J. Am. Chem. Soc. 1993, 115, 5302

Total Synthesis of (+)-Lactacystin, the First Non-Protein Neurotrophic Factor

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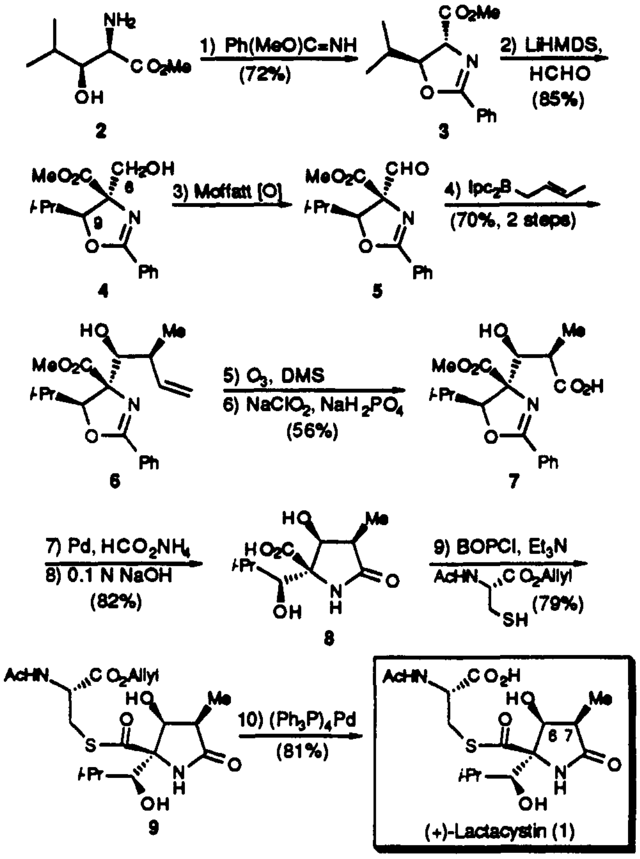
Received March 25, 1993

Neurotrophic agents such as nerve growth factor (NGF), a well-characterized protein, are required for the survival and function of neurons.[1] In 1991 we reported the isolation and characterization of the first non-protein neurotrophic factor, (+)-lactacystin (**1**), a novel sulfur-containing γ-lactam produced by a culture broth of *Streptomyces* sp. OM-6519.[2] Lactacystin induces neuritogenesis and causes a transient increase in the intracellular cAMP level in Neuro 2A neuroblastoma cells[2a] and is also active against Sarcoma 180.[3] Corey and Reichard recently reported the first total synthesis of **1**.[4] Herein we describe a concise alternative approach, designed to afford easy access to both the natural product and a variety of analogues. Key steps in the elaboration of the lactam moiety include the stereoselective hydroxymethylation of oxazoline **3** and an asymmetric allylboration which introduces the hydroxyl and methyl substituents at C(6) and C(7), respectively (Scheme I).

As our point of departure, 2(*R*),3(*S*)-β-hydroxyleucine methyl ester[5] (**2**) was treated with methyl benzimidate to furnish the *trans*-disubstituted oxazoline **3**.[6,7] Aldol condensation with formaldehyde via the Seebach protocol[8] then gave **4**[6] exclusively (85% yield, >98% de); the stereochemical assignment was secured by ^{1}H NOE studies.[9] Moffatt oxidation[10] afforded aldehyde **5**,[6] which was subjected without purification to allylboration with (*E*)-crotyldiisopinocampheylborane as described by Brown.[11] The desired β-methyl homoallylic alcohol **6**[6] was thus obtained in 70% yield from **4** after chromatography on silica gel.

Conversion of **6** to carboxylic acid **7**[6] entailed ozonolysis and reductive workup (dimethyl sulfide) followed by selective oxidation ($NaClO_4$, NaH_2PO4, 2-methyl-2-butene; 56% yield from **6**).[12] The key γ-lactam **8**[6] could be elaborated in 82% yield by catalytic transfer hydrogenation[13] of **7** and ester hydrolysis. For the transformation of **8** to **1** we employed a two-step sequence first devised by Corey.[4] The amino side chain was incorporated via thioesterification of **8** with bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOPCl)[14] and *N*-acetyl-L-cysteine allyl ester (79%). Finally, deallylation of **9**[6] [$Pd(PPh_3)_4$, HCOOH, Et_3N] gave pure (+)-lactacystin (**1**)[6] in 81% yield as colorless needles. The successful construction of (+)-lactacystin was confirmed by detailed comparison of the synthetic and natural compounds (400-MHz ^{1}H and 100-MHz ^{13}C NMR, IR, HRMS, optical rotation, melting and mixed melting points, and TLC in four solvent systems).

Scheme I



In summary, the development of an economic and versatile synthetic approach to **1** (10 steps, 13% overall yield) should permit the preparation of useful quantities of (+)-lactacystin and its analogues, greatly facilitating the ongoing pharmacological studies of neurotrophic factors.

Acknowledgment. Financial support of the National Institutes of Health (Institute of Neurology, Communicative Disorders and Stroke) through grant 18254 is gratefully acknowledged. We also thank Drs. Noritaka Chida and James J.-W. Duan for helpful discussions and Professor Corey for a preprint of their manuscript.

Supplementary Material Available: Characterization data for **1**–**9** (IR, ^{1}H and ^{13}C NMR, MS, mp, optical rotation) (4 pages). Ordering information is given on any current masthead page.

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(1) (a) Perez-Polo, J. R. In *Cell Culture in the Neurosciences*; Bottenstein, J. E., Sato, G., Eds.; Plenum Press: New York, 1985; pp 95–123. (b) Hefti, F.; Weiner, W. J. *Ann. Neurol.* **1986**, *20*, 275–281. (c) Barde, Y.-A. *Neuron* **1989**, *2*, 1525–1534.

(2) (a) Ōmura, S.; Fujimoto, T.; Otoguro, K.; Matsuzaki, K.; Moriguchi, R.; Tanaka, H.; Sasaki, Y. *J. Antibiot.* **1991**, *44*, 113–116. (b) Ōmura, S.; Matsuzaki, K.; Fujimoto, T.; Kosuge, K.; Furuya, T.; Fujita, S.; Nakagawa, A. *J. Antibiot.* **1991**, *44*, 117–118.

(3) Unpublished results of Dr. K. Komiyama of The Kitasato Institute, to be described in detail elsewhere.

(4) Corey, E. J.; Reichard, G. A. *J. Am. Chem. Soc.* **1992**, *114*, 10677–10678.

(5) Jung, M. E.; Jung, Y. H. *Tetrahedron Lett.* **1989**, *48*, 6637–6640.

(6) The structure assigned to each new compound is in accord with its infrared, 270-MHz ^{1}H NMR, and 67.5-MHz ^{13}C NMR spectra, as well as appropriate parent ion identification by high-resolution mass spectrometry. In addition, analytical samples of **1**–**4**, **6**, **8**, and **9** gave satisfactory C and H combustion analyses.

(7) Moss, R. A.; Lee, T. B. K. *J. Chem. Soc., Perkin Trans. 1* **1973**, 2778–2781.

(8) Seebach, D.; Aebi, J. D. *Tetrahedron Lett.* **1983**, *24*, 3311–3314.

(9) Irradiation of the C(9) proton (lactacystin numbering) led to 1.7 and 1.4% NOE enhancements of the C(6) methylene protons in **4**.

(10) (a) Pfitzner, K. E.; Moffatt, J. G. *J. Am. Chem. Soc.* **1965**, *87*, 5661. (b) Pfitzner, K. E.; Moffatt, J. G. *J. Am. Chem. Soc.* **1965**, *87*, 5670.

(11) Brown, H. C.; Bhat, K. S. *J. Am. Chem. Soc.* **1986**, *108*, 293–294.

(12) Corey, E. J.; Myers, A. G. *J. Am. Chem. Soc.* **1985**, *107*, 5574–5576.

(13) Bieg, T.; Szeja, W. *Synthesis* **1985**, 76–77.

(14) Tung, R. D.; Rich, D. H. *J. Am. Chem. Soc.* **1985**, *107*, 4342–4343.

0002-7863/93/1515-5302$04.00/0