Published in final edited form as:

Bioorg Med Chem Lett. 2006 July 15; 16(14): 3855–3858.

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

John S. Schneekloth Jr.^b, John L. Sanders^b, John Hines^a, and Craig M. Crews^{a,b,c*}

^aDepartment of Molecular, Cellular, and Developmental Biology, Yale University, PO Box 208103, New Haven, CT 06520-8103, USA

^bDepartment of Chemistry, Yale University, PO Box 208103, New Haven, CT 06520-8103, USA

^cDepartment of Pharmacology, Yale University, PO Box 208103, New Haven, CT 06520-8103, USA

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.

Keywords

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

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 IC₅₀ 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-hydroxydo-decanoic acid synthon **6**. Solid phase synthesis of the peptide chain was a particularly attractive strategy, as it would allow us to access analogs

*Corresponding author. Tel.: +1 203 432 9364; fax: +1 203 432 6161; e-mail: craig.crews@yale.edu.

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 phosphonoacetate in 85% yield. Sharpless asymmetric dihydroxylation⁶ of **4** was carried out using AD-Mix- β with excess methane sulfonamide in a 1:1 *t*-BuOH/H₂O 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/H₂O 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

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 beads¹³ **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/H₂O.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 IC₅₀ 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 μ M 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.

Neurotrophic activity is defined here as neurite out-growth in response to the NGF present in the conditioned medium.²⁰ Importantly, Figure 2D indicates that fellutamide **B** does induce the secretion of NGF from L-M cells. In an identical assay, 50 μ M **10** was equally effective in inducing NGF secretion (not shown). These data indicate that even though **10** is significantly less cytotoxic than **2**, the simplified analog retains neurotrophic activity. While the capacity of

these compounds to induce NGF secretion is intriguing, it should be noted that concentrations necessary to achieve the neurotrophic effect are currently still above the IC₅₀ value for cytotoxicity. However, it should be noted that the secretion of NGF in this assay can be attributed directly to the action of fellutamide on the L-M cells. In a similar assay, conditioning of medium by treatment of L-M cells with other general toxic agents such as ion channel inhibitors (veratridine and ouabain)²¹ or an actin binding molecule (cytochalasin D)²² did not produce neurite outgrowth in PC12 cells.

In conclusion, we have completed the first chemical synthesis of the natural product fellutamide B (**2**) and an *N*-octanoyl analog (**10**). Both compounds were verified to be cytotoxic and induce NGF secretion in L-M cells. Fellutamide B behaves in a manner similar to previous reports. Simplification of the lipophilic tail of fellutamide B in **10** significantly decreases the cytotoxicity of the compound, but the analog retains its neurotrophic activity. As other peptide aldehydes are known to inhibit proteases,²³ including the proteasome,²⁴ it is possible that protease or proteasome inhibition could be a potential mechanism of action for these compounds. Work is ongoing in our laboratory to elucidate the specific intra-cellular protein target of fellutamide B and its mechanism of NGF induction.

Acknowledgments

J.S.S. gratefully acknowledges the American Chemical Society, Division of Medicinal Chemistry and Aventis Pharmaceuticals for a predoctoral fellowship. We acknowledge Stanley Lo for help with initial experiments. We acknowledge the NIH (GM062120) for funding.

References and notes

1. Pettus TRR, Chen XT, Danishefsky SJ. *J. Am. Chem. Soc.* 1998;120:12684.
2. Cho YS, Carcache DA, Tian Y, Li YM, Danishefsky SJ. *J. Am. Chem. Soc.* 2004;126:14358. [PubMed: 15521747]
3. Waters SP, Tian Y, Li YM, Danishefsky SJ. *J. Am. Chem. Soc.* 2005;127:13514. [PubMed: 16190712]
4. Shigemori H, Wakuri S, Yazawa K, Nakamura T, Sasaki T, Kobayashi J. *Tetrahedron* 1991;47:8529.
5. Yamaguchi K, Tsuji T, Wakuri S, Yazawa K, Kondo K, Shigemori H, Kobayashi J. *Biosci. Biotechnol. Biochem* 1993;57:195. [PubMed: 7763492]
6. Becker H, Sharpless KB. *Angew. Chem., Int. Ed* 1996;35:448.
7. Kim BM, Sharpless KB. *Tetrahedron Lett* 1989;30:655.
8. Gao Y, Sharpless KB. *J. Am. Chem. Soc.* 1988;110:7538.
9. Dale JA, Mosher HS. *J. Am. Chem. Soc.* 1973;95:512.
10. Gupta P, Naidu SV, Kumar P. *Tetrahedron Lett* 2004;45:9641.
11. General procedure for SPPS coupling: H-Leu-H NovaSyn® beads (1.0 g, 0.23 mmol) were added to an oven-dried solid phase peptide synthesis reactor vessel and allowed to soak three times under DMF in 10 min cycles. To the dried beads was added a solution of a carboxylic acid (0.64 mmol), 243 mg HBTU (0.64 mmol), 86 mg HOBt (0.64 mmol), and 0.14 mL *N*-methylmorpholine in 4 mL DMF. The reaction was agitated with nitrogen for 35 min, and the solution was drained from the vessel. After washing the beads with DMF, the reaction was repeated for 35 min (to ensure complete conversion) and the beads were drained and washed with DMF. Deprotection was carried out using the general procedure described below, after which the next coupling was immediately carried out.
12. General procedure for SPPS Fmoc deprotection: beads were covered with 20% piperidine in DMF and reacted for 2 min. The solution was drained, and the process repeated twice to ensure complete deprotection. The beads were washed five times with DMF to remove excess piperidine.
13. H-Leu-H NovaSyn® beads were obtained from Novabiochem (<<http://www.novabiochem.com>>).
14. General procedure for global deprotection: beads were washed with DMF five times and dichloromethane five times. The beads were covered with neat trifluoroacetic acid, and reacted for 30 min, after which the solution was drained. The process was repeated until no yellow color appeared in the eluent, after which the beads were rinsed five times with dichloromethane.

15. General procedure for cleavage from resin: the beads were covered with a solution of 60:40:0.1 H₂O/MeCN/TFA and agitated for 30 min. The solution was collected, and the process repeated three times. The combined eluents were lyophilized to afford the desired product.
16. Spectral data for fellutamide B (**2**): ¹H NMR δH (500 MHz; DMSO-*d*₆) 0.7–0.9 (m, 9H), 1.2–1.4 (m, 16H), 1.46 (m, 2H), 1.55 (m, 1H), 1.67 (m, 1H), 1.92 (m, 1H), 2.00 (m, 2H), 2.05 (m, 2H), 2.40 (m, 1H), 2.49 (m, 1H), 3.94 (m, 1H), 4.06 (m, 1H), 4.12 (m, 1H), 4.43 (m, 1H), 6.68 (br s, 1H), 6.82 (br s, 1H), 7.17 (br s, 1H), 7.41 (br s, 1H), 8.00 (m, 2H), 8.24 (br s, 1H), 9.28 (s, 1H). ¹³C NMR δC (125 MHz; DMSO-*d*₆) 13.8, 21.3, 22.0, 23.0, 23.9, 28.7, 29.0, 29.1, 31.3, 31.4, 36.9, 43.5, 49.8, 52.4, 56.6, 67.5, 171.0, 171.2, 171.8, 171.9, 201.6.
17. Spectral data for octanoyl fellutamide (**10**): ¹H NMR δH (500 MHz; DMSO-*d*₆) 1.05 (m, 9H), 1.47 (m, 2H), 1.35 (m, 8H), 1.51 (m, 3H), 1.62 (m, 1H), 1.75 (m, 1H), 2.10 (m, 4H), 2.41 (m, 2H), 4.04 (m, 1H), 4.19 (m, 1H), 4.50 (m, 1H), 6.77 (br s, 1H), 6.94 (br s, 1H), 7.21 (br s, 1H), 7.41 (br s, 1H), 8.06 (d, *J* = 7.2, 1H), 8.12 (d, *J* = 7.2, 1H), 8.31 (d, *J* = 6.6, 1H), 9.35 (br s, 1H). ¹³C NMR δC (125 MHz; DMSO-*d*₆) 14.3, 21.7, 22.4 (2C, *i*-Pr), 23.4, 24.3, 25.5, 28.8, 29.0, 31.5, 31.6, 36.1, 36.6, 36.9, 50.2, 52.7, 57.0, 171.5, 172.1, 172.9, 174.1, 202.0.
18. [³H]thymidine incorporation: cells were seeded into 96-well plates at a density of 2000–3500 cells/well. The medium was replaced after 24 h with fresh medium containing various concentrations of fellutamide B or octanoyl-fellutamide B. After 20 h, the cells received 2 μCi/well of [³H]thymidine, and after another 4 h were detached from the wells and passed through glass fiber filters. The filters were transferred to vials and the amount of radioactivity incorporated into the cells was quantified by scintillation counting. The resulting data were analyzed using PRISM software (GraphPad Software).
19. Neuronal cell differentiation: L-M cells were grown to confluency and incubated for 24 h in serum-free Medium 199 containing DMSO (vehicle) or fellutamide B or octanoyl fellutamide. This conditioned medium was then collected, filtered, and dialyzed against RPMI 1640 medium at 4 °C for 24 h. Horse serum and fetal bovine serum were then added to the dialyzed conditioned medium to a final concentration of 2% and 1%, respectively. Finally, the conditioned medium was applied to undifferentiated PC12 cells that had been plated onto collagen. After 48 h, the PC12 cells were photographed and neurite outgrowth assessed.
20. Obin M, Mescio E, Gong X, Hass AL, Joseph J, Taylor A. J. Biol. Chem 1999;274:11789. [PubMed: 10206996]
21. Shimojo RY, Iwaoka WT. Toxicology 2000;154:1. [PubMed: 11118665]
22. Castilla MA, Carmelo C, Gazapo RM, Martin O, Gonzalez-Pacheco FR, Tejedor A. Life Sci 2000;6:1003. [PubMed: 10954034]
23. Ede NJ, Eagle SN, Wickham G, Bray AM, Warne B, Shoemaker K, Rosenberg S. J. Pept. Sci 2000;6:11. [PubMed: 10674715]
24. Myung J, Kim KB, Crews CM. Med. Res. Rev 2001;21:245. [PubMed: 11410931]

Figure 1.
Structures of fellutamides A and B.

Figure 2. PC12 cells differentiate in response to fellutamide B-conditioned medium. (A) Undifferentiated PC12 cells. (B) PC12 cells treated with DMSO-conditioned medium, 48 h. (C) PC12 cells treated with NGF (100 ng/mL), 48 h. (D) PC12 cells treated with 50 μ M fellutamide B-conditioned medium, 48 h.

Scheme 1.

Reagents and conditions: (a) LiOH, triethyl phosphonoacetate, THF, reflux, 4 h, 85%, 95:5 *E:Z*; (b) AD-Mix- β , MeSO₂NH₂, 1:1 *t*-BuOH/H₂O, 0 °C, 36 h, 80%, 90% ee; (c) SOCl₂, pyridine, 0 °C, 30 min, 99%; (d) NaIO₄, 1 mol % RuCl₃, 3:1 MeCN/H₂O, rt, 30 min, 90%; (e) NaBH₄, DMA, 0 °C to rt, 1 h, then 20% aq HCl, rt, 18 h, 72%; (f) LiOH, MeOH/H₂O/THF 1:1:1, rt, 3 h, 97%.

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/H₂O, 30 min; (g) octanoic acid, HBTU, HOBT, 35 min.

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

Cell line	Fellutamide B (2) (nM)	<i>N</i> -Octanoyl fellutamide (10) μ M
KB (human epithelial carcinoma)	349 ($\pm$ 31.5)	4.83 ($\pm$ 0.20)
PC12 (rat pheochromocytoma)	437 ($\pm$ 26.0)	1.67 ($\pm$ 0.23)
L-M (mouse fibroblast)	482 ($\pm$ 81.5)	3.33 ($\pm$ 0.39)

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