Total Synthesis

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Syntheses and Biological Evaluation of Iriomoteolide 3a and Analogues**

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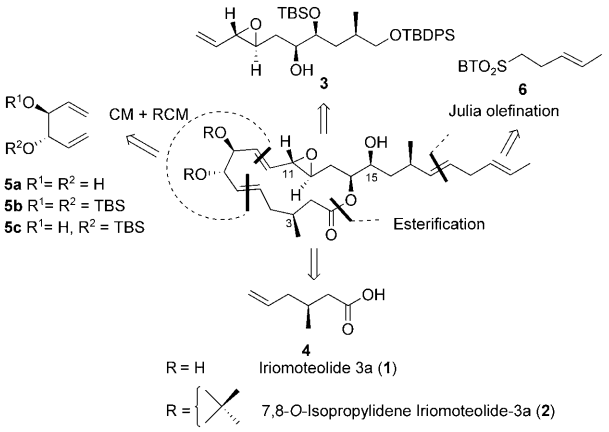
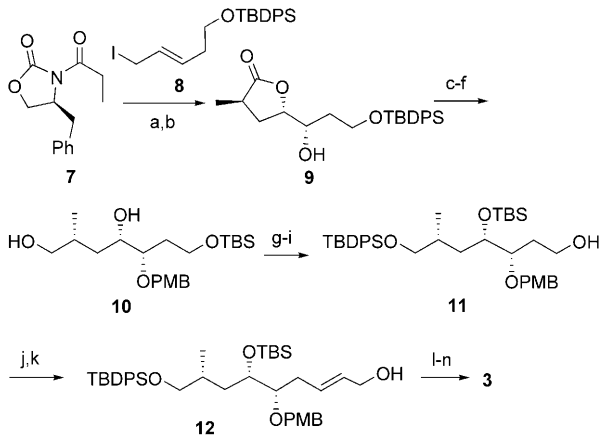
Amphidinium species are an extremely prolific source of marine secondary metabolites.[1] Structurally unique polyketides such as amphidinolides, caribenolide I, and amphidinolactones have fostered the interest of chemists not only as challenging targets for total synthesis but also because of their potent anticancer activity.[2] Recently, the *Amphidinium* strain HYA024 was found to produce cytotoxic compounds such as iriomoteolides 1a–c,[3] and a rare 15-membered macrolide, iriomoteolide 3a (**1**).[4] With a novel carbon framework comprising eight stereogenic centers, four of them in allylic positions, compound **1** represents the first member of a unique and unprecedented 15-membered macrolide class. Compound **1** represents the first member of a unique and unprecedented 15-membered macrolide class. In addition, the preliminary physiological properties disclosed for **1** and its 7,8-*O*-isopropylidene derivative **2** are very promising, showing potent cytotoxicity against lymphoma cell lines in the low nanomolar range.[4]

To confirm the assigned structure, further evaluate its biological activity, and determine whether its cellular targets are related to those of larger congeners such as amphidinolides,[5] substantial quantities of these compounds are required. Our retrosynthetic approach to **1** involved four major disconnections, which revealed key fragments **3**–**6** as summarized in Scheme 1. Fragment **6** was planned to be incorporated at the end of our synthetic sequence by a Julia–Kocienski olefination because of its widely recognized performance in the elaboration of such sensitive settings and also to ensure a flexible late-stage diversification of the parent compound. An intermolecular esterification was envisioned to assemble fragments **3** and **4**. Finally, we hypothesized that the C_2-symmetry of the diol precursor of fragment **5** could be advantageously used to construct the 1,5-diene upon ring closure by a cross-metathesis (CM)/ring-closing metathesis (RCM) approach. We were relying on the excellent results achieved by the Grubbs-type carbene complexes in both CM and RCM processes with the expectation that the formation of a medium-sized ring would also be *E*,*E* stereoselective (Scheme 1).



Scheme 1. Retrosynthetic analysis for iriomoteolide 3a (**1**).

The required building block **3** (Scheme 2) was prepared by alkylation of the Evans oxazolidinone **7**[6] with iodide **8**.[7]



Scheme 2. a) $Na[N(SiMe_3)_2]$, THF, −78 °C, 85%; b) ADmix-α, $MeSO_2NH_2$, *t*BuOH/H_2O, 0 °C, 83% (94% *de*); c) $PMBNHCCl_3$, CSA, CH_2Cl_2, RT, 89%; d) TBAF, THF, 89%; e) TBSCl, imidazole, DMF, 0 °C, 94%; f) $LiBH_4$, Et_2O, 91%; g) TBDPSCl, imidazole, CH_2Cl_2, 0 °C, 90%; h) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 98%; i) PPTS, EtOH, RT, 82%; j) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, −78 °C→RT, then Ph_3PCHCO_2Me, CH_2Cl_2, RT, 91%; k) DIBAL-H, CH_2Cl_2, −78 °C, 88%; l) *t*BuOOH, $Ti(OiPr)_4$, (+)-DIPT, CH_2Cl_2, MS (4 Å), 94%, (92% *de*); m) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, −78 °C→RT, then $[Ph_3PCH_3]Br$, $Na[N(SiMe_3)_2]$, THF, 73%; n) DDQ, CH_2Cl_2, pH 7 buffer, RT, 85%. CSA = camphorsulfonic acid, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, DIBAL-H = diisobutylaluminium hydride, DIPT = diisopropyl tartrate, DMF = *N*,*N*-dimethylformamide, DMSO = dimethylsulfoxide, MS = molecular sieves, PMB = *para*-methoxybenzyl, PPTS = pyridinium *para*-toluenesulfonate, TBAF = tetra-*n*-butylammonium fluoride, TBDPS = *tert*-butyldiphenylsilyl, TBS = *tert*-butyldimethylsilyl.

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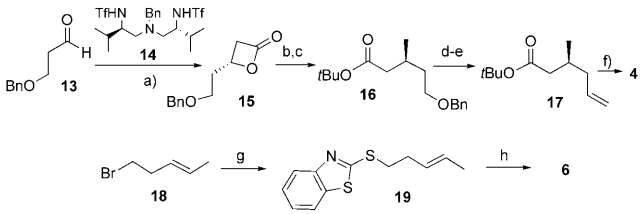
Asymmetric dihydroxylation of the double bond and subsequent spontaneous lactonization to release the chiral auxiliary afforded **9** with excellent selectivity (d.r. > 20:1). The free hydroxy group in **9** was protected as p-methoxybenzyl ether, and the TBDPS protecting group on the primary alcohol was exchanged for a TBS group.[8] Reductive opening of the lactone afforded diol **10**. A series of protecting group manipulations delivered alcohol **11** in excellent yield. Intermediate **12** was obtained after a reaction sequence including an oxidation, a Wittig reaction with (carbamethoxymethylene)triphenylphosphorane, and a DIBAL-H reduction of the methyl ester. Sharpless asymmetric epoxidation[9] allowed the installation of the required oxirane with high stereochemical control (> 92% *de*). Straightforward oxidation-state and protecting group manipulation led to the alkene moiety in fragment **3** which is necessary for the envisioned metathesis reaction.

The route to fragment **4** commenced with the catalytic asymmetric cyclocondensation of 4-(benzyloxy)-1-butanal (**13**)[10] with acetyl bromide using triamine ligand **14**[11] by a modification of the procedure previously reported by Nelson et al. (Scheme 3).[12] Dimethyl cuprate, generated in situ, was reacted with **15** to afford the corresponding acid, which was readily transformed into *tert*-butyl ester **16**. Hydrogenolysis of the benzyl protecting group and subsequent oxidation of the primary alcohol gave the corresponding aldehyde, which was then subjected to a Wittig olefination to provide compound **17** in good yield.[13] Hydrolysis of the *tert*-butyl ester under acidic conditions delivered fragment **4** in 40% overall yield after six steps. Fragment **6** was prepared from (*E*)-5-bromo-2-pentene (**18**)[14] in two steps by halogen displacement to give sulfide **19** and then chemoselective oxidation, using H_2O_2/Na_2WO_4, to give sulfone **6** (Scheme 3).[15] Analogues of fragment **5** (Scheme 1) were synthesized from L-tartaric acid according to known procedures.[16]

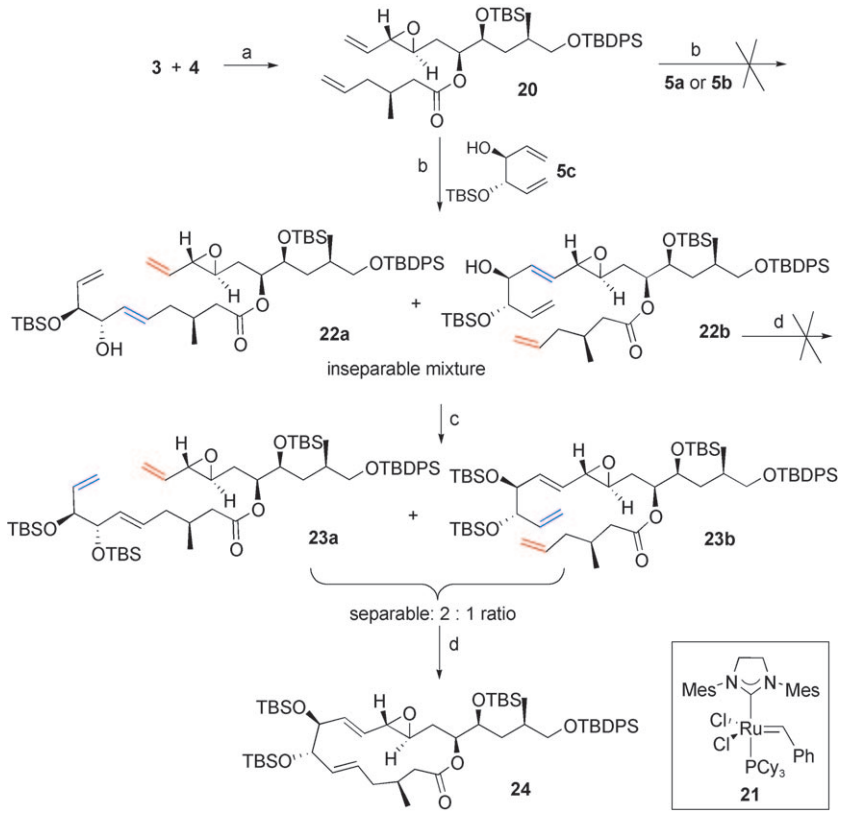
Esterification of acid **4** with alcohol **3** was cleanly effected using EDC as activating agent in the presence of 4-pyrrolidinopyridine (Scheme 4). Completion of the macrolide required the assembly of compound **20** with fragment **5** by a CM/RCM sequence using ruthenium complex **21**.[17] When **5a** was used, a complex mixture of products was obtained, which was probably a result of the high reactivity of the diol. In contrast, under the same reaction conditions, olefin **5b** was recovered unreacted, and **20** underwent both RCM to give a 10-membered ring lactone and also self-immolative CM.[18] We hypothesized that the steric hindrance imposed by the TBS group in **5c** could prevent the coordination of the ruthenium catalyst **21** to the substrate and thereby prevent the CM reaction on its neighboring double bond.[19] Remarkably, CM between **20** and



Scheme 3. a) Me_2AlCl, AcBr, iPr_2EtN, CH_2Cl_2, −78 °C, 94%; b) CuBr, Me_2S, MeMgBr, THF, −50 °C, 68%; c) Boc_2O, DMAP, *t*BuOH, RT, 82%; d) Pd/C (10 mol%), H_2, MeOH, RT; e) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, −78 °C→RT, then $[Ph_3PCH_3]Br$, *n*BuLi, −78 °C→RT, 80% over 3 steps; f) trifluoroacetic acid, CH_2Cl_2, RT, 72%; g) BTSH, NaH, DMF, RT, 80%; h) Na_2WO_4 (10 mol%), H_2O_2, RT, 75%. Ac = acetyl, Boc = *tert*-butoxycarbonyl, BTSH = 1-phenyl-1*H*-benzothiazol-5-thiol.

five equivalents of **5c** afforded an inseparable mixture of regioisomers **22a** and **22b** in 49% yield (Scheme 4).

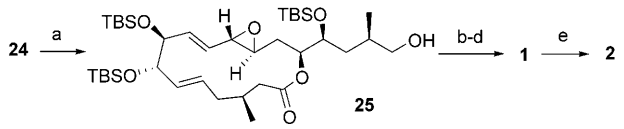
Surprisingly, when the mixture of **22a** and **22b** was submitted to classical RCM conditions (**21**, 0.005 M in toluene, RT or 60 °C), the desired products were obtained in low yields, in addition to the RCM product of **20** and dimeric **5c**. In our view, the ruthenium–carbene complex might react first with the terminal double bonds present in **22a** and **22b** (shown in red in Scheme 4), but the steric hindrance of the double bond close to the silyl group prevents the desired RCM event. Instead, the ruthenium–carbene complex reacted with the internal double bond close to the free hydroxy group (shown



Scheme 4. a) EDC·HCl, 4-pyrrolidinopyridine, CH_2Cl_2, RT, 72%; b) **5c** (5 equiv), **21** (5 mol%), toluene, 50 °C, 49%; c) TBSOTf, 2,6-lutidine, THF, 0 °C, 80%; d) **21** (12 mol%), toluene, RT, 76%. EDC = 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide.

in blue) to produce a ring-contracted homologue of **20** and released **5c**, which then dimerized. To circumvent this problem, we decided to silylate the mixture of **22a** and **22b** with the hope that upon the first cycloaddition of the ruthenium catalyst to the less hindered double bond (red), the remaining available double bonds of the molecule would have similar steric constraints.[20] To our delight, the mixture of **23a** and **23b** underwent clean RCM under the above-mentioned reaction conditions to give compound **24** as a single isomer in 72% overall yield.

The access to compound **24** enabled us to test the critical chemoselective removal of the primary OTBDPS group in the presence of three secondary OTBS groups.[21] After extensive experimentation, we found that an excess of ammonium fluoride in methanol[22] resulted in a slow but clean conversion of the starting material into the desired product **25**. Oxidation of the primary alcohol with Dess–Martin periodinane and subsequent Julia–Kocienski olefination[23] with sulfone **6** afforded the immediate precursor of iriomoteolide 3a in 76% yield over two steps (Scheme 5). Notably, this last transformation was highly *E* stereoselective (>93:7) and the



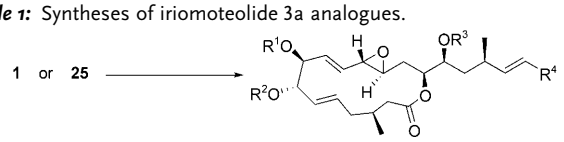
Scheme 5. a) NH_4F, MeOH, RT, 58%; b) DMP, CH_2Cl_2, RT; c) **6**, K[N-($SiMe_3$)$_2$], THF, 0°C, 93:7 *E*/*Z*, 76% (over 2 steps); d) TBAF, THF, RT, 86%; e) 2,2-dimethoxypropane, PPTS, CH_2Cl_2, 20%. DMP = Dess–Martin periodinane.

mild conditions preserved the stereochemical integrity of the intermediate α-branched aldehyde.[24] Final removal of the three silyl groups was achieved with TBAF to afford iriomoteolide 3a (**1**) (Scheme 5), whose analytical and spectroscopic properties were in good accordance with the published data.[4] 7,8-*O*-isopropylidene derivative **2** was obtained by treatment of **1** with 2,2-dimethoxypropane in the presence of pyridinium *para*-toluenesulfonate.[4]

A systematic structural editing of the natural product became our next immediate goal. First, the hydroxy groups in **1** were fully acetylated to afford compound **26** (Table 1, entry 1). As originally planned, the side chain was used for structural diversification. Starting from alcohol **25**, and after Dess-Martin periodinane oxidation, longer (**27**) and shorter (**29**) side chains were assembled through Julia and Wittig olefination reactions, respectively (Table 1, entries 2 and 4). The macrolides **27** and **29** were deprotected using TBAF in THF to afford triols **28** and **30**, respectively (Table 1, entries 3 and 5).

The growth inhibitory activities of compounds **1**, **2**, **26**, **28**, and **30** were investigated on two different human cancer cell lines: DAUDI (lymphoma) and HL-60 (leukemia) using the alamarBlue fluorometric assay (Table 2).[18,25] Synthetic **1** and **2** showed high potency against lymphoma cell lines (GI_{50} = 80 and 48 nM, respectively) confirming the preliminary results reported in the isolation paper.[4] However, the activity of peracetylated derivative **26** dramatically decreased (GI_{50} = 737 nM). The introduction of a truncated side chain (**30**) also compromised the antiproliferative activity even at 10 μM concentration. Noticeably, a more lipophilic pendant chain (**28**) afforded similar levels of potency as **1** and **2**, thus highlighting the importance of the lateral chain for the cytotoxicity of these molecules. A similar pattern was observed for HL-60 with activities in the low μM range. In light of these results, we were able to deduce the following trends: first, the enhanced activity of compound **28** compared to that of **30** could be explained by simple increase in lipophilicity, which might facilitate the cell penetration of the molecule. This fact is partially confirmed by the higher activity of acetonide **2** compared to that of parent compound **1**. Second, as peracetylated **26** showed very low cytotoxicity compared to **1** and **2**, we conclude that other factors might also influence the activity of these compounds and the presence of the free OH group on C15 is important for the interaction with their biological targets.

Table 1: Syntheses of iriomoteolide 3a analogues.



Entry		Reaction conditions[18]	R^1–R^4	Product (yield %)
1	**1**	Ac_2O, Pyridine	$R^1 = R^2 = R^3 = Ac$, $R^4 = trans\text{-}CH_2CHCHCH_3$	**26** (quant.)
2	**25**[a]	Na[N($SiMe_3$)$_2$], $C_7H_{15}SO_2PT$[b,c]	$R^1 = R^2 = R^3 = TBS$, $R^4 = C_6H_{13}$	**27** (72)
3	**27**	TBAF (4 equiv)[d]	$R^1 = R^2 = R^3 = H$, $R^4 = C_6H_{13}$	**28** (71)
4	**25**[a]	Na[N($SiMe_3$)$_2$], [Ph_3PCH_3]Br[b]	$R^1 = R^2 = R^3 = TBS$, $R^4 = H$	**29** (75)
5	**29**	TBAF (4 equiv)[d]	$R^1 = R^2 = R^3 = R^4 = H$	**30** (88)

[a] DMP, CH_2Cl_2, RT (quant.). [b] Reaction performed in THF at −78°C. [c] PT = 1-phenyl-1*H*-tetrazol-5-thiol. [d] Reaction performed in THF at 25°C.

Table 2: Antiproliferative activity of **1** and analogues (**2**, **26**, **28**, **30**) in the alamarBlue fluorimetric assay.[a]

Cell line	**1**	**2**	**26**	**28**	**30**
DAUDI	0.080	0.048	0.737	0.083	n.d.
HL-60	2.6	2.0	n.d.	2.8	n.d.

[a] GI_{50} values in μM. n.d. = not determined; no activity was observed up to a concentration of 10 μM.

In summary, we report the first total synthesis of iriomoteolide 3a (**1**), which confirmed the absolute configuration of this potent cytotoxic macrolide and provided sufficient quantities for additional biological evaluation. The key ring-closure to construct the 15-membered ring macrocycle relies on a highly *E*,*E* stereoselective cross-metathesis/ring-closing metathesis sequence. Through a modular synthetic approach, we have synthesized a small collection of non-natural derivatives of **1**, and tested their antiproliferative activity, revealing the suitable sites for structural modifications in the original core. Additional chemical editing of this

promising structure, and studies to elucidate both, its mode of action and cellular targets, are currently underway.

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