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Stereoselective Synthesis of the Phorboxazole A Macrolide by Ring-Closing Metathesis

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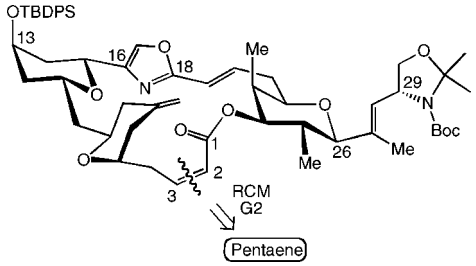
ABSTRACT



Described is a regio- and stereoselective ring-closing metathesis (RCM) to form the C2–C3 alkene of the macrolide-containing domain of phorboxazole A. This work demonstrates a dramatic effect of reaction solvent on RCM product (*E*/*Z*)-selectivity. This process offers an alternative assembly of the macrolide-containing domain of phorboxazole A, one of the most potent anticancer agents known.

Phorboxazoles A and B (Scheme 1) are potent cytostatic natural products originally isolated from the Indian Ocean sponge *Phorbas* sp. collected in 1993 off Western Australia.[1a] More recently, phorboxazole A has also been isolated from a 1996 collection of the Western Australian Indian Ocean sponge *Raspailia* sp.[2] This suggests that these natural products may ultimately be biosynthesized by a currently unidentified symbiotic marine microorganism. Despite these natural sources, laboratory synthesis remains the most reliable route to access the phorboxazoles.

The phorboxazoles exhibit S-phase cytostatic activity against a broad spectrum of human cancer cell lines.[1] Synthetic phorboxazole A[3] was also reported to induce cancer cell apoptosis.[4] Moreover, the phorboxazoles display unique modes of action with respect to currently viable cancer therapeutics.[1,4,5] Several total syntheses have yielded these natural products[3,6] and their structural variants.[7] SAR studies indicated that elements of both the macrolide and side chain domains of phorboxazole A are necessary for potent cytostatic activity.[7a,c] The macrolide has been assembled using

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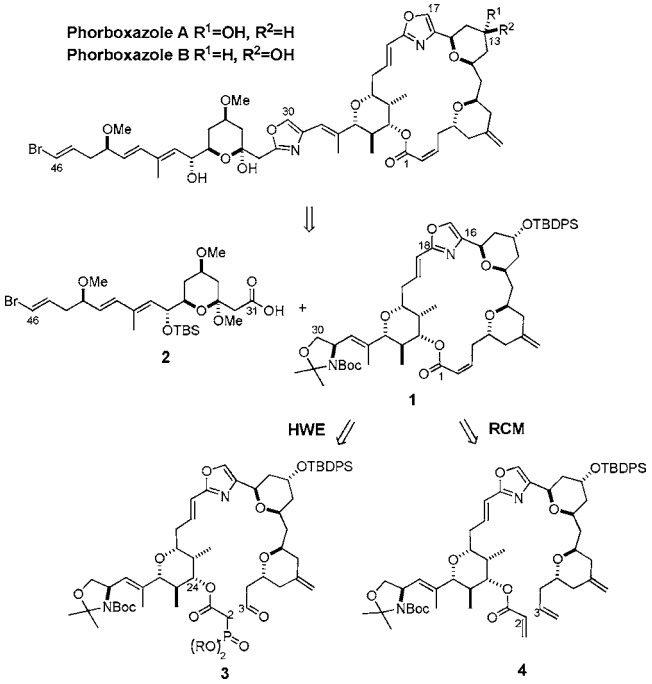
Scheme 1. Phorboxazoles and Intermediates



an intramolecular Still−Gennari reaction[8] to form predominantly the (2*Z*)-alkene in all of the published total syntheses of phorboxazole A and in the most recently published assembly of phorboxazole B.[3,6b−f] The original synthesis of phorboxazole B exploited the semireduction of a 2,3-alkyne to provide the (2*Z*)-alkene in higher geometrical selectivity.[6a] Described here is a remarkably regio- and stereoselective ring-closing metathesis[9] (RCM) to form the C2 alkene of the macrolide-containing domain (**1**)[3] of phorboxazole A. This work demonstrates a dramatic effect of reaction solvent on RCM product (*E*/*Z*)-geometry and can provide the (2*Z*)-alkene almost exclusively.

The stereochemistry and conformation of the natural products' (2*Z*)-macrolide were originally determined on the basis of NMR spectroscopy[1a] and subsequently corroborated by X-ray crystallography of synthetic material.[3] Alteration of phorboxazole A's (2*Z*)-acrylate moiety and macrolide conformation by alkene saturation to give 2,3-dihydrophorboxazole A[10] resulted in a dramatic loss of cytotoxic activity.[4] The unnatural (2*E*)-isomer[11] has more recently been reported to have 30−80-fold diminished in vitro cancer cell line growth inhibitory activity.[7c] Hence, the question of whether our convergent strategy[3] could be adapted to improve the synthetic efficiency of macrolide formation via an RCM process (Scheme 1) was coupled with the issue of C2 alkene geometrical selectivity.

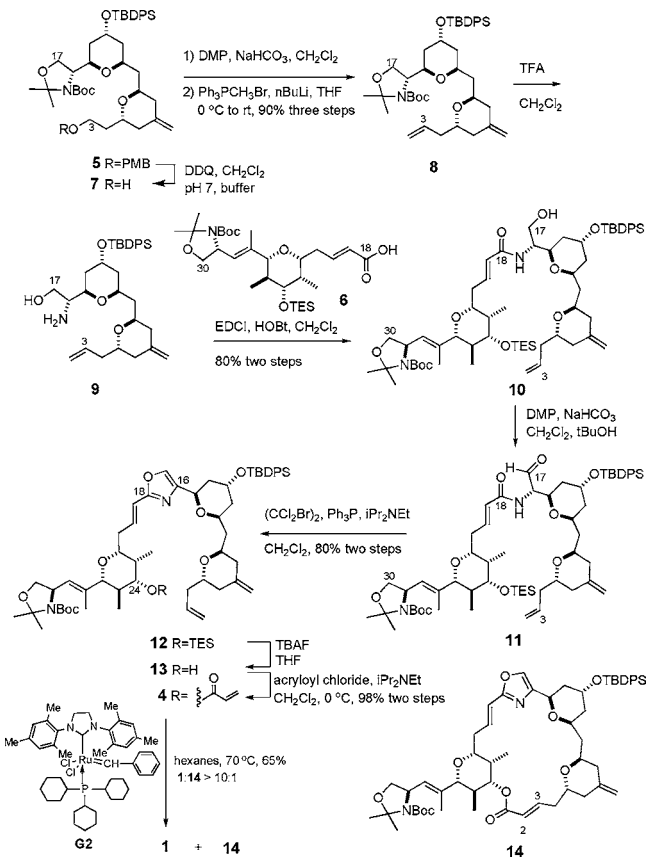
The original approach to the phorboxazole architecture utilized synthetic fragments representing C3−C17 and C18−C30 for macrolide construction.[3] These intermediates were first combined via de novo formation of the C16−C18 oxazole moiety. Subsequent elaboration of the C24 hydroxyl and the C3 terminus provided C24 phosphonoacetates bearing a C3 aldehyde (Scheme 1). Intramolecular Horner−Wadsworth−Emmons reactions under a variety of conditions generated the macrolide in variable (2*E*)/(2*Z*) ratios (Table 1). It was initially found that the (2*E*)-acrylate **14** (Scheme 2) could be formed in high yield and geometrical selectivity under thermodynamically controlled[12] HWE conditions (Table 1, entry 1).[3] In contrast, an ca. 4:1 ratio of (2*Z*)-**1** to (2*E*)-**14** macrolides was obtained using intramolecular Still−Gennari reactions (entries 2−4).[3,8,11,13] Although the key coupling processes of oxazole and acrylate formation have been satisfactorily optimized, the extensive functional group

Table 1. HWE Conversions of **3** to (2*Z*)-**1** and (2*E*)-**14**[a]

entry	**3**, R	temp (°C)	time (h)	yield (%)	**1**/**14**
1	$Et^{(b),3}$	25	1.5	69	∼1:20
2	$Et^{(c),3,13}$	25	>5	∼50	4:1
3	$CF_3CH_2^{(c),3,13}$	−40 to −5	5	77	4:1
4	$CF_3CH_2^{(c),6b,11}$	25	3	93	4:1

[a] Reagents applied: (b) $^iPr_2NEt$, LiCl, CH_2Cl_2;[12] (c) K_2CO_3, toluene, 18-crown-6.[8] The structure of **14** is given in Scheme 2.

Scheme 2. Synthesis and RCM of **4**



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manipulations required for the deployment of the original C3−C17 and C18−C30 fragments for these couplings have prompted the exploration of alternative strategies.

To explore an RCM-based macrolide assembly for phorboxazole A, our established C3−C17 bispyran **5**[14] and C18−C30 acid **6**[3] building blocks (Scheme 2) could be employed with only minor functional group modifications. First, the C3 PMB ether of **5** was selectively cleaved with DDQ[15] and the resultant primary alcohol **7** was oxidized with the Dess−Martin periodinane reagent[16] to yield the C3 aldehyde. Wittig olefination provided terminal alkene **8**. Exposure of the latent C16,17 vicinal amino alcohol from *N*-Boc-*N*,*O*-acetonide **8** was accomplished in one step by treatment with TFA in CH_2Cl_2 to give **9**. The secondary amine of **9** was joined with C18 carboxylic acid **6**[3] via EDCI-mediated amide formation[17] to yield the C17 hydroxy, C16 amide **10**. Optimized Dess−Martin oxidation[11,16] of the C17 alcohol gave aldehyde **11**, poised for dehydrative oxazole formation.[18] Inspired by variations[19] of Crimmin's cyclodehydration protocol,[18] α-amido aldehyde **11** was subjected to a single manipulation using halogen electrophile-activated Ph_3P and Hünig's base in CH_2Cl_2 to generate oxazole **12** in 80% yield from **10**.[11] This procedure provides a uniquely efficient conversion of an α-*N*-acyl serine derivative into a 2,4-disubstituted oxazole. Selective cleavage of the C24 TES ether of **12** was accomplished with TBAF to yield **13**. The hindered C24 hydroxyl group of **13** was acylated using acryloyl chloride and Hünig's base to form the C24 acrylic acid ester **4**.

It was expected that cross-metathesis of acrylate **4** at high dilution would favor intramolecular C2−C3 double bond formation due, in part, to the steric hindrance of the C7 and the C27−C28 alkenes and conjugation of the C19−C20 alkene. RCM studies began with addition of the second-generation Grubbs catalyst (G2, Scheme 2)[9b,20] to a dilute solution of **4** in toluene at reflux. Macrolides (2*Z*)-**1** and (2*E*)-**14** were obtained in a 1:1.7 ratio and in 70% combined yield (Table 2, entry 1). Repeating the RCM of **4** in toluene at 70 °C for 1 h at higher catalyst loading gave an improved yield with diminished *E*-selectivity (Table 2, entry 2). Attempts to perform the RCM of **4** in CH_2Cl_2 at reflux gave no detectable RCM products.

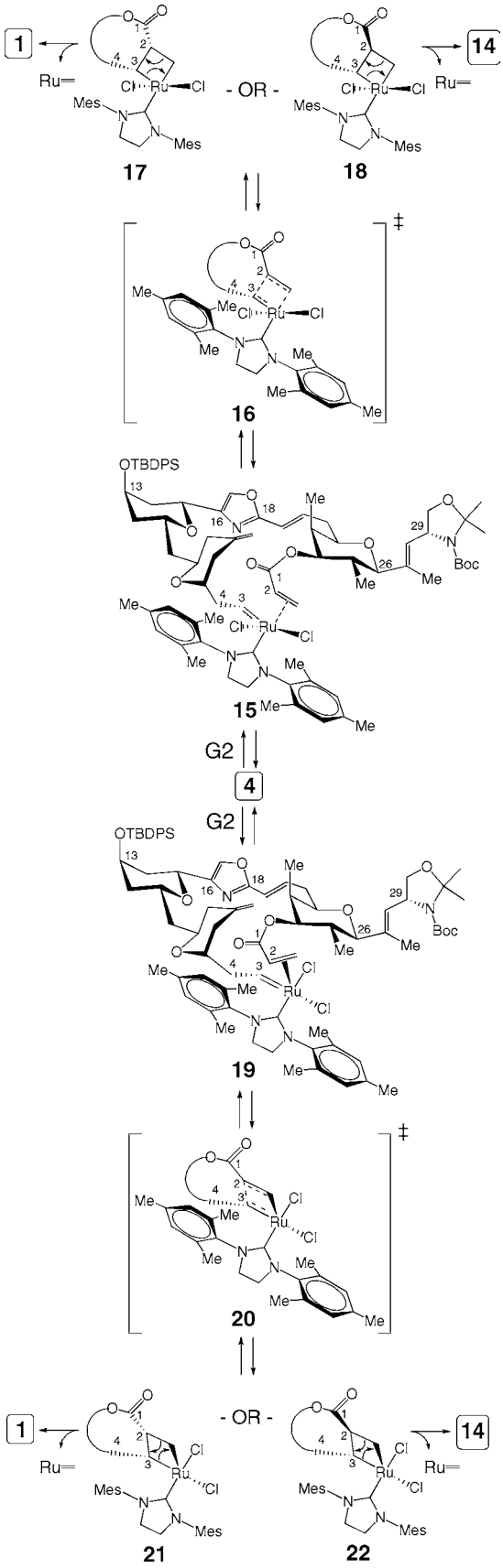
Table 2. Solvent and Temperature Effects on RCM of **4**[a]

entry	solvent	temp (°C)	time (h)	catalyst (mol %)	yield (%)	**1/14**
1	toluene	110	0.4	15	70	1:1.7
2	toluene	70	1	25	~90	1:1.2
3	hexanes	70	1	25	65	>10:1
4	pentane	36	3	25	<10	nd
5	CH_2Cl_2	40	16	25	0	nd
6	CH_2Cl_2	70	3	25	nd	nd

[a] Results shown represent ca. three replicate experiments for each solvent entry. nd = not determined.

The failure of **4** to undergo cyclization in CH_2Cl_2 using the G2 catalyst may reflect an important influence of solvent

Scheme 3. Partitioning of **4** between **1** and **14** via RCM



polarity, not just temperature. To explore this hypothesis, **4** was subjected to additional RCM conditions with varying solvent and temperature (Table 2). Repeating the reaction of **4** in CH_2Cl_2 using a sealed flask at 70 °C led to little conversion (analyzed by TLC), even after several hours and with up to 1 equiv of catalyst. Attempted RCM of **4** in *n*-pentane at a temperature and catalyst loading similar to those used initially with CH_2Cl_2 yielded only minor amounts (TLC) of macrolides after 3 h. Prolonged reaction time in *n*-pentane resulted in a colored reaction solution, an observation that is consistent with catalyst degradation. In contrast, a remarkable result was obtained when the RCM reaction of **4** was performed in hexanes at 70 °C. A combined yield of macrolide formation comparable to that obtained in toluene at 110 °C was achieved after 1 h (45 min catalyst addition plus an additional 15 min). However, the RCM of **4** in hexanes at 70 °C was highly *Z*-selective [>10:1, (*2Z*)-**1**/(*2E*)-**14**] as determined by ^{1}H NMR spectroscopy. Only trace amounts of **4** remained in the hexane reaction mixture at 1 h. A prolonged reaction time in hexanes did not improve the yield or change the distribution of macrolide geometrical isomers but led to darkening of the reaction mixture.

The ratios of (2*E*/Z)-macrolides **1** and **14** did not change measurably (^{1}H NMR spectroscopy) when the isolated mixtures were resubjected to refluxing toluene in the presence (25 min) or absence (3 h) of the G2 catalyst. Similarly, prolonged treatment of a 1.7:1.0 ratio of **14** to **1**, respectivley, to the G2 catalyst in hexanes at 70 °C did not appreciably change the ratio of **14** to **1**. These results indicate that once the metallocyclobutane intermediates collapse to generate macrolides **1** and **14** the newly generated C2 alkenes are unlikely to reengage in cross-metathesis (Scheme 3), undergo equilibration under the reaction conditions, or lead to selective degradation of one of the geometrical isomers. Hence, the (2*E*/Z)-selectivities observed in the RCM products reflect initial kinetic preferences that vary among the conditions used.

The basis of the dramatic differences observed in kinetic selectivities between RCM of **4** in toluene and hexanes is not understood but may reflect the partitioning of **4** among *cis*- and *trans*-metallocyclobutanes. Although a solvent-dependent, substrate conformational preference may be operative, the effect of solvent polarity on differentially stabilizing lateral (cf. **19**, Scheme 3) vs axial (**15**) alkene coordination with Ru in the G2 catalyst is expected to be large.[21] Specifically, toluene would better stabilize the more polar lateral alkene coordination (**19**, *cis*-chlorides) and subsequent metallocyclobutane-forming transition state (cf. **20**) than would hexanes. The much less polar axial coordination (cf. **15**, *trans*-chlorides) and derived transition state (**16**) would benefit less from the more polar solvent. Whether a solvent-dependent correlation exists between lateral (**20**) vs axial (**16**) transition states and *cis*- (cf. **17**, **21**) vs *trans*- (cf. **22**, **18**) metallocyclobutane generation with the G2 catalyst and substrate **4** remains to be determined.[22] Further optimization of this *Z*-selective RCM may involve lowering the catalyst loading, performing the reaction at higher concentration, and surveying alternative catalysts and solvents.

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Supporting Information Available: Experimental procedures and characterization data for **1**, **4**, **7**, **8**, **10**, **12**, and **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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