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Leptostatin: A synthetic hybrid of the cytotoxic polyketides callystatin A and leptomycin B

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Abstract—Four stereoisomeric hybrids of the polyketide natural products callystatin A and leptomycin B have been prepared by parallel synthetic routes involving chiral allenylstannane methodology. Like their natural counterparts, these hybrids exhibit nanomolar levels of cytotoxicity toward HCT-116 human colon cancer cells.

Natural products of the polyketide family have attracted a great deal of attention in recent years because of their high levels of activity against various human tumor cell lines.[1] A number of the more potent members with cytotoxicities in the nanomolar to picomolar range feature a branched chain of 17–19 carbons attached to the α′ position of an α-pyranone terminus. The relatively simple structures of these compounds, their scarce availability, and high levels of biological activity render them attractive targets for total synthesis. Within the past several years syntheses of leptomycin B,[2] callystatin A,[3] leptofuranin D,[4] kazusamycin A,[5] and ratjadone[6] have been reported (see Fig. 1). Typically the stereochemical issues surrounding such efforts have been well resolved by existing aldol and allylmetal methodology.[7] As a possible candidate for development as an antitumor drug, callystatin A presents a particularly attractive profile in consideration of its more modest structural complexity and its reported IC_{50} value of 5 pM against KB tumor cells. Not surprisingly then, no fewer than eight total syntheses have been reported to date along with numerous segment assemblages.[3]

A significant reoccurring feature among these natural products, and one that is essential to high levels of bioactivity, is the α-pyranone terminus. Several studies support the notion that the α,β-unsaturated lactone is the pharmacophore for callystatin A, leptomycin B, and ratjadone. Kobayashi and co-workers performed structure–activity relationship studies on callystatin A and found that analogs lacking the pyranone double bond showed a dramatic decrease in activity against KB cells.[8] In addition, both a nitro Michael adduct[9] and a saturated lactone[10] derivative of leptomycin showed greatly diminished activity against HeLa cells. Furthermore, ratjadone analogs lacking the lactone carbonyl exhibited no biological activity against three different cell lines.[11] The idea of the lactone as the pharmacophore also applies to compounds that have an α,β-unsaturated lactone but are not members of the leptomycin family. For example, a recent study by Boger and co-workers on fostriecin, a novel polyketide α-pyranone with significant antitumor activity, showed a 200-fold decrease in activity upon reduction of the conjugated double bond.[12] Additional studies conducted on both leptomycin and ratjadone showed that the mechanism of action is a Michael addition to the α,β-unsaturated lactone by a cysteine residue in the protein CRM1, which is involved in the export of proteins from the nucleus to the cytoplasm.[1,13]

We recently completed total syntheses of callystatin A and leptofuranin D by parallel nonaldol routes in which an α-pyranone progenitor was attached to the intact branched polyketide chain.[4,14] The successful application of the methodology employed in these syntheses

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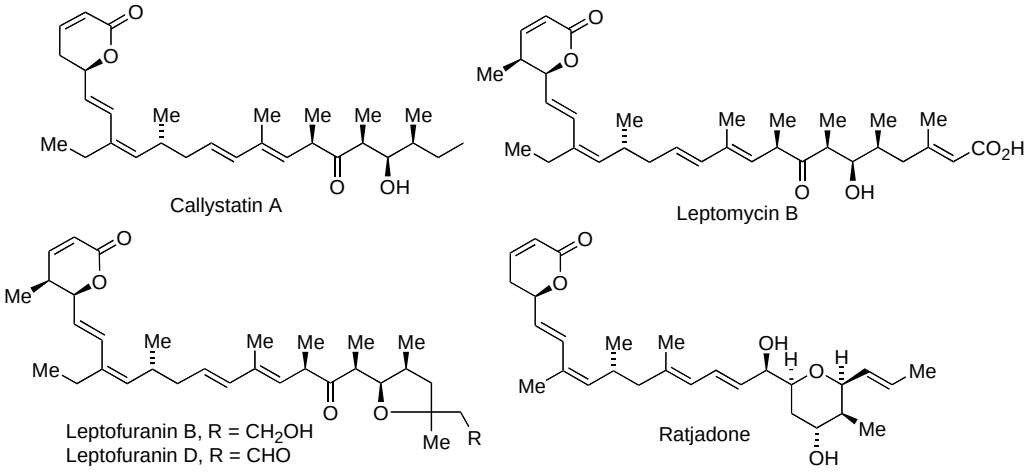


Figure 1. Cytotoxic polyketide natural products with an α-pyranone terminus.

prompted our speculation on the possibility of interchanging the pyranone segment of leptomycin and its congeners with the acyclic polyketide chain of callystatin A to produce a synthetic nonnatural hybrid 'leptostatin.' The concept was particularly appealing since by simple variations on our allenylstannane methodology[15] we could produce all possible stereoisomers ('leptostatin 1–4' in Fig. 2) of the pyranone segment and thereby probe a previously unexplored correlation of lactone stereochemistry and biological activity.

We prepared the C6–C22 segment of the aforementioned leptostatin analogs as previously described.[14] For the pyranone segment we employed variations on the methodology used in our synthesis of leptofuranin D.[4] Accordingly, $MgBr_2$-promoted addition of the allenylstannane (*M*)-**1** to the *p*-methoxybenzyloxy aldehyde **2** afforded a roughly 3:1 mixture of the syn and anti-adducts (*S*,*R*)-**3** and (*S*,*S*)-**3**. The use of $SnCl_4$ in this reaction led to the same two adducts as a 1:3 mixture.[15] By using the allenylstannane (*P*)-**1** in the addition reaction we obtained the enantiomeric syn and anti-adducts (*R*,*S*)-**3** and (*R*,*R*)-**3**. At this stage, the syn and anti-adducts were not separable (see Scheme 1).

Removal of the pivalic ester group of **3** with DIBAL-H led to the isomeric diols **4**, which were readily separated by flash chromatography. Lindlar hydrogenation[16] of the derived TBS ethers **5** followed by PMB removal

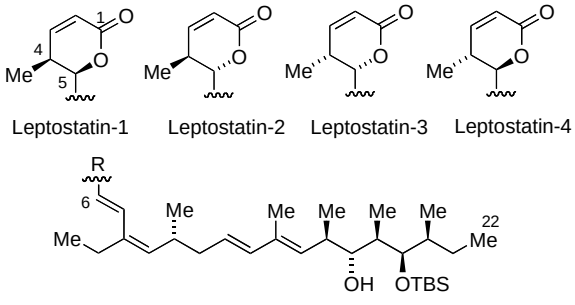
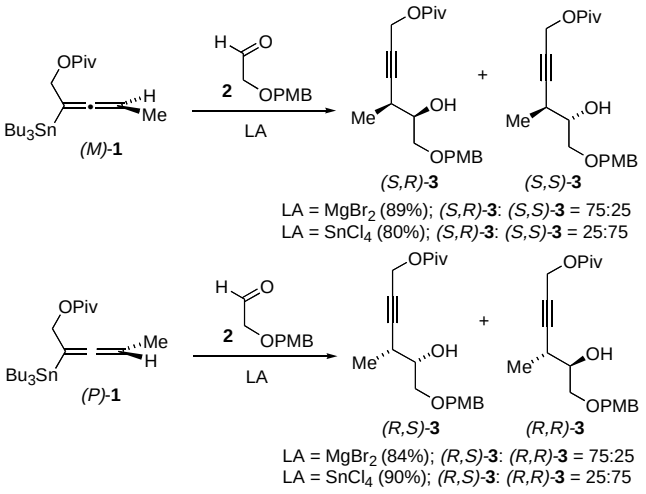


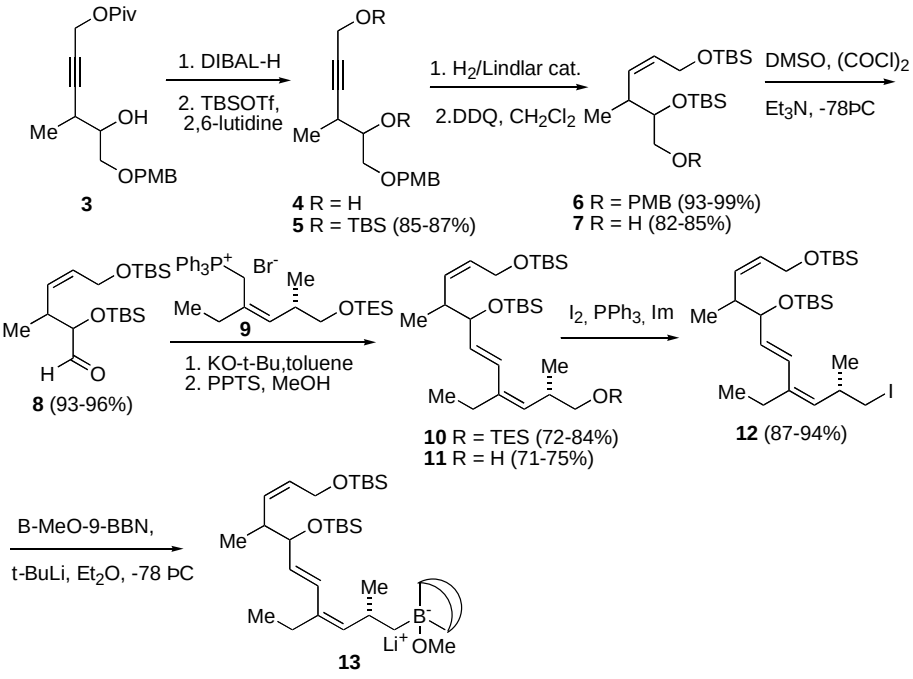
Figure 2. Nonnatural hybrids of callystatin A and leptomycin.



Scheme 1. Synthesis of four stereoisomeric pyranone precursors.

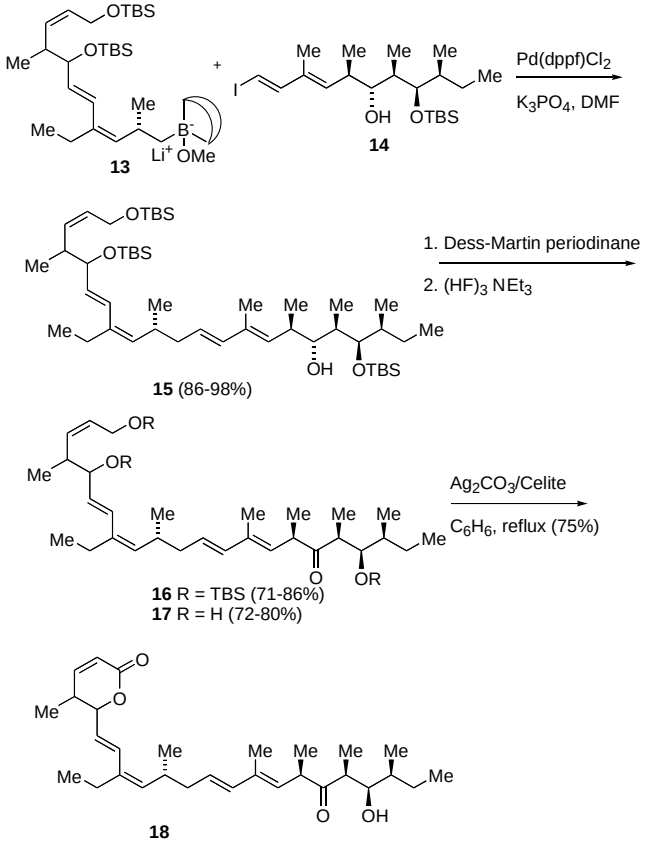
from ethers **6** afforded the unsaturated alcohols **7** in high overall yield for all diastereomers. Swern oxidation of these alcohols produced the aldehydes **8** representing the C1–C6 pyranone segment of the targeted leptostatins. Wittig condensation with the phosphonium reagent **9** was smoothly effected with KO–*t*-Bu as the base to yield the (*E*,*Z*)-dienes **10** contaminated by trace amounts (4–10%) of inseparable (*E*,*E*)-isomers. These impurities were gradually removed through chromatographic purification of succeeding intermediates. Cleavage of the terminal TES grouping of **10** and iodinolysis of the resulting alcohol **11** gave iodides **12**, which were directly converted to the borates **13** in situ (see Scheme 2).

Attachment of the acyclic polyketide segment **14** of callystatin A to the pyranone precursor segments **13** was efficiently effected through Suzuki coupling with $Pd(dppf)Cl_2$ as the precatalyst. The closing steps of the syntheses entailed oxidation of the alcohols **15** with the Dess–Martin periodinane reagent and deprotection of the silyl ethers **16** with a HF–NEt_3 complex to form the diols **17**. The final conversion of diols **17** to



Scheme 2. Synthesis of the four stereoisomeric C1–C11 fragments of leptostatins 1–4.

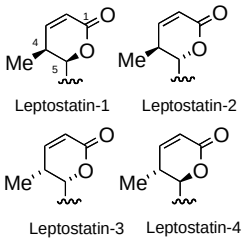
leptostatins 1–4 was effected by means of Fetizon's reagent.[17] This method proved to be faster and produced yields of lactones higher than those of the previously employed MnO_2 methodology (see Scheme 3).[14]



Scheme 3. Completion of the leptostatin 1–4 syntheses.

The IC_{50} values of the four leptostatin isomers and callystatin A against human HCT-116 colon cancer cells are summarized in Table 1. Leptostatins-1 and 3 are syn isomers and leptostatins-2 and 4 are anti. It will be noted that the introduction of a methyl substituent at C4 significantly diminishes cytotoxicity relative to callystatin A. Interestingly, the C4/C5 configuration of the isomer with the highest activity (leptostatin-3) is enantiomeric to that of leptomycin and its congeners. Kobayashi and Kalesse found that altering the C5 stereocenter in callystatin and ratjadone, respectively, decreased the activity against KB[8] and HEPG2 tumor cells.[11] It is important to note that the lactone in these studies lacks the methyl group found in both leptomycin and leptostatin; the added methyl group may cause

Table 1. Cytotoxicity toward HCT-116 cells



Compound	IC_{50} (nM)[a]
Leptostatin-1	3.0
Leptostatin-2	2.0
Leptostatin-3	0.2
Leptostatin-4	30
Callystatin A	0.03

[a]Average of two determinations.

different interactions and thus lead to different activity profiles.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.09.084.

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