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Neurotrophic peptide aldehydes: Solid phase synthesis of fellutamide B and a simplified analog

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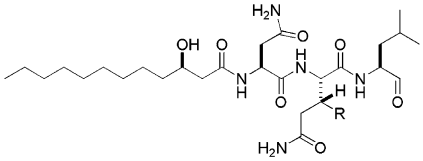
Abstract—A combination of solid phase and solution phase synthetic methods have been used to complete the total synthesis of the neurotrophic lipopeptide aldehyde fellutamide B (**2**). The β-hydroxy aliphatic tail was prepared by regioselective reductive opening of a cyclic sulfate, and later coupled to a solid phase resin. The synthetic compound was then examined in cytotoxicity and nerve growth factor (NGF) induction assays. A simplified analog of fellutamide B also showed activity.

Interest in neurotrophic small molecules has increased significantly in recent years.[1–3] It is hoped that the study of such low molecular weight factors will provide further insight into the biochemical mechanisms of neuronal cell proliferation/differentiation and lead to drug candidates for neurodegenerative conditions such as Alzheimer's disease. As part of a program directed toward the total synthesis of biologically active natural products and the identification of their relevant intracellular binding protein(s), we became interested in the group of lipopeptide aldehydes known as the fellutamides.

Despite displaying potent activity in both the NGF induction and cytotoxicity assays, the fellutamide class of natural products have attracted little attention since their isolation in 1991.[4] Fellutamides A (**1**) and B (**2**) were originally identified for their cytotoxic properties, and later found to stimulate NGF synthesis and secretion.[5] The structures of fellutamides A and B were elucidated by degradation studies, but have not yet been confirmed by total synthesis. In an effort to better understand the mechanism of action of these compounds, we initiated a program directed toward synthesizing fellutamide B, studying its neurotrophic activity, and identifying its target binding protein. In this letter, we report the total synthesis of fellutamide B, a simplified analog, and confirmation of both molecules' biological activities.

As both fellutamides A and B were reported to have comparable IC_{50} values for cytotoxicity, we initially pursued the synthesis of the more accessible fellutamide B (**2**). It was envisioned early on that a solid phase construction of the peptide chain would be facilitated by a solution phase preparation of the (*R*)-(−)-3-hydroxydodecanoic acid synthon **6**. Solid phase synthesis of the peptide chain was a particularly attractive strategy, as it would allow us to access analogs of the natural product by coupling different amino acids to the resin. Additionally, the mild resin cleavage conditions would be unlikely to epimerize the sensitive aldehyde functionality (See Fig. 1).

Our route to the requisite β-hydroxy side chain is described below (Scheme 1). Decanal (**3**) was homologated to α,β-unsaturated ester **4** by means of a Horner–Wadsworth–Emmons condensation with triethyl phospho-



R = OH, fellutamide A (**1**)
R = H, fellutamide B (**2**)

Figure 1. Structures of fellutamides A and B.

Keywords: Natural product; Peptide aldehyde; Nerve growth factor (NGF); Synthesis.
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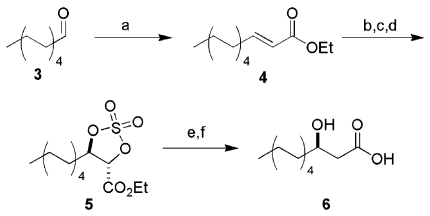
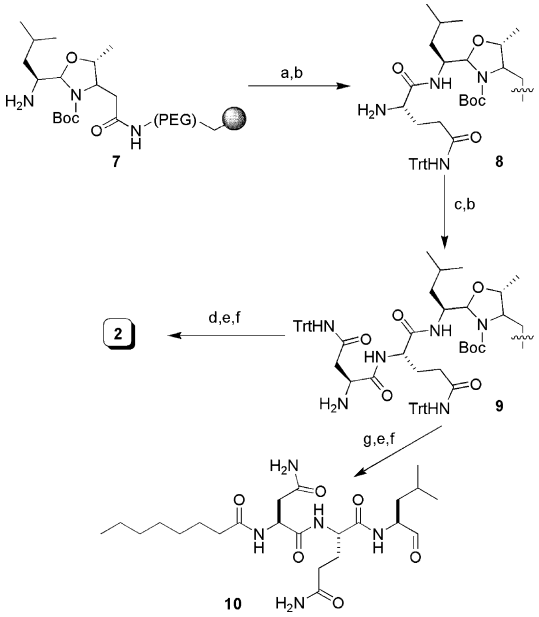


Scheme 1. Reagents and conditions: (a) LiOH, triethyl phosphonoacetate, THF, reflux, 4 h, 85%, 95:5 *E*:*Z*; (b) AD-Mix-β, $MeSO_2NH_2$, 1:1 *t*-BuOH/H_2O, 0 °C, 36 h, 80%, 90% ee; (c) $SOCl_2$, pyridine, 0 °C, 30 min, 99%; (d) $NaIO_4$, 1 mol % $RuCl_3$, 3:1 MeCN/H_2O, rt, 30 min, 90%; (e) $NaBH_4$, DMA, 0 °C to rt, 1 h, then 20% aq HCl, rt, 18 h, 72%; (f) LiOH, MeOH/H_2O/THF 1:1:1, rt, 3 h, 97%.

noacetate in 85% yield. Sharpless asymmetric dihydroxylation[6] of **4** was carried out using AD-Mix-β with excess methane sulfonamide in a 1:1 *t*-BuOH/H_2O mixture in 80% yield. The resultant diol was treated with thionyl chloride, and the cyclic sulfite was immediately oxidized to the sulfate by treatment with sodium periodate and catalytic ruthenium(III) chloride.[7] Cyclic sulfate **5** was isolated in 79% yield over three steps. Regioselective reductive cleavage of sulfate **5** was affected by treatment with sodium borohydride, and acidic workup provided the β-hydroxy ester.[8] At this stage, the enantiomeric excess of the asymmetric dihydroxylation was confirmed to be 90% by conversion of the alcohol to its corresponding Mosher's ester.[9] Lithium hydroxide mediated saponification of the ester in a MeOH/THF/H_2O mixture provided (*R*)-(−)-3-hydroxydodecanoic acid **6** in 70% over two steps. The preparation of acid **6** was accomplished in six steps with 47% overall yield, closely following the methods previously reported in a synthesis of sulfobacin A.[10]



Scheme 2. Reagents and conditions: (a) Fmoc-Gln(Trt)-OH, HBTU, HOBt, 35 min; (b) 20% piperidine in DMF, 2 min; (c) Fmoc-Asn(Trt)-OH, HBTU, HOBt, 35 min; (d) **6**, HBTU, HOBt, 35 min; (e) TFA, 30 min; (f) 0.1:40:60 TFA/MeCN/H_2O, 30 min; (g) octanoic acid, HBTU, HOBt, 35 min.

With acid **6** in hand, we next attempted to complete the solid phase synthesis of fellutamide B (Scheme 2). Using Fmoc solid phase peptide synthesis (SPPS) procedures,[11,12] leucinal loaded beads[13] **7** were coupled first with protected glutamine (Fmoc-Gln(Trt)-OH), followed by protected asparagine (Fmoc-Asn(Trt)-OH), and finally with (*R*)-(−)-3-hydroxydodecanoic acid (**6**). Global deprotection of the side chain protecting groups with anhydrous trifluoroacetic acid (TFA)[14] was followed by cleavage of the peptide from the resin with 0.1% TFA in 2:3 MeCN/H_2O.[15] Fellutamide B was isolated in analytically pure form after lyophilization from the resin cleavage solution. Synthetic fellutamide B was found to be identical in all respects to the isolated natural product.[16]

Encouraged by our success with fellutamide B, we next prepared an *N*-octanoyl analog of fellutamide B **10** using an analogous strategy (Scheme 2).[17] This analog contains a shorter lipophilic tail and lacks the β-hydroxy amide functionality present in the natural product. It was hoped that this analog would allow us to determine the importance of the *N*-acyl chain for NGF induction.

Having prepared the two aldehydes **2** and **10**, we examined their biological activities. Cytotoxicity IC_{50} values were determined for three cell lines (Table 1).[18] In our hands, fellutamide B exhibited a potency similar to earlier reports.[4,5] To our surprise, the *N*-octanoyl analog **10** was approximately an order of magnitude less cytotoxic than **2** against all cell lines tested, suggesting that modification of the lipophilic tail has a significant effect on the activity of these compounds.

With data in hand regarding the cytotoxicity of the two compounds, we next examined the capacity of both aldehydes to induce NGF secretion (Fig. 2).[19] In this assay, L-M cells were treated with 50 μM fellutamide B or octanoyl fellutamide for 24 h to allow for drug-induced NGF secretion, after which the medium was removed, filtered, and dialyzed. The conditioned medium was then added to rat preneuronal PC12 cells for 48 h, after which the cells were examined for neurite outgrowth.

Table 1. Cytotoxicity assay results for fellutamide B (**2**) and octanoyl fellutamide analog **10**[a]

Cell line	Fellutamide B (**2**) (nM)	*N*-Octanoyl fellutamide (**10**) μM
KB (human epithelial carcinoma)	349 (±31.5)	4.83 (±0.20)
PC12 (rat pheochromocytoma)	437 (±26.0)	1.67 (±0.23)
L-M (mouse fibroblast)	482 (±81.5)	3.33 (±0.39)

[a] Values are means of three to five experiments, standard error is given in parentheses.