influence these cellular responses.

A stability check on all compounds after an extended (6 months) stay in DMSO revealed that all compounds were stable except in the case of compound **21** and **3** in which the methoxy group at position 7 was eventually replaced by a hydroxyl group to give **23** and **24**, respectively, most likely due to moisture in the DMSO. Compound **23** underwent further rearrangement to compound **26** as outlined in Scheme 3.

Interestingly, a comparison between the three experiments performed for compound **21** indicated a significant drop in biological activity for experiment 3, suggesting that the rearrangement

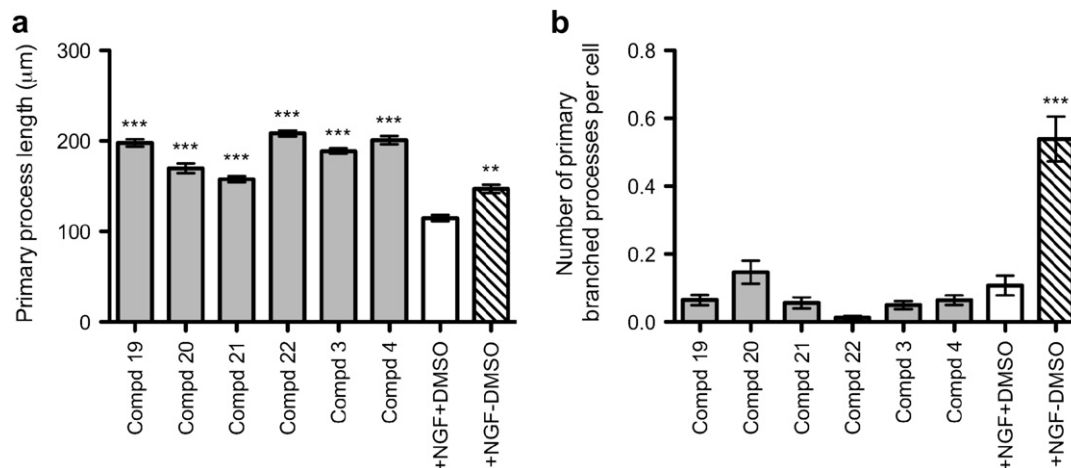
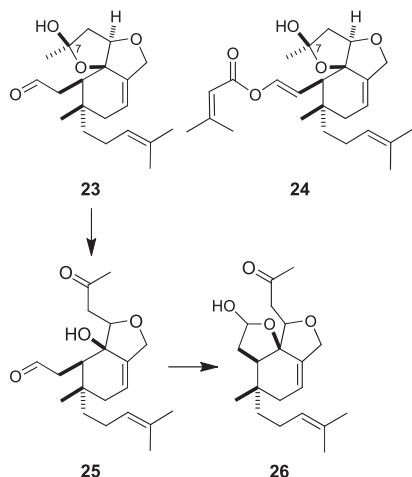


Figure 5. (a) Quantitative analysis of primary process length and (b) number of primary branched processes per cell at 72 h. Data are presented as mean $\pm$ SE (n=3). Statistical significance was tested against control+NGF+DMSO using a one-way ANOVA followed by Bonferroni post hoc means comparison with ** $p < 0.01$ and *** $p < 0.001$.



Scheme 3. Hydroxyl derivatives **23** and **24** derived from compounds **21** and **3**, respectively. Hemiacetal **23** further rearranges to lactol ketone **26**.

described in Scheme 3 occurred between experiment 2 and 3 (Fig. 6). All statistical analyses were therefore calculated using only experiment 1 and 2 for compound **21**. Compound **24**, however, did not rearrange further and the data obtained for compound **3** showed no decrease in activity and all three data points were included.

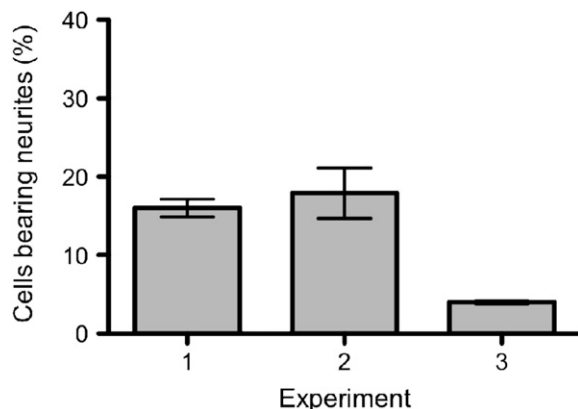


Figure 6. Experiments conducted for compound **21** on the percentages of cells bearing neurites. Data are presented as mean $\pm$ SE (n=1).

Evaluation of these derivatives suggests that there is no adverse effect on the biological activity with the replacement of the methoxy group, but a considerable decrease in activity was observed when the tricyclic core is replaced with a lactol ketone structure. Therefore, an oxygen at position 7 and the tricyclic core may be of importance in conferring biological activity.

3. Conclusion

Various compounds containing the tricyclic core of neovibsanin A were synthesized and evaluated for neurotrophic activity. The diastereomer (**3**) of neovibsanin A (**1**) markedly promoted neuritegenesis in a synergistic manner with NGF. Further synthetic exploration to increase solubility, stability and drug-likeness will be reported in due course.

4. Experimental

4.1. Chemistry

4.1.1. General. ^1H and ^{13}C NMR spectra were recorded on Bruker AV300 (300.13 MHz; 75.47 MHz), AV400 (400.13 MHz; 100.62 MHz), DRX500 (500.13 MHz; 125.76 MHz) and AV900 (900.13 MHz; 226.36 MHz) instruments in deuteriochloroform (CDCl_3) or hexadeuteriobenzene (C_6D_6) unless otherwise stated. Coupling constants are given in hertz and chemical shifts are expressed as δ values in parts per million. IR spectra were measured on a Perkin Elmer FTIR spectrometer (Spectrum 2000) with a Smiths detection (DuraSampler II). GC/MS data were recorded on a Shimadzu GC-17A Ver.3, mass-spectrometer: MS QP5050A, ionisation at 70 eV, column: DB-5ms 30 m 0.25 mm, carrier-gas: He, total flow 32.2 mL/min, column flow 1.3 mL/min, injector temperature: 250 $^\circ\text{C}$, standard program: 2 min at 100 $^\circ\text{C}$, followed by a temperature increase of 16 $^\circ\text{C}/\text{min}$ and held at 250 $^\circ\text{C}$ for 10 min. Low resolution electrospray ionization mass spectrometry measurements (LRESIMS) were recorded in positive ionization mode on a Bruker Esquire HCT (High Capacity 3D ion trap) instrument with a Bruker ESI source. High resolution electrospray ionization (HRESIMS) accurate mass measurements were recorded in positive mode on a Bruker MicroTOF-Q (quadrupole-Time of Flight) instrument with a Bruker ESI source. Accurate mass measurements were carried out with external calibration using sodium formate as a reference calibrant. High and low resolution EI mass spectral data were obtained on a Finnigan MAT900. Column chromatography was undertaken on silica gel (Flash Silica gel

230–400 mesh), with distilled solvents. Moisture sensitive experiments were conducted in oven or flame-dried glassware under an atmosphere of argon. Anhydrous solvents used in moisture sensitive reactions were dried according to Perin and Armarego, 'Purification of laboratory solvents', third Ed. THF was freshly distilled from sodium/benzophenone. Melting points were determined on a Fisher Johns melting point apparatus and are uncorrected. Fine chemicals were purchased from the Aldrich Chem. Co.

4.1.1.1. 3-Methyl-3-(4-methylpent-3-enyl) cyclohexanone (**6**).

Following the procedure developed by Heim,²³ magnesium turnings (4.62 g, 190 mmol) were stirred under high vacuum for 5 min before an argon atmosphere was introduced. Iodine crystals (15 mg) were added followed by freshly distilled THF (15 mL). 5-Bromo-2-methyl-2-pentene (14.1 g, 127 mmol), a portion (2 mL) of which was added directly to the THF suspension to initiate the reaction, was dissolved in anhydrous THF (40 mL) and the solution added dropwise to the above suspension over 20 min with heating. The mixture was stirred at rt until completion of the reaction (monitored by TLC). In a second flask, CuI (602 mg, 3.17 mmol) was suspended in anhydrous THF (25 mL). The solution was then cooled to -20°C and the Grignard solution added via syringe over 10 min. After 40 min at -20°C 3-methyl-2-cyclohexenone (**5**) (6.5 mL, 57.1 mmol, 0.9 equiv) in THF (20 mL) was added dropwise at -20°C . The reaction was stirred at -20°C for 30 min, then at rt for 1 h. The reaction was quenched by the dropwise addition of saturated NH_4Cl solution (50 mL) and the phases separated. The aqueous solution was extracted with Et_2O (4×100 mL) and the combined organic layers were washed with brine (50 mL) and dried over MgSO_4 . After removal of the solvent in vacuo the residue was subjected to vacuum distillation ($66^{\circ}\text{C}/0.02$ mmHg) yielding the title compound as a yellow oil (9.20 g, 83%). IR ν_{max} (ATR) 2961, 2917, 2876, 1712, 1681, 1453, 1380, 1227 cm^{-1} ; δ_{H} (300 MHz, CDCl_3) 0.92 (3H, s, Me), 1.23–1.29 (2H, m, CCH_2CH_2), 1.51–1.67 (2H, m, CH_2), 1.58 (3H, br s, =CMe), 1.66 (3H, br d, J 1.0 Hz, =CMe), 1.81–1.96 (4H, m, 2CH_2), 2.09 (1H, d, J 13.5 Hz, $\text{COCH}_3\text{H}_b\text{C}$), 2.19 (1H, d, J 13.5 Hz, $\text{COCH}_3\text{H}_b\text{C}$), 2.25 (2H, t, J 6.8 Hz, CH_2), 5.02–5.09 (1H, m, $\text{CH}=\text{CMe}_2$); δ_{C} (75 MHz, CDCl_3) 17.6, 22.1, 22.2, 24.9, 25.7, 35.9, 38.6, 41.0, 41.7, 53.7, 124.3, 131.6, 212.1; GC/MS: m/z (%): 194 [M^+] (14), 179 (11), 161 (11), 151 (55), 133 (6), 112 (44), 111 (100), 109 (75), 97 (34), 83 (31), 69 (76), 55 (81), 41 (78).

4.1.1.2. 5-Methyl-5-(4-methylpent-3-enyl)-2-cyclohexenone (**7**).

Based on the procedure reported by Heim²³, a mixture of *o*-iodoxybenzoic acid³⁰ (23.2 g, 82.9 mmol) and *N*-methyl morpholine *N*-oxide (11.6 g, 86.3 mmol) was dissolved in DMSO (65 mL) and heated to 45°C . 3-Methyl-3-(4-methylpent-3-enyl)cyclohexanone (**6**) (6.70 g, 34.5 mmol) was added at 45°C and the mixture heated at that temperature for 18 h. On cooling the mixture was extracted with petroleum ether (6×80 mL) and the combined extracts washed with brine (200 mL). The combined organic phase was dried over MgSO_4 and the solvent was removed in vacuo to give the title compound (5.17 g, 78%) as a pale yellow oil, which was carried to the next step without further purification. IR ν_{max} (ATR) 2964, 2916, 2874, 1678, 1384, 1247, 734 cm^{-1} ; δ_{H} (300 MHz, CDCl_3) 1.01 (3H, s, Me), 1.35 (2H, dd, J 9.8, 7.3 Hz, CCH_2CH_2), 1.57 (3H, br s, =CMe), 1.65 (3H, br d, J 1.1 Hz, =CMe), 1.93 (2H, dt, J 15.0, 7.3 Hz, CCH_2CH_2), 2.16 (1H, dddd, J 18.8, 4.5, 1.9, 1.0 Hz, $\text{CCH}_3\text{H}_b\text{CH}=\text{C}$), 2.23 (1H, d, J 15.9 Hz, $\text{COCH}_3\text{H}_b\text{C}$), 2.31 (1H, ddd, J 18.8, 3.6, 2.4 Hz, m, $\text{CCH}_3\text{H}_b\text{CH}=\text{C}$), 2.33 (1H, d, J 15.9 Hz, $\text{COCH}_3\text{H}_b\text{C}$), 5.01–5.08 (1H, m, $\text{CH}=\text{CMe}_2$), 6.00 (1H, dt, J 10.0, 1.9 Hz, $\text{COCH}=\text{CH}$), 6.83 (1H, ddd, J 10.0, 4.5, 3.6, $\text{COCH}=\text{CH}$); δ_{C} (75 MHz, CDCl_3) 17.6, 22.4, 24.8, 25.7, 36.6, 38.2, 41.6, 50.2, 124.0, 129.1, 131.8, 148.2, 199.9; GC/MS: m/z (%): 192 [M^+] (16), 149 (25), 121 (35), 109 (100), 95 (22), 81 (41), 69 (51), 55 (36), 41 (72).

4.1.1.3. 2-Hydroxymethyl-5-methyl-5-(4-methylpent-3-enyl)cyclohex-2-enone (**8**). Based on the procedure reported by Porzelle,²⁹ sodium dodecyl sulfate (772 mg, 2.68 mmol) and 4-(dimethylamino)pyridine (3.76 g, 30.7 mmol) were added to a mixture of distilled water (25.8 mL) and compound **7** (5.15 g, 26.8 mmol). After stirring for 15 min, formalin (36.0 mL) was added and stirred for 16 h at rt. The mixture was then quenched with brine (50 mL) and extracted with EtOAc (2×70 mL). The combined organic layers were dried over MgSO_4 and the solvent was removed in vacuo. The residue was purified by column chromatography (ethyl acetate/petroleum ether, 1:2) to give the titled compound (3.32 g, 56%) as a light yellow oil. IR ν_{max} (ATR) 3600–3100 (br), 2963, 2923, 2873, 1666, 1380, 1027 cm^{-1} ; δ_{H} (500 MHz, CDCl_3) 1.01 (3H, s, Me), 1.34 (2H, dd, J 9.5, 7.5 Hz, CCH_2CH_2), 1.57 (3H, s, =CMe), 1.65 (3H, s, =CMe), 1.88–1.97 (2H, m, CCH_2CH_2), 2.21 (1H, dddd, J 19.0, 4.7, 2.6, 1.3 Hz, $\text{CCH}_3\text{H}_b\text{CH}=\text{C}$), 2.27 (1H, dd, J 16.1, 1.1 Hz, $\text{COCH}_3\text{H}_b\text{C}$), 2.32–2.38 (1H, m, $\text{CCH}_3\text{H}_b\text{CH}=\text{C}$), 2.35 (1H, d, J 16.1 Hz, $\text{COCH}_3\text{H}_b\text{C}$), 2.41 (1H, t, J 6.5 Hz, OH), 4.25 (2H, ddd, J 6.5, 2.6, 1.1 Hz, CH_2OH), 5.01–5.06 (1H, m, $\text{CH}=\text{CMe}_2$), 6.76 (1H, ddt, J 4.7, 3.8, 1.1 Hz, $\text{COCR}=\text{CH}$); δ_{C} (125 MHz, CDCl_3) 17.6, 22.4, 24.7, 25.6, 36.7, 38.0, 41.3, 50.3, 60.6, 124.1, 131.7, 137.5, 144.2, 200.4; GC/MS: m/z (%): 222 [M^+] (9), 204 (14), 189 (10), 164 (8), 161 (15), 139 (22), 121 (86), 109 (53), 97 (22), 83 (18), 69 (49), 55 (39), 41 (100).

4.1.1.4. 2-[(*tert*-Butyldimethylsilyloxy)methyl]-5-methyl-5-(4-methylpent-3-enyl)cyclohex-2-enone (**9**). Imidazole (1.33 g, 19.5 mmol) was added to a solution of compound **8** (2.17 g, 9.80 mmol) in anhydrous CH_2Cl_2 (40 mL) followed by the addition of *tert*-butyldimethylsilyl chloride (2.21 g, 14.7 mmol) under an argon atmosphere. The reaction mixture was stirred for 30 min before being diluted with CH_2Cl_2 (30 mL) and washed with saturated NaHCO_3 solution (70 mL), saturated NH_4Cl solution (70 mL) and then brine (70 mL). The organic layer was dried over MgSO_4 and concentrated in vacuo. The residue was then purified by column chromatography (Et_2O /petroleum ether, 1:10) to give the titled compound (3.18 mg, 97%) as a light yellow oil. IR ν_{max} (ATR) 2956, 2930, 2858, 1673, 1254, 1059, 835, 776 cm^{-1} ; δ_{H} (400 MHz, CDCl_3) 0.05 (6H, s, SiMe_2), 0.90 (9H, s, SiCMe_3), 0.99 (3H, s, Me), 1.33 (2H, dd, J 9.6, 7.5 Hz, CCH_2CH_2), 1.57 (3H, s, =CMe), 1.65 (3H, br s, =CMe), 1.86–1.99 (2H, m, CCH_2CH_2), 2.17–2.25 (1H, m, $\text{CCH}_3\text{H}_b\text{CH}=\text{C}$), 2.23 (1H, dd, J 15.8, 1.1 Hz, $\text{COCH}_3\text{H}_b\text{C}$), 2.32 (1H, d, J 15.8 Hz, $\text{COCH}_3\text{H}_b\text{C}$), 2.35 (1H, ddd, J 18.7, 6.4, 3.1 Hz, $\text{CCH}_3\text{H}_b\text{CH}=\text{C}$), 4.34 (2H, ddd, J 4.8, 2.4, 2.4 m, CH_2OSi), 5.00–5.09 (1H, m, $\text{CH}=\text{CMe}_2$), 6.78–6.85 (1H, m, $\text{COCR}=\text{CH}$); δ_{C} (100 MHz, CDCl_3) -5.4 (2C), 17.6, 18.4, 22.4, 24.8, 25.6, 25.9 (3C), 36.7, 38.0, 41.5, 50.5, 59.9, 124.2, 131.7, 137.5, 141.5, 199.2; HRMS (ESI+) m/z found 359.2377; $\text{C}_{20}\text{H}_{36}\text{NaO}_2\text{Si}$ requires 359.2382.

4.1.1.5. Ethyl 2-{3-[(*tert*-butyldimethylsilyloxy)methyl]-6-methyl-6-[4-methylpent-3-enyl]-2-oxocyclohex-3-enyl}acetate (**10**) & (**11**).

To a stirred solution of diisopropylamine (331 mg, 450 μL , 3.27 mmol) in anhydrous THF (30 mL) was added *n*-butyl lithium (1.47 M in hexane, 2.23 mL, 3.27 mmol) at 0°C under an argon atmosphere. The reaction mixture was stirred at this temperature for 15 min. The solution was then cooled to -50°C , to which was added the protected alcohol (1.00 g, 2.98 mmol) dissolved in anhydrous THF (2 mL) and the reaction mixture stirred at -50°C for 30 min. A solution of ethyl iodoacetate (764 mg, 3.57 mmol) in anhydrous THF (2 mL) was added and the mixture was stirred at -50°C for 20 min before allowing the mixture to warm to rt and then heated at 45°C for 17 h. The reaction was quenched by pouring the reaction mixture into a saturated NH_4Cl solution (20 mL) containing ice. The mixture was extracted with Et_2O (3×30 mL) and washed with saturated NaHCO_3 solution (40 mL), distilled water (40 mL) and brine (40 mL). The combined organic phase was dried over MgSO_4 and the solvent removed in vacuo. The

residue was then purified by silica gel column chromatography (Et₂O/petroleum ether, 1:15) to give the titled compounds **10** (468 mg, 37%, mp 54–55 °C) and **11** (304 mg, 24%, mp 50–51 °C) as crystalline solids. Compound **10**: IR $\nu_{\max}$ (ATR) 2957, 2930, 2858, 1737, 1661, 1169, 1119, 1099, 837, 775 cm⁻¹; δ_{H} (300 MHz, CDCl₃) 0.04 (6H, s, SiMe₂), 0.81 (3H, s, Me), 0.89 (9H, s, SiCMe₃), 1.25 (3H, t, *J* 7.1 Hz, OCH₂CH₃), 1.30–1.42 (2H, m, CCH₂CH₂), 1.58 (3H, d, *J* 0.9 Hz, =CMe), 1.66 (3H, d, *J* 0.9 Hz, =CMe), 1.89–2.05 (2H, m, CCH₂CH₂), 2.11–2.22 (1H, m, CCH₂H_bCH=), 2.22 (1H, dd, *J* 16.1, 3.2 Hz, COCH₂H_bCH), 2.54–2.65 (1H, m, CCH₂H_bCH=), 2.57 (1H, dd, *J* 16.1, 9.7 Hz, COCH₂H_bCH), 3.03 (1H dd, *J* 9.7, 3.2 Hz, COCH₂CHCO), 4.14 (2H, qd, *J* 7.1, 1.4 Hz, OCH₂CH₃), 4.20–4.45 (2H, m, CH₂OSi), 5.00–5.11 (1H, m, CH=CMe₂), 6.80 (1H, ddd, *J* 6.3, 4.4, 2.1 Hz, COCR=CH); δ_{C} (75 MHz, CDCl₃) –5.5 (2C), 14.2, 17.6, 18.4, 20.1, 22.1, 25.7, 25.9 (3C), 28.9, 37.6, 39.5, 41.2, 52.1, 59.9, 60.5, 123.8, 132.0, 136.9, 140.1, 173.2, 199.5; HRMS (ESI⁺) *m/z* found 445.2760; C₂₄H₄₂NaO₄Si requires 445.2750. Compound **11**: IR $\nu_{\max}$ (ATR) 2960, 2930, 2887, 2857, 1736, 1673, 1174, 1116, 1102, 855, 835, 774 cm⁻¹; δ_{H} (400 MHz, CDCl₃) 0.03 (6H, s, SiMe₂), 0.88 (9 Hs, SiCMe₃), 1.00–1.09 (1H, m, CCH₂H_bCH₂), 1.05 (3H, s, Me), 1.23 (3H, t, *J* 7.1 Hz, OCH₂CH₃), 1.24–1.33 (1H, m, CCH₂H_bCH₂), 1.52 (3H, s, =CMe), 1.61 (3H, s, =CMe), 1.69–1.90 (2H, m, CCH₂CH₂), 2.25 (1H, dd, *J* 16.0, 3.8 Hz, COCH₂H_bCH), 2.26 (1H, ddd, *J* 18.8, 6.2, 3.2 Hz, CCH₂H_bCH=), 2.44 (1H, dd, *J* 18.8, 5.7 Hz, CCH₂H_bCH=), 2.63 (1H, dd, *J* 16.0, 9.3 Hz, COCH₂H_bCH), 2.95 (1H, dd, *J* 9.3, 3.8 Hz, COCH₂CHCO), 4.12 (2H, qd, *J* 7.1, 2.1 Hz, OCH₂CH₃), 4.22–4.39 (2H, m, CH₂OSi), 4.91–4.99 (1H, m, CH=CMe₂), 6.71–6.78 (1H, m, COCR=CH); δ_{C} (100 MHz, CDCl₃) –5.5 (2C), 14.1, 17.5, 18.3, 22.7, 25.6, 25.8, 25.9 (3C), 29.0, 33.0, 36.7, 39.7, 55.3, 59.9, 60.5, 123.9, 131.7, 137.6, 139.6, 173.3, 199.2; HRMS (ESI⁺) *m/z* found 445.2745; C₂₄H₄₂NaO₄Si requires 445.2750.

4.1.1.6. 7-[(tert-Butyldimethylsilyloxy)methyl]-4-methyl-7a-(5-methyl-1,3,5-dithiazinan-2-yl)-4-(4-methylpent-3-enyl)-3,3a,4,5-tetrahydrobenzofuran-2(7aH)-one (12). *n*-BuLi (1.49 M in hexane, 2.50 mL, 3.73 mmol) was added dropwise to a solution of 5-methyl-1,3,5-dithiazine (521 mg, 3.86 mmol) in anhydrous THF (10 mL) at –78 °C and warmed to rt over 1 h and cooled again to –78 °C. A solution of compound **10** (1.05 g, 2.49 mmol) in anhydrous THF (3 mL) was added at –78 °C and stirred for 120 min at –78 °C. The reaction was quenched by pouring the reaction mixture into a saturated NH₄Cl solution (50 mL) containing ice. The mixture was extracted with Et₂O (3×50 mL) and the organic layer was washed with distilled water (25 mL) and brine (25 mL). The combined organic phase was dried over MgSO₄ and the solvent removed in vacuo. Petroleum spirit was added and the flask cooled in the freezer and a white precipitate was formed, which was isolated by filtration to give compound **12** (730 mg, 57%) as a white solid (mp 160–162 °C). [Found: C, 61.12; H, 9.17; N, 2.74; S 12.53. C₂₆H₄₅NO₃S₂Si requires C, 61.01; H, 8.86; N, 2.74; S 12.53%]. IR $\nu_{\max}$ (ATR) 2926, 2882, 2856, 1765, 1096, 836, 780, 558 cm⁻¹; δ_{H} (500 MHz, CDCl₃) 0.07 (3H, s, SiMe), 0.08 (3H, s, SiMe), 0.76 (3H, s, Me), 0.91 (9H, s, SiCMe₃), 1.23 (1H, ddd, *J* 13.7, 11.1, 6.4 Hz, CCH₂H_bCH₂), 1.36 (1H, ddd, *J* 13.7, 10.3, 6.2 Hz, CCH₂H_bCH₂), 1.60 (3H, s, =CMe), 1.67 (3H, s, =CMe), 1.88–2.05 (4H, m, CCH₂CH₂, CCH₂CH=), 2.46 (1H, dd, *J* 18.9, 1.8 Hz, COCH₂H_bCH), 2.52 (3H, s, NMe), 2.83 (1H, dd, *J* 10.2, 1.8 Hz, COCH₂CH), 3.04 (1H, dd, *J* 18.9, 10.2 Hz, COCH₂H_bCH), 4.05 (1H, dd, *J* 13.3, 1.4 Hz, CH₂H_bOSi), 4.11 (1H, dd, *J* 13.3, 1.2 Hz, CH₂H_bOSi), 4.28 (1H, dq, *J* 14.0, 1.7 Hz, S₁CH₂H_bN), 4.41 (1H, dq, *J* 14.0, 2.0 Hz, S₂CH₂H_bN), 4.59 (1H, d, *J* 13.5 Hz, S₁CH₂H_bN), 4.62 (1H, d, *J* 13.5 Hz, S₂CH₂H_bN), 4.80 (1H, s, CHS), 5.05–5.11 (1H, m, CH=CMe₂), 6.19–6.24 (1H, m, COCR=CH); δ_{C} (100 MHz, CDCl₃) –5.3, –5.2, 17.6, 18.5, 18.9, 22.0, 25.7, 26.0 (3C), 31.6, 35.1, 35.3, 37.4, 41.7, 45.7, 60.3, 60.9 (2C), 62.1, 87.4, 124.2, 127.9, 131.8, 133.4, 176.1; HRMS (ESI⁺) *m/z* found 512.2695; C₂₆H₄₅NNaO₃S₂Si requires 512.2683.

4.1.1.7. 7-[(tert-Butyldimethylsilyloxy)methyl]-4-methyl-4-(4-methylpent-3-enyl)-2-oxo-2,3,3a,4,5,7a-hexahydrobenzofuran-7a-carbaldehyde (13). A mixture of **12** (104 mg, 0.203 mmol), CaCO₃ (203 mg, 2.03 mmol) and mercury(II) perchlorate hydrate (203 mg, 0.448 mmol) in THF (4 mL) and demineralized water (1 mL) was stirred at rt for 5 min and quenched by adding saturated NaHCO₃ solution (4 mL). The mixture was filtered through Celite and washed with ether (10 mL). The filtrant was washed with saturated NaHCO₃ solution (5 mL), brine (5 mL), dried over MgSO₄ and the solvent removed in vacuo. The residue was then purified by silica gel column chromatography (Et₂O/petroleum ether, 1:1) affording the aldehyde as a low melting off-white solid (60 mg, 73%). IR $\nu_{\max}$ (ATR) 2955, 2930, 2858, 1776, 1724, 1254, 1115, 835, 776 cm⁻¹; δ_{H} (300 MHz, CDCl₃) 0.02 (3H, s, SiMe), 0.03 (3H, s, SiMe), 0.84 (3H, s, Me), 0.85 (9H, s, SiCMe₃), 1.13–1.28 (1H, m, CCH₂H_bCH₂), 1.29–1.45 (1H, m, CCH₂H_bCH₂), 1.57 (3H, s, =CMe), 1.65 (3H, s, =CMe), 1.90 (2H, q, *J* 7.9 Hz, CCH₂CH₂), 2.01 (1H, ddd, *J* 17.5, 5.5, 1.3 Hz, CCH₂H_bCH=), 2.11 (1H, dt, *J* 17.5, 2.5 Hz, CCH₂H_bCH=), 2.33–2.58 (3H, m, COCH₂CH, COCH₂CH), 4.08 (1H, dd, *J* 12.2, 0.7 Hz, CH₂H_bOSi), 4.32 (1H, d, *J* 12.2 Hz, CH₂H_bOSi), 5.02 (1H, m, CH=CMe₂), 6.02–6.16 (1H, m, COCR=CH), 9.38 (1H, s, CHO); δ_{C} (100 MHz, CDCl₃) –5.7, –5.6, 17.6, 18.3, 19.6, 22.0, 25.6, 25.8 (3C), 29.4, 34.0, 35.0, 40.5, 43.4, 63.9, 87.3, 123.6, 129.5, 132.0, 132.2, 175.3, 195.5; HRMS (ESI⁺) *m/z* found 429.2423; C₂₃H₃₈NaO₄Si requires 429.2432.

4.1.1.8. (E)-7-[(tert-Butyldimethylsilyloxy)methyl]-4-methyl-4-(4-methylpent-3-enyl)-7a-(3-oxobut-1-enyl)-3,3a,4,5-tetrahydrobenzofuran-2(7aH)-one (14). A mixture of the aldehyde (300 mg, 0.737 mmol) and 1-(triphenylphosphoranyliden) acetone (500 mg, 1.57 mmol) in anhydrous CHCl₃ was heated at reflux for 5 d. The solvent was removed from the reaction mixture, and the remaining material was purified by column chromatography (Et₂O/petroleum ether, 1:1). The titled compound **14** was isolated (300 mg, 94%) as a slightly yellow liquid, which solidified (mp 80–82 °C); δ_{H} (300 MHz, CDCl₃) 0.02 (6H, d, *J* 0.7 Hz, SiMe₂), 0.87 (9H, s, SiCMe₃), 0.89 (3H, s, Me), 1.18–1.31 (1H, m, CCH₂H_bCH₂), 1.36–1.49 (1H, m, CCH₂H_bCH₂), 1.56 (3H, s, =CMe), 1.66 (3H, d, *J* 0.9 Hz, =CMe), 1.83–1.98 (2H, m, CCH₂CH₂), 2.02–2.11 (2H, m, CCH₂CH=), 2.24 (3H, s, COMe), 2.31–2.61 (3H, m, COCH₂CH, COCH₂CH), 4.06 (1H, ddd, *J* 14.4, 2.0, 1.8, CH₂H_bOSi), 4.17 (1H, ddd, *J* 14.4, 2.0, 1.8, CH₂H_bOSi), 4.92–5.15 (1H, m, CH=CMe₂), 5.98–6.19 (1H, m, COCR=CH), 6.35 (1H, d, *J* 15.8 Hz, COCH=CH), 6.64 (1H, d, *J* 15.8 Hz, COCH=CH); δ_{C} (100 MHz, CDCl₃) –5.5, –5.4, 17.7, 18.3, 21.9, 22.0, 25.6, 25.9 (3C), 28.5, 30.5, 34.2, 34.5, 40.3, 48.4, 61.4, 84.7, 123.6, 124.8, 128.4, 132.2, 133.2, 146.3, 175.2, 197.0; HRMS (ESI⁺) *m/z* found 469.2736; C₂₆H₄₂NaO₄Si requires 469.2745.

4.1.1.9. Methyl 2-[2-methoxy-2,8-dimethyl-8-(4-methylpent-3-en-1-yl)-3,3a,5,7,8,9-hexahydro-2H-furo[3,2-c]isobenzofuran-9-yl]acetate 19 & 20. Compound **14** (100 mg, 0.224 mmol) was dissolved in anhydrous MeOH (20 mL) and cooled to 0 °C before freshly opened concentrated (98%) H₂SO₄ (59 μ L) was slowly added dropwise. The solution was sealed under argon and placed in a refrigerator overnight for 17 h. The reaction was quenched by pouring the reaction mixture into an ice cold saturated NaHCO₃ solution (50 mL). The mixture was extracted with CH₂Cl₂ (3×50 mL) and the organic layer was washed with brine (50 mL), dried over MgSO₄ and the solvent removed in vacuo. The residue was then purified by silica gel column chromatography (Et₂O/petroleum spirit, 1:5) to give compounds **19** (47 mg, 55%) and **20** (15 mg, 18%) as colourless oils. Compound **19**: IR $\nu_{\max}$ (ATR) 2958, 2934, 2883, 1730, 1163, 1095, 1063, 1028, 969, 875, 841 cm⁻¹; δ_{H} (500 MHz, CDCl₃) 0.95 (3H, s, Me), 1.19–1.33 (2H, m, CCH₂CH₂), 1.45 (3H, s, OMe), 1.56 (3H, s, =CMe), 1.65 (3H, d, *J* 0.7 Hz, =CMe), 1.81 (1H, dd, *J* 14.5, 5.9 Hz, OCH₂H_bC), 1.87–1.94 (3H, m, CCH₂CH₂, CCH₂H_bCH=), 2.20–2.26

(1H, m, CCH₃H_bCH=), 2.23 (1H, d, *J* 14.5 Hz, OCHCH_aH_bC), 2.25 (1H, dd, *J* 7.4, 3.3 Hz, COCH₂CH), 2.36 (1H, dd, *J* 17.1, 7.4 Hz, COCH_aH_bCH), 2.43 (1H, dd, *J* 17.1, 3.3 Hz, COCH_aH_bCH), 3.19 (3H, s, OMe), 3.65 (3H, s, ester OMe), 4.10 (1H, dd, *J* 10.1, 0.9 Hz, =CCH_aH_bO), 4.43 (1H, d, *J* 5.9 Hz, OCHCH₂C), 4.59 (1H, ddt, *J* 10.1, 3.1, 3.1 Hz, =CCH_aH_bO), 5.01–5.06 (1H, m, CH=CMe₂), 5.62–5.66 (1H, m, OCH₂C=CH); δ_C (101 MHz, CDCl₃) 17.6, 21.5, 22.1, 22.5, 25.7, 29.9, 35.1, 37.8, 41.6, 43.2, 47.3, 48.9, 51.8, 70.3, 87.8, 90.3, 108.8, 121.4, 124.4, 131.4, 138.0, 174.4; HRMS (ESI+) *m/z* found 401.2291; C₂₂H₃₄NaO₅ requires 401.2298. Compound **20**: IR $\nu_{\max}$ (ATR) 2953, 2924, 2856, 1737, 1375, 1173, 1116, 1090, 1028 cm⁻¹; δ_H (500 MHz, CDCl₃) 0.97 (3H, s, Me), 1.17–1.37 (2H, m, CCH₂CH₂), 1.50 (3H, s, OMe), 1.55 (3H, s, =CMe), 1.65 (3H, s, =CMe), 1.77–1.87 (2H, m, CCH₂CH₂), 1.88 (1H, dddd, *J* 18.7, 4.4, 3.1, 1.7 Hz, CCH_aH_bCH=), 1.93 (1H, dd, *J* 14.7, 4.8 Hz, OCHCH_aH_bC), 2.19–2.25 (1H, m, CCH_aH_bCH=), 2.22 (1H, dd, *J* 7.3, 2.5 Hz, COCH₂CH), 2.26 (1H, d, *J* 14.7 Hz, OCHCH_aH_bC), 2.41 (1H, dd, *J* 18.7, 2.5 Hz, COCH_aH_bCH), 3.07 (1H, dd, *J* 18.7, 7.3 Hz, COCH_aH_bCH), 3.31 (3H, s, OMe), 3.66 (3H, s, ester OMe), 4.22 (1H, dd, *J* 11.0, 0.7 Hz, =CCH_aH_bO), 4.42 (1H, d, *J* 4.8 Hz, OCHCH₂C), 4.40–4.45 (1H, m, =CCH_aH_bO), 5.01–5.06 (1H, m, CH=CMe₂), 5.60–5.63 (1H, m, OCH₂C=CH); δ_C (126 MHz, CDCl₃) 17.5, 21.8, 22.1, 23.9, 25.7, 28.8, 35.3, 37.7, 41.1, 41.4, 47.3, 49.6, 51.8, 69.8, 88.7, 91.2, 110.6, 120.5, 124.4, 131.5, 138.5, 175.1; HRMS (ESI+) *m/z* found 401.2304; C₂₂H₃₄NaO₅ requires 401.2298.

4.1.1.10. 2-[2-Methoxy-2,8-dimethyl-8-(4-methylpent-3-en-1-yl)-3,3a,5,7,8,9-hexahydro-2H-furo[3,2-c]isobenzofuran-9-yl]acetaldehyde **21 & **22**.** To a solution of compounds **19** and **20** (118 mg, 0.312 mmol) in anhydrous Et₂O (10 mL) at rt was added LiAlH₄ (35.5 mg, 0.936 mmol) in one portion and stirred under an argon atmosphere for 10 min. Sodium sulfate dodecahydrate (300 mg) was added to the reaction until the suspension turned white in colour. The slurry was then filtered and washed with Et₂O (30 mL). The filtrate was then concentrated in vacuo to give a pale yellow oil (104 mg, 95%). The crude was considered sufficiently pure to be used in the next step without further purification. The above crude was stirred at rt in a solution of anhydrous CH₂Cl₂ and pyridine (1:1, 10 mL) under an argon atmosphere and Dess–Martin periodinane (212 mg, 0.50 mmol), dissolved in CH₂Cl₂ and pyridine (1:1, 10 mL), was added in one portion. The reaction was stirred until completion (TLC, ~4 h), then filtered through a plug of cotton wool and silica, and washed with CH₂Cl₂ (20 mL). The filtrate was then concentrated in vacuo and then diluted with Et₂O (20 mL) and washed with brine (20 mL), dried over MgSO₄ and the solvent removed in vacuo. The residue was then purified by silica gel column chromatography (Et₂O/petroleum spirit, 1:5) to give compounds **21** (46 mg, 42%) and **22** (13 mg, 12%) as colourless oils. Compound **21**: IR $\nu_{\max}$ (ATR) 2934, 1722, 1379, 1222, 1139, 1096, 1065, 1025, 982, 878, 845 cm⁻¹; δ_H (500 MHz, C₆D₆) 1.01 (3H, s, Me), 1.11 (2H, dd, *J* 9.1, 7.9 Hz, CCH₂CH₂), 1.24 (3H, s, OMe), 1.56 (1H, dd, *J* 14.4, 6.3 Hz, OCHCH_aH_bC), 1.57 (3H, s, =CMe), 1.64–1.70 (1H, m, CCH_aH_bCH=), 1.69 (3H, s, =CMe), 1.73–1.87 (2H, m, CCH₂CH₂), 1.87–1.93 (1H, m, CCH_aH_bCH=), 2.14–2.19 (3H, m, COCH₂CH, COCH₂C), 2.37 (1H, d, *J* 14.4 Hz, OCHCH_aH_bC), 3.09 (3H, s, OMe), 4.15 (1H, d, *J* 10.0 Hz, =CCH_aH_bO), 4.40 (1H, d, *J* 6.3 Hz, OCHCH₂C), 4.84 (1H, *J* 10.0, 2.9, 1.9 Hz, =CCH_aH_bO), 5.09–5.14 (1H, m, CH=CMe₂), 5.28–5.32 (1H, m, OCH₂C=CH), 9.35–9.36 (1H, m, CHO); δ_C (101 MHz, C₆D₆) 17.7, 21.5, 22.5, 22.7, 25.8, 35.1, 38.0, 40.8, 40.9, 42.1, 47.6, 48.6, 70.6, 88.3, 90.7, 108.9, 121.0, 125.0, 131.2, 138.9, 200.6; HRMS (ESI+) *m/z* found 371.2192; C₂₁H₃₂NaO₄ requires 371.2193. Compound **22**: IR $\nu_{\max}$ (ATR) 2960, 2922, 1719, 1463, 1380, 1261, 1226, 1091, 1024, 800 cm⁻¹; δ_H (400 MHz, C₆D₆) 1.02 (3H, s, Me), 1.11 (1H, ddd, *J* 13.8, 11.9, 5.3 Hz, CCH_aH_bCH₂), 1.18 (1H, ddd, *J* 13.8, 11.9, 5.5 Hz, CCH_aH_bCH₂), 1.44 (3H, s, OMe), 1.55 (3H, s, =CMe), 1.56–1.62 (1H, m, CCH_aH_bCH=), 1.69 (3H, s, =CMe), 1.73–1.88 (2H, m, CCH₂CH₂), 1.88–1.94 (1H, m, CCH_aH_bCH=), 1.91 (1H, dd, *J* 14.7, 4.7 Hz, OCHCH_aH_bC), 2.22 (1H, dd,

J 20.1, 2.1 Hz, COCH_aH_bCH), 2.28 (1H, d, *J* 14.7 Hz, OCHCH_aH_bC), 2.40 (1H, dd, *J* 7.7, 2.1 Hz, COCH₂CH), 3.18 (3H, d, *J* 0.6 Hz, OMe), 3.28 (1H, dd, *J* 20.1, 7.7 Hz, COCH_aH_bCH), 4.13 (1H, dd, *J* 10.7, 1.0 Hz, =CCH_aH_bO), 4.43 (1H, dt, *J* 10.7, 2.2 Hz, =CCH_aH_bO), 4.49 (1H, d, *J* 4.7 Hz, OCHCH₂C), 5.08–5.14 (1H, m, CH=CMe₂), 5.19–5.23 (1H, m, OCH₂C=CH), 9.51–9.55 (1H, m, CHO); δ_C (101 MHz, C₆D₆) 17.6, 22.3, 22.4, 23.9, 25.8, 35.0, 37.7 (2C), 40.1, 41.8, 47.4, 49.4, 69.9, 89.0, 91.5, 110.9, 120.2, 125.0, 131.2, 139.3, 201.1; HRMS (ESI+) *m/z* found 371.2193; C₂₁H₃₂NaO₄ requires 371.2193.

4.1.1.11. 4,5-bis-epi-Neovibsanin A (3**).** To aldehyde **21** (10.0 mg, 28.7 μ mol) dissolved in anhydrous toluene (0.6 mL) under an argon atmosphere was added 4-(*N,N*-dimethylamino)pyridine (4.21 mg, 34.5 μ mol) and 3-methylbut-2-enoic anhydride (6.29 mg, 34.5 μ mol). The mixture was heated under microwave irradiation for 24 h (maximum temperature 110 °C, 250 W). On cooling, Et₂O (5 mL) was added and the organic layer washed with saturated NaHCO₃ solution (5 mL). The aqueous phase was extracted with Et₂O (5 mL), dried over Na₂SO₄ and concentrated in vacuo. Column chromatography (Et₂O/petroleum spirit, 1:4, with 1% triethylamine) provided 4,5-bis-epi-neovibsanin A (**3**) (3.7 mg, 30%) as a mixture of unresolvable *E/Z* (5:1) diastereomers (colourless oil); δ_H (900 MHz, C₆D₆) 1.22 (3H, s, Me), 1.17–1.37 (2H, m, CCH₂CH₂), 1.32 (3H, s, OMe), 1.35 (3H, d, *J* 1.0 Hz, =CMe'), 1.63 (3H, s, =CMe), 1.67 (3H, s, =CMe), 1.77–1.80 (1H, m, CCH_aH_bCH=), 1.82 (1H, d, *J* 10.9 Hz, CHCH=CHO), 1.84 (1H, dd, *J* 14.0, 5.9 Hz, OCHCH_aH_bC), 1.86–1.90 (1H, m, CCH_aH_bCH=), 1.90–1.98 (2H, m, CCH₂CH₂), 2.03 (3H, d, *J* 1.0 Hz, =CMe'), 2.44 (1H, d, *J* 14.0 Hz, OCHCH_aH_bC), 3.14 (3H, s, OMe), 4.23 (1H, d, *J* 10.0 Hz, =CCH_aH_bO), 4.31 (1H, d, *J* 5.8 Hz, OCHCH₂C), 4.89 (1H, ddd, *J* 10.0, 4.9, 3.0 Hz, =CCH_aH_bO), 5.17–5.20 (1H, m, CH=CMe₂), 5.33–5.34 (1H, m, OCH₂C=CH), 5.64 (1H, dt, *J* 2.6, 1.3 Hz, OCOCH=CMe₂), 5.70 (1H, dd, *J* 12.7, 10.9 Hz, CHCH=CHO), 7.36 (1H, d, *J* 12.7 Hz, CHCH=CHO); δ_C (226 MHz, C₆D₆) 17.7, 20.2, 21.4, 22.0, 22.6, 25.8, 27.0, 34.9, 38.2, 42.8, 47.6, 48.3, 48.7, 71.0, 89.2, 90.9, 109.4, 111.5, 115.2, 120.7, 125.5, 130.8, 137.1, 139.0, 159.6, 163.2; HRMS (ESI+) *m/z* found 453.2620; C₂₆H₃₈NaO₅ requires 453.2611.

4.1.1.12. 4,5-bis-epi-neovibsanin B (4**).** Aldehyde **22** was subjected to the same conditions as above to provide 4,5-bis-epi-neovibsanin B (**4**) in 14% yield as a mixture of *E/Z* (3:2) diastereomers (colourless oil). Further careful chromatography gave a 10:1 *E/Z* mixture that was used for characterisation. IR $\nu_{\max}$ (ATR) 1731, 1643, 1378, 1132, 1078, 955, 839 cm⁻¹; δ_H (900 MHz, C₆D₆) 1.23 (1H, ddd, *J* 13.6, 11.8, 4.7 Hz, CCH_aH_bCH₂), 1.24 (3H, s, Me), 1.33 (3H, d, *J* 1.2 Hz, =CMe'), 1.41 (1H, ddd, *J* 13.6, 11.8, 4.7 Hz, CCH_aH_bCH₂), 1.49 (3H, s, OMe), 1.65 (3H, s, =CMe), 1.67 (3H, s, =CMe), 1.74–1.78 (1H, m, CCH_aH_bCH=), 1.81 (1H, d, *J* 11.2 Hz, CHCH=CHO), 1.83–1.87 (1H, m, CCH_aH_bCH=), 1.95–2.01 (2H, m, CCH₂CH₂), 2.03 (3H, d, *J* 1.1 Hz, =CMe'), 2.30 (1H, d, *J* 14.0 Hz, OCHCH_aH_bC), 2.37 (1H, dd, *J* 14.0, 4.6 Hz, OCHCH_aH_bC), 3.28 (3H, s, OMe), 4.08 (1H, d, *J* 4.6 Hz, OCHCH₂C), 4.20 (1H, dd, *J* 11.0, 1.1 Hz, =CCH_aH_bO), 4.53 (1H, ddd, *J* 11.0, 5.5, 3.4 Hz, =CCH_aH_bO), 5.20–5.23 (1H, m, CH=CMe₂), 5.23–5.25 (1H, m, OCH₂C=CH), 5.57–5.58 (1H, m, OCOCH=CMe₂), 6.02 (1H, dd, *J* 12.5, 11.2 Hz, CHCH=CHO), 7.42 (1H, d, *J* 12.5 Hz, CHCH=CHO); δ_C (226 MHz, C₆D₆) 17.7, 20.2, 20.9, 22.6, 24.1, 25.8, 26.9, 35.1, 38.2, 42.8, 47.3, 47.6, 49.5, 70.5, 90.0, 90.6, 110.9, 112.2, 115.6, 119.9, 125.6, 130.8, 136.8, 139.2, 158.9, 163.3; HRMS (ESI+) *m/z* found 453.2619; C₂₆H₃₈NaO₅ requires 453.2611.

4.2. Neurite outgrowth screening method

PC12 cells were plated (2 coverslips per condition) at a density of 0.5 × 10⁵ cells/well on 0.0015% poly-L-ornithine (Sigma, USA) coated glass coverslips in 24 well plates and cultured in growth medium [DMEM; 10% normal horse serum, 5% foetal calf serum,

penicillin (50 U/mL), streptomycin sulfate (50 mg/mL)]. Twenty-four hours after plating, growth medium was replaced with differentiation medium [DMEM; 1.5% normal horse serum, penicillin (50 U/mL), streptomycin sulfate (50 mg/mL)] containing test compounds (40 μ M) and NGF (20 ng/mL) (Millipore, USA). Compounds were dissolved in DMSO (Sigma) to a stock concentration of 3 mM and diluted to a final concentration of 40 μ M (1.33% DMSO) in culture medium. After a further 48 h, medium was replaced with fresh differentiation medium containing test compounds and NGF at the above concentrations. At indicated times, cells were fixed in 4% paraformaldehyde and immunolabelled with mouse-*anti*- β III tubulin (Tuj 1) (Covance, USA, 1:2000) for 30 min at room temperature. After several washes in PBS/0.1% TritonX-100, cells were incubated with Alexa Fluor 568 goat-*anti*-mouse secondary antibody (Molecular Probes, USA, 1:1000) and 4',6-diamidino-2-phenylindole (DAPI) for 30 min at room temperature. After several washes in PBS/0.1% TritonX-100, coverslips were mounted using Prolong Gold (Invitrogen, USA). Images were captured on an AxioImager (Zeiss, Germany) using epifluorescence optics. Ten images were selected randomly under the microscope for each coverslip.

Neuronal differentiation was assessed by presence, length and number of processes on cells with processes greater than one and a half cell body diameter defined as neurites. The proportion of neurite-positive cells to total cells in randomly selected fields were examined and analysed using NeuronJ to obtain process length and number of branched processes. Data from three repeated experiments were pooled with approximately 1000 cells for each condition examined and data were further analysed using one-way ANOVA followed by Bonferroni post hoc tests against the controls +NGF+DMSO; or *t*-tests between 48 h and 72 h timepoints.

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Supplementary data

Supplementary data associated with this article can be found in online version at doi:10.1016/j.tet.2010.06.056

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