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Synthesis of a natural product-inspired eight-membered ring lactam library via ring-closing metathesis

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Abstract

We have prepared a novel speculative eight-membered lactam demonstration library based on the skeletal structure of the potent antitumor marine natural product octalactin A. The basic scaffold was readily constructed in a convergent fashion via ring-closing metathesis chemistry from the corresponding diene amides. A cursory examination of the biological properties of the library validates the relevance and significance of these structures.

Keywords

Eight-membered; Natural product; Lactam; Library; Ring-closing metathesis; Medium-ring; Octalactin; Parallel synthesis

Libraries of compounds based on natural products have gained in importance in recent years.^{1,2} Although the structures contained in these libraries tend to be fairly diverse, one class of compound remains to be fully exploited, namely, the monocyclic medium-ring.^{3,4} An interesting natural product from the standpoint of structural architecture and biological profile is the powerful antitumor marine natural product octalactin A, **1** (Fig. 1).^{5,6} This fascinating natural product which features a rare saturated eight-membered lactone core continues to receive considerable attention in total synthesis efforts from many laboratories.⁷

We have previously constructed this lactone by means of an especially facile and direct lactonization from the corresponding seco acid ('zip-up' approach),^{7a,8} and by an equally facile ring-closing metathesis (RCM) route ('zip-down' approach) to afford the unsaturated oxocene.⁹ These enabling technologies prompted us to investigate the feasibility of generating a speculative eight-membered ring library inspired by octalactin A.

In order to simplify the synthesis, we elected in the present work to construct a simpler lactam scaffold that at a minimum retained the eight-membered core feature of the octalactins. The strategy is depicted in Scheme 1.

The unsaturated lactam scaffold **2** would be constructed by means of RCM. The diene amides **3** would result from the condensation of the racemic Fmoc-protected 3-amino-5-hexenoic acid **4** and the sublibrary of various racemic secondary allylic amines **5**. The allylic amines in turn would be derived from vinyl Grignard addition to the Schiff bases. By using readily prepared racemic components, a mixture of diastereomers **2** would be obtained resulting in a larger library with a relative minimum of effort and cost. Separation of the diastereomeric scaffolds would precede derivitization of the primary amines, thus generating the desired lactam library.

The allylic amine sublibrary was prepared as shown in Scheme 2. Reaction of a series of commercially available benzaldehydes **6a-h** and benzyl amines **7a-f** in ether at room temperature in the presence of magnesium sulfate¹⁰ gave after filtration the Schiff bases in nearly quantitative yield in all cases.

Addition of vinyl Grignard with BF₃·OEt₂ at –78 °C for 12 h gave a representative 13-member sublibrary in 67–87% yield (Fig. 2).

Synthesis of the racemic Fmoc-protected aminohexenoic acid component **4** was achieved using a modified literature procedure (Scheme 3).^{11,12} Addition of allylmagnesium chloride to the acylpyridinium salt formed between 4-methoxypyridine and phenylchloroformate gave the allyl-substituted dihydropyridone carbamate **9** in 90% isolated yield.

Methanolysis of the carbamate to give **10** was achieved in 86% yield. Oxidative cleavage with sodium periodate, followed by basic hydrolysis of the intermediate formamide, and finally Fmoc protection of the resulting primary amine gave **4** in 45% overall yield from **10** on a 5-g scale.

Several methods for the condensation of **4** and **5** were attempted, including DCC coupling,¹³ HATU-mediated coupling,¹⁴ activation of the carboxylic acid as its pentafluorophenyl (Pfp) ester,¹⁵ and the in situ generated acid chloride with oxalyl chloride and catalytic DMF.¹⁶ Of these attempts, only the last method (Scheme 4) gave consistent and reliable yields (61–96%) of the amides with our components.

Construction of the eight-membered lactam scaffolds via RCM was investigated next. Although we previously showed that the monocyclic oxocenes in the octalactin series could be obtained via RCM at only 40 °C,⁹ no ring closure with the amides **3a-m** was observed at temperatures up to 80 °C. We attribute this observation to an unfavorable amide rotamer population present at these temperatures. Gratifyingly, RCM was eventually effected with 15 mol% of the Grubbs' second-generation catalyst in refluxing toluene for 5–12 h to give a diastereomeric 1:1 separable mixture of lactams **2** in yields ranging from 55% to 93% (Scheme 5). There was no observed cyclization preference for either amide diastereomer, except in the case of **3a** which failed to cyclize, presumably due to the sterically hindered *o*-bromobenzaldehyde moiety. In this manner 24 diastereomerically pure eight-membered lactam scaffolds were obtained on a 20–50 mg scale.

The stereochemical assignments for the lactam diastereomers were made on the basis of NOE experiments. It was noted that for the high *R_f* diastereomers, an NOE was observed between the methine proton at C7 and one of the benzylic protons attached to the amide nitrogen which led to an assignment of the *cis* isomer. This effect was absent in all of the low *R_f* compounds; however, in these structures, an NOE between the methine proton at C7 and one of the methylene protons at C2 led to the assignment of the *trans* isomer. This remarkable correlation between *R_f* and the relative stereochemical configuration was seen throughout the entire series.

Two scaffolds, namely, **2i-trans** and **2l-cis**, were selected for preparation on a larger scale (about 1 gram) for use in derivitization of the unmasked primary amine. Removal of the Fmoc

group¹⁷ was accomplished with TBAF in THF/MeOH (1:1)¹⁸ (Scheme 6) to afford the corresponding amines **11i** and **11l** in quantitative yield.

These amines were then reacted with a series of 15 reagents **12a–o** (Fig. 3), such as acyl chlorides, sulfonyl chlorides, and isocyanates, to give a library of amides, sulfonamides, and ureas.

Reaction of the amines with the reagents **12a–o** with DIEA followed by scavenging the excess reagents with PS-trisamine¹⁹ (Scheme 7) gave 19 of 30 possible products, which were further purified by chromatography.

The isolated yields ranged from 27% to 100% (Table 1). These derivatives combined with the original scaffolds and afforded a 43-component unsaturated eight-membered lactam library.

To our knowledge this represents the first such library based on a monocyclic medium-ring scaffold.

Finally, of the 19-member derivitized library members synthesized, seven were randomly selected for an initial antiproliferative biological screen with murine L1210 lymphocytic leukemia, human HL-60 promyelocytic leukemia, and murine Pan02 pancreatic ductal adenocarcinoma cell lines. The results are summarized in Table 2.

Although the antiproliferative activities of these compounds are far from matching those of established anticancer drugs such as daunorubicin (DAU) and mitoxantrone (MITOX), which, under similar experimental conditions, consistently inhibit the growth of various tumor cell lines in the low nanomolar range, they nevertheless validate the success and relevance of these medium-ring libraries. Additionally, the entire demonstration library has already been submitted to the NIH MLSCN screening network for more expansive biological evaluation. The outcome of these studies will be reported as the results become available and posted on Pub-Chem (<http://pubchem.ncbi.nlm.nih.gov/>).

In summary we have prepared a novel, biologically relevant natural product-inspired eight-membered lactam library using a ring-closing metathesis strategy combined with late-stage amine derivitization with common pharmacophores. Other libraries based on medium-ring templates are in progress and will be reported as developments warrant.

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Figure 1.
Octalactin A.

Scheme 1.
Retrosynthetic analysis of the lactam scaffold.

Scheme 2.
 Synthesis of amine sublibrary **5a-m**.

Figure 2.
Allylic amines **5a-m**.

Scheme 3.
Synthesis of Fmoc-protected 3-amino-2-hexenoic acid **4**.

Scheme 4.
Diene amide formation via the acid chloride.

Scheme 5.
RCM reaction to give lactam library **2b-m**.

Scheme 6.
Fmoc deprotection with TBAF.

Figure 3.
Representative electrophiles for derivitization.

Scheme 7.
Generation of the eight-membered lactam library.

Table 1Yields of primary amine derivatives shown in Scheme 7^a

Electrophile	Primary amine 11i	Primary amine 11l
12a	13a 0%	14a 0%
12b	13b 100%	14b 74%
12c	13c 98%	14c 62%
12d	13d 51%	14d 62%
12e	13e 0%	14e 0%
12f	13f 0%	14f 59%
12g	13g 32%	14g 27%
12h	13h 96%	14h 51%
12i	13i 54%	14i 52%
12j	13j 71%	14j 54%
12k	13k –	14k 42%
12l	13l –	14l 64%
12m	13m –	14m 100%
12n	13n –	14n 0%
12o	13o –	14o 38%

^a Isolated yields after chromatographic purification.

Table 2

Antiproliferative activity of novel eight-membered lactams inspired by octalactin A in various tumor cell lines in vitro^a

Compound	L1210 cells (day 4) IC ₅₀ ^b (μM)	HL-60 cells (day 4) IC ₅₀ (μM)	Pan02 cells (day 4) IC ₅₀ (μM)
13b	27.1 (±2.4)	54.7 (±7.2)	40.6 (±3.8)
13c	25.2 (±1.9)	40.1 (±0.8)	34.9 (±3.7)
13d	40.1 (±3.2)	na ^c	—
13g	37.9 (±2.2)	na	—
13h	11.0 (±0.6)	na	—
13i	8.5 (±0.8)	na	—
13j	15.4 (±1.4)	na	—

^aConcentrations of compound required to inhibit by 50% (IC₅₀ values) the metabolic activity of L1210, HL-60, and Pan02 tumor cells, using MTS:PMS assay at day 4 in vitro. IC₅₀ values were calculated from linear regression of the slopes of the log-transformed concentration survival curves.

^bMeans ± SD (*n* = 3).

^cValue not available because the compound is unable to inhibit below 50% when tested at 62.5–156.25 μM.