Total Synthesis

An Efficient Synthesis of Lactacystin β-Lactone**

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Proteasomes are proteins within cells that are responsible for protein degradation. The 20S proteasome, a 700-kDa protein that consists of 14 distinct subunits, is implicated in the ubiquitin proteasome pathway (UPP).[1] UPP, present in all eukaryotic cells, is essential for the normal turnover of cellular proteins and for the removal of damaged or misfolded

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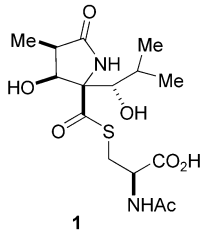
[**] We thank the EPSRC and GlaxoSmithKline for funding this project. AstraZeneca, Pfizer, and Novartis are thanked for generous unrestricted funding.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

 DOI: 10.1002/anie.200453843

proteins.[1] Another vital role of UPP is the processing and degradation of regulatory proteins that control cell growth, transcriptional activation, and metabolism.[1] In view of this significant biological role, the 20S proteasome has become an important biological target in many drug-discovery programs.

Omura et al.[2,3] reported the isolation and characterization of (+)-lactacystin (**1**) in 1991 and showed it to be a novel



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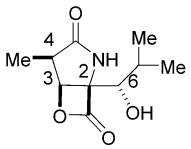

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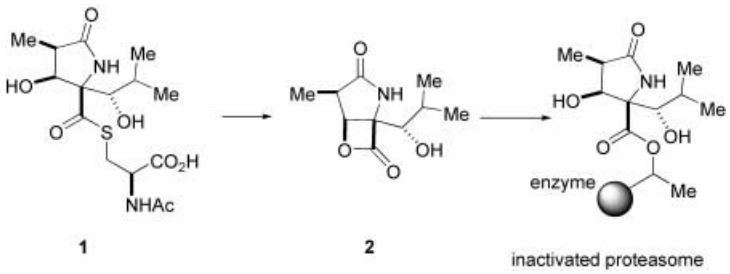
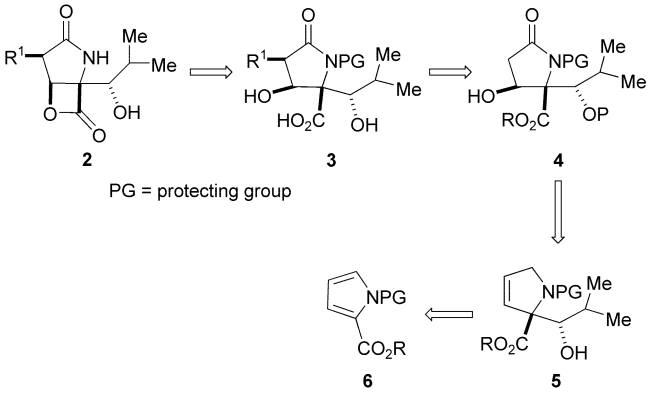
γ-lactam produced by a culture broth of *Streptomyces* sp. OM-6519. As lactacsytin inhibits the 20S proteasome, there has been a flurry of synthetic approaches towards the synthesis of this interesting molecule and analogues thereof.[4–10]

Key work by Corey, Schreiber, and co-workers,[11,12] Huber and co-workers,[13] and Dick et al.[14] clearly defined the mechanism of inhibition displayed by (+)-lactacystin (**1**). These investigations showed that (+)-lactacystin (**1**) is, in fact, a prodrug for (+)-lactacystin β-lactone (**2**), formed by the loss of *N*-acetylcysteine (Scheme 1). Once inside a cell, the lactam **2** then acylates the proteasome, causing inhibition.



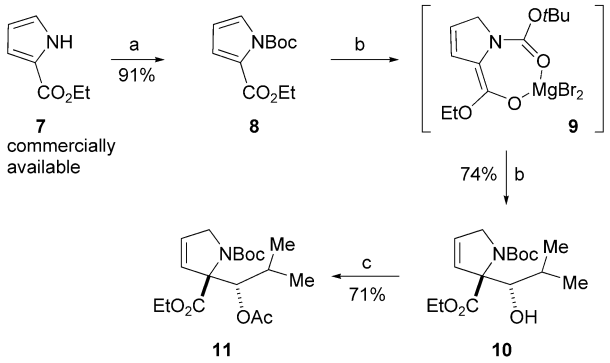
Scheme 1. (+)-Lactacystin (**1**) is a prodrug for (+)-lactacystin β-lactone (**2**). Once inside a cell, **2** acylates the 20S proteasome, causing inhibition.

Structure–activity relationship (SAR) studies by Corey and co-workers[11,15–21] and by Adams and co-workers[9] showed that the SAR requirements for proteasome inhibition were rather stringent. There is an absolute requirement for the β-lactone ring to be present, and the stereochemical fidelity at C2, C3, and C6 cannot be compromised without losing biological activity. The isopropyl group attached to C6 is also important for inhibition. Thus, the only replaceable group is the methyl group at C4.[10] All the syntheses of lactacystin β-lactone (**2**) reported so far introduce the methyl group at C4 at a relatively early stage; therefore, these strategies are not ideal for the easy production of analogues.[9,22] Our work addresses this issue and a retrosynthetic analysis of such an approach is shown in Scheme 2.



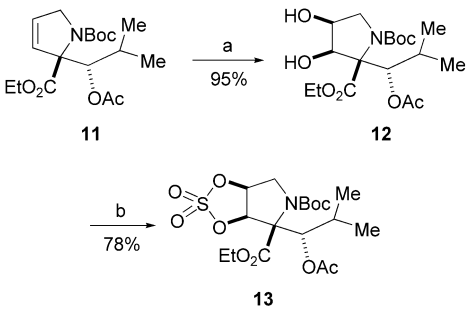
Scheme 2. Retrosynthetic analysis of **2**.

Herein we describe a short alternative approach to (±)-lactacystin β-lactone (**2**) through a diastereoselective reductive aldol reaction of Boc-protected pyrrole **8** that we have recently developed.[23] The reaction of pyrrole **8** in the presence of $MgBr_2$ led to an *anti* selectivity greater than 20:1 (Scheme 3): This selectivity has its origins in the formation of *Z* enolate **9**.[23] Subsequent protection of the *anti* aldol adduct (±)-**10** as an acetate following a standard protocol proceeded to yield **11** (Scheme 3).



Scheme 3. Reagents and conditions: a) $(Boc)_2O$, DMAP, Et_3N, CH_2Cl_2; b) Li, DBB, THF, $(MeOCH_2CH_2)_2NH$, $MgBr_2$, isobutyraldehyde; c) Ac_2O, pyridine, DMAP. Boc = *tert*-butoxycarbonyl, DMAP = 4-dimethylaminopyridine, DBB = 4,4′-di-*tert*-butylbiphenyl.

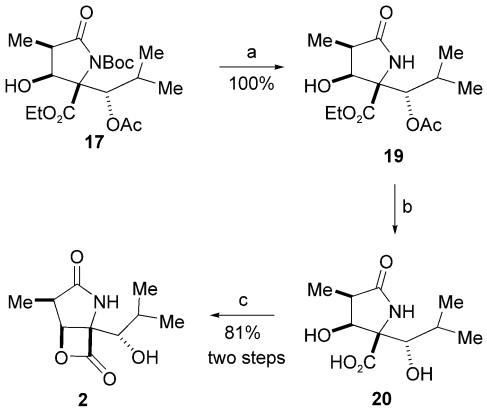
A key step in our synthesis was the diastereoselective dihydroxylation of **11** (Scheme 4). Treatment of **11** with catalytic OsO_4 and NMO (3 equiv) in acetone/water (4:1) afforded diol **12** as a single diastereoisomer in an average yield of 65%. However, by changing the dihydroxylation conditions to those reported by Poli[24] (cat. OsO_4, $Me_3NO{\cdot}2H_2O$ (3 equiv) in CH_2Cl_2), the diol **12** was produced in an excellent yield of 95%. The stereochemistry of the diol **12** was proven by a two-step conversion into the crystalline cyclic sulfate **13**, the structure of which was determined by X-ray diffraction analysis.[25] The X-ray crystal structure of **13** reveals that the isopropyl group effectively shields one face of the ring. Importantly, reagents therefore approach **11** from the face *syn* to the ester functionality.



Scheme 4. Reagents and conditions: a) cat. OsO_4, $Me_3NO{\cdot}2H_2O$, CH_2Cl_2; b) 1. $SOCl_2$, Et_3N, CH_2Cl_2; 2. cat. $RuCl_3{\cdot}xH_2O$, $NaIO_4$, MeCN/CCl_4/H_2O.

Two crucial steps of our total synthesis were the regioselective deoxygenation at C4 and subsequent diastereoselective (*syn*) introduction of the methyl group at C4. To this end, several strategies were investigated. The C4-OH of diol **12** was selectively converted into a bromide or iodide by selective mesylation and S_N2 displacement of the mesylate with lithium bromide or zinc iodide. Unfortunately, the resulting halide functionalities could not be displaced with any nucleophile that we examined.[26] An alternative approach was therefore sought. A selective Mitsunobu reaction led to the conversion of the C4-OH functionality of **12** into iodide **14** (Scheme 5). The regioselectivity observed in the Mitsunobu reaction was as expected because displacement of the C3 (neopentyl) hydroxy group is slow. The resulting iodide **14** was deiodinated through a recently reported method for producing (catalytic) indium hydride in situ.[27] The use of indium hydride instead of the traditional tributyltin hydride obviated the need for extensive purification of the product. Next, the C3-OH functionality of **15** was protected with a triethylsilyl group (TES), the product was oxidized with catalytic RuO_4 to form a lactam, and the TES group was then removed to furnish **16**.

The second key step then followed when we introduced the methyl group at C4 with LDA (2 equiv) and methyl iodide (Scheme 5). Gratifyingly, the major alkylation adduct **17** was formed with a similar selectivity to that of the dihydroxylation step (see above) and had the requisite stereochemistry at C4 for lactacystin β-lactone (**2**). The stereochemistry of both **17** and **18** was assigned by NOE studies.[28] Finally, cleavage of the *tert*-butoxycarbonyl group of **17** with TFA in CH_2Cl_2 led to the formation of lactam **19** in quantitative yield. Basic hydrolysis of the ethyl ester gave acid **20**, which was used without purification to give **2** (Scheme 6). The spectroscopic data of compound **2** was identical to that reported in the literature.

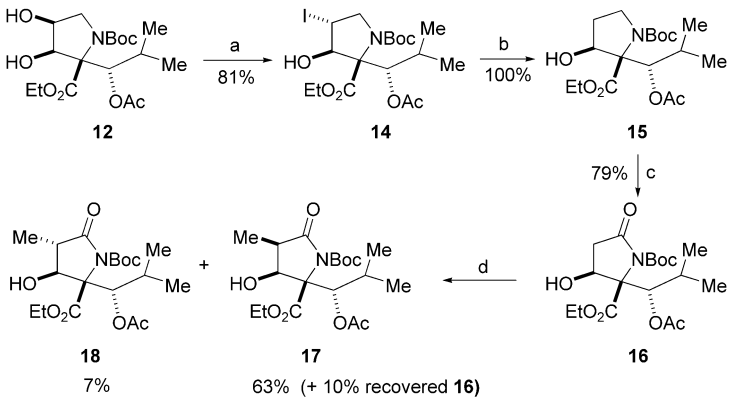


Scheme 6. Reagents and conditions: a) CF_3CO_2H, CH_2Cl_2; b) NaOH (aqueous 0.5 M); c) BOPCl, Et_3N, CH_2Cl_2. BOPCl = bis(2-oxo-3-oxazolidyl)phosphinic chloride.

In conclusion, a short synthesis of (±)-lactacystin β-lactone (**2**) was completed in only 13 steps starting from commercially available pyrrole **7**. The final product was isolated in 14% overall yield. The advantage of our strategy is centered around the introduction of the methyl group at C4 at a late stage of the synthesis, thereby making our route easily amenable to the production of analogues.

Received: January 26, 2004 [Z53843]

Keywords: aldol reaction · heterocycles · natural products · reduction · total synthesis



Scheme 5. Reagents and conditions: a) PPh_3, DBAD, MeI, benzene; b) cat. $InCl_3$, $NaBH_4$, MeCN; c) 1. TESCl, imidazole, DMAP, CH_2Cl_2; 2. cat. $RuCl_3{\cdot}xH_2O$, m-$NaIO_4$, CCl_4/MeCN/H_2O; 3. HF·py, THF, pyridine; d) LDA, HMPA, MeI, THF. DBAD = di-*tert*-butyl azodicarboxylate, TES = triethylsilyl, py = pyridine, LDA = lithium diisopropylamide, HMPA = hexamethyl phosphoramide.

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[28] In compound **17**, irradiation of 3-H led to an NOE enhancement of 4-H, but not of the methyl group at C4. In compound **18**, irradiation of the 3-H led to NOE enhancements of both 4-H and of the methyl group at C4.

