

Published in final edited form as:

Org Lett. 2012 December 21; 14(24): 6242–6245. doi:10.1021/ol302999v.

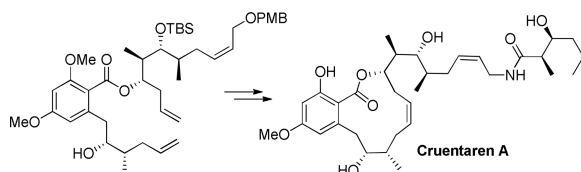
Synthesis of Cruentaren A

 Bhaskar Reddy Kusuma[†], Gary E. L. Brandt[†], and Brian S. J. Blagg

Department of Medicinal Chemistry, The University of Kansas, 1251 Wescoe Hall Dr, Malott 4070, Lawrence, Kansas 66045-7562

Brian S. J. Blagg: bblagg@ku.edu

Abstract



Cruentaren A, an antifungal benzolactone produced by the myxobacterium *Byssovorax cruenta*, is highly cytotoxic against various human cancer cell lines and a highly selective inhibitor of mitochondrial F-ATPase. A convergent and efficient synthesis of cruentaren A is reported, based upon a diastereoselective alkylation, a series of stereoselective aldol reactions utilizing Myers' pseudoephedrine propionamide, an acyl bromide-mediated esterification and a ring-closing metathesis (RCM) as the key steps. The RCM reaction was applied for the first time towards the total synthesis of cruentaren A, which led to a convergent and efficient synthesis of the natural product.

Cruentaren A and its isomeric analogue, cruentaren B, have been identified as the first novel structures isolated from the fermentation broths of *Byssovorax cruenta* by Hofle *et al.*^{1,2} Cruentaren A (**1**) was the major natural product, which was isolated through bioassay guided fractionation that focused upon the identification of novel antifungal and cytotoxic agents. While cruentaren A demonstrated potent cytotoxic effects (IC₅₀ of 8.3 nM against L929 mouse fibroblasts), its closely related derivative, cruentaren B, was only marginally active.³

Cruentaren A is a member of the growing class of benzolactone natural products that contains a resorcinol derived 12-membered macrocyclic lactone and an *N*-acylallylamine side chain (Figure 1).⁴ Despite structural similarities to other natural products of this class, cruentaren A possesses a unique mechanism of action. This natural product induces cytotoxicity through selective inhibition of F-ATPase, but is devoid of inhibitory activity against other ATPases.^{1–3}

Due to the interesting architecture and a unique biological profile, cruentaren A has attracted significant attention from the synthetic community. Four successful total syntheses and numerous approaches to fragments have been reported.^{5,6,7,8} In fact, all published syntheses rely upon the ring-closing alkyne metathesis (RCAM) reaction as a key step, followed by Lindlar reduction to introduce the *cis*-olefin that is present within the macrocyclic ring. Due

Correspondence to: Brian S. J. Blagg, bblagg@ku.edu.

[†]Both authors contributed equally to this work.

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to the publication of prior existing routes, we pursued an alternate method that does not rely upon the RCAM in an effort to ultimately produce analogs that are not easily obtained through other routes. Herein, we report the total synthesis of cruentaren A that utilizes the RCM reaction for construction of the desired *cis*-macrocyclic product.

Retrosynthetic analysis of cruentaren A (**1**), utilizing the RCM reaction for construction of the 12-membered lactone, required fragments **2** and **3** as relevant synthons. Weinreb amide **5** could then be reacted with the benzylic anion of **6** to provide ketone intermediate **4**, which would enable subsequent metathesis to form **2**. Likewise, esterification of secondary alcohol **7** would provide substrate **6** and after RCM, the macrocycle. Staudinger ligation between the allylazide of **2** and acid **3** was envisioned to occur before global deprotection to provide the natural product. Successful stereochemical induction with Myers' pseudoephedrine chiral auxiliary would be required throughout this synthetic strategy. Specifically, the synthesis of allyl alcohol **7** was envisioned to depend upon Myers' pseudoephedrine to construct all three stereocenters through sequential reactions (Figure 2). Myers' pseudoephedrines are readily available from inexpensive, commercially available reagents *via* a simple one step process and furthermore, these chiral auxiliaries can be elaborated into a variety of useful entities through well-described synthetic protocols.⁹

In the event, alkylation of (*S,S*)-pseudoephedrine propionamide **9** with *cis*-allyl bromide **8**¹⁰ proceeded smoothly under Myers' optimized conditions¹¹ to provide the diastereomerically enriched methyl amide **10** (>20:1), which upon subsequent lithium aluminium hydride reduction gave the corresponding aldehyde, **11**. Induction of stereochemistry at C-16 and C-17 was accomplished through use of a zirconium-mediated aldol reaction⁹ between the (*Z*)-enolate of propionamide **12** and aldehyde **11** to give diastereomerically pure **13** (Scheme 1).

Carboxylic acid **14** was accessed by mild hydrolysis of **13** followed by conversion to the Weinreb amide **15** using the (1-Cyano-2-ethoxy-2-oxoethylideneaminoxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) coupling reagent. COMU is a 3rd generation uronium-type coupling reagent that is commonly used for solution-phase peptide synthesis; the by-products produced with COMU are water soluble and easily removed. Furthermore, COMU exhibits a less hazardous safety profile than benzotriazole-based reagents and prevents racemization during the coupling event.¹² The secondary alcohol of **15** was protected as the *tert*-butyldimethylsilyl ether before subsequent reduction of the amide to give aldehyde **17**. Upon treatment of **17** with *in situ* generated Soderquist's allylborane intermediate that was generated upon exposure of allyl Grignard to **18**,¹³ a highly enantio-enriched homoallylic alcohol **7** was produced with excellent facial selectivity.

Stereochemical assignment of advanced intermediate **7** was accomplished by Mosher's ester analysis,¹⁴ which validated the desired *syn* stereochemistry of the 1,3-diol relationship for **7**. To determine the relative stereochemical relationship of the C-16 methyl group, acid-mediated TBS hydrolysis and subsequent acetonide protection of the resultant 1,3-diol enabled formation of **19**, which was shown to exhibit the correct stereochemical relationship as determined by Rynchnovsky's method (Scheme 2).¹⁵

The synthetic route to provide Weinreb amide fragment **5** was straightforward and provided a rapid means to generate significant quantities of this intermediate for the optimization of latter steps. Alkylation of propionamide **12** with allyl bromide under Myers' conditions generated α -methyl amide, **20** (dr >20:1). Hydrolysis and subsequent COMU-mediated coupling produced Weinreb amide **5** without loss of enantioenrichment on multi gram scale. A similar zirconium-mediated aldol reaction between propionamide **9** and butanal as

described above to provide amide **22** followed by hydrolysis, gave carboxylic acid **3** without diastereomeric loss. The successful COMU-mediated coupling reaction¹⁶ suggested that the hydroxyl group of compound **3** did not require protection. Therefore, the free alcohol of **3** was left unprotected (Scheme 3).

Construction of the macrocyclic ring involved esterification of allylic alcohol **7** with commercially available benzoic acid **22**. Synthesis of ester **6** proved to be challenging and unsuccessful results were obtained following the methods reported by Furstner and Vintonyak for the related alkynyl derivative.^{4,5} After a significant investment of time and effort, synthesis of ester **6** was finally achieved upon treatment of acid **2** with oxalyl bromide (COBr)₂, DIPEA, and catalytic DMF in DCM at 0 °C for 30 min, followed by the addition of alcohol **3** and 4-(dimethylamino)pyridine (DMAP). Complete conversion to desired ester **6** occurred within 10 min in high yield. Alkylation of **6** with Weinreb amide **5** proceeded by generation of the benzylic anion of **6** to produce advanced intermediate **4** in reasonable yield (Scheme 4).

Prior to RCM of the terminal olefins, stereoselective reduction of ketone **4** was considered. Attempts to affect stereoselective reduction of ketone **4** under various conditions enlisting the CBS reagent were unsuccessful as **4** was highly resistant to reduction under these constraints. However, reduction of ketone **4** utilizing Noyori's asymmetric transfer hydrogenation conditions proved successful.^{17,18} In the event, ketone **4** was treated with sodium formate and catalytic RuCl[(S,S)-Tsdpen](p-cymene) in DMF and water to generate alcohol **23** in quantitative yield and reasonable diastereomeric selectivity (~5:1). Unfortunately, the epimeric product was not separable via column chromatography at this stage (Scheme 4).

Consequently, a mixture of diastereomeric alcohols (**23**) was subjected to RCM catalysis, and gratifyingly, the *cis*-olefin containing macrocycle was obtained in diastereomerically pure form, and the alcohol epimers were readily separable *via* column chromatography. Unfortunately, an unexpected side reaction did occur, in which *cis* to *trans* isomerization of the allylic ether side chain was observed. Several solvents, including THF, benzene, and methanol were screened, however, the most minimal isomerization occurred in DCM. The optimized reaction conditions included the addition of 5 mol% of catalyst at 0 °C to a 0.5 mM solution of alcohol **23** in DCM, followed by warming to 20 °C. RCM was complete after 3.5 h, but required treatment with saturated aqueous potassium carbonate (K₂CO₃) for catalyst deactivation in order to prevent additional isomerization during work-up. These reaction conditions furnished the epimerically pure *cis*-macrocyclic product, **24**, in 79% yield as a 4:1 (*cis:trans*) mixture of the olefinic side chain isomers. Compound **24** was then sequentially TBS-protected at C-8 to generate **25**, followed by PMB-removal to give allyl alcohol **26**.

Conversion of **26** to cruentaren A, and completion of the total synthesis involved four additional manipulations (Scheme 5). The allylic alcohol of **26** was converted to the corresponding allyl-azide (**2**) upon treatment with Zn(N₃)₂(pyridine)₂,¹⁹ diisopropyl azodicarboxylate and triphenylphosphine. One-pot azide reduction and COMU-mediated amide formation was achieved *via* Staudinger ligation conditions to provide the protected variant of cruentaren A (**27**) as a single diastereomer. Cleavage of the C-3 methyl ether was achieved by subjecting compound **27** to boron trichloride in DCM at low temperature, followed by removal of the corresponding bis(TBS-ether) with HF-pyridine in acetonitrile to furnish cruentaren A (**1**). The spectroscopic data for synthetic cruentaren A (**1**) were in agreement with data reported for the natural product.¹

In summary, a convergent and efficient total synthesis of cruentaren A has been described with a longest linear sequence of 18 steps. The synthesis is dependent upon the use of Myers' diastereoselective alkylation, a series of stereoselective aldol reactions utilizing pseudoephedrine propionamide, a Soderquist allylation, an acyl bromide-mediated esterification, and RCM as the key steps. Additional studies for cruentaren A and analogues are currently under investigation with the goal of more thoroughly understanding the role F-ATPase plays in the maturation of nascent polypeptides.^{20,21,22} The results from such studies will be disclosed in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors gratefully acknowledge the support of this project by NIH (CA109265). G.E.L.B. acknowledges the Madison and Lila Self Graduate Fellowship and the American Foundation for Pharmaceutical Education for financial support.

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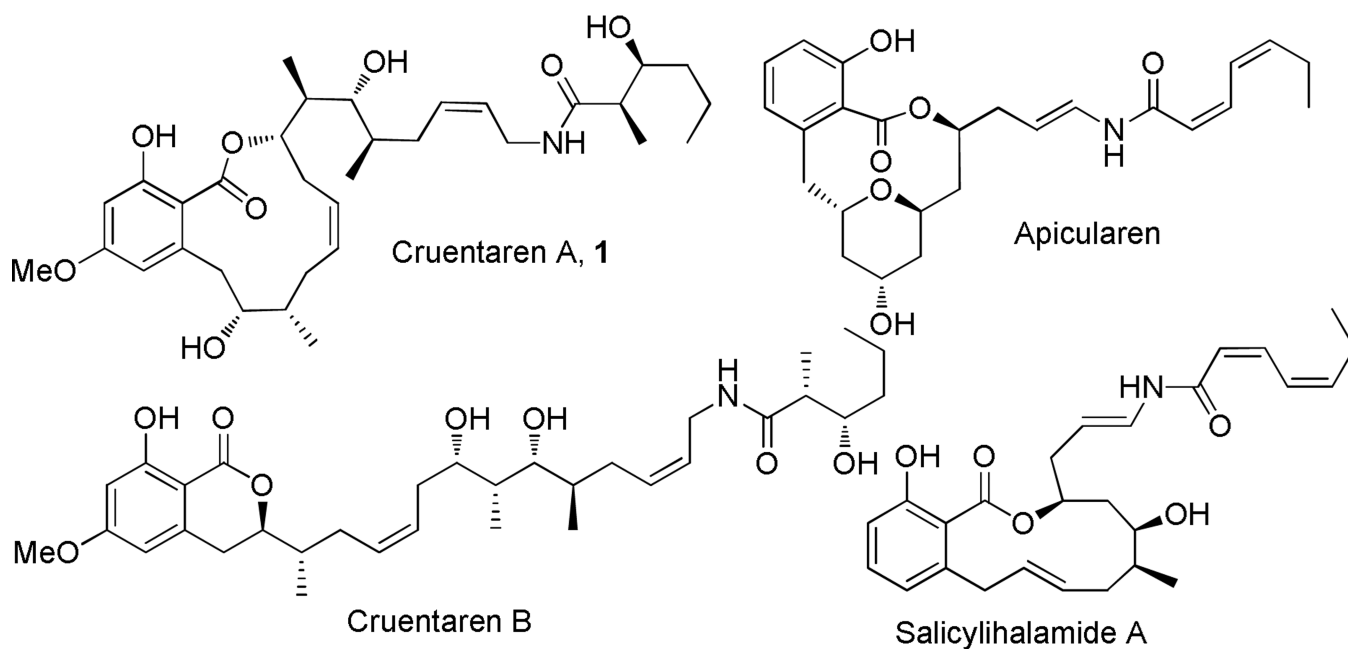


Figure 1.
Benzolactone natural product family.

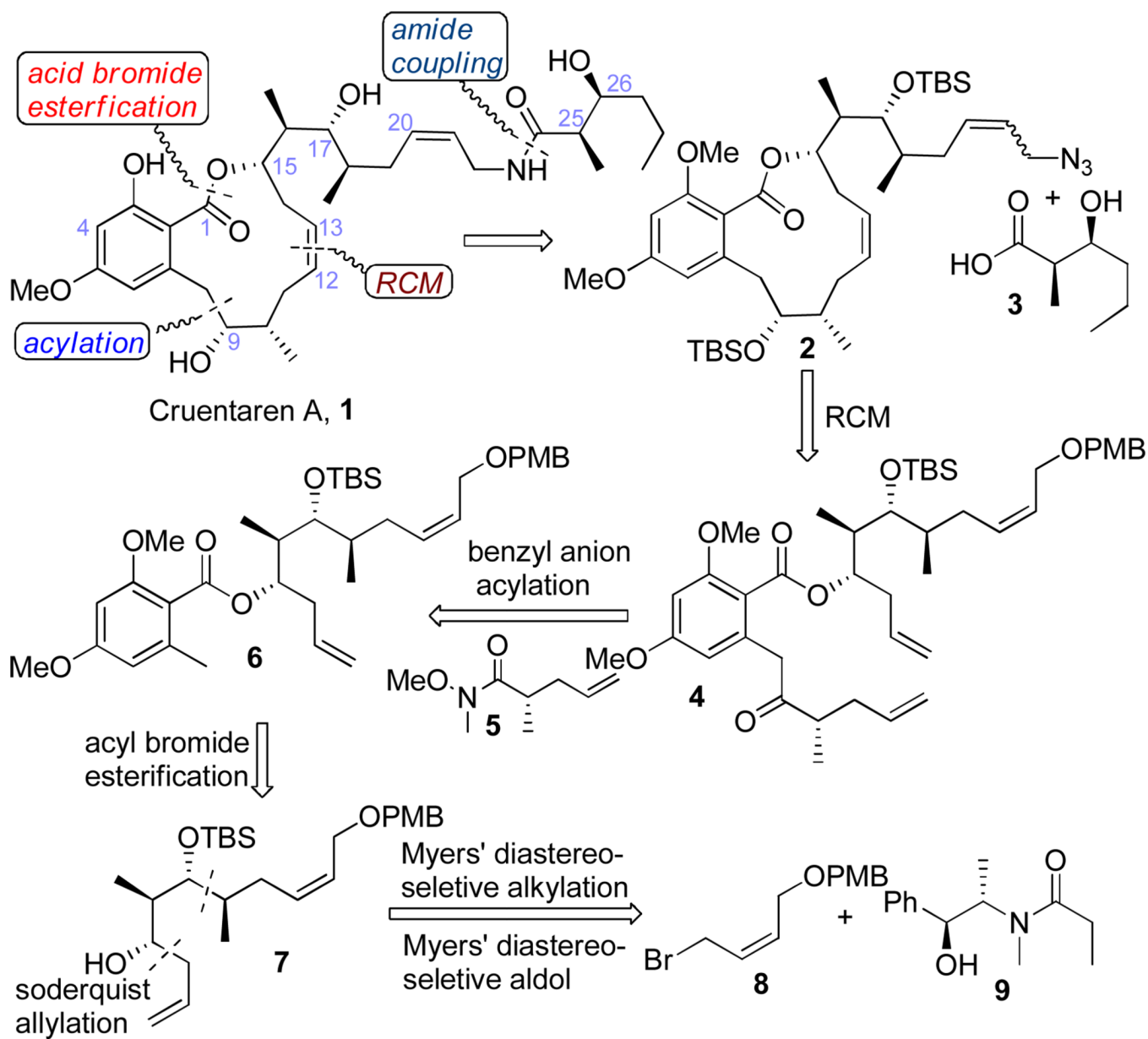
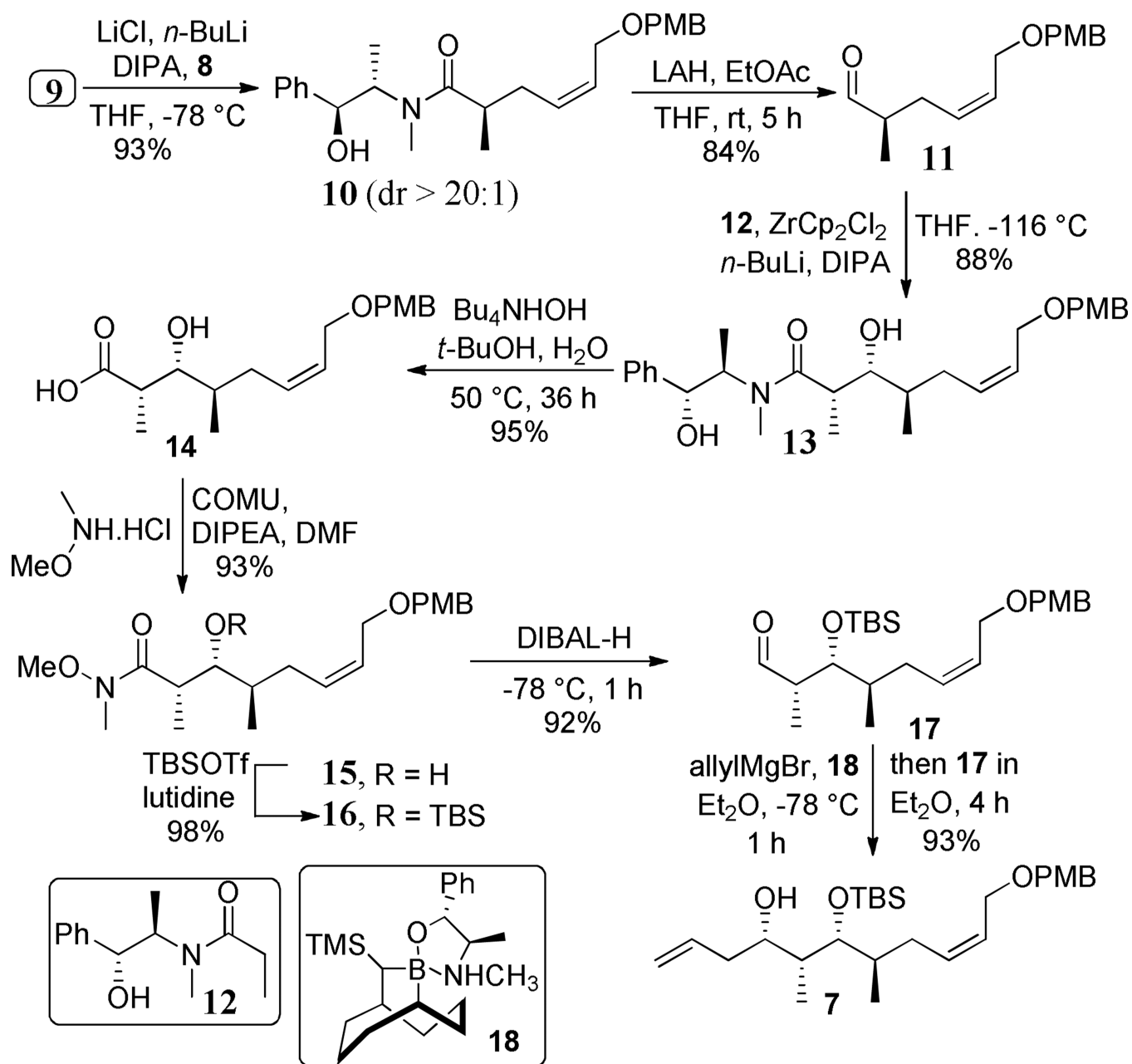
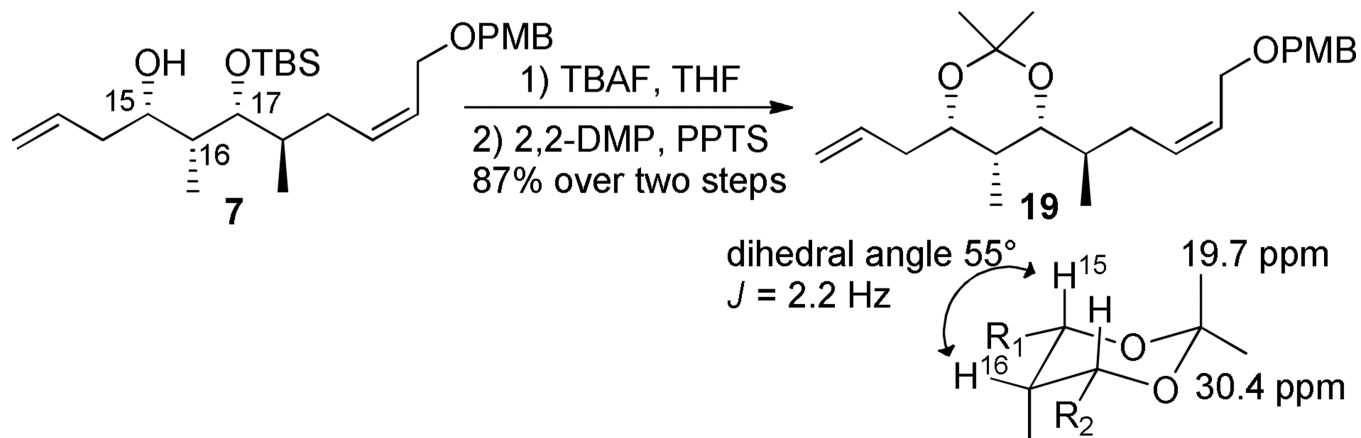


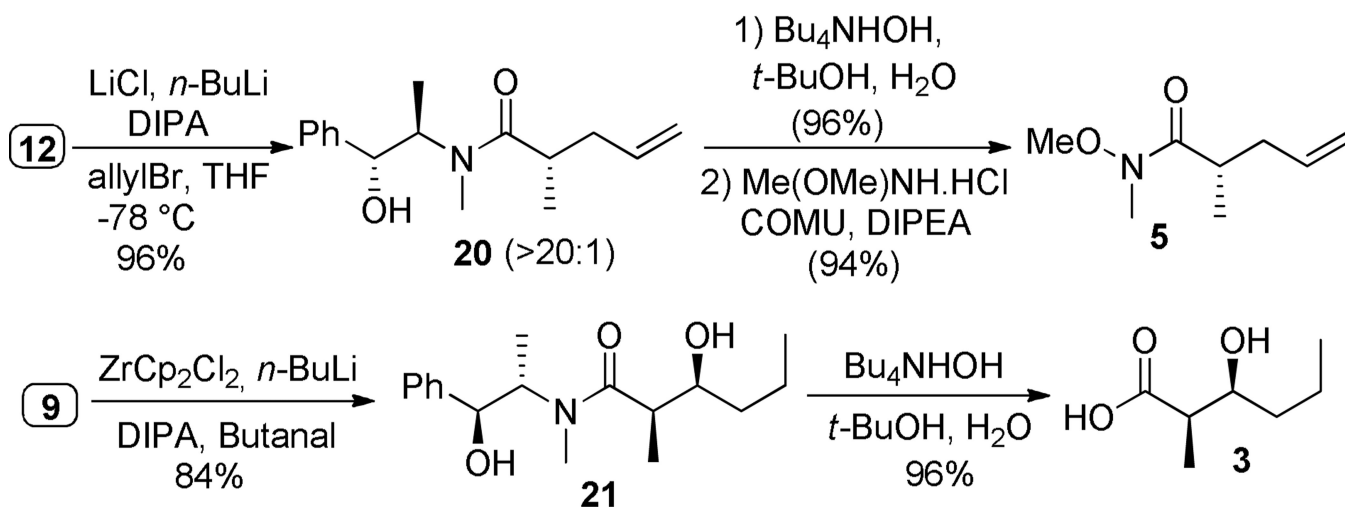
Figure 2.
Retrosynthetic analysis of cruentaren A (1).



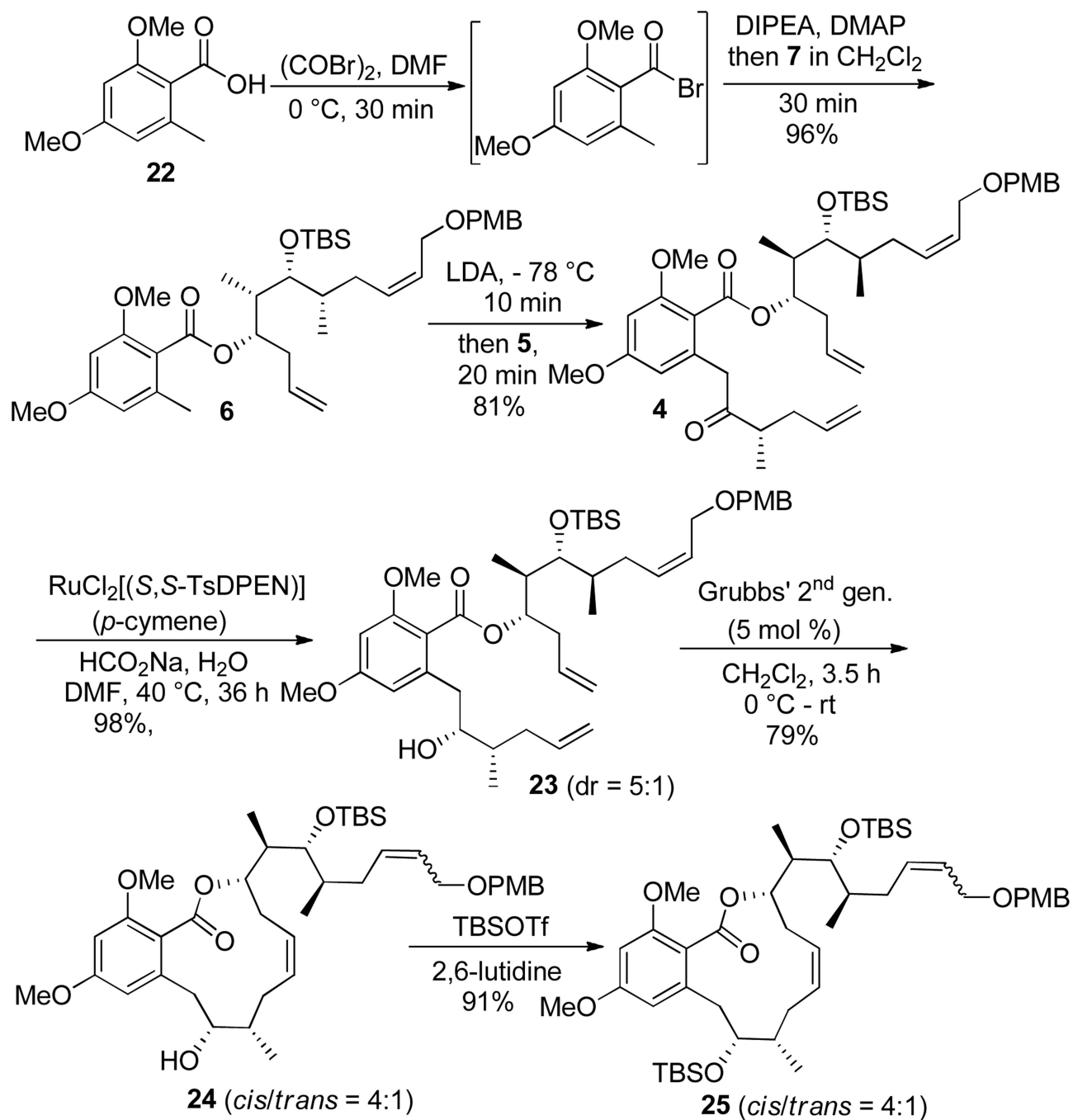
Scheme 1.
 Synthesis of allylic alcohol **7**.



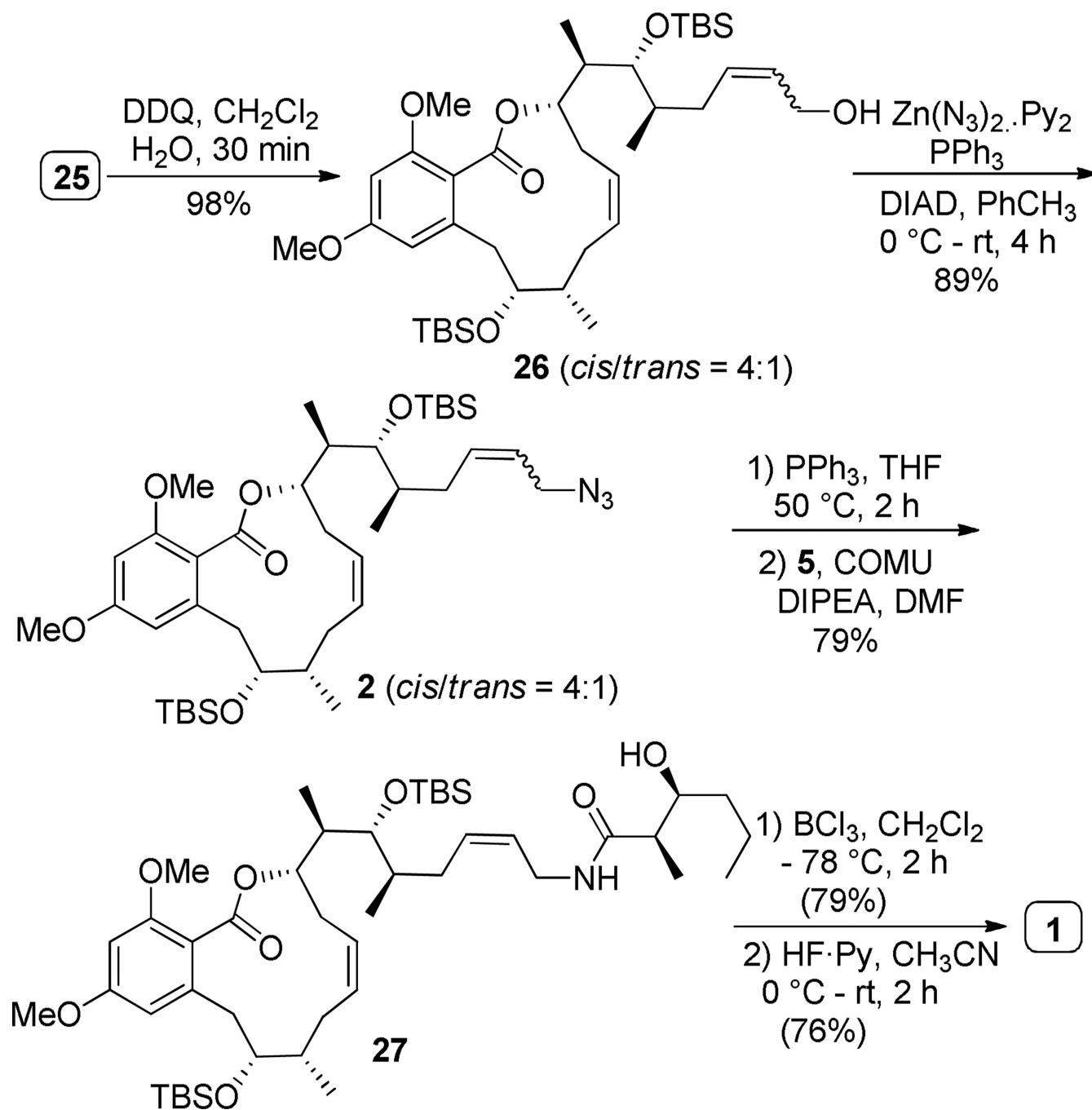
Scheme 2.
Stereochemistry confirmation at C-16 carbon.



Scheme 3.
Synthesis of the Weinreb amide **5** and side chain acid **3**.



Scheme 4.
Synthesis of key macrolactone **25**.



Scheme 5.
Conversion of **25** to cruentaren A (**1**).