



# Karlotoxin synthetic studies: concise synthesis of a C(42–63) B-ring tetrahydropyran fragment



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## ARTICLE INFO

### Article history:

Received 27 August 2013

Revised 19 September 2013

Accepted 21 September 2013

Available online 28 September 2013

### Keywords:

Polyketide

Karlotoxin

Tetrahydropyran

D-Mannose

Julia–Kocienski olefination

## ABSTRACT

Starting from natural D-mannose, a C(42–63) B-ring tetrahydropyran fragment in karlotoxin 2 has been prepared via a common THP intermediate in a concise manner. E-Selective Julia–Kocienski olefination efficiently assembled a C(51–63) chlorodiene subunit and a C(42–50) tetrahydropyran segment.

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Karlotoxins (KmTxS) are a family of linear polyketides isolated from a toxic phytoplankton, the dinoflagellate *Karlodinium micrum*.<sup>1</sup> These toxins are believed to possess hemolytic, cytotoxic, and ichthyotoxic activities.<sup>2</sup> A recent study also demonstrated that the toxins possess a unique, strong binding affinity to cholesterol,<sup>3</sup> which is one of the major components of lipid rafts. Since the lipid rafts, a cholesterol-rich membrane domain, have important clinical implications in major human diseases such as cancer, HIV (human immunodeficiency virus), TB (tubercle bacillus), and neurological disorders, the study of KmTx–cholesterol interactions may help to reveal further mechanistic aspects for the chemopreventive and drug design.<sup>4</sup> The scarcity of this class of natural products is the key limitation to further biological evaluation. Indeed, more than 50 years of isolation efforts by a number of researchers globally has provided only several 1–10 mg batches of toxin to date which has been utilized to characterize in vitro pharmacology and the structure of the molecule.

Recently, the absolute configuration of KmTx2 (**1**), the first complete structure determination among the congeners, was successfully assigned by our groups (Fig. 1).<sup>5</sup> The KmTx2 reveals unique structural features, including highly oxygenated C(32–49)

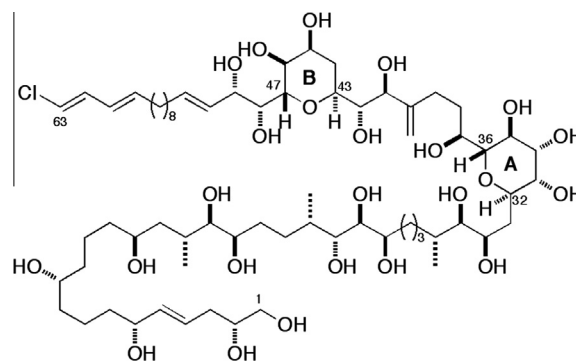


Figure 1. Karlotoxin 2 (**1**).

bis-tetrahydropyran core fragment, in conjunction with a long, irregular C(1–31) polyol arm as well as a C(50–63) lipophilic chlorotriene unit, with a total of 28 stereogenic centers. Such potent biological activities as well as novel molecular complexity of the toxins engaged our interest in the synthesis of KmTx2 and the analogues.

Interestingly, the KmTx2 shares structural similarities to amphidinol 3 (AM3)<sup>6</sup> and their bis-tetrahydropyran core units nearly identical with an exception of a few substitution patterns.

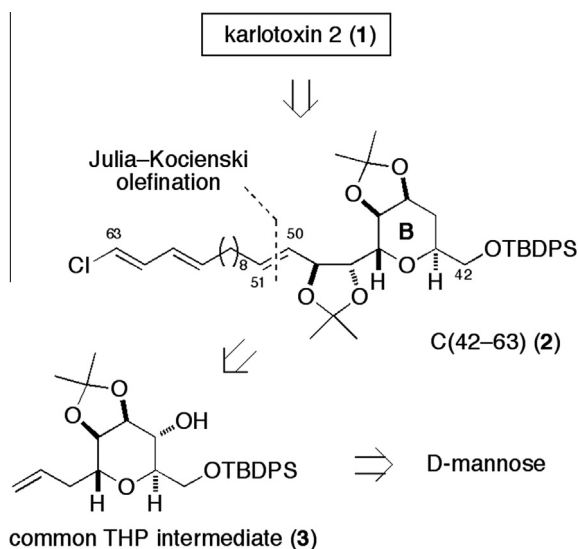
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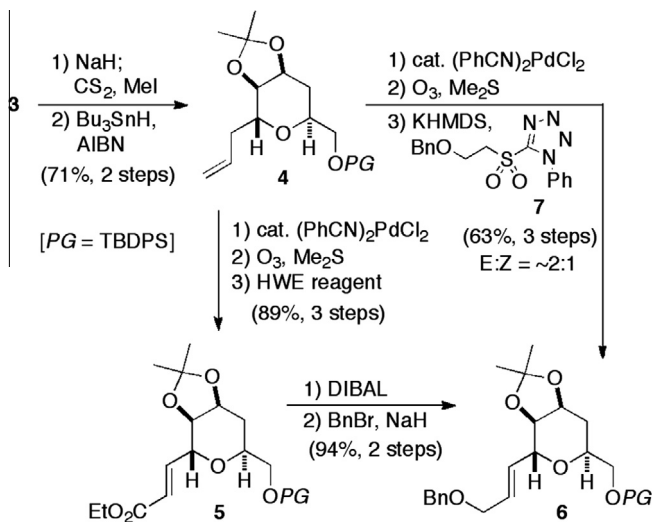
Importantly the absolute configuration of these two fragments is opposite and still remains uncertain.<sup>7</sup> Although several synthetic groups have been actively involved in the synthesis of AM3,<sup>8–13</sup> the recent structure revisions in 2008<sup>13a</sup> and 2013<sup>13c</sup> unfortunately hinder their progress. Thus, the synthesis of KmTx2 would also serve to confirm the proposed absolute configuration as well as supply the toxin for further biological study.

Our retrosynthetic plan disconnects KmTx2 into three major fragments: two THP fragments (A and B) and a polyol chain fragment. Further disconnections of both THPs lead to a known common THP intermediate (**3**) that can be readily prepared from natural D-mannose.<sup>14a</sup> In this Letter, we describe a concise stereo-selective approach leading to a C(42–63) B-ring THP segment (**2**) employing the Julia–Kocienski olefination as a key reaction (Scheme 1). Note: during the preparation of this manuscript, a partly similar approach to construct the *ent*-THP fragment of AM3 from D-mannose has been reported by Reymond et al.<sup>11f</sup>

To begin, based on the literature procedures,<sup>14</sup> the common THP intermediate **3** was prepared from D-mannose in a five-step reaction series. For subsequent construction of the B-ring THP framework (Scheme 2), a free hydroxyl group in **3** was removed by



Scheme 1. Retrosynthetic analysis of KmTx 2.



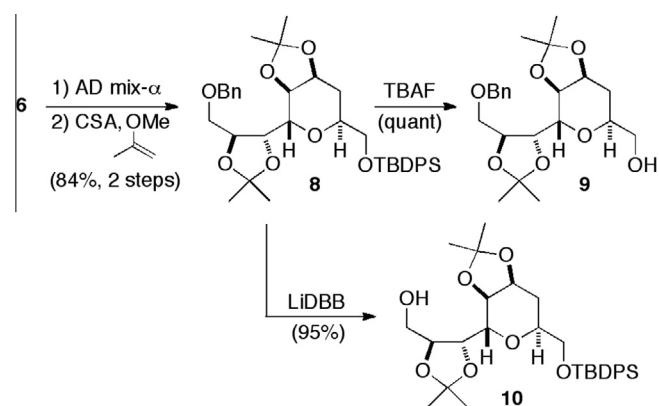
Scheme 2. Synthesis of **6**.

standard Barton–McCombie deoxygenation<sup>15</sup> which led to **4** (71% over two steps). A Pd(II)-catalyzed alkene isomerization of **4** followed by ozonolytic cleavage of the internal alkene provided corresponding aldehyde. Treatment of the crude aldehyde with sulfone **7** under Julia–Kocienski olefination conditions afforded desired  $\alpha,\beta$ -unsaturated ester **6** (63% over three steps); however, the (*E*)-selectivity was mediocre (*E*:*Z* = ~2:1) and the *E*/*Z* mixture was difficult to separate. To avoid this drawback, following the ozonolysis the aldehyde was treated with a Horner–Wadsworth–Emmons (HWE) reagent and (*E*)- $\alpha,\beta$ -unsaturated ester **5** was predominantly obtained in 89% yield over three steps. Following DIBAL reduction of the ester, the resulting alcohol was protected by a benzyl group. Pure *E*-isomer **6** was provided in high yields (94% over two steps).

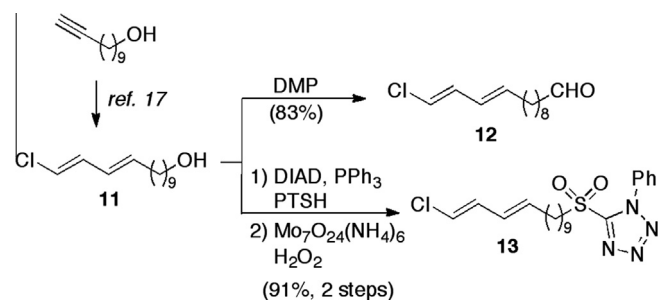
Sharpless asymmetric dihydroxylation of **6** using AD mix- $\alpha$  and subsequent acetonide protection of diol gave **8** (Scheme 3). The stereoconfiguration of **8** was confirmed by leading to the enantiomer of Crimmins' AM3 THP intermediate **9** which was spectroscopically identical to the literature values except the sign of the optical rotation [ $\alpha$ ]<sub>D</sub><sup>20</sup> –3.75° (c 0.40, CH<sub>2</sub>Cl<sub>2</sub>), lit.: [ $\alpha$ ]<sub>D</sub><sup>23.5</sup> +3.76° (c 3.3, CH<sub>2</sub>Cl<sub>2</sub>).<sup>12</sup> Reductive debenzoylation of **8** with lithium di-*tert*-butylbiphenylide (LiDBB)<sup>16</sup> afforded alcohol **10** in 95% yield.

The preparation of the side chain fragment (C51–C63) was readily accomplished (Scheme 4). The starting chlorodiene **11** was prepared from a commercially available undec-10-yn-1-ol based on two-step literature procedures (lit.: 50%).<sup>17</sup> Since the degree of *E*-selectivity in Julia olefination is often substrate dependent and unpredictable,<sup>8b,9b</sup> both aldehyde **12** and sulfone **13** were accordingly prepared. DMP oxidation of **11** gave aldehyde **12** in 83% yield. One-pot Mitsunobu reaction of **11** with 1-phenyl-1*H*-tetrazolyl sulfone (PTSH) followed by molybdate-promoted oxidation afforded sulfone **13** in 91% yield.

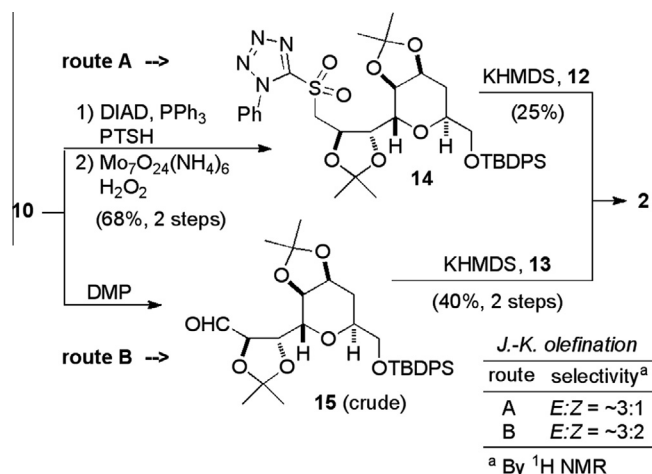
Likewise, THP alcohol **10** was also converted into sulfone **14** (68%) and aldehyde **15** (Scheme 5). Those fragments were finally assembled by the Julia–Kocienski reaction. Although both approaches (routes A and B) afforded desired olefination product



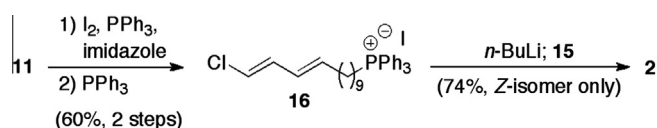
Scheme 3. Synthesis of **10**.



Scheme 4. Synthesis of chlorodiene fragments.



Scheme 5. Julia–Kocienski olefination.



Scheme 6. Wittig reaction.

(*E*)-**2** as a major isomer (up to *E*:*Z* = ~3:1), route B (40%, two steps) provided a higher yield of **2** than route A (17%, three steps). (*E*)-**2** and (*Z*)-**2** were only separable by HPLC.

As an alternative to construct the C50–C51 double bond, olefin metathesis and Wittig reactions were then examined. Unfortunately, the metathesis approach was unsuccessful and also, as expected, the standard Wittig olefination exclusively gave the undesired *Z*-isomer of **2** in 74% yield (Scheme 6). The Schlosser modification of the Wittig reaction often produces an *E*-isomer from an unstabilized ylide,<sup>18</sup> but, due to the technical complexity in small scale operations, our attempt failed.

In summary, a C(42–63) B-ring THP fragment (**2**) of KmTx2 was concisely prepared from a readily available common THP intermediate **3** in a 12-step reaction series (11% overall yield, 83% per step). The Julia–Kocienski olefination, particularly between an aldehyde **15** and a sulfone **13**, effectively afforded **2**. Further investigations toward the synthesis of karlotoxins are currently underway in our laboratories.

## Acknowledgments

We thank Dr. Amal Dass, Mr. Nuwan Kothalawala, Ms. Amanda Waters, and Dr. Naohito Abe for the analytical assistance. The

project described was supported by Grant Number 5P20R021929 from the National Center for Research Resources. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health. The University of Mississippi also supported this research.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.tetlet.2013.09.104>.

## References and notes

- Place, A. P.; Bowers, H. A.; Bachvaroff, T. R.; Adolf, J. E.; Deeds, J. R.; Sheng, J. *Harmful Algae* **2012**, *14*, 179, and references therein.
- Deeds, J. R.; Terlizzi, D. E.; Adolf, J. E.; Stoecker, D. K.; Place, A. R. *Harmful Algae* **2002**, *1*, 169.
- Adolf, J. E.; Krupatkina, D.; Bachvaroff, T.; Place, A. R. *Harmful Algae* **2007**, *6*, 400.
- (a) Simons, K.; Eehalt, R. J. *Clin. Invest.* **2002**, *110*, 597; (b) Palmer, C. P.; Mahen, R.; Schnell, E.; Djamgoz, M. B. A.; Aydar, E. *Cancer Res.* **2007**, *67*, 11166; (c) Mutoh, T. *J. Neurol. Transl. Neurosci.* **2013**, *1*, 2.
- Peng, J.; Place, A. R.; Yoshida, W.; Anklin, C.; Hamann, M. T. *J. Am. Chem. Soc.* **2010**, *132*, 3277.
- Murata, M.; Matsuoka, S.; Matsumori, N.; Paul, G. K.; Tachibana, K. *J. Am. Chem. Soc.* **1999**, *121*, 870.
- (a) Swasono, R. T.; Kanemoto, M.; Matsumori, N.; Oishi, T.; Murata, M. *Heterocycles* **2010**, *82*, 1359; (b) Manabe, Y.; Ebine, M.; Matsumori, N.; Murata, M.; Oishi, T. *J. Nat. Prod.* **2012**, *75*, 2003.
- (a) Paquette, L. A.; Chang, S.-K. *Org. Lett.* **2005**, *7*, 3111; (b) Chang, S.-K.; Paquette, L. A. *Synlett* **2005**, 2915; (c) Bedore, M. W.; Chang, S.-K.; Paquette, L. A. *Org. Lett.* **2007**, *9*, 513.
- (a) de Vicente, J.; Betzemeier, B.; Rychnovsky, S. D. *Org. Lett.* **2005**, *7*, 1853; (b) de Vicente, J.; Huckins, J. R.; Rychnovsky, S. D. *Angew. Chem., Int. Ed.* **2006**, *45*, 7258; (c) Huckins, J. R.; de Vicente, J.; Rychnovsky, S. D. *Org. Lett.* **2007**, *9*, 4757.
- (a) Flamme, E. M.; Roush, W. R. *Org. Lett.* **2005**, *7*, 1411; (b) Hicks, J. D.; Flamme, E. M.; Roush, W. R. *Org. Lett.* **2005**, *7*, 5509; (c) Hicks, J. D.; Roush, W. R. *Org. Lett.* **2008**, *10*, 681.
- (a) Dubost, C.; Marko, I. E.; Bryans, J. *Tetrahedron Lett.* **2005**, *46*, 4005; (b) Cossy, J.; Tsuchiya, T.; Ferrie, L.; Reymond, S.; Kreuzer, T.; Colobert, F.; Jourdain, P.; Marko, I. E. *Synlett* **2007**, 2286; (c) Colobert, F.; Kreuzer, T.; Cossy, J.; Reymond, S.; Tsuchiya, T.; Ferrie, L.; Marko, I. E.; Jourdain, P. *Synlett* **2007**, 2351; (d) Cossy, J.; Tsuchiya, T.; Reymond, S.; Kreuzer, T.; Colobert, F.; Marko, I. E. *Synlett* **2009**, 2706; (e) Rival, N.; Hazelard, D.; Hanquet, G.; Kreuzer, T.; Bensoussan, C.; Reymond, S.; Cossy, J.; Colobert, F. *Org. Biomol. Chem.* **2012**, *10*, 9418; (f) Bensoussan, C.; Rival, N.; Hanquet, G.; Colobert, F.; Reymond, S.; Cossy, J. *Tetrahedron* **2013**, *69*, 7759.
- Crimmins, M. T.; Martin, T. J.; Martinot, T. A. *Org. Lett.* **2010**, *12*, 3890.
- (a) Oishi, T.; Kanemoto, M.; Swasono, R.; Matsumori, N.; Murata, M. *Org. Lett.* **2008**, *10*, 5203; (b) Kanemoto, M.; Murata, M.; Oishi, T. *J. Org. Chem.* **2009**, *74*, 8810; (c) Ebine, M.; Kanemoto, M.; Manabe, Y.; Konno, Y.; Sakai, K.; Matsumori, N.; Murata, M.; Oishi, T. *Org. Lett.* **2013**, *15*, 2846.
- (a) Nicolaou, K. C.; Hwang, C.-K.; Duggan, M. E. *J. Am. Chem. Soc.* **1989**, *111*, 6682; (b) Arya, P.; Barkley, A.; Randell, K. D. *J. Comb. Chem.* **2002**, *4*, 193; (c) Timmons, S. C.; Jakeman, D. L. *Org. Lett.* **2007**, *9*, 1227.
- Barton, D. H. R.; McCombie, S. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1574.
- Freeman, P. K.; Hutchinson, L. L. *J. Org. Chem.* **1980**, *45*, 1924.
- Chen, X.; Millar, J. G. *Synthesis* **2000**, 113.
- (a) Schlosser, M.; Christmann, K. F. *Angew. Chem., Int. Ed. Engl.* **1966**, *5*, 126; (b) Wang, Q.; Deredas, D.; Huynh, C.; Schlosser, M. *Chem. Eur. J.* **2003**, *9*, 570.