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Enantiospecific Formal Total Synthesis of the Tumor and GSK-3β Inhibiting Alkaloid, (−)-Agelastatin A

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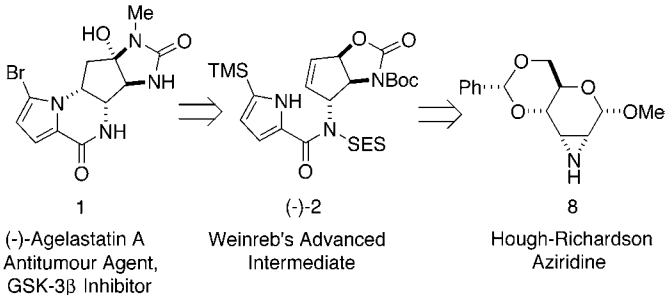
ABSTRACT



An enantiospecific total synthesis of Weinreb's advanced intermediate 2 for (−)-agelastatin A has been achieved from the Hough−Richardson aziridine 8. Noteworthy reactions in our sequence include the highly regioselective *trans*-diaxial ring-opening of 8 with azide ion to set up the vicinal diamido functionality present within (−)-2 and the Grubbs−Hoveyda ring-closing metathesis (RCM) reaction that was used to construct its cyclopentene core.

In 1993, Pietra and co-workers at the University of Trento reported their isolation of the novel oroidin alkaloid, (−)-agelastatin A, from a collection of the axinellid sponge *Agelas dendromorpha*.[1] They deduced its absolute stereostructure through a combination of spectral methods that included, inter alia, CD spectroscopy and a new application of the exciton-coupling technique to diamides.[1a,b]

Of special interest were their observations that (−)-agelastatin A potently inhibited the proliferation of a human KB nasopharyngeal cancer cell line at low drug concentrations (IC_{50} = 0.075 μg/mL) and that it inhibited the growth of an L1210 murine tumor cell line.[1c] (−)-Agelastatin A was also found to prolong the life-expectancy of mice with L1210 leukemia when repeatedly administered intraperitoneally at doses of 2.6 mg/kg, although no antitumor effects were noted when it was given intravenously.[1c] The antitumor mechanism of (−)-agelastatin A has yet to be elucidated.

Although (−)-agelastatin A does not inhibit casein kinase 1, CDK1/cyclin B, or CDK5/p25, it has been found to selectively inhibit GSK-3β (glycogen synthase kinase-3β) with an IC_{50} of 12 μM.[2]

GSK-3β hyperphosphorylates the microtubule-binding protein, tau. Hyperphosphorylated tau proteins are major components of the neurofibrillary tangles observed in Alzheimer's disease (AD) and are now believed to be key players in the onset of this disease.[3]

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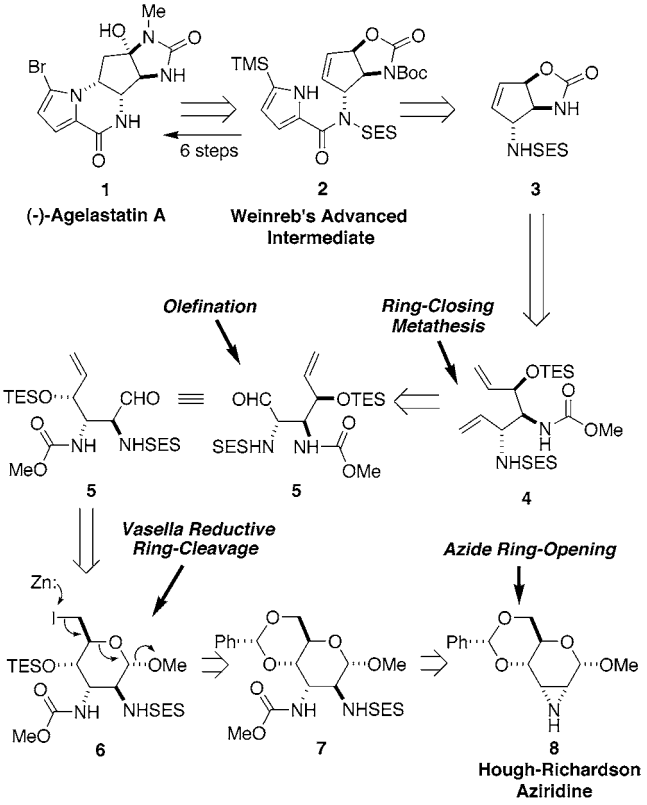
GSK-3β also plays a central role in the Wnt/β-catenin/TCF cell-signaling pathway.[3b,4] Wnts are secreted glycoproteins that bind to the Frizzled/Frizzled-2-type receptors on the surfaces of cells to activate the protein Dishevelled (Dv1), which inhibits GSK-3β.[4] Active Wnt signaling causes the up-regulation and accumulation of free cytosolic β-catenin, which subsequently translocates to the nucleus where it complexes with members of the TCF (T-cell factor) family of transcription factors, to activate the expression of genes involved in cell growth and proliferation. Target genes for β-catenin/TCF-mediated transcription include include *c*-myc,[5] cyclin D1,[6,7] matrilysin,[8] TCF1,[9] the multidrug resistance 1 (MDR1) gene,[10] *c*-jun, fra-1, the urokinase-type plasminogen activator receptor,[11] and osteopontin.[12] Another recently confirmed target is the peroxisome proliferator-activated receptor (PPAR) δ-gene.[13]

It is now well established that functionally inactivating mutations to GSK-3β cause an accumulation of β-catenin and that this in turn activates certain tumorigenic promoters involved in melanoma and colon cancer such as TCF4.[4] The observation that (−)-agelastatin A can *selectively* inhibit GSK-3β and yet still function as a powerful antitumor agent is therefore quite remarkable and makes this a molecule of enormous biological interest.

Importantly, this observation suggests that it might be possible to design new and more potent small-molecule GSK-3β inhibitors that will be highly selective and non-tumorigenic. Inhibitors of deregulated GSK-3β activity could potentially serve as drugs[14] for treating neurodegenerative diseases such as AD or for preventing neuronal apoptosis after stroke. They might also function as novel insulin mimetics, because insulin activates a protein cascade (PI3-Kinase/PKB) that inhibits GSK-3β.[14b] It was with the preparation of novel GSK-3β inhibitors in mind that we recently embarked on an enantioselective total synthesis of (−)-**1** and its analogues.

Scheme 1. Retrosynthetic Planning for (−)-Agelastatin A



At the outset of our studies, Weinreb's group had already published an elegant total synthesis of racemic agelastatin A[15] that employed a novel hetero Diels−Alder cycloaddition reaction and a Sharpless/Kresze allylic amination[16] sequence for assembly of the cyclopentane core. Feldman and Saunders subsequently reported an enantioselective route to (−)-agelastatins A and B that exploited an unusual vinylcarbene C−H insertion reaction for carbocycle construction.[17] O'Brien's group at the University of York[18] have also been active in this area, having reported a concise asymmetric synthesis of an *N*-tosylated C-ring fragment that could prove to be useful for a future total synthesis of the natural product.

In our approach to (−)-agelastatin A (Scheme 1), we hoped to prepare one of Weinreb's advanced intermediates (**2**) in

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optically pure form and, thereafter, to use Weinreb's synthetic end-game to access the target itself. Weinreb's team had previously converted racemic **2** into (±)-agelastatin A by a six-step protocol that looked highly amenable to analogue construction. It seemed logical therefore to capitalize on their past successes.

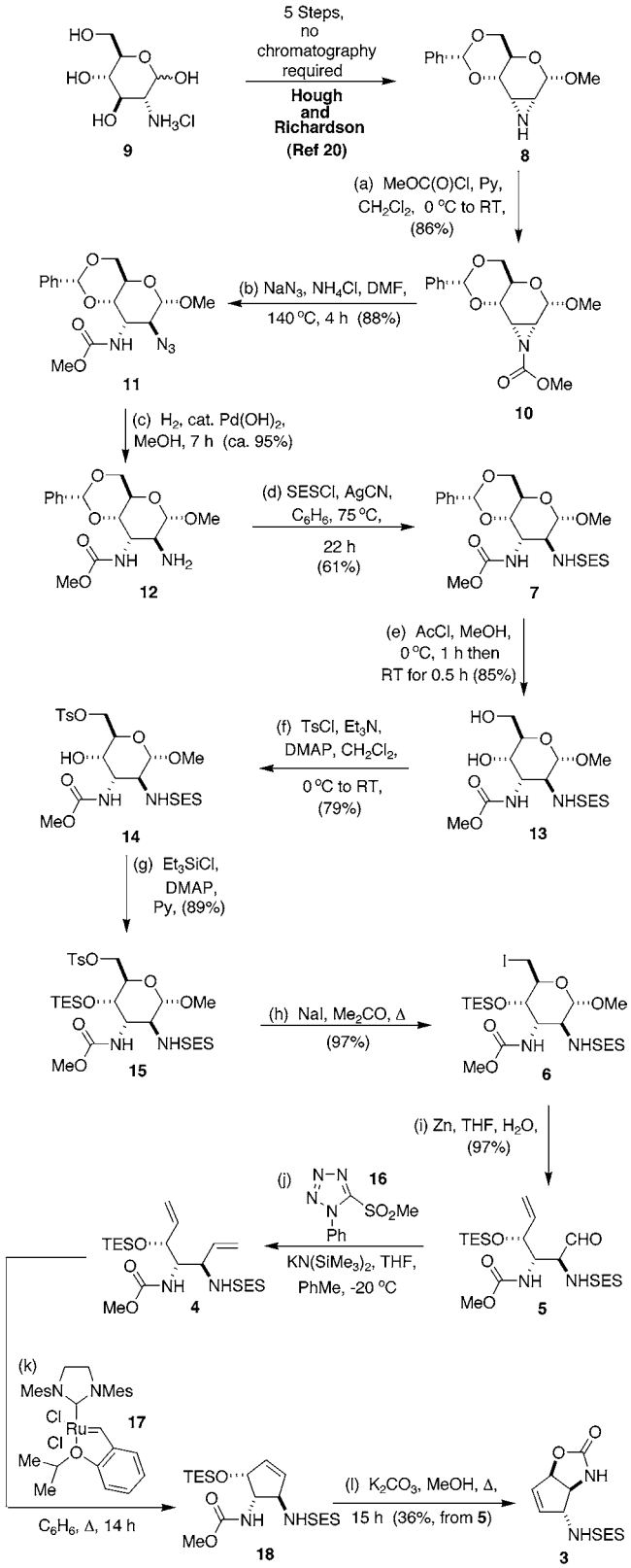
Our strategy for accessing the enantiopure pyrroloamide **2** would selectively *N*-acylate the oxazolidinone **3** with Boc_2O prior to attaching the TMS-pyrrole-carboxylic acid residue (Scheme 1). A ring-closing metathesis reaction would be used to construct the cyclopentene ring system of **3**, while diene **4** would be assembled from the aldehyde **5** by Wittig methylenation. A Vasella reductive ring-opening[19] was envisaged for accessing **5** from **6**; the latter, in turn, would be derived from aziridine **8**[20] by a multistep pathway that would involve aziridine acylation with methyl chloroformate and Furst−Plattner ring-opening[21] of the product with azide ion. Potentially, the latter sequence could completely control the stereochemistry of the two vicinal amido groupings in **7** and nicely set the stage for the chemistry that would follow. With this in mind, we now present our synthesis of (−)-**2** in full.

Our route to diene **4** set off from the known aziridine **8**[20] (Scheme 2). The latter is readily available on a large scale, without recourse to chromatography, through the procedure of Hough and Richardson.[20] Significantly, *N*-acyl derivatives of **8** show a marked preference for undergoing trans-diaxial ring-opening reactions with strong nucleophiles to give 3-amino sugars with the α-D-*altro*-configuration.[21] As a result, we predicted that when the *N*-methylcarbamate **10** was reacted with sodium azide in hot DMF, we would obtain **11** as the major reaction product. In the event, a smooth and highly regioselective aziridine ring-opening occurred at 140 °C using just 4 equiv of NaN_3. Compound **11** was isolated as the sole reaction product in 88% yield.

The next two steps of the synthesis were hydrogenolysis of the azide group in **11** and protection of the crude amine **12** with trimethylsilylethylsulfonyl chloride (SES−Cl)[22] and silver cyanide in benzene at 75 °C. Significantly, this new protocol for introducing the SES group gave significantly higher yields of **7** (58−61% from **11**) than more traditional amine-catalyzed protocols, which were all much slower and more problematic with respect to the occurrence of side-reactions.

The most satisfactory means of detaching the *O*-benzylidene acetal from **7** treated it with anhydrous HCl in MeOH for 1.5 h. Diol **13** was thereafter selectively *O*-tosylated in 79% yield with tosyl chloride, DMAP, and Et_3N in CH_2Cl_2. The secondary alcohol of **14** was then *O*-silylated with triethylsilyl chloride and DMAP in pyridine to obtain **15**, which was converted into the iodide **6** by nucleophilic displacement with NaI in acetone at reflux. A Vasella

Scheme 2. Synthetic Route to Oxazolidinone (−)-**3**[a]



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reductive ring-opening[19] on **6** (see Scheme 1) with zinc dust in aqueous THF subsequently procured aldehyde **5** (Scheme 2).

To our great dismay, the Wittig methylenation of **5** with $Ph_3P{=}CH_2$ was unsuccessful under all the conditions that we studied. Tebbe and Peterson olefinations with $Cp_2Ti{=}CH_2$ and Me_3SiCH_2MgCl, respectively, also failed to olefinate **5**. Eventually, we found that Kocienski's modification of the Julia olefination protocol was effective in this capacity;[23] this reacted the anion of tetrazolyl sulfone **16** with aldehyde **5** to produce the diene **4** directly. Although it was not usually possible to remove all of the tetrazole by-product from diene **4** on a preparative scale, this impurity did not adversely affect the subsequent RCM reaction to obtain **18**.

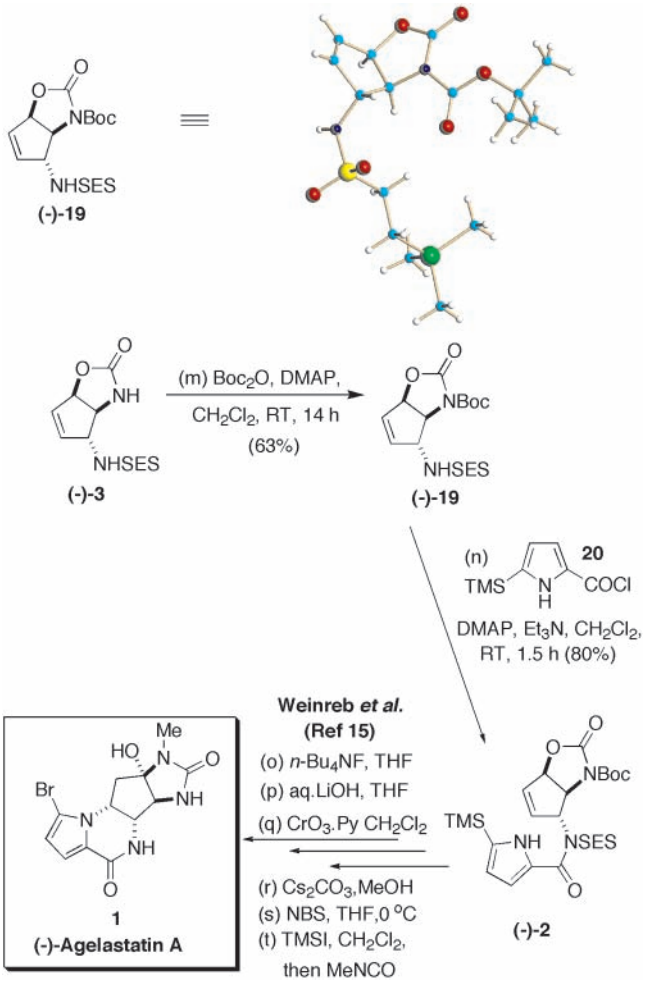
The recently developed Hoveyda−Grubbs ruthenium alkylidene **17**[24] was by far the most convenient and effective catalyst for effecting this ring-closure. It very cleanly converted **4** into **18**, notwithstanding the presence of the urethane and sulfonamido groupings within **4**, which often deactivate RCM catalysts of earlier vintage. Yet again, it still proved to be difficult to remove all of the tetrazole byproduct from **18** by chromatography. Slightly impure **18** was therefore heated with K_2CO_3 in MeOH at reflux for 2 h to obtain the pure oxazolidinone **3** in 36% overall yield for the three steps from aldehyde **5**.

We were then faced with the challenging issue of having to chemoselectively *N*-acylate the oxazolidinone nitrogen of **3** in the presence of the sulfonamido functionality to obtain (−)-**19** (Scheme 3). Fortunately, this could be achieved reasonably cleanly and efficiently (in 63% yield) using Boc_2O and DMAP in CH_2Cl_2. Racemic **19** had previously featured as an advanced intermediate in Weinreb's total synthesis of (±)-agelastatin A, and the ^1H and ^{13}C NMR spectra of our optically pure (−)-**19** matched those recorded by these workers for (±)-**19** in the same solvent. Our enantiopure **19** also had a large negative $[\alpha]_D$ (−88° at *c* 0.22 in CH_2Cl_2), and its relative and absolute stereostructure were further confirmed by X-ray crystallography (see Scheme 3).

As expected from Weinreb's work on (±)-**19**, the *N*-acylation of (−)-**19** with acid chloride **20** proceeded satisfactorily, delivering (−)-**2** in 80% yield. Our new route to (−)-**2** thus constitutes a fully stereocontrolled enantiospecific formal total synthesis of (−)-agelastatin A.

Currently, we are attempting to use the Weinreb end-game to prepare multigram quantities of (−)-agelastatin A, as well as a host of analogues for further biological testing. The chemical biological results of these efforts will be the subject of future publications.

Scheme 3. Formal Total Synthesis of (−)-Agelastatin A



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Supporting Information Available: Full experimental procedures and detailed spectral data of all new compounds, copies of 500 MHz ^1H and 125 MHz ^{13}C NMR spectra, and HRMS spectra and X-ray crystallographic analysis/data for (−)-**19**. This material is available free of charge via the Internet at http://pubs.acs.org.

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