Marine Natural Products

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Total Synthesis of the Marine Diterpenoid Blumiolide C

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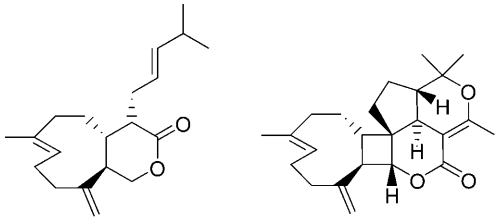
Natural products of marine origin have become increasingly important lead structures for drug discovery. Several such compounds or analogues derived from marine natural product leads have been advanced to clinical trials in humans.[1] However, the structural diversity represented by natural products from marine sources, whose molecular architectures are often distinct from those obtained from terrestrial organisms, have only been exploited for drug discovery purposes to a limited degree.[1] In this context one of the major limitations is the often insufficient availability of material from the natural source, and in many cases the development of an efficient total synthesis of a biologically active marine natural product represents an important prerequisite for the comprehensive evaluation of its medical potential.

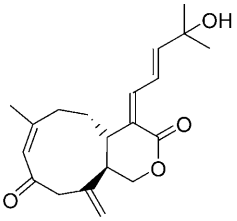
Diterpenoids derived from soft corals of the genus *Xenia* exhibit a wide range of biological activities, including antiproliferative,[2] antiangiogenic,[3] or antibacterial[4] effects. The common structural denominator of these compounds is a nine-membered carbocyclic ring, which is generally fused to a dihydropyran, a δ-lactone, or a cyclobutane moiety, thus leading to three major classes of *Xenia* diterpenoids which have been termed the xenicins (or xenicans), xeniolides, and xeniaphyllans, respectively.[5] Despite the interesting biological activity of several *Xenia* diterpenoids, synthetic efforts in this area have been sparse and the potential of *Xenia* diterpenoids to serve as lead structures for drug discovery has remained largely unexplored. To the best of our knowledge total syntheses have only been reported for two different members of the group, namely coraxeniolide A[6] and antheliolide[7]. (Also see reference [8]).

To gain a better understanding of the biological potential of *Xenia* diterpenoids and to establish a basis for future structure-activity relationship (SAR) studies, we embarked on the total synthesis of a number of these structurally unique natural products. Herein we report on the first total synthesis



Coraxeniolide A

Antheliolide



Blumiolide C (1)

of the *Xenia* diterpenoid blumiolide C (**1**), which was isolated in 2005 by El-Gamal et al. from the soft coral *Xenia blumi* and reported to exhibit potent in vitro antiproliferative activity (ED_{50} values of 1.5 μM and 0.6 μM against the human colon cancer cell line HT-29 and the mouse P-388 leukemia line, respectively).[9] Structurally, blumiolide C (**1**) is distinct from the majority of *Xenia* diterpenoids because of the presence of a *Z*, rather than the commonly found *E*, double bond as part of the nine-membered ring.

As illustrated in Scheme 1 our retrosynthesis of **1** initially led to the disconnection into the synthon **I-5** and an unsaturated C4-side chain aldehyde that would be connected in an aldol reaction/elimination sequence (although the stereochemical outcome of this process was unclear at this point). The exocyclic double bond in **I-5** was to be installed by olefination of the corresponding C11-keto lactone. The latter would be obtained from diene **I-4** through ring-closing olefin metathesis (RCM),[10] which represents the key strategic step in our approach to blumiolide C (**1**). Whereas this RCM-based strategy was highly appealing at the conceptual level, the successful implementation of this approach was much less certain as no literature precedent existed prior to our own work, for the direct construction of *trans*-fused bicyclo[7.4.0] systems by an RCM-mediated closure of a nine-membered ring.[11,12] The stereogenic center at C4a was envisioned to be created by substrate controlled 1,4-cuprate addition onto α,β-unsaturated δ-lactone **I-3**, which would be derived from diene **I-2** by a second RCM reaction. Lastly, the stereocenter at C11a was to be set through a diastereoselective aldol reaction, thus leading to O-protected 3-hydroxy-propanal **I-1a** and a suitable chiral crotonyl derivative **I-1b** as the ultimate precursors.

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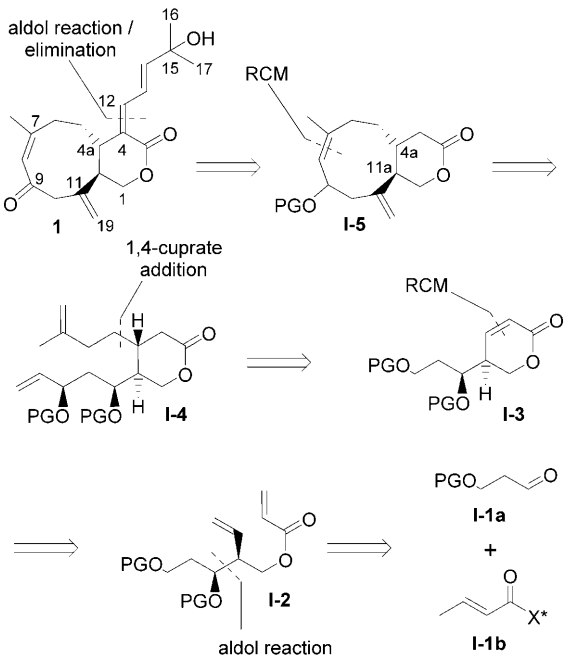
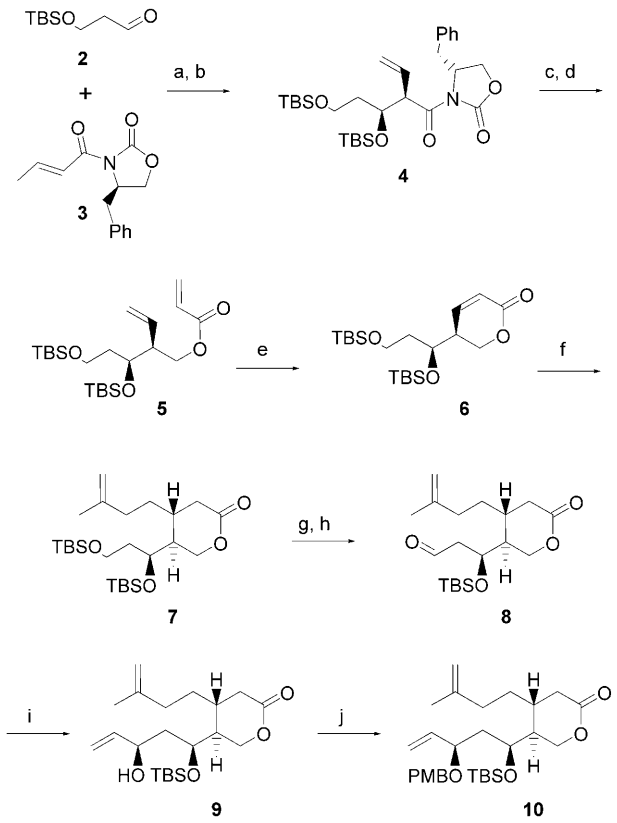


Scheme 1. Retrosynthesis of blumiolide C (**1**). PG = protecting group or H. Protecting groups may vary independently.



Scheme 2. a) Bu_2BOTf, Et_3N, CH_2Cl_2, −78 °C→RT, 1.5 h, 95%; b) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 1 h, 93%; c) $LiBH_4$, MeOH (1 equiv), Et_2O, 0 °C→RT, 1.5 h, 77%; d) CH_2=CHC(O)Cl, Et_3N, DMAP, CH_2Cl_2, 0 °C, 1 h, 75%; e) Grubbs II, CH_2Cl_2, reflux overnight, 92%; f) CH_2=C(CH_3)CH_2CH_2MgI, CuI, Et_2O, −78 °C, 90%; g) CSA, MeOH/CH_2Cl_2 (1:1), 0 °C, 30 min, 90%; h) DMSO, $(COCl)_2$, Et_3N, −78 °C, 93%; i) CH_2=CHMgCl, $ZnCl_2$, THF, −30 °C, 18 h, 62%, d.r. 5:1; j) PMBOC(=NH)CCl_3, cyclohexane, PPTS (3 mol%), 0 °C→RT, 20 h, 68%. Tf = trifluoromethanesufonyl, DMAP = 4-dimethylaminopyridine, CSA = (+)-camphorsulfonic acid, DMSO = dimethysulfoxide, PMB = *para*-methoxybenzyl, PPTS = pyridinium *p*-toluenesulfonate.

The departure point for the implementation of this strategy was the diastereoselective aldol reaction of TBSO-substituted propanal **2** with *E*-crotonyl-oxazolidinone **3**, which gave the desired aldol product in excellent yield (95%)[13] (Scheme 2). After protection of the secondary hydroxy group as a TBS ether (to give **4**) the chiral auxiliary was reductively cleaved with $LiBH_4$, and the newly formed primary alcohol was acylated with acryloyl chloride. Treatment of the resulting ester **5** with the second generation Grubbs catalyst in refluxing CH_2Cl_2 overnight[14] furnished α,β-unsaturated lactone **6** as the substrate for the stereoselective attachment of the C5–C7 fragment. To our delight, the substrate-controlled 1,4-addition of the mixed cuprate, derived from CH_2=C(CH_3)CH_2CH_2MgI and CuI, to lactone **6** in ether at −78 °C[15] proceeded with excellent diastereoselectivity to provide *trans*-product **7** in 90% yield as the only isolable isomer. Subsequent deprotection of the primary hydroxy group with CSA in a mixture of MeOH and CH_2Cl_2 (1:1) followed by Swern oxidation of the resulting free alcohol gave aldehyde **8** (84% based on **7**). Unexpectedly, the attempted addition of vinyl Grignard reagents to **8** was low yielding and accompanied by the formation of side products; however, this problem was overcome by the use of in situ prepared divinyl zinc,[16] which provided the desired allylic alcohol **9** in satisfactory yield as a 5:1 mixture of isomers (which was inconsequential, as C9 in the natural product, which corresponds with the newly formed chiral center in **9**, is present in the keto oxidation state). Reaction of **9** with *p*-methoxybenzyl trichloroacetimidate gave the bisprotected diol **10**.

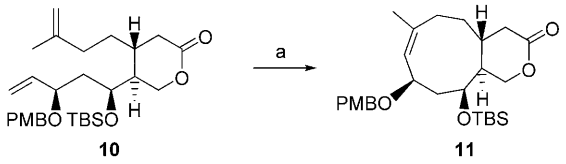
With dienes **9** and **10** in hand the stage was now set for the investigation of the crucial ring closure reaction to establish the oxabicyclo[7.4.0]tridecene framework of blumiolide C (**1**). Given the lack of precedence for the RCM-based formation of nine-membered rings in bicyclic[7.4.0] systems, a number of preliminary experiments were conducted, in order to establish a basis for the final optimization cycle. These experiments clearly indicated a need for the protection of the hydroxy group at C9 (blumiolide C numbering) for the RCM step, as none of the desired cyclic products were obtained from **9** under a variety of reaction conditions. The most promising results were obtained with **10** in combination with 50 mol% of the second generation Hoveyda–Grubbs catalyst.[17] The final set of optimization experiments with this catalyst loading are summarized in Table 1. The most favorable results were obtained upon prolonged exposure of diene **10** to the catalyst at elevated temperature in toluene, which furnished the desired oxabicyclo[7.4.0]tridecene derivative **11** in 66% yield as a single isomer (Scheme 3, Table 1).

Having established efficient access to the oxabicyclic framework of **1**, the next challenge consisted in the introduction of the exocyclic methylene moiety at C11. Initial attempts

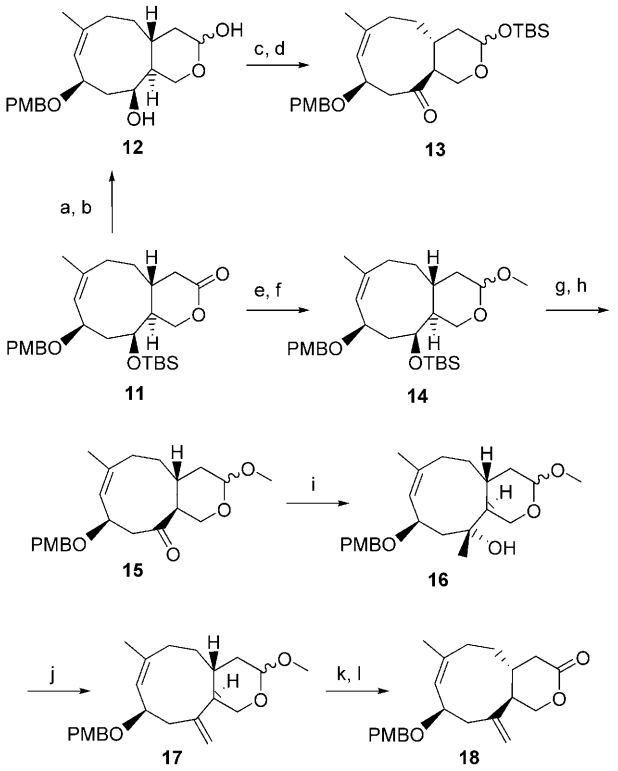
Table 1: Optimization of reaction conditions for the RCM-based cyclization of diene **10** (Scheme 3).

T [°C]	*t*	Solvent	Additive	Yield [%][a]
60	2 d	toluene	BQ[b]	0[d]
90	3 d	toluene	BQ[b]	66
90	3 d	toluene	–	66
160[c]	1.5 h	toluene	–	60
190[c]	1 h	*o*-$Cl_2C_6H_4$	–	28[d]
100[c]	30 min	*o*-$Cl_2C_6H_4$	–	0[d]

[a] Yields of isolated products. [b] BQ = *p*-benzoquinone. [c] Microwave irradiation. [d] Varying amounts of starting material were isolated.



Scheme 3. a) [**10**] = 1.5 mM, Hoveyda–Grubbs II catalyst, 50 mol%. For other conditions see Table 1.



Scheme 4. a) TBAF, THF, 0°C→RT, 85%; b) DIBAL-H, THF, −78°C, 96%; c) TBSOTf, 2,6-lutidine, −78°C, 85%; d) DMP, RT, 88%; e) DIBAL-H, CH_2Cl_2, −78°C; f) MeOH, PPTS (5 mol%), RT, 1 h, 73% (two steps, d.r. 1:1); g) TBAF (2 equiv), THF, RT, overnight, 90%; h) TPAP, NMO, 4 Å M.S., CH_2Cl_2, 0°C, 1 h, 80%; i) MeMgBr, Et_2O, −30°C, 30 min, 75%; j) Martin's sulphurane, CH_2Cl_2, 0°C→RT, 30 min, 86%; k) 48% aq HF, MeCN/THF (5:1), RT, 2.5 h; l) TPAP, NMO, CH_2Cl_2, 4 Å M.S., RT, 30 min, 73% (two steps). TBAF = tetrabutylammonium fluoride, DIBAL-H = diisobutylaluminum hydride, DMP = Dess–Martin periodinane, TPAP = tetrapropylammonium peruthenate, NMO = 4-methylmorpholin-*N*-oxide, M.S. = molecular sieves.

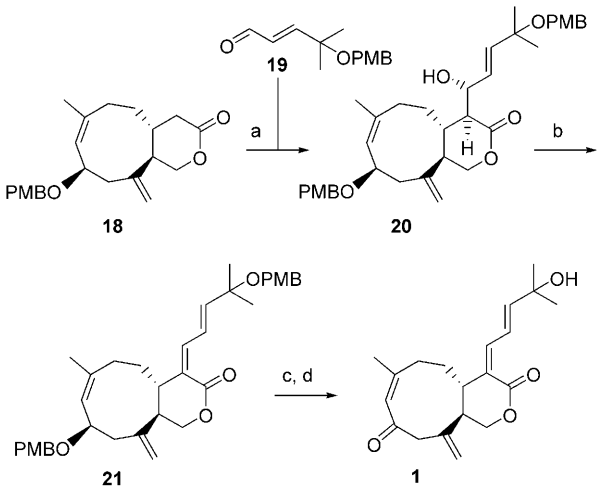
at the installation of this C11–C19 double bond involved the direct methylenation of the ketone derived from **11** by removal of the TBS ether and subsequent oxidation of the resulting secondary hydroxy group. Unfortunately, the conversion of the C11-keto group into the corresponding exocyclic methylene moiety proved to be exceedingly difficult and none of the different olefination methods investigated (Wittig, Tebbe,[18] Nysted,[19] or the Huang[20] reagents) delivered any of the desired olefin. Similar difficulties were encountered by Leumann and co-workers in their synthesis of coraxeniolide A;[6a] however, in the coraxeniolide A system Tebbe methylenation of the C11-keto group in the nine-membered ring was possible if the six-membered ring included a methyl or silyl acetal rather than the natural ester moiety. In contrast, TBS acetal **13** (Scheme 4) failed to undergo methylenation either under Tebbe or Petasis[21] conditions.

Gratifyingly, however, the investigation of indirect methylenation methods revealed that **13** could be converted into the desired olefin through an addition/elimination sequence, and in subsequent experiments methyl acetal **15** (Scheme 4) was found to be an even more favorable substrate in this process. Thus, addition of MeMgBr to **15** proceeded cleanly and diastereoselectively to provide tertiary alcohol **16** in 75% yield.[22] Regioselective formation of the exocyclic double bond was then achieved by treatment of **16** with Martin's sulphurane,[23] which produced the desired alkene **17** in excellent yield (86%). Subsequent restoration of the lactone functionality proceeded smoothly and gave the key bicyclic intermediate **18** (corresponding to **I-5** in Scheme 1) in 73% yield for the two-step sequence from **17** (i.e. acetal hydrolysis and oxidation of the resulting lactol).

After the successful construction of its bicyclic core structure, including the incorporation of the exocyclic methylene moiety at C11, the final challenge in the synthesis of blumiolide C (**1**) was the stereoselective introduction of the α,β/δ,γ-unsaturated side chain at C4. (It should be noted that no precedence for the stereoselective introduction of a C4–C12 double bond in xeniolides exists in the literature, as the coraxeniolide A side chain is connected to the bicyclic core by a C–C single bond).

As illustrated in Scheme 5, the attachment of the C4-side chain to intermediate **18** involved an aldol reaction with aldehyde **19**,[24] which proceeded in a highly diastereoselective manner, albeit in somewhat moderate yield (50%).[25] After extensive optimization of the reaction, the stereospecific *syn* dehydration of aldol adduct **20** was achieved in excellent yield using DCC in the presence of $CuCl_2$.[26] Subsequent deprotection of the resulting diene **21** with DDQ and followed by DMP oxidation of the secondary hydroxy group delivered blumiolide C (**1**) in 58% yield (from **21**).

The spectroscopic properties of synthetic (+)-**1** were in good agreement with the literature data for natural (+)-blumiolide C (^1H and ^{13}C NMR, HRMS, IR, and optical rotation).[27] Ultimate structural proof was established by a single crystal X-ray crystallographic analysis of **1**, which confirmed both the *Z*, *E* geometry of the unsaturated side



Scheme 5. a) LDA (3 equiv), THF, −78 °C, then **19**, 2 h, 50%; b) neat DCC, $CuCl_2$, RT, overnight, 90%; c) DDQ, CH_2Cl_2/phosphate buffer (20:1), RT, 68%; d) DMP, CH_2Cl_2, RT, 1 h, 85%. LDA = lithiumdiisopropylamide, DCC = *N*,*N*′-dicyclohexylcarbodiimide, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

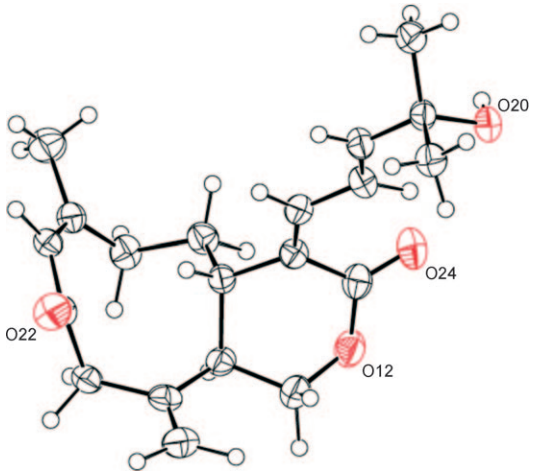


Figure 1. X-ray crystal structure of synthetic blumiolide C (**1**). Ellipsoids include 50% of the electron density.

chain, as well as the *trans* fusion of the six- and nine-membered rings (Figure 1).[28]

On the basis of a preliminary assessment of its in vitro antiproliferative activity in HCT-116 and A549 human cancer cells, (+)-**1** inhibits human cancer cell growth with μM activity (HCT-116 cells: $IC_{50} = 13.8$ μM; A549 cells: 33% growth inhibition at 10 μM), although the compound appears to be somewhat less active than what has been reported in the literature.[9] It remains to be seen, whether these differences may be caused (at least partly) by differences in the experimental conditions employed, including the specific cell lines investigated. Future work will show whether other *Xenia* diterpenoids or, in particular, synthetic analogues of **1** may possess enhanced antiproliferative activity.

In summary, the first enantioselective total synthesis of the *Xenia* diterpenoid blumiolide C (**1**) has been accomplished in a total of 27 steps (24 steps for the longest linear sequence) and 1% overall yield. The synthesis is built around the unprecedented construction of the [7.4.0]oxabicylic ring system through the RCM-based formation of a nine-membered ring as the key enabling step. Other crucial elements of our approach to **1** include an Evans aldol reaction, a highly diastereoselective mixed cuprate addition to an α,β-unsaturated δ-lactone, and a stereospecific dehydration reaction with DCC/$CuCl_2$ to introduce the side chain at C4.

The chemistry developed in the course of our work on blumiolide C (**1**) provides the foundation for the future synthesis of other natural *Xenia* diterpenoids as well as the preparation of synthetic analogs of **1** for SAR studies and target finding.

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Keywords: medium-sized rings · metathesis · natural products · terpenoids · total synthesis

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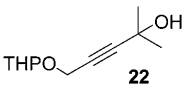

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[25] The relative configuration of the aldol adduct **20** was deduced from the geometry of the double bond after the *syn*-elimination reaction, while the absolute configuration at C4 was assumed to be *S* based on the work of Leumann[6a] and Corey.[6b]
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[27] The ^{1}H NMR spectrum of synthetic blumiolide C corresponds exactly with the published data.[9] For the ^{13}C NMR spectrum of synthetic **1** 17 out of 20 signals match with the literature data within 0.1 ppm. Two carbonyl signals deviate by 0.6 ppm, which could simply be a consequence of different measuring conditions. (See the Supporting Information). For the signal at 130.3 ppm in our spectrum the corresponding literature signal is reported at 133.0 ppm. We assume this to be a publication error in reference [9]. **1**: HRMS: calc. for $[M+Na]^{+}$: 353.1723, found 353.1728; lit. HRFABMS calc. for $[M]$: 331.1902, found 331.1905. $[\alpha]_D^{25} = +50.0°$ ($c = 0.4\ g\,cm^{-3}$, $CHCl_3$); lit. $[\alpha]_D^{25} = +66.0°$ ($c = 0.4\ g\,cm^{-3}$, $CHCl_3$).
[28] CCDC 703479 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

