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Total Synthesis of Phorboxazole A via *de Novo* Oxazole Formation: Convergent Total Synthesis

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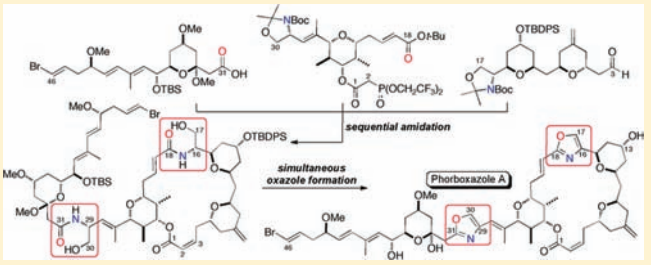
Supporting Information

ABSTRACT: The phorboxazoles are mixed non-ribosomal peptide synthase/polyketide synthase biosynthetic products that embody polyketide domains joined via two serine-derived oxazole moieties. Total syntheses of phorboxazole A and analogues have been developed that rely upon the convergent coupling of three fragments via biomimetically inspired *de novo* oxazole formation. First, the macrolide-containing domain of phorboxazole A was assembled from C3−C17 and C18−C30 building blocks via formation of the C16−C18 oxazole, followed by macrolide ring closure involving an intramolecular Still−Genarri olefination at C2−C3. Alternatively, a ring-closing metathesis process was optimized to deliver the natural product's (2*Z*)-acrylate with remarkable geometrical selectivity. The C31−C46 side-chain domain was then appended to the macrolide by a second serine amide-derived oxazole assembly. Minimal deprotection then afforded phorboxazole A. This generally effective strategy was then dramatically abbreviated by employing a total synthesis approach wherein both of the natural product's oxazole moieties were installed simultaneously. A key bis-amide precursor to the bis-oxazole was formed in a chemoselective one-pot, bis-amidation sequence *without the use of amino or carboxyl protecting groups*. Thereafter, both oxazoles were formed from the key C18 and C31 bis-*N*-(1-hydroxyalkan-2-yl)amide in a simultaneous fashion, involving oxidation−cyclodehydrations. This synthetic strategy provides a total synthesis of phorboxazole A in 18% yield over nine steps from C3−C17 and C18−C30 synthetic fragments. It illustrates the utility of a synthetic design to form a mixed non-ribosomal peptide synthase/polyketide synthase biosynthetic product based upon biomimetic oxazole formation initiated by amide bond formation to join synthetic building blocks.



BACKGROUND AND INTRODUCTION

Phorboxazoles A (**1**) and B (**2**) are remarkable marine natural products that embody unique structures and biological activities (Figure 1).[1] Their combination of novel structure and potent cytostatic and apoptotic activities has made the phorboxazoles prime targets of chemical synthesis, with eight total syntheses published to date.[2] This activity has been fostered by the fact that the biogenetic source of the phorboxazoles has yet to be identified. Thus, laboratory synthesis remains the best source of the natural products and their non-natural analogues.

In designing and refining the total synthesis of the phorboxazole A, we found it instructive to consider the plausible biosynthetic assembly of these non-ribosomal peptide synthase−polyketide synthase products. Structurally, the phorboxazoles appear to contain two serine (C15−C17 and C28−C30) amide-derived oxazole rings linking three separate (C1−C14, C18−C27, and C31−C46) polyketide domains. Whereas the terminal C31−C46 and C1−C14 domains derive from consecutive acetate condensations, the intervening fragment incorporates three consecutive propionates (C22−C27) and two acetates (C18−C21). Oxazoles occur widely among biologically active natural products, where they may be biosynthesized via cyclodehydration−oxidation processes from

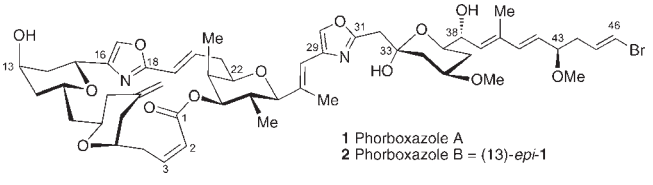


Figure 1. Phorboxazole A and B.

serine or threonine amides.[3] Little or nothing is known about the specific biosynthesis of the phorboxazoles, but general characteristics may be extrapolated from other non-ribosomal peptide synthase−polyketide synthase products.[3] In the case of the phorboxazoles, this may involve an initial linear polyacetate assemblage to attain a C31−C46 polyketide synthase (PS) cystine thiol ester intermediate that undergoes *trans*-acylation with the amino group of a serine peptidyl carrier protein (PCP) thiol ester of a non-ribosomal peptide synthase (NRPS) complex (Scheme 1). The intermediate *N*-acyl serine thiol ester may undergo immediate cyclodehydration catalyzed by a heterocyclization domain of the

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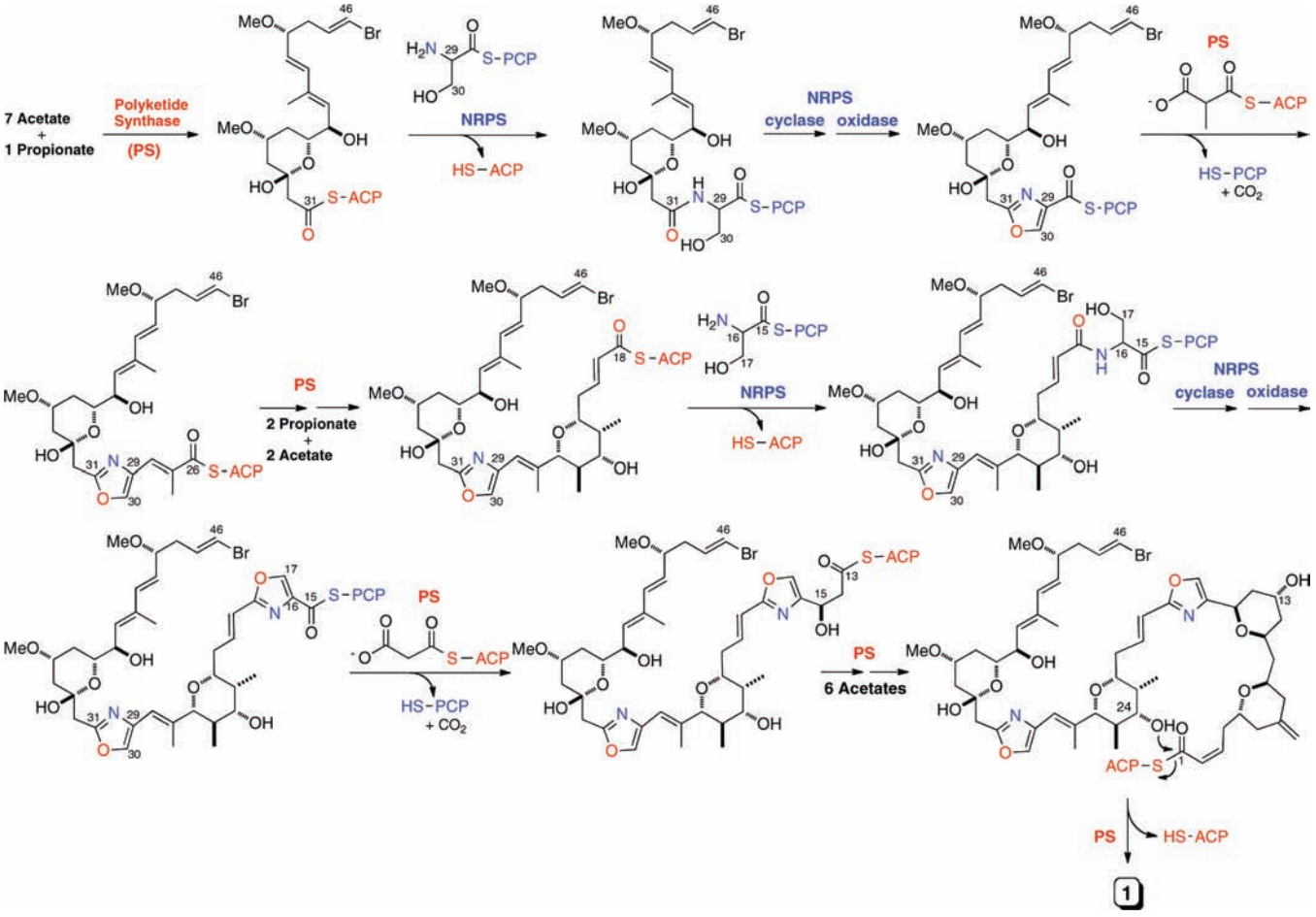
Scheme 1. Putative Biosynthesis of Phorboxazole A (1)[a]



[a] Three polyketide domains are assembled by polyketide synthases with intervening transfer to and from NRPS-bound serine residues. Abbreviations: NRPS, non-ribosomal peptide synthase; PS, polyketide synthase; ACP, acyl carrier protein; PCP, peptidyl carrier protein.

NRPS, followed by subsequent oxidation to an oxazole utilizing a flavin mononucleotide cofactor. The NRPS-processed serine-derived thioester would then rejoin the polyketide synthetic machinery by *S*-acyl transfer to a 2-methyl malonyl-derived propionate enolate via a Claisen condensation to give a PS-bound C26−C46 intermediate. Iterative C18−C25 PS-mediated polyketide extension would precede incorporation of the C15−C17 serine unit, again via *trans*-acylation with the amino group of a serine PCP thiol ester. NRPS protein module-mediated cyclodehydration−oxidation would form the C16−C18 oxazole before the second cystine-derived PCP thiol ester is transferred back to the PS via Claisen condensation. Alternatively, although seemingly less likely, the intermediate C29−C31 serine amide may be retained until the incorporation of the second serine moiety at C16−C18 and both oxazoles generated from a bis-amide intermediate. Sequential linear incorporation of the final seven acetates and intervening skeletal modifications would complete the carbon backbone of the phorboxazoles. Finally, macrolactonization would then release the phorboxazole scaffold from the PS. The presumed biosynthesis of the phorboxazoles is highly linear (C46 → C1), involving the sequential incorporation of 22 two- and three-carbon backbone units.

Among the laboratory syntheses of the phorboxazoles that have been reported to date,[2] only the first approach[2a] features amide-based fragment couplings and subsequent *de novo* oxazole formation inspired by the putative biosynthesis. In contrast with the linearity of combining two or three carbons in each carbon−carbon bond-forming step to biosynthetically construct the 46 carbons of the phorboxazole backbone, we embraced a convergent synthetic strategy toward **1**. Three fully elaborated major fragments, representing the ketide-derived portions of **1**, were joined via the *sequential de novo* formation of the C16−18 and C29−31 oxazole moieties from carboxylic acids and vicinal amino alcohols (Scheme 2), with an intervening (2*Z*)-acrylate formation.[2a] This involved amide formation followed by oxidation−cyclodehydration of the *N*-(1-hydroxyalkan-2-yl)amide in each case.[4,5]

The genesis and optimization of tri-component convergent assemblies of phorboxazole A and analogues that are reliant upon the three key C3−C17, C18−C30, and C31−C46 building blocks[6] are detailed here. These span two types of fragment coupling sequences, with two variations of generating the (2*Z*)-acrylate within the first: (*i*) assembly of the C1−C30 macrolide-containing domain via C16−C18 oxazole formation followed by the stereoselective generation of the (2*Z*)-acrylate, and then appendage of the C31−C46 side-chain domain via C29−C31 oxazole synthesis (*oxazole−acrylate−oxazole* assembly sequence; the (2*Z*)-acrylate of the C1−C30 macrolide-containing domain could be installed by either an intramolecular Horner−Wadsworth−Emmons (HWE) reaction or ring-closing metathesis

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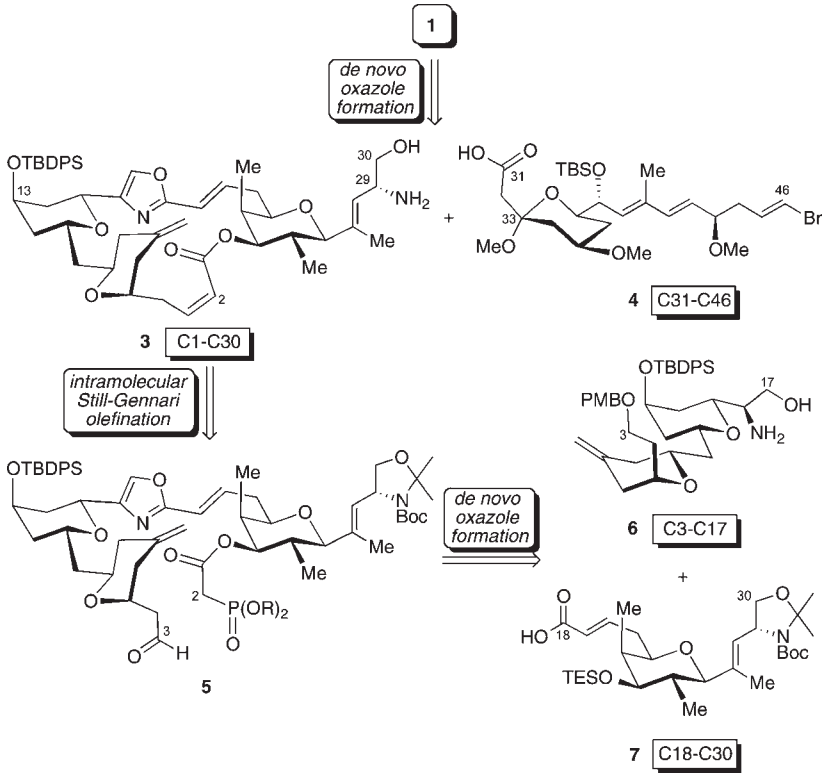
Scheme 2. Original Retrosynthetic Dissection of Phorboxazole A (1)



(RCM)), or (*ii*) combination of the C3−C17 and C18−C30 fragments via intermolecular HWE generation of the (2*Z*)-acrylate followed by attachment of the C31−C46 side-chain domain via sequential amide coupling and the final *simultaneous formation* of both oxazole moieties from a bis-amide intermediate (*HWE−acrylate−bis-oxazole* assembly sequence).

RESULTS AND DISCUSSION

1. Total Synthesis of Phorboxazole A by Stepwise Oxazole Formation (Oxazole−Acrylate−Oxazole) . *1.1. Assembly of the C1−C30 Macrolide-Containing Domain (Oxazole−HWE).* The first total synthesis of a phorboxazole utilized the *oxazole−acrylate−oxazole* assembly sequence. This proceeded through phorboxazole A intermediate **3**, which was prepared by an intramolecular HWE reaction of aldehyde-phosphonate **5**. In turn, **5** was prepared by coupling of the C3−C17 vicinal amino alcohol **6**[6] with the C18−C30 carboxylic acid **7**[6] by C16−C18 amidation−oxazole formation. The *de novo* oxazole formation was founded upon Crimmin's cyclodehydration protocol of *N*-acyl-α-amino-ketones using halonium-activated triphenylphosphine (Ph_3P, CCl_4)[4] (Scheme 3).

Amide formation between **6** and **7** was carried out in 87% yield using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (EDCI·MeI)[7] and HOBt (Scheme 3).[8] Dess−Martin periodinane[9] oxidation of the resulting hydroxy-amide **8** gave intermediate aldehyde **9**, which underwent optimized cyclodehydration upon treatment with PPh_3, $(BrCCl_2)_2$, and *i*-Pr_2NEt in CH_2Cl_2 to give the oxazole **10** in 91% yield. Previously we had reported[2a] formation of **10** in 77% overall yield from hydroxy-amide **8** using the reaction conditions specified by Wipf.[5] This involved a two-step, one-pot protocol of cyclodehydration of **9** to a bromooxazoline using Ph_3P, 1,2-dibromo-1,1,2,2-tetrachloroethane [$(BrCCl_2)_2$], and 2,6-*tert*-butyl-4-methylpyridine in CH_2Cl_2, followed by dilution with CH_3CN and addition of DBU to eliminate HBr and generate the oxazole. Wipf had suggested that the alternative use of Ph_3P, I_2, and triethylamine base may favor oxazole formation via an alternative acylimino carbene.[5a] With the use of PPh_3, $(BrCCl_2)_2$, and *i*-Pr_2NEt in CH_2Cl_2, evidence for the formation of intermediate bromooxazolines was observed under our optimized conditions in forming the C28−C31 oxazole (TLC, ESI-MS), but for the C16−C18 oxazole such intermediates were not observed. This may reflect subtle differences in reactivity due to the differential exocyclic alkene substitution of the C16−C18 oxazole (vinyl substituted at C18) versus the C29−C31 (vinyl substituted at C29). A key feature that contributed to the success of the cyclodehydration process was rapid and minimal processing of the intermediate aldehyde **9** by Dess−Martin periodinane oxidation in the presence of *tert*-butanol and $NaHCO_3$.[10] The optimized amide coupling−oxazole formation protocol efficiently coupled the two major fragments and provided oxazole **10** in 79% overall yield from **7**.

As a prelude to macrolide closure via an intramolecular HWE reaction, the C24 silyl ether was converted into phosphonoacetates and the C3 terminus into an aldehyde. This was initiated by selective desilylation of the C24 silyl ether of **10** and acylation of the resultant secondary alcohol **11** with either the bis(2,2,2-trifluoroethyl)phosphonoacetic acid or the diethyl analogue to afford **12** or the diethylphosphonoacetate, respectively. The C3 PMB ethers were then cleaved, and the primary alcohols were oxidized to the corresponding aldehydes **5** or **13**.

Intramolecular HWE reactions under a variety of conditions generated the macrolides in variable ratios of geometrical isomers

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Scheme 3. C1−C30 Macrolide Closure via *Oxazole−HWE* Sequence

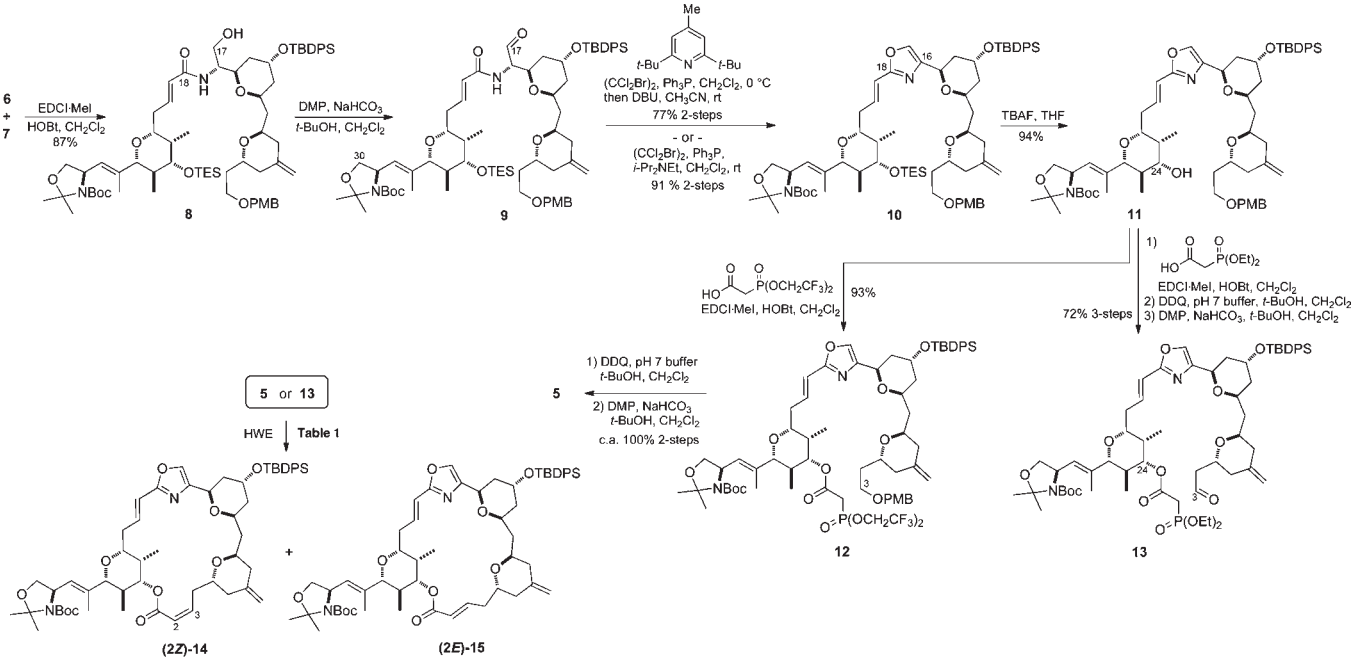


Table 1. Divergent Selectivity of (2*Z*)-14 and (2*E*)-15 Formation in the Intramolecular HWE Reactions[a]

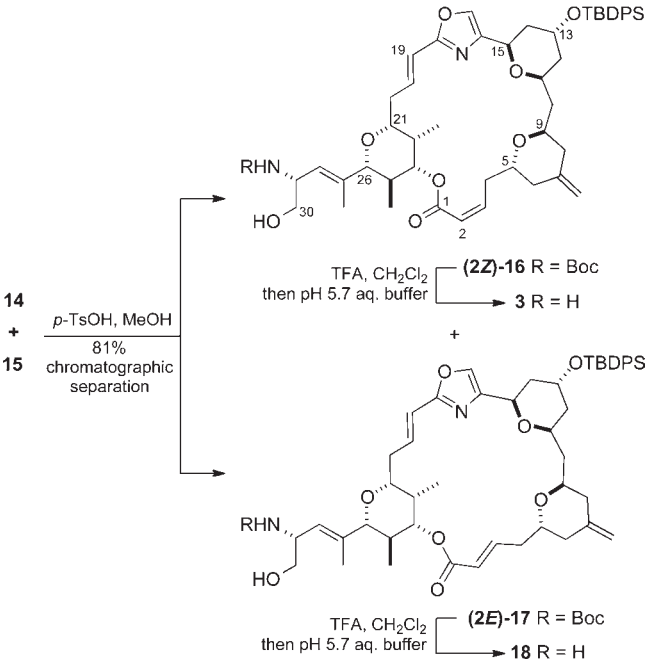
entry	phosphonate	reagents[a]	temp (°C)	time (h)	yield (%)	14:15
1	**13**	b	25	1.5	69	∼1:20
2	**13**	c	25	>5	∼50	4:1
3	**5**	c	−40 to −5	5	77	4:1
4	**5**	c	25	3	93	4:1

[a] Reagents applied: (b) *i*-Pr_2NEt, LiCl, CH_2Cl_2; (c) K_2CO_3, toluene, 18-crown-6.

(2*Z*)-**14**/(2*E*)-**15** (Table 1).[2a,8,11] An initial hypothesis was that the (2*Z*)-acrylate configuration of the natural products **1** and **2** might have been favored thermodynamically within the geometrical constraints of the macrolides. To test this, the diethyl phosphonoacetate **13** was subjected to thermodynamically controlled Masamune−Roush HWE conditions,[12] which afforded the (2*E*)-acrylate **15** in high yield and geometric selectivity (Table 1, entry 1). This provided the first indication that the (2*E*)-acrylate was clearly favored thermodynamically over the natural products' (2*Z*)-isomer.[2a] The (2*Z*)-acrylate of the phorboxazoles seems to be essential for potent cytostatic activity, as both the 2,3-dihydrophorboxazole A[13,14] and (2*E*)-phorboxazole A[11−15] are relatively inactive. In contrast, **13** gave a ca. 4:1 ratio of chromatographically difficult to separate macrolides **14** and **15** in modest combined yield under the Still−Gennari conditions using K_2CO_3, toluene, and 18-crown-6[16] (Table 1, entries 2 and 3).[8] The same geometrical ratio of macrolide acrylates was obtained, but in higher yields, using the bis-trifluorethyl phosphonoacetate **5** for the intramolecular HWE macrolide closure.[2a] At lower temperature (−40 to −5 °C), the combined yield was ca. 77%, which was remarkably improved to 93% by performing the reaction process at room temperature.[2e,11]

In preparation for appending the C31−C46 domain via C29 amide formation, the acetonide group protecting the vicinal

Scheme 4. Synthesis of the Macrolide-Containing Amino Alcohol 3



amino alcohol of the mixture of **14** and **15** was removed under controlled acidic conditions to yield the corresponding primary alcohols **16** and **17**, without any apparent isomerization of the (2*Z*)-acrylate (Scheme 4). Fortuitously, the (2*Z*)-acrylate **16** was crystalline (Figure 2),[2a] which provided an opportunity to verify the complete structure of this most advanced intermediate. X-ray crystallographic analysis not only confirmed the relative stereochemistry but also indicated that the macrolide-containing

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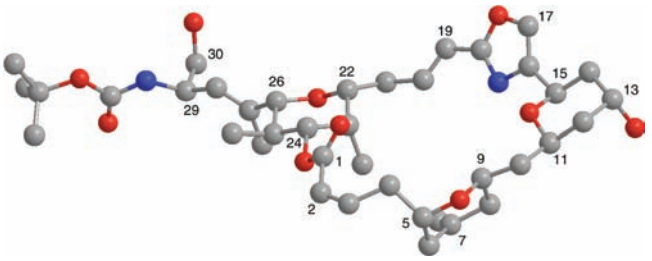
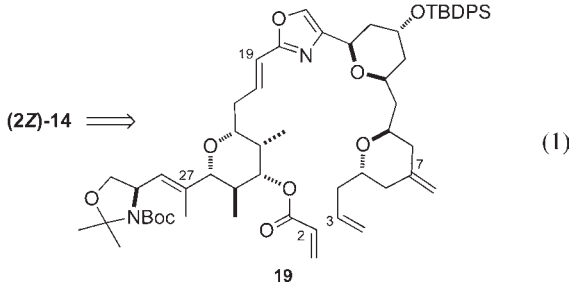


Figure 2. X-ray structure of compound **16**.[2a] The C13 OTBDPS group is omitted for clarity.

intermediate **16** adopted the same conformation in the solid state as **1** did in solution, as originally assigned by Molinski.[1] The absolute configuration of macrolide component **6** derived from D-serine, whereas that of **7** arose from L-phenylalanine via Evans's oxazolidinone (the C29 stereogenic center was inconsequential).[6] The (2*E*)-acrylate **17**, however, evaded extensive crystallization attempts. Finally, the *N*-Boc groups were removed from separated **16** and **17** to provide the corresponding ammonium salts of **3** and **18**, respectively.

This original assembly of macrolide **3** spanned 10 steps and ca. 42% overall yield in the longest linear sequence from **6** and **7**. Moreover, it supported the first total synthesis of phorboxazole A and the generation of informative analogues that indicated several structural requirements for potent biological activities,[15] as well as preliminary cellular target identification.[17] Although the two key coupling processes — oxazole and acrylate formation — had been individually optimized, the moderate stereoselectivity in the biologically essential (2*Z*)-acrylate prompted the exploration of an alternative strategy.

1.2. Assembly of the Macrolide-Contaning Domain (Oxazole—RCM). A variation on the macrolide closure involved formation of the C2—C3 alkene via RCM.[18,19] This made geometrical selectivity an important issue, given the general configurational promiscuity of RCM processes involving large rings. The RCM approach to **14** necessitated the incorporation of a C3 terminal alkene and C24 acrylate ester in pentaene **19** (eq 1). These terminal alkenes were expected to preferentially react over the more substituted olefins at C7, C19, and C27 with Ru-based RCM catalysts.[20]



(1)

The C3 alkene could be readily installed before combination of the C3—C17 bis-oxane domain **22** and C18—C30 central oxane-containing domain **7**[6] by C16—C18 oxazole formation (Scheme 5). Subsequent acrylation of the C24 alcohol would provide the pentaene precursor **19** for the RCM process. Therefore, the C3 PMB ether of **20**[6] was selectively cleaved with DDQ,[21] and the resultant primary alcohol was oxidized with the Dess—Martin periodinane reagent[9] to yield the corresponding C3 aldehyde. Wittig olefination then provided terminal alkene **21** in good overall yield from **20**. To set up oxazole formation, deprotection of the latent C16,C17 vicinal amino-alcohol from *N*-Boc, *N,O*-acetonide **21** was accomplished efficiently in one step by treatment with HCl in MeOH to give **22**. The secondary amine of **22** was combined with the C18 carboxylic acid **7** via EDCI-mediated amide formation[7] to yield C16 hydroxy-amide **23**. Optimized oxidation/cyclodehydration processes provided oxazole **24** in 87% yield from **23**. Selective cleavage of the hindered C24 TES ether of **24** was accomplished with a stoichiometrically controlled application of TBAF to yield the C24 alcohol. Thereafter, acylation of the encumbered secondary C24 alcohol was achieved at low temperature by brief treatment with premixed acryloyl chloride and Hünig's base in CH_2Cl_2 to give the pentaene RCM precursor **19** in 98% yield.

The initial RCM studies were performed using the second-generation Grubbs catalyst **25**[20] (G2, Scheme 5) in a dilute solution of **19** in toluene at reflux. Macrolides (2*Z*)-**14** and (2*E*)-**15** were obtained in a 1:1.7 ratio, respectively, and in 70% combined yield (Table 2, entry 1). Repeating the RCM of **19** 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 **19** in CH_2Cl_2 at reflux gave no detectable RCM products. It was hypothesized that the failure of **19** to undergo RCM cyclization in CH_2Cl_2 using the G2 catalyst may reflect an important influence of solvent polarity, not just temperature. To explore this hypothesis, **19** was subjected to additional RCM conditions with varying solvents and temperatures (Table 2). Repeating the reaction of **19** 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 G2 reagent. Attempted RCM of **19** 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 only resulted in catalyst degradation. In contrast, a remarkable result was obtained when the RCM reaction of **19** 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 **19** in hexanes at 70 °C was highly *Z*-selective [>10:1, (2*Z*)-**14**/(2*E*)-**15**], as determined by ^{1}H NMR spectroscopy. Only trace amounts of **19** 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.

The ratios of (2*Z*)-**14** and (2*E*)-**15** did not change measurably (^{1}H NMR spectroscopy) when the isolated mixtures were re-subjected to refluxing toluene in the presence (25 min) or absence (3 h) of the G2 catalyst. Similarly, prolonged treatment of the mixture of **14** and **15** with the G2 catalyst in hexanes at 70 °C did not appreciably change the ratio of **14** to **15**. These results indicated that, once the metallocyclobutane intermediates collapse to generate macrolides **14** and **15**, the newly generated C2 alkenes were unlikely to re-engage in cross-metathesis, 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 successful RCM reaction of pentaene **19** in hexanes allowed a direct comparison of synthetic routes to the macrolide containing phorboxazole intermediate. The Still—Gennari approach provided (2*Z*)-**14** and (2*E*)-**15** as a 4:1 mixture in ca. 50% yield over nine steps, whereas the RCM approach generated (2*Z*)-**14** and (2*E*)-**15** as a >10:1 mixture in ca. 40% yield over 10 steps from **20**.[22] Although the RCM chemistry did not

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Scheme 5. Assembly of the C1−C30 Macrolide via *Oxazole−RCM* Sequence

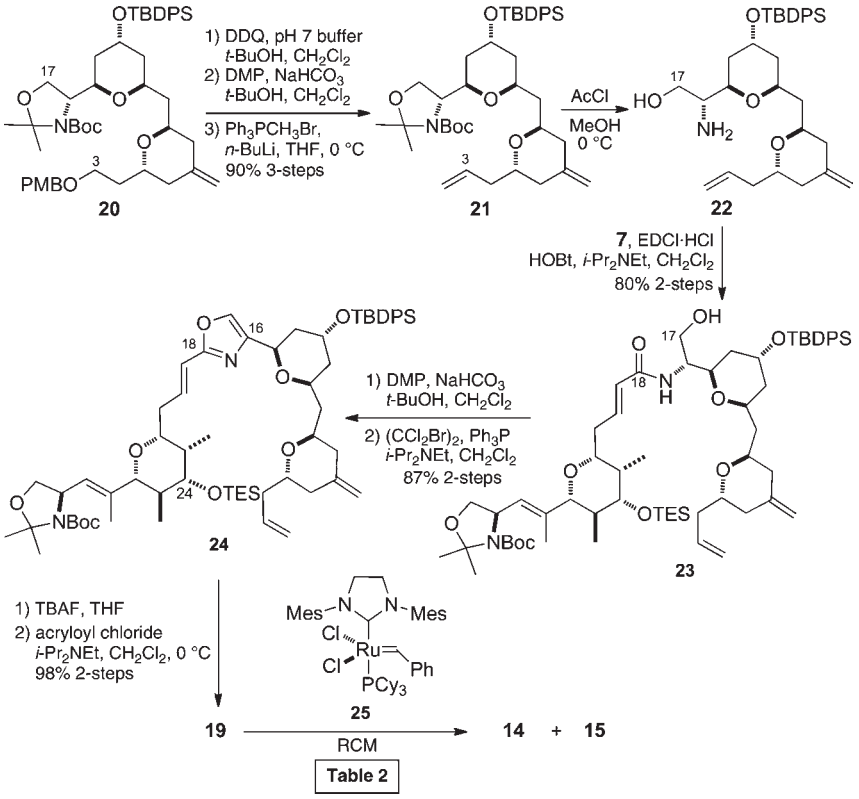

Table 2. Solvent and Temperature Effects on RCM of 19[a]

entry	solvent	temp (°C)	time (h)	catalyst (mol %)	yield (%)	14:15
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	*n*-pentane	36	3	25	<10	ND
5	CH_2Cl_2	40	16	25	0	—
6	CH_2Cl_2	70	3	25	ND	ND

[a] ND = not determined.

enhance the synthetic access to (2*Z*)-**14** from **20** and **7**, it did reveal an intriguing product geometrical dependence on reaction solvent. The basis of the dramatic differences observed in kinetic selectivities between RCM of **19** in toluene and hexanes was not fully understood but may reflect the partitioning of **19** among *cis*- and *trans*-metallocyclobutanes.[18]

In summary, the phorboxazole macrolide-containing domain represented by advanced synthetic intermediate **3**, which bears only a single protecting group (that at C13-*O*), was prepared most efficiently from components **6** and **7** via *de novo* oxazole formation and an intramolecular Still−Gennari olefination. The longest linear sequence to **3** began with the amino acid serine and proceeded through the derived Garner's aldehyde[23] in the preparation of both **6** and **7**.[6] Thus, **3** was prepared in 21 steps and ca. 13% overall yield in the longest linear sequence from Garner's aldehyde.[24]

1.3. Completion of Phorboxazole A via the Oxazole−Acrylate−Oxazole Sequence. Upon completion of the synthesis of the macrolide, the coupling between the C31−46 fragment **4**[6] and the macrolide-containing amino alcohol **3** (Scheme 2) was examined. The ammonium salt generated *in situ* by treatment of (2*Z*)-**16** was coupled with carboxylic acid **4** using (7-azabenzotriazol-1-yl)-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate (PyAOP)[25] and Hünig's base (Scheme 6). The amide coupling gave an unexpectedly low yield of 30% of hydroxy-amide **26** along with a side product, trifluoroacetamide **30**, in 50% yield (Scheme 7).[13] The unsatisfactory results led to a survey of amide coupling conditions and reagents, including carbodiimdes, phosphonium salts, uronium salts, and phosphinic chloride. Unfortunately, neither pre-activating carboxylic acid **4** by the coupling reagents nor pre-mixing **4** and the ammonium salt improved the yield of the hydroxy-amide **26**. All the attempted conditions gave approximately the same yield of side product **30**. During the attempts to obtain the free amine **3**, trifluoroacetamide **30** was formed instantaneously upon base treatment of the ammonium salt obtained from **16**. The result indicated the formation of the intermediate trifluoroacetate **29** in the TFA treatment of **16**. Under basic conditions, migration of the trifluoroacetyl group from the C30 alcohol to the C29 amine would give trifluoroacetamide **30**. To avoid this problem, 1 M HCl, 10% H_2SO_4, and 2 M TsOH·H_2O were examined for the cleavage of the Boc protecting group from the C29 amine. Although these acids in dioxane were sufficient for the Boc cleavage, the (2*Z*)-acrylate could be isomerized to the (2*E*)-configuration. The best yield (66%) of hydroxy-amide **26** was obtained using EDCI·HCl-mediated amide coupling of **4** with the *in situ* neutralized ammonium hydrochloride salt of **3**, which was generated via brief exposure of **16** to 4 M HCl in dioxane. On the other hand, hydrolysis of the C30 trifluoroacetate of intermediate **29** was achieved by washing it with weak acidic aqueous phosphate buffer (pH 5.5−5.8) to give vicinal

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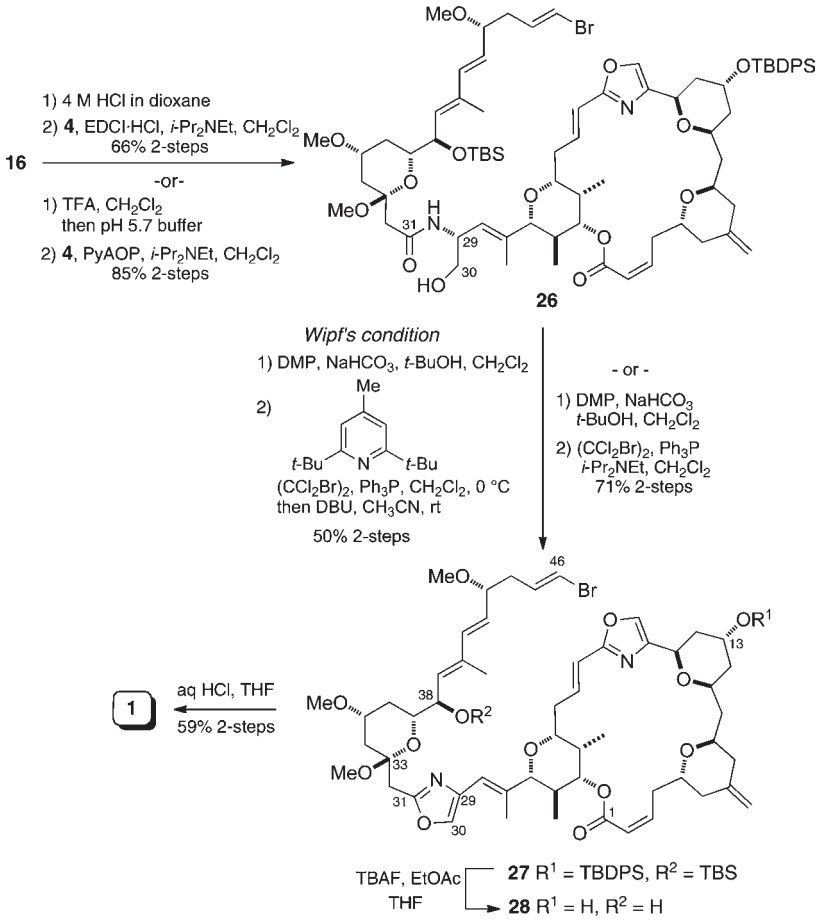
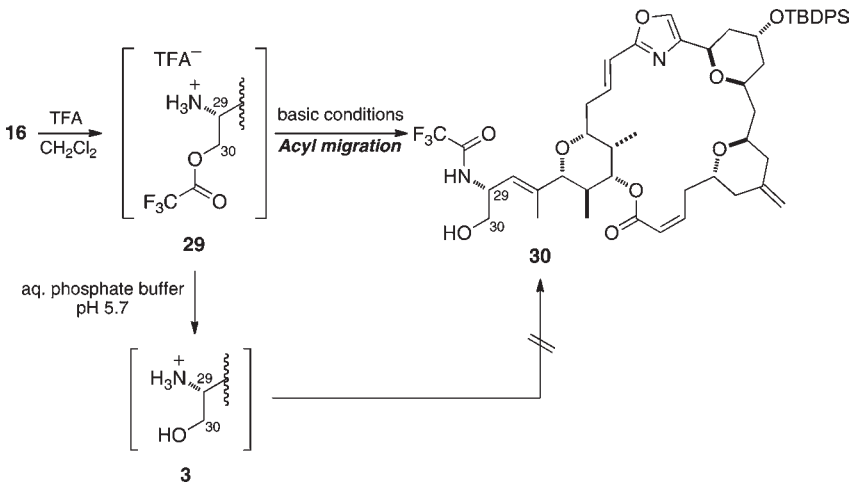
Scheme 6. C29−C31 Oxazole Formation and Completion of Phorboxazole A



Scheme 7. Unexpected Acylation



hydroxy-amine **3** (Scheme 7), presumably as an ammonium phosphate salt. PyAOP-mediated amide coupling between **3** and **4** afforded hydroxy-amide **26** in 85% yield.

In the original total synthesis, the yield of C29−C31 oxazole formation via the oxidation−cyclodehydration protocol under Wipf's condition[5b] was significantly lower (33%) than the C16−C18 oxazole formation (77%).[2a] The intermediate C30 β,γ-unsaturated aldehyde obtained from hydroxy-amide **26** was considered unstable in both acidic and basic conditions and could isomerize to the corresponding α,β-unsaturated aldehyde in the prolonged oxidation and/or the cyclodehydration reactions. Extensive effort was applied to optimize both the oxidation of the C30 alcohol of

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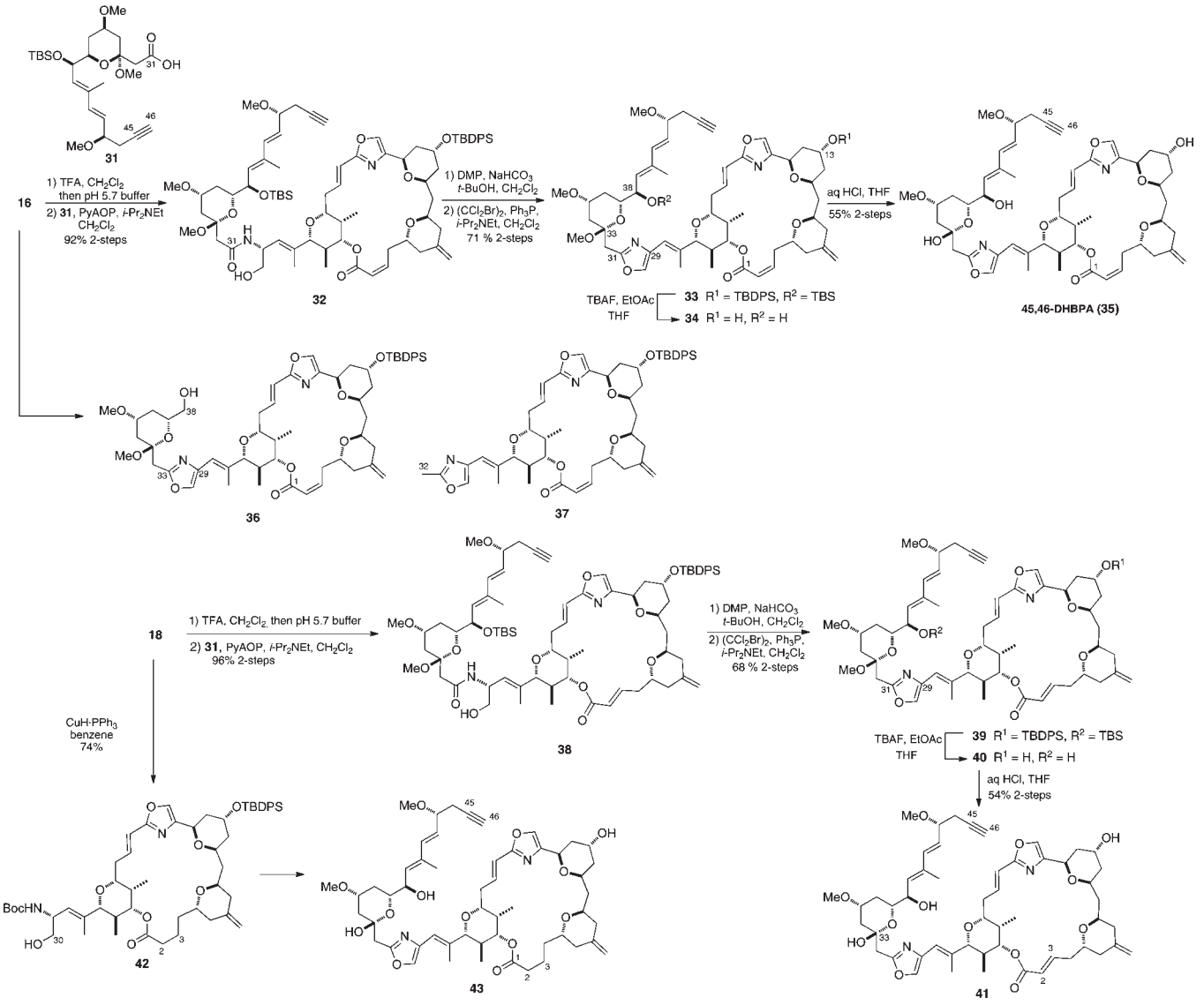
Scheme 8. Syntheses of Some Phorboxazole Analogues



hydroxy-amide **26** and the subsequent phosphonium-salt-mediated cylcodehydration of the resulting *N*-acyl-α-amino-aldehyde. The aldehyde could be obtained efficiently with $NaHCO_3$-buffered Dess−Martin periodinane in CH_2Cl_2 containing *t*-BuOH. Subsequent cyclodehydration under Wipf's conditions [(a) PPh_3/$(BrCCl_2)_2$/4-methyl-2,6-di-*tert*-butylpyridine, (b) CH_3CN, DBU][5b] at room temperature led to an improved 50% yield of oxazole **27** (Scheme 6). We found that cyclodehydration of the aldehyde obtained from **26** was best performed using an excess of Hünig's base with PPh_3 and $(BrCCl_2)_2$ in CH_2Cl_2 for a prolonged reaction time, which gave oxazole **27** in 71% yield. The use of the strong base DBU and more polar solvent CH_3CN in Wipf's protocol could be avoided in converting the intermediate bromooxazoline into the desired oxazole.

The final task was the deprotection of the C13 and C38 alcohols and the C33 hemiketal in bisoxazole **27**. Predictably, the TBDPS group resident upon the C13 axial hydroxyl group was found to be much more stable than the TBS ether at C38. Consequently, a large excess of TBAF and a long reaction time were required to cleave both C13 and C38 silyl ethers. To prevent base-induced saponification of the macrolide ester, ethyl acetate was used as co-solvent to consume residual hydroxide in the commercial THF solution of TBAF. The resulting diol **28** was then treated with aqueous HCl in THF to hydrolyze the C33 ketal to give phorboxazole A (**1**) in 59% yield over the final two deprotection steps (Scheme 6).

1.4. Synthesis of Phorboxazole Analogues. The original tricomponent coupling *oxazole−acrylate−oxazole* route to **1** was also applied to the generation of several structural analogues to gain preliminary insight into phorboxazole structure−activity relationships.[15] Beginning with the macrolide-containing synthetic intermediate **16**, the C31−C46 terminal vinyl bromide **4** was substituted with the corresponding C45,C46 alkyne **31** (Scheme 8) for generation of the side-chain oxazole moiety. The methyl ester of the terminal alkyne served previously as a precursor to the *trans*-vinyl bromide for the synthesis of **1**.[6] However, avoiding the hydrostannation−bromination sequence provided a slight gain in mass throughput while retaining a

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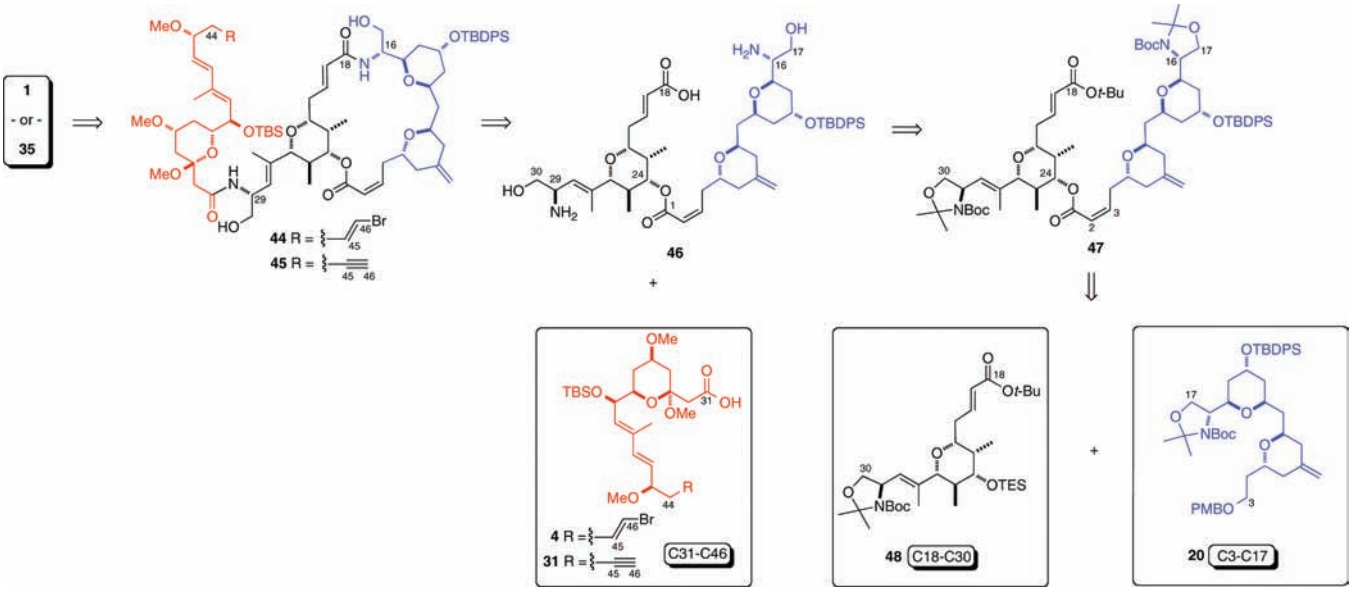
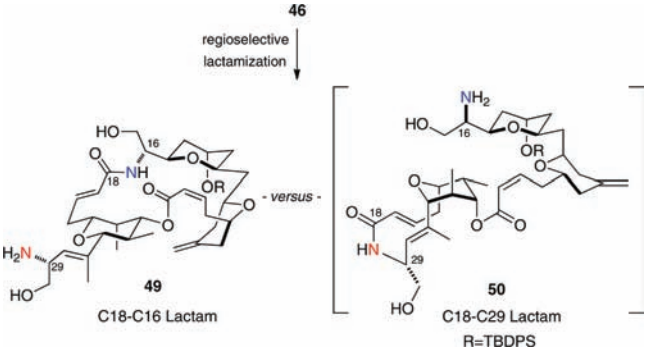
Scheme 9. Fragment Coupling Design via Simultaneous Oxazole Formation



synthetically versatile functional handle at the phorboxazole terminus. Paralleling the steps used in the synthesis of **1**, the coupling of amino alcohol **3** (derived from carbamate **16**) with alkyne-carboxylic acid **31** led directly to the semi-deprotected cyclic C33 ketal **34** and fully deprotected hemiketal **35** [(2*Z*)-45,46-dehydrobromophorboxazole, (2*Z*)-45,46-DHBPA]. Alternatively, other carboxylic acids were substituted for the C31−C46 carboxylate intermediate to form the C29−C30 oxazole moiety. This led to truncated analogues **36** and **37**, formally representing the C1−C38 and abbreviated C1−C32 portions of **1** (Scheme 8).[13] Substitutions involving the (2*E*)-macrolide intermediate **18** were also pursued. These involved the generation of the analogous (2*E*)-45,46-DHBPA (**41**) and its C33 semiprotected analogue **40**.[11] Additionally, selective reduction of the C2-alkene of **18** using Stryker's reagent paved the way to the total synthesis of 2,3-dihydro-45,46-DHBPA.[13] Interestingly, the cytostatic/cytotoxic biological activities for these analogues indicated that only minor structural modifications are tolerated in maintaining potent activities.[14,15] Specifically, among the analogues illustrated in Scheme 8, only **34** and **35** retained appreciable activities. Access to terminal alkyne **34** has allowed derivatization via Sonogashira couplings with fluorescent and biotin-containing moieties. The derived conjugates have been useful as cellular probes to study the phorboxazoles' mechanisms and modes of action.[17,26]

2. Total Synthesis of Phorboxazole A via Simultaneous Oxazole Formation (*HWE−Acrylate−Bis-oxazole*). Phorboxazole A (**1**) and the biologically active analogue 45,46-DHBPA (**35**) were obtained via a biomimetic sequential oxazole formation process, but with many transformations intervening between the assembly of each oxazole in **1** and **35**. A total of 14 steps were required to merge the three fully protected major building blocks (C3−C17, C18−C30, and C31−C46)[6,22] to obtain the bis-oxazoles **27** and **33**. Alternatively, we aspired to implement an abbreviated fragment coupling strategy that would deliver the complete phorboxazole architecture via the *simultaneous* formation of both oxazoles from C18,C31-bis-amide intermediates (i.e., **44**, Scheme 9) in a two-step oxidation−cyclodehydration process. This would provide a substantial gain in synthetic efficiency while greatly expanding the versatility of the original amide-based tri-component coupling approach that allows streamlined generation of analogues. For this, we anticipated that the C16,C29-diamino-C18-carboxylic acid **46** would preferentially form a C18−C16 lactam **49** over an alternative C18−C29 lactam **50** upon C18 carboxylate activation (Scheme 10). This key regioselective macrolactamization would leave the C29 amino group uniquely free for subsequent amidation with the C31−C46 side-chain carboxylate (i.e., **4**). Diamino acid **46** would derive from bis-carbamate-*tert*-butyl ester **47**, which in turn is the product of merging C3−C17 and C18−C30 macrolide precursors **20**[6] and **48**,[27] respectively. The *tert*-butyl ester in **47** was designed for a one-step entry to diamino acid **46** via an acid-promoted deprotection of *five* functional groups. In addition to enhancing synthetic efficiency, the designed generation of the phorboxazoles' bis-oxazoles from bis-amide intermediates also reflected an abbreviation of the biogenetic sequence of the natural products' assembly.

Scheme 10. Regioselective Macrolactamization



To implement the simultaneous oxazole formation strategy, our established C3−C17 bis-oxane **20**[6] (Scheme 2) and C18−C30 *tert*-butyl ester **48**[27] building blocks could be employed with minor functional group modifications to generate C3-aldehyde **52** and C24-phosphonoacetate **54**, respectively (Scheme 11). An

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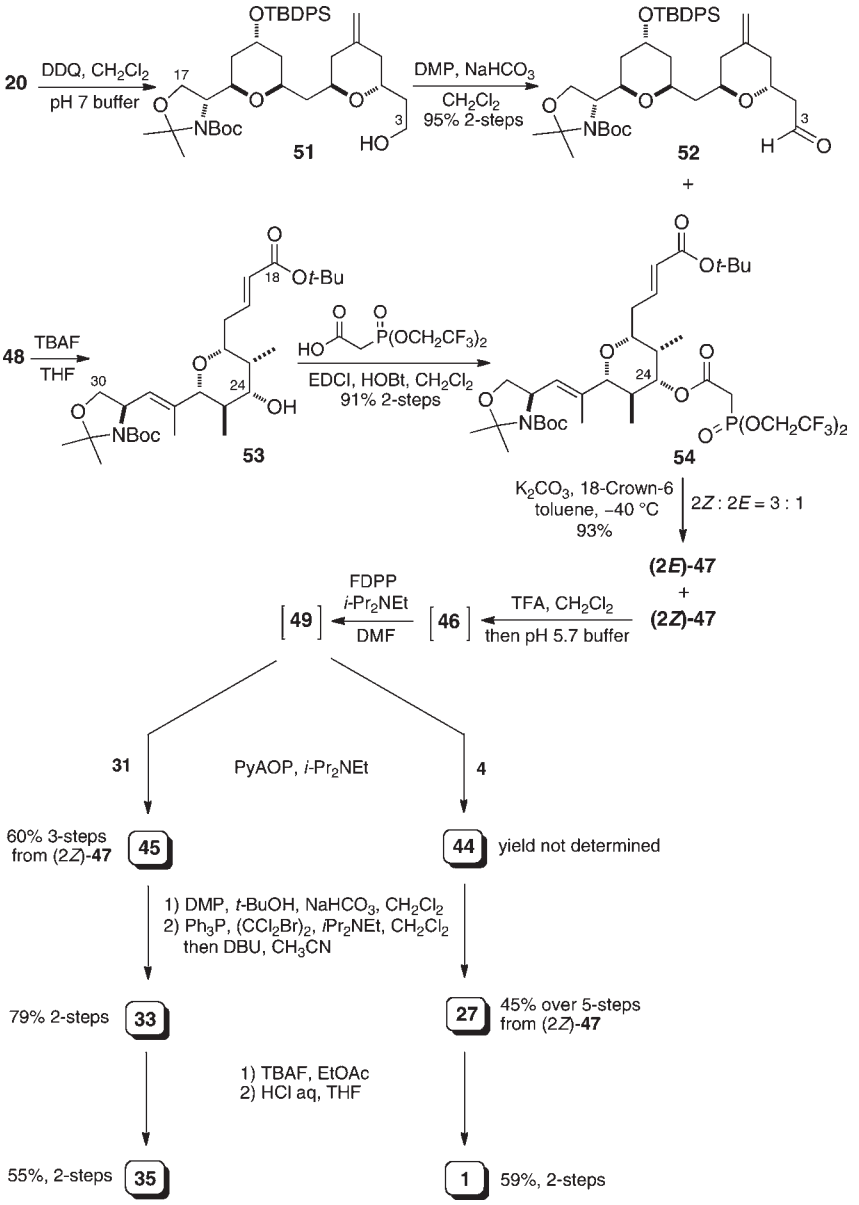
Scheme 11. Total Synthesis of 1 and 35 via Simultaneous Oxazole Formation



intermolecular Still−Gennari olefination[16] between **52** and **54** provided the C1−C30 acrylate **47** as a ca. 3:1 ratio of 2*Z*:2*E* isomers. Treatment of separated (2*Z*)-**47** with trifluoroacetic acid in dichloromethane liberated the C16 and C29 amino groups, the C17 and C30 hydroxyl groups, and the C18 carboxylic acid. A subsequent wash with pH 5.7 aqueous phosphate buffer gave **46** as its bis-ammonium salt. After an extensive survey of amide coupling reagents and solvents,[19] the regioselective lactam formation between the C16 amine and the C18 carboxylic acid of **46** was accomplished using pentafluorophenyl diphenylphosphinate (FDPP)[28] in DMF. Upon complete conversion of **46** to lactam **49** (ESI-MS monitoring, 5 h), the PyAOP[22] pre-activated C31 carboxylic acids **4** or **31**[6] were added to the provide the bisamides **44** or **45** (Scheme 9) in ca. 60% isolated yield over three steps.

Oxidation of the C17,C30 diol **44** or **45** to the corresponding bis-(*N*-acyl-α-amino-aldehyde) was accomplished with the $NaHCO_3$-buffered Dess−Martin periodinane reagent with the additive *t*-BuOH. After a rapid wash with saturated aqueous $NaHCO_3$ and $Na_2S_2O_3$, the labile bis-(*N*-acyl-α-amino-aldehyde) was obtained in sufficient purity by extraction with ether. Cyclodehydration of the bis-(*N*-acyl-α-amino-aldehyde) using our optimized condition (PPh_3/$(BrCCl_2)_2$/Hünig's base in CH_2Cl_2) led to rapid formation of the oxazole and bromooxazoline (ESI-MS and TLC analysis). Changing the solvent to CH_3CN and adding of DBU following Wipf's protocol[5b] accelerated the dehydrobromination to efficiently yield the bis-oxazole **27** or **33** in 45% and 47% yields, respectively, from (2*Z*)-**47**. Thereafter, desilylation and hydrolysis of the C33-*O*-methyl ketal yielded hemiketals **1** and **35**. This synthetic strategy provided a total synthesis of **1** in 18% yield over nine steps from macrolide components **20** and **48**.

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CONCLUSION

Coupled with the development of efficient routes to the three-component C3−C17, C18−C30, and C31−C46[6] building blocks and their structural variants, the novel coupling strategy involving intermolecular Still−Gennari olefination to form the C2−C3 alkene, sequential one-pot amidation, and simultaneous oxidative−cyclodehydrative oxazole formation provides for the rapid generation of phorboxazole A and its biologically active analogues (e.g., **35**). This delivers phorboxazole A in unprecedented chemical efficiency: ca. 6% overall yield spanning 19 steps in the longest linear sequence from the serine-derived Garner's aldehyde, which was used to prime the preparation of the C18−C30 fragment **48**. Moreover, this work demonstrates the utility of a synthetic strategy involving the *simultaneous* formation of more than one oxazole from *N*-acyl vicinal amino alcohols for complex molecule synthesis. The convergent tri-component design lends efficiency to the generation of structural analogues that may vary in each of the three domains.

ASSOCIATED CONTENT

Supporting Information. Experimental details and characterization data for compounds **1**, **5**, **8**−**12**, mixture of **14** and **15**, **16**, **17**, **19**, **21**, **23**, **24**, **26**−**28**, **32**−**35**, **38**−**41**, **44**, **45**, **47**, **48**, and **51**−**54**, including ^{1}H and ^{13}C NMR spectra of key compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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