Synthesis of (+)-Obafluorin, a β-Lactone Antibiotic

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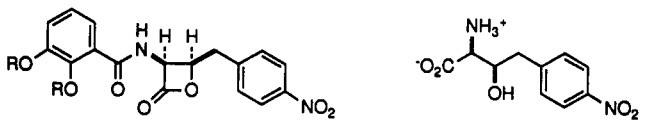
Summary: Optically pure obafluorin (1), an antibacterial agent from *Pseudomonas fluorescens*, was synthesized via lactonization of *N*-[(2-nitrophenyl)sulfenyl]-(2*S*,3*R*)-2-amino-3-hydroxy-4-(4-nitrophenyl)butanoic acid (6), which was prepared in a stereospecific manner from 4-nitrophenylacetaldehyde and (*S*)-1-benzoyl-2-(*tert*-butyl)-3-methyl-4-imidazolidinone (**4a**).

Compounds having an α-amino-β-lactone moiety are not only useful synthetic intermediates for preparation of amino acid derivatives[1-3] but also occur naturally as microbial metabolites with antibiotic activity.[4,5] One of the first of these to be isolated[5a] is obafluorin (1) from *Pseudomonas fluorescens* (ATCC 39502). Its relatively simple structure,[5b] which resembles the monobactam class of β-lactam antibiotics, belies its unprecedented, albeit moderate, biological activity.[5] Considerable attention has also focused on the mechanism of its biosynthesis,[6] which may involve the β-substituted serine **2a** as an intermediate.[6b] Very recently, Rao et al. reported the synthesis of racemic diacetylobafluorin[7] (3) employing cyclization and protection–deprotection methodology similar to that described previously by our group.[1a] It remains unclear whether the acetyl groups can successfully be removed from **3** to give **1** because of the instability of pure obafluorin[5b] and the general sensitivity of such α-amino-β-lactone derivatives to base and strong nucleophiles.[1] In the present work, we describe the first synthesis of optically pure obafluorin (1) by a method that permits great flexibility in variation of the side chains at C-3 and the nitrogen of the β-lactone moiety.



1 R = H

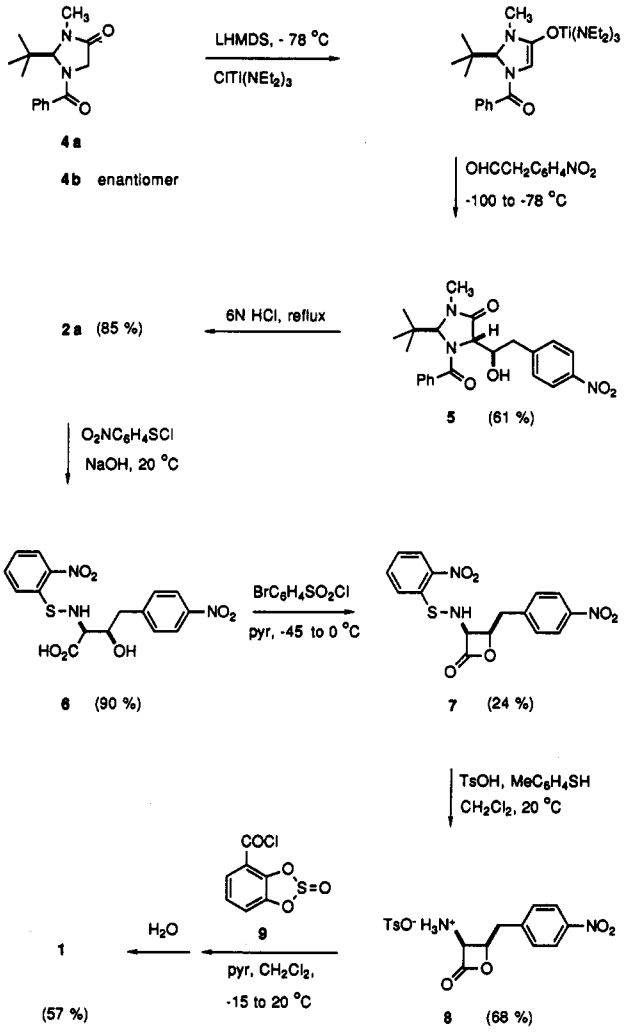
3 R = Ac

2a

2b enantiomer

The imidazolidinone **4a** devised by Seebach and coworkers[8] is an excellent chiral glycine synthon for asymmetric aldol reactions (Scheme I).[9] Treatment with lithium hexamethyldisilazane at −78 °C generates the corresponding enolate, which reacts with 4-nitrophenylacetaldehyde[10] to give the expected adduct **5**. However, this material appears to readily undergo epimerization in the reaction mixture, which upon hydrolysis then gives variable yields of a mixture of **2a** and its erythro isomer (typically 4:1). Although the reasons for the unusual behavior of this reaction are still undetermined, it may be due to the acidity and sensitive nature of the 4-nitrophenylacetaldehyde moiety. Schöllkopf and co-workers documented the use of chloro[tris(dimethylamino)]titanium for enhancement of threo vs erythro diastereoselectivity in aldol condensations of bis-lactim ether enolates.[11] In an analogous procedure, treatment of the

Scheme I



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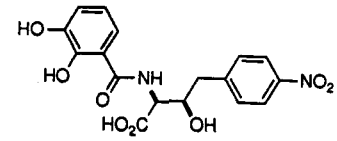
lithium enolate of **4a** with chloro[tris(diethylamino)]titanium[11b] presumably gives a titanium enolate. Slow addition of 4-nitrophenylacetaldehyde[10] to the reaction mixture at −100 °C, with subsequent warming to −78 °C and quenching with aqueous ammonium chloride solution, increases the yield of the aldol reaction (61%) and produces only the threo isomer within detection limits. Acidic hydrolysis of the condensation product **5** and purification by ion-exchange chromatography (AG 50W-X8, H^+) affords (2*S*,3*R*)-2-amino-3-hydroxy-4-(4-nitrophenyl)butanoic acid (**2a**) in an overall 52% yield from **4a** with no detectable trace (by NMR, TLC) of the erythro diastereomer. The optical purity of **2a** was verified by preparation of the methyl ester of its (*S*)-camphanamide derivative and comparison of the spectral data with a similar derivative of (2*R*,3*S*)-2-amino-3-hydroxy-4-(4-nitrophenyl)butanoic acid (**2b**). The latter was prepared analogously to **2a** from (*R*)-1-benzoyl-2-(*tert*-butyl)-3-methyl-4-imidazolidinone (**4b**). The ^1H NMR spectra of the diastereomeric (*S*)-camphanamide methyl esters are easily distinguishable and show that within detection limits (ca. 1%) each derivatized product contains only one optical isomer.

Protection of the amino acid **2a** with the (2-nitrophenyl)sulfenyl group[1a,12] to form **6** followed by cyclization via carboxyl group activation with 4-bromophenylsulfonyl chloride in pyridine[1a] gives the N-protected β-lactone **7** (24%). The yield of the latter step is disappointingly low, but **7** is the only material easily isolable by standard chromatographic purification; the rest of the reaction mixture consists of very polar side products. In previous work we had demonstrated that removal of nitrogen protecting groups from α-amino-β-lactones under carefully controlled acidic conditions provides the corresponding salts, which can be acylated.[1a] In order to avoid difficulties with deprotection of the phenolic hydroxyls of the highly sensitive obafluorin molecule, the acid chloride **9**[13] was chosen as an acylating agent. The cyclic sulfite moiety hydrolyzes very readily upon exposure to water, and **9** is available in a single step by reaction of thionyl chloride with 2,3-dihydroxybenzoic acid.[13] Thus, treatment of **7** with 4-thiocresol and 4-toluenesulfonic acid[1a,14] generates the stable tosylate salt **8** (68%) of the parent oxetanone. Acylation[1a] with the acid chloride **9**[13] and aqueous workup produces optically pure (+)-obafluorin (**1**) (57% yield after reversed-phase HPLC purification). As expected,[5b] obafluorin (**1**) decomposes upon standing in aqueous acetonitrile to the hydrolyzed product **10**. The partly decomposed material can be repurified by rapid HPLC (isocratic elution, 55% acetonitrile–water) to give pure obafluorin ($[\alpha]_D$ +43° (*c* = 0.03, MeCN)), which can be stored dry satisfactorily for some weeks at −15 °C under an inert atmosphere. The optical rotation differs from the literature value[5b] ($[\alpha]_D$ +116° (*c* = 0.1, MeCN)), possibly because of experimental error in measurement of the rotation at low concentration. To confirm this, the hydrolysis product **10** was further cleaved under acidic conditions to release the free amino acid. Purification by ion exchange chromatography, derivatization to the (*S*)-camphanamide methyl ester as described for **2a** and **2b**, and analysis of the ^1H NMR spectrum reveals signals corresponding solely to the derivative of **2a**, thereby verifying the optical integrity of **1**. The remaining spectral data for **1** agrees with published values,[5b] and the compound displays potent antibacterial activity against *Staphylococcus aureus* strains in preliminary microbiological tests. Further studies on the synthesis, biological activity, and mechanism of antibacterial action of modified obafluorin analogues are in progress.



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Supplementary Material Available: Experimental procedures (7 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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