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Phorboxazole synthetic studies: design, synthesis and biological evaluation of phorboxazole A and hemi-phorboxazole A related analogues

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ABSTRACT

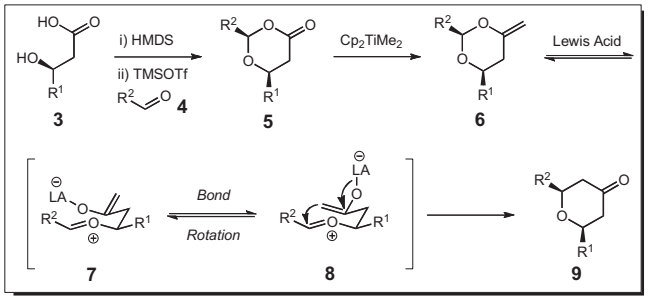
The design, synthesis and biological evaluation of a new phorboxazole analogue, comprising an acetal replacement for the **C**-ring tetrahydropyran of the natural product and carrying a potency-enhancing C(45–46) vinyl chloride side chain, is described. In addition, the synthesis of (+)-hemi-phorboxazole A and a series of related hemi-phorboxazole A analogues has been achieved. The new acetal ring replacement analogue displayed activity comparable to that of the parent natural product against HCT-116 (colon) cells (IC_{50} 2.25 ng/mL). Equally important, the phorboxazole analogue and two related hemi-phorboxazole A congeners exhibited significant antifungal activity when assayed against pathogenic *Candida albicans* strains.

1. Introduction

The seminal report by Searle and Molinski in 1995, disclosing the isolation, structural elucidation and remarkable cytotoxicity exhibited by (+)-phorboxazoles A and B (**1** and **2**), ensured that these architecturally complex marine natural products would attract considerable attention from the synthetic and bioorganic communities.[1] To date eight different research groups have collectively achieved the total syntheses of either (+)-phorboxazole A or B;[2] numerous related synthetic ventures have also been reported.[3]

In 1997, we initiated research in the phorboxazole area, with our first-generation total synthesis of (+)-phorboxazole A (**1**) recorded in 2001.[2c] The cornerstone of this synthetic venture entailed a modified Petasis–Ferrier union/rearrangement tactic (Scheme 1),[4] designed and developed specifically to orchestrate the stereocontrolled elaboration of the **A**- and **C**-ring *cis*-tetrahydropyrans embedded in the phorboxazole macrolide.

The Petasis–Ferrier union/rearrangement, comprises three steps: (**A**) generation of a dioxanone (cf. **5**) by condensation of a bis-silylated β-hydroxy acid and an aldehyde, (**B**) olefination utilizing either the Petasis[5] or Tebbe[6] protocol, and (**C**) Lewis acid-promoted rearrangement of the derived enol ether (**8**) to yield the 2,6-*cis*-disubstituted tetrahydropyranone (**9**). In addition to the total synthesis of (+)-phorboxazole A, we have also validated this versatile synthetic tactic for the construction of other complex, biologically active natural products, to include (+)-zampanolide (**10**),[7] (+)-dactylolide (**11**),[8] (+)-spongistatin 1 (**12**),[9] (−)-kendomycin (**13**),[10] (−)-clavosolide A (**14**)[11] and (−)-okilactomycin (**15**) (Fig. 1).[12]



Scheme 1.

In the phorboxazole area, the Petasis–Ferrier union/rearrangement tactic was proposed for the construction of the C(11–15) (**C**-ring) and C(22–26) (**A**-ring) 2,6-*cis*-tetrahydropyrans (Scheme 2). Initially we envisioned that the **C**-ring tetrahydropyran would arise via union of the oxazole-containing β-hydroxy acid (+)-**20** and aldehyde (−)-**21**; however treatment of the derived enol acetal (cf. **27**, Scheme 3) with Me_2AlCl, the Lewis acid demonstrated in model studies to be optimal for the rearrangement, failed to deliver

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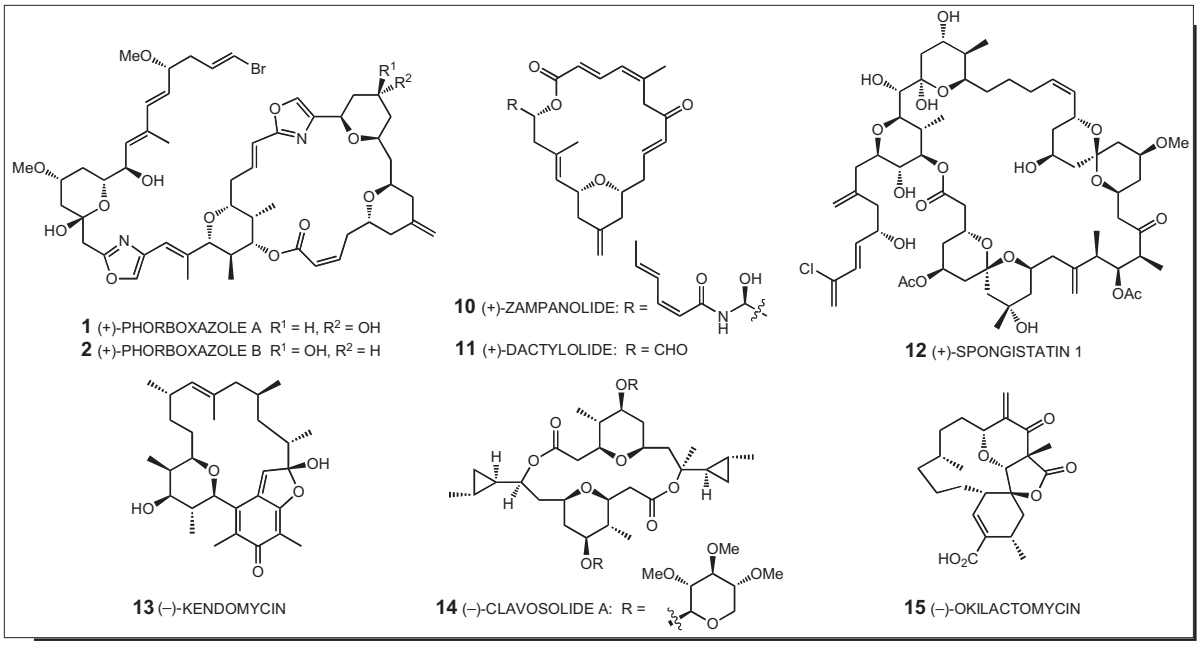
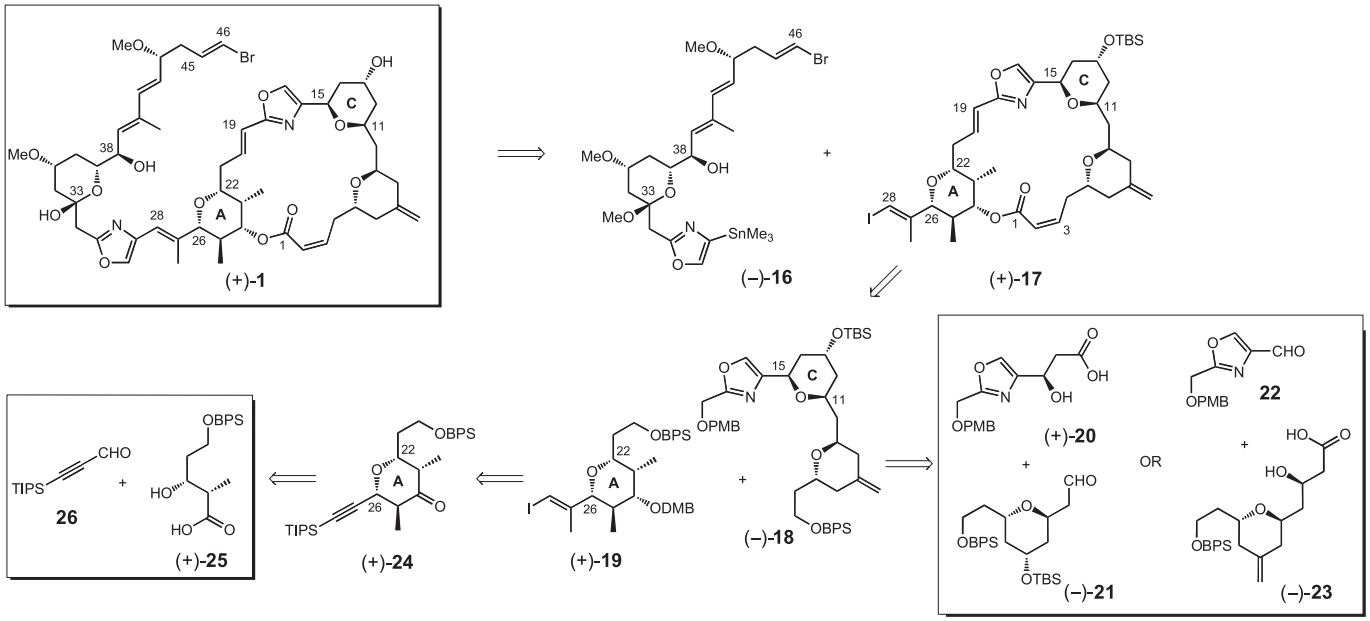


Fig. 1. Petasis–Ferrier union/rearrangement tactic in natural product synthesis.

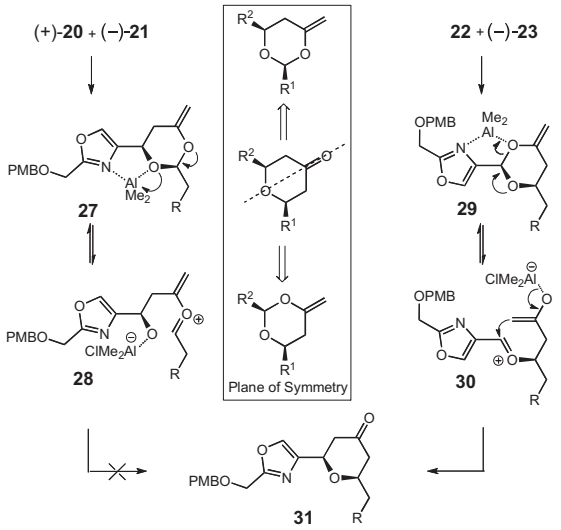
any of the required tetrahydropyranone (**31**). This result was presumed to be due to undesired chelation of the Me_2AlCl between the oxazole nitrogen and the Lewis basic acetal oxygen—a problem that was overcome by simply switching the β-hydroxy acid and aldehyde coupling partners [cf. **22** and (−)-**23**].[2c]

Thus by exploiting the inherent, albeit important from a strategic perspective, pseudo symmetry of the Petasis–Ferrier union/rearrangement, a new substrate **29** was envisioned (Scheme 3), wherein coordination to the oxazole nitrogen would direct approach of the Lewis acid to the requisite site on the acetal to permit rearrangement to the C(11–15) tetrahydropyranone (**31**). In the event tetrahydropyranone **31** was obtained in three steps and 52% overall yield from β-hydroxy acid (−)-**23**.

For preparation of the C(22–26) **A**-ring, extension of the Petasis–Ferrier union/rearrangement tactic was required to permit introduction of the equatorial methyl group at C(25). In this case, condensation of β-hydroxy acid (+)-**25** with propargylic aldehyde **26** was followed by conversion to the ethylidene acetal (**32**, Scheme 4) utilizing a Julia type-II olefination protocol.[13] Ethylidene acetal **32**, observed as a 1:1 mixture of *E*/*Z* isomers, was then subjected to the Petasis–Ferrier rearrangement. To our surprise, tetrahydropyranone (+)-**24** was isolated as a single diastereomer, possessing the desired 2,6-*cis*-disubstitution, in 91% yield. While we initially had envisioned that the *Z*-aluminum enolate (**34**) would lead, via a least motion mechanism, to the desired stereochemical outcome (+)-**24**, were the *E*-enolate to follow a similar pathway an undesired isomer (**37**)



Scheme 2.

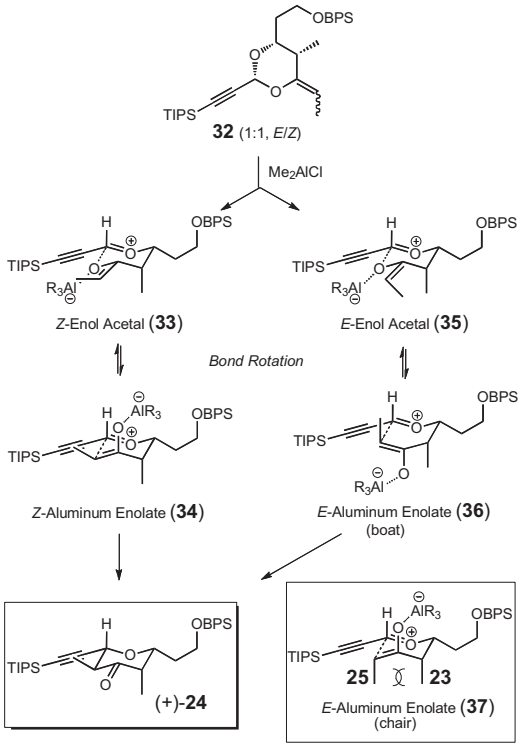


Scheme 3.

would result. However in the event, to avoid an unfavorable 1,3-diaxial interaction between C(23) and C(25) methyl group, the *E*-enolate is presumed to adopt a boat-like conformation (cf. **36**), which upon rearrangement delivers the desired C(25) equatorial methyl group in (+)-**24** (Scheme 4).

Achievement of our first-generation synthesis, featuring the Petasis–Ferrier union/rearrangement, as outlined in Scheme 2, was followed in 2005 by completion of a more efficient, scalable, second-generation total synthesis, providing (+)-phorboxazole A in 4.2% overall yield requiring a longest linear sequence of 24 steps.[14]

Since completion of the second-generation synthesis, our efforts have focused on the design, synthesis and biological evaluation of



Scheme 4.

phorboxazole A analogues.[15] Two regions of the phorboxazole skeleton were selected for variation: (**A**) the side chain terminus,[15a] and (**B**) tetrahydropyran to acetal ring replacements.[15b] With respect to variation at C(45–46) of the side chain, we reported in 2005 the discovery of a new, remarkably potent analogue, (+)-46-chlorophorboxazole **41**, demonstrating picomolar activity across a range of cancer cell lines.[15a] In addition, we have examined the effect, on biological activity, of replacement of the **C**-ring tetrahydropyran, with the synthetically simplified, but geometrically similar **C**-ring cyclic acetal by synthesis of analogue (+)-**42** (Fig. 2).[15b]

Notably, Wender et al. introduced the strategy of tetrahydropyran to acetal ring replacements as part of their elegant bryostatin program.[16] When applied in the phorboxazole area, only modest loss in potency was observed for congener (+)-**42**, featuring an acetylene at the terminus of the side chain, when compared to the parent natural product, (+)-phorboxazole A (**1**).[15b]

More recently (2009) we reported the first total synthesis of the related (+)-phorboxazole A truncated variant, (+)-hemi-phorboxazole A (**43**; Fig. 3),[17] isolated earlier that year by Dalisay and Molinski.[18] Hemi-phorboxazole A (**43**), the first example of a natural phorboxazole congener reported since the original isolation of phorboxazoles A and B in 1995, derives from the same marine sponge (*Phorbas* sp.), that not only provides the phorboxazoles, but also the unrelated phorbasides A–F[19] and muironolide A.[20] Hemi-phorboxazole A is, however, some 10 000 times less abundant than phorboxazoles A and B (0.07 vs 400 ppm, respectively). In what proved to be a remarkable structural elucidation, only 16 μg of the natural product were available to the Molinski group.[18]

Having earlier completed a second-generation, scalable synthesis of (+)-phorboxazole A, a facile two-step synthesis of (+)-hemi-phorboxazole A (**43**), utilizing an available, late-stage intermediate was readily achieved, thus demonstrating the importance of scalable syntheses of biologically important natural products, an emerging theme of our laboratory.

2. Results and discussion

2.1. Design and synthesis of phorboxazole analogues

We report here a full account of the design, synthesis and biological evaluation of a new phorboxazole analogue (**44**), comprising a **C**-ring acetal replacement, in combination with the potency-enhancing C(45–46) vinyl chloride side chain (Fig. 4). The question was, would it be possible to rescue the level of cytotoxicity lost upon simplification of the tetrahydropyran to an acetal ring by introducing the potency-enhancing vinyl chloride side chain present in analogue (+)-**41**?

We envisioned that **44** could be prepared via union of side chain (−)-**45**[15c] with vinyl iodide (+)-**46a**[15b] (Scheme 5). The synthetic route to the requisite side chain, featuring the C(46) chloride substituent, that was developed specifically for a prespective large-scale preparation of (+)-46-chlorophorboxazole **41**, would facilitate introduction of the vinyl chloride moiety early in the synthetic sequence, thereby avoiding late-stage manipulation of the side chain terminus, a tactic that proved necessary during elaboration of the bromide substituent in the natural product.[2c] Macrolide (+)-**46a** was thus prepared in accord with our previously reported synthetic route to (+)-**42** (Fig. 2).[15b]

We began with the Stille union[21] of side chain (−)-**45**[15c] to vinyl iodide (+)-**46a**[15b] to provide the complete carbon skeleton of phorboxazole analogue **44** (Scheme 6). Although the yield was at best modest (35%), sufficient material was available to proceed. Toward this end, hydrolysis of the methyl ketal at C(33), by treatment with lithium tetrafluoroborate, followed by removal of the TIPS group at C(38) furnished (+)-**44** in 84% yield for the two steps. For congener (+)-**48**, the TIPS group of (+)-**47** was removed (TBAF)

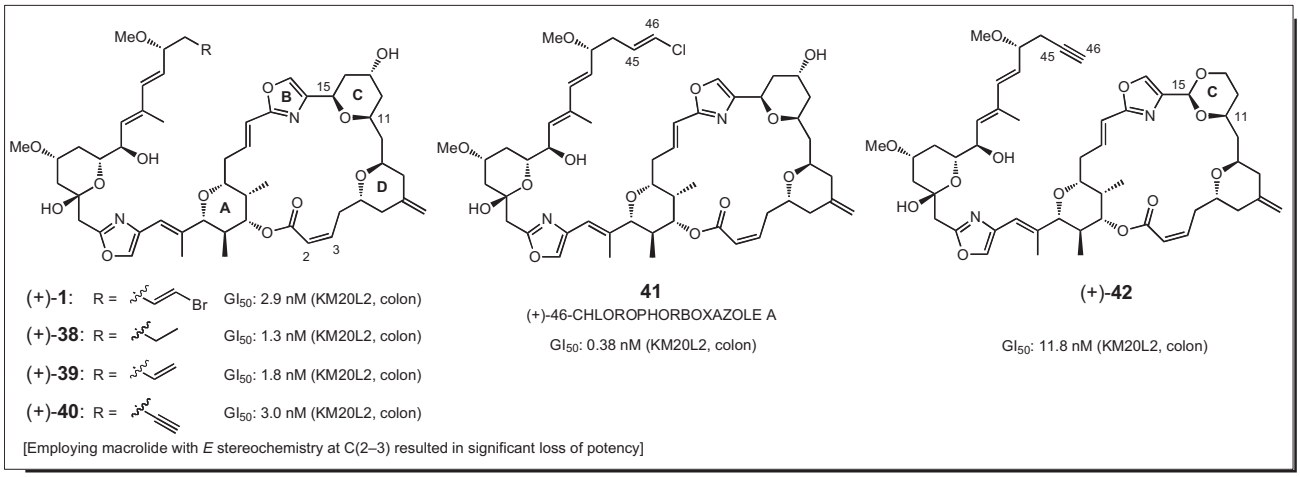


Fig. 2. Phorboxazoles A and B and analogues.

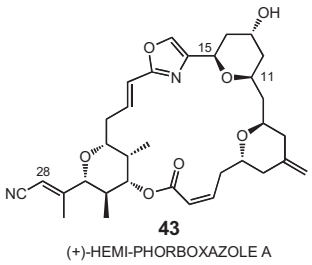


Fig. 3. Hemi-phorboxazole A.

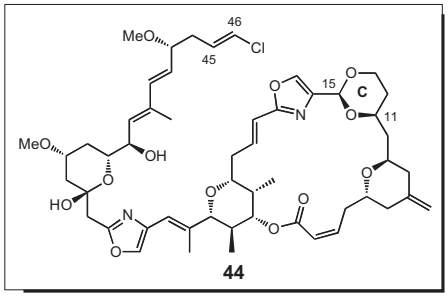


Fig. 4. Proposed phorboxazole analogue.

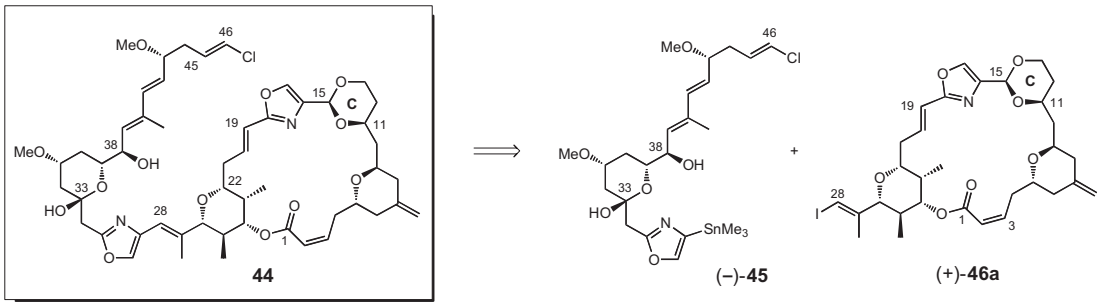
to reveal the free hydroxyl at C(38), with the C(33) mixed methyl ketal still intact. As will be presented later, the phorboxazole A analogue [(+)-**44**] displayed activity comparable to that of the parent natural product (+)-phorboxazole A (**1**).

2.2. (+)-Hemi-phorboxazole A: synthesis and analogue design

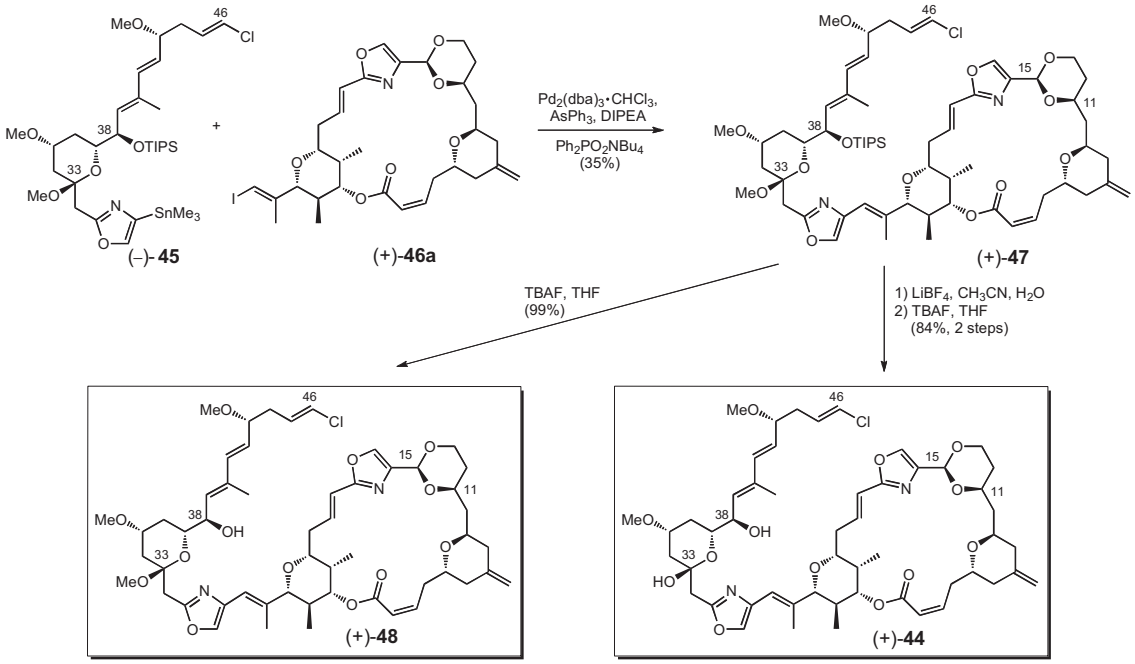
The total synthesis of (+)-hemi-phorboxazole A (**43**) was envisioned to be achieved also utilizing the advanced vinyl iodide (+)-**17**, available from our second-generation phorboxazole A synthesis.[14] To this end, removal of the TBS-ether at C(13) by exposure to tetrabutylammonium fluoride (TBAF), followed by palladium-catalyzed cyanation[22] utilizing tributyltin cyanide, provided totally synthetic (+)-hemi-phorboxazole A (**43**) in 84% yield for the two steps (Scheme 7).[17] Pleasingly, synthetic (+)-hemi-phorboxazole (**43**) was identical in all respects, including the complete relative stereochemistry and assigned absolute configuration, to that of the isolated natural product.

Having achieved the synthesis of hemi-phorboxazole A, we turned to hemi-phorboxazole analogue synthesis. Initially, we envisioned two congeners featuring ring replacements within the phorboxazole macrolide: (**A**) replacement of the **C**-ring tetrahydropyran with an acetal [cf. (+)-**49a**] similar to phorboxazole analogue (+)-**44**, and (**B**) a bis-ring replacement comprising an acetal for the **C**-ring tetrahydropyran and a phenyl ring for the ***B***-ring oxazole [cf. (−)-**50a**] (Fig. 5).

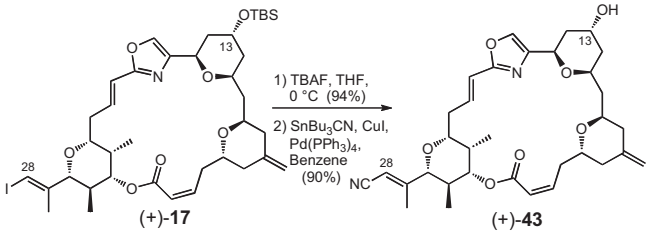
We also recognized from previous experience, that the Horner–Emmons macrocyclization would not be highly stereoselective,



Scheme 5.



Scheme 6.



Scheme 7.

thus presenting the opportunity to vary the macrolide conformation with the *E* configuration of the C(2–3) olefin. Taken together these analogues were designed with the goal of introducing synthetic simplicity, while maintaining the potentially important macrolide conformation of the natural product derived by molecular modeling (Fig. 6).

Acetal replacement congener (+)-**49a** was prepared in one step from our previously reported macrolide, vinyl iodide (+)-**46a**[15b] (Scheme 8). Specifically, treatment of (+)-**46a** with tributyltin cyanide, employing palladium catalysis, afforded nitrile (+)-**49a** in 90% yield.

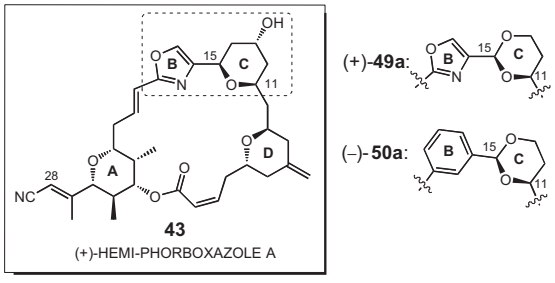


Fig. 5. Hemi-phorboxazole A and analogues.

For the bis-ring replacement congener [(–)-**50a**] we envisioned vinyl iodide **51a** to comprise an advanced intermediate. From the synthetic perspective, macrolide **51a** would be obtained via union of aldehyde (+)-**52** with phosphonium salt **53** (Scheme 9). Fragment **53** in turn, containing the key C(11–15) *cis*-acetal, would derive via condensation of aldehyde **54** with diol (–)-**55**. Both aldehyde (+)-**52** and diol (–)-**55** were prepared according to the synthetic routes reported previously.[14,15]

We began the synthesis of analogue **50a** with the preparation of the requisite aryl aldehyde **54**, available in two steps from com-

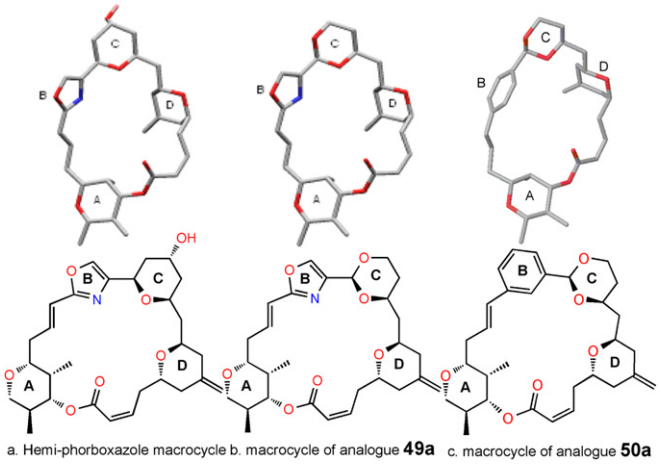
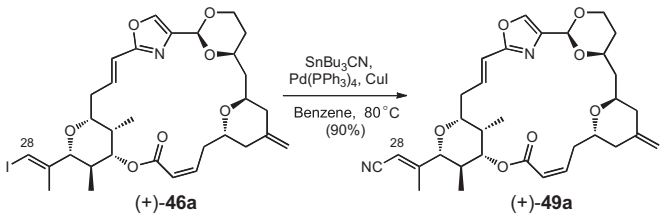


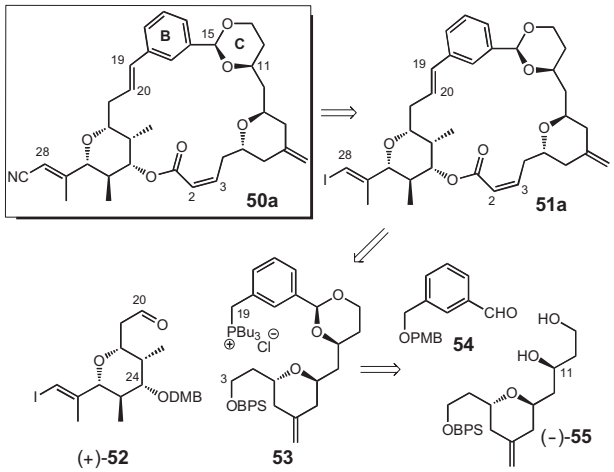
Fig. 6. Conformation of macrocycle.

mercially available 1,3-benzenedimethanol (Scheme 10).[17] Subsequent condensation of **54** with diol (–)-**55** in the presence of catalytic 10-camphorsulfonic acid furnished *cis*-acetal (+)-**56** in 94% yield as a single diastereomer (^{1}H NMR). With (+)-**56** in hand, removal of the PMB group at C(19) followed by conversion of the resultant alcohol to the corresponding chloride and generation of the phosphonium salt, by treatment with tri-*n*-butylphosphine in DMF, provided (–)-**53** in 87% yield for the three steps. Next, union of



Scheme 8.

(−)-**53** with aldehyde (+)-**52**, to furnish the C(19–20) olefin, was achieved by Wittig reaction.[23] The optimum reaction conditions, utilizing KO^{t}Bu as base in a THF/toluene solvent mixture, provided styrene **57** in 94% yield as a mixture of *E*/*Z* isomers (4:1). This mixture was carried through a three-step sequence involving



Scheme 9.

removal of the BPS-ether at C(3), oxidation of the resultant alcohol to furnish the corresponding aldehyde, and removal of the DMB group to reveal the C(24) secondary alcohol, to provide hydroxyaldehyde **58** in 58% yield for the three steps. Macrocyclization of **58**, employing the Still–Gennari modified-Horner–Emmons olefination protocol,[24] then furnished the (2*Z*,19*E*) macrolide (−)-**51a** in 44% yield. The corresponding (2*E*,19*E*) and (2*E*,19*Z*) macrolides (**51b** and **51c**, respectively) were also isolated as a mixture (4:1) in 15% yield. Finally, introduction of the cyanide at C(28) was achieved, as with the generation of (+)-**49a**, by palladium-catalyzed reaction of vinyl iodide (−)-**51a** with tributyltin cyanide to furnish the desired bis-ring replacement congener (−)-**50a** in 93% yield.

Based on the synthetic route described above, additional hemiphorboxazole analogues were designed featuring two variations: (**A**) the macrolide conformation, with changes in olefin geometry at C(2–3) and C(19–20); and (**B**) the substituent at C(28) (Fig. 7).

Since the Wittig reaction of aldehyde (+)-**52** with phosphonium salt (−)-**53** furnished a mixture of C(19–20) isomers (4:1=*E*/*Z*), variation of the alkene stereochemistry at this point could also be readily achieved. That is, advanced styrene **57** was carried through the remainder of the synthetic sequence as a mixture of olefin isomers, with separation achieved after macrocyclization. Thus, exposure of the mixture of **51b** and **51c**, isolated from macrocyclization of **58** (cf. Scheme 10), to tributyltin cyanide in the presence of palladium provided (−)-**50b** and (−)-**50c** in 71% and 19% yields, respectively (Scheme 11). Importantly, all three isomers of **50** [(2*Z*,19*E*), (2*E*,19*E*) and (2*E*,19*Z*)], as well as those of the vinyl iodide precursor **51** [e.g., the (2*E*,19*E*) and (2*E*,19*Z*) isomers] could be separated by preparative HPLC, and thus were submitted for biological evaluation. In addition, preparation of the (2*E*,19*E*) isomer of congener **49** (cf. **49b**) was achieved in an analogous manner, from (+)-**46b**, as outlined in Scheme 12.

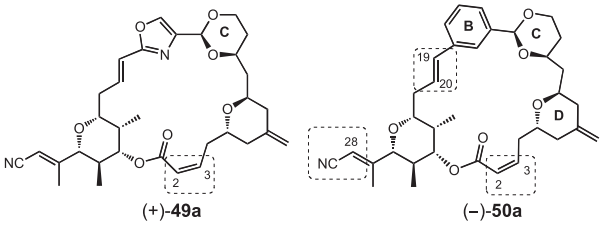
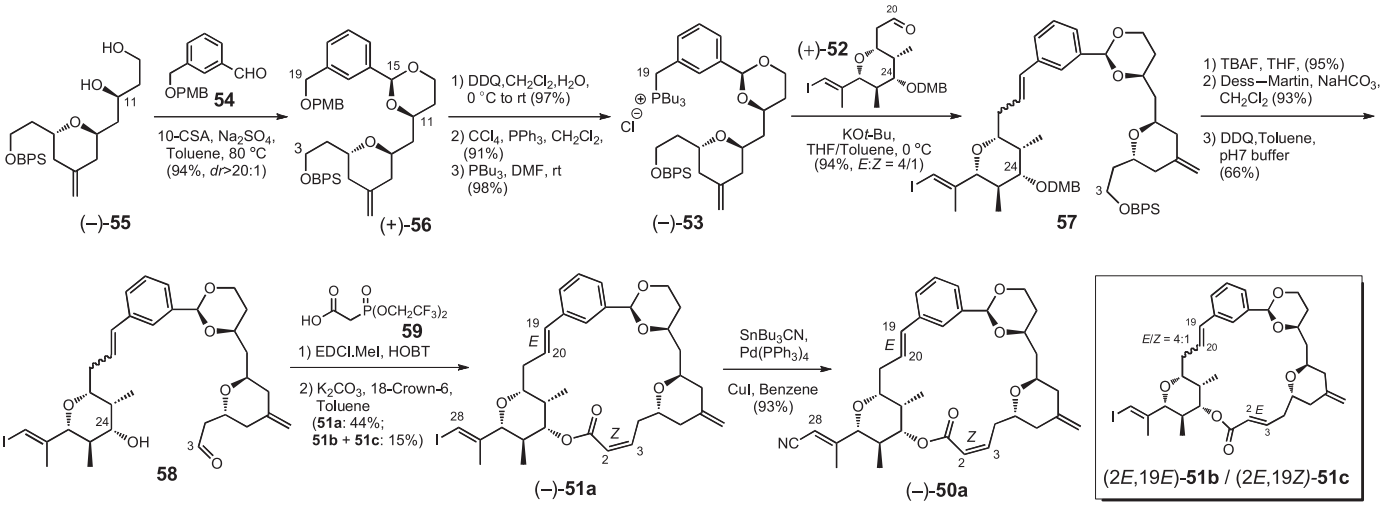
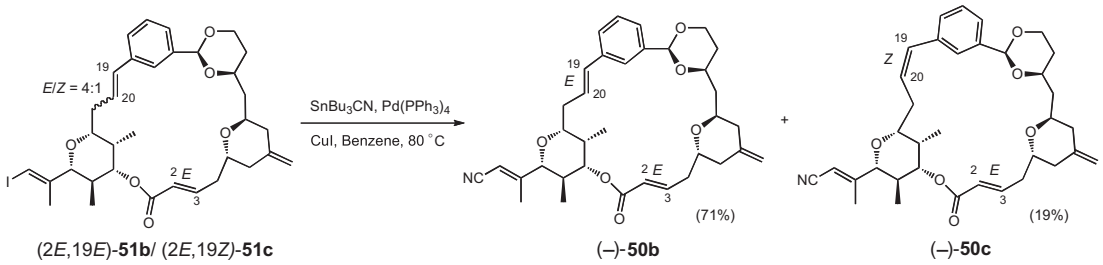


Fig. 7. Sites of variation for further analogues of hemi-phorboxazole A.

Finally, we turned attention to variation of the substituent at C(28), employing analogue (−)-**50a**. To this end, Stille coupling of vinyl iodide (−)-**51a** with appropriate tributylstannyl agents permitted introduction of furan, oxazole, thiophene and thiazole moieties, respectively, at C(28). Congeners (+)-**60**, (+)-**61**, (+)-**62** and (+)-**63** were obtained in yields of 73–90% (Scheme 13).

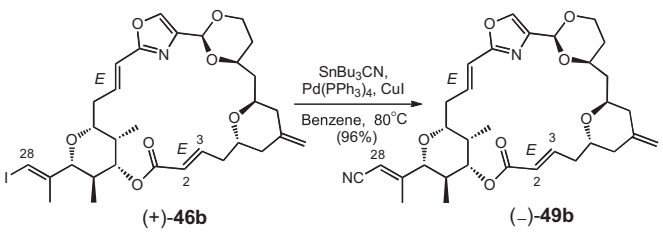


Scheme 10.



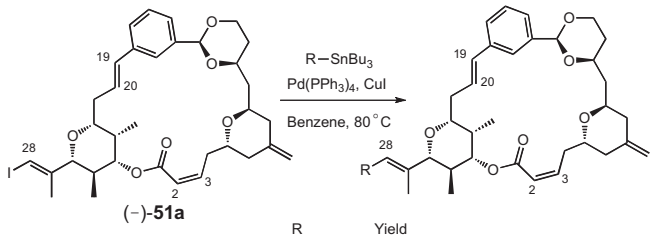
(2*E*,19*E*)-**51b**/ (2*E*,19*Z*)-**51c** (–)-**50b** (–)-**50c**

Scheme 11.



(+)-**46b** (–)-**49b**

Scheme 12.



(–)-**51a**

	R	Yield
(+)-**60**:		90%
(+)-**61**:		76%
(+)-**62**:		73%
(+)-**63**:		76%

Scheme 13.

2.3. Biological results

All phorboxazole and hemi-phorboxazole congeners were assayed for tumor cell growth inhibitory activity against HCT-116 (colon) cells (Table 1). Hemi-phorboxazole A [(+)-**43**] and congeners (+)-**49a** and (−)-**50a** were also assayed against SK-BR-3 (breast) cells. Pleasingly, phorboxazole congener (+)-**44**, comprising an acetal for the **C**-ring tetrahydropyran in conjunction with the C(46) chloride, exhibited good cytotoxicity with an IC_{50} value of 2.25 ng/mL. This result, which is comparable to that of (+)-phorboxazole A (entry 1, Table 1), is particularly encouraging from a synthetic standpoint as both structural alterations simplify the synthetic route, when compared to that of the parent natural product. Interestingly, congener (+)-**48**, with the methyl ketal at C(33), displays a significant reduction in potency (92.6 ng/mL) indicating the importance of the free hydroxyl at C(33).

Hemi-phorboxazole A [(+)-**43**], the truncated version of phorboxazole A, not surprisingly given the earlier observation by Forsyth et al.[26] that truncation of the side chain at C(32) or at C(38) leads to complete loss of cytotoxicity, proved inactive against both HCT-116 and SK-BR-3 cell lines. Interestingly however, hemi-phorboxazole analogue (−)-**50a**, possessing bis-ring replacement, displayed moderate cytotoxicity.

Table 1
Cytotoxicity of phorboxazole analogues against HCT-116 and SK-BR-3 cells[a]

Compound	HCT-116 IC_{50} (ng/mL)[b]	SK-BR-3 IC_{50} (ng/mL)[c]
(+)-Phorboxazole A (**1**) [d]	0.71	2.0
(+)-Hemi-Phorboxazole A (**43**) [d]	>6200	>6200
(+)-**49a** [d]	>6200	>6200
(−)-**50a** [d]	207	258
(+)-**44**	2.25	
(+)-**48**	92.6	
(−)-**51a**	>6250	
(−)-**51b**	>6250	
(−)-**51c**	>6250	
(+)-**46a**	>6250	
(+)-**46b**	>6250	
(−)-**50b**	>6250	
(−)-**50c**	>6250	
(−)-**49b**	1540	
(+)-**61**	5440	
(+)-**62**	5960	

[a] MTS cytotoxicity endpoint.[25]
[b] Human colon tumor line, ATCC CCL-247.
[c] Human breast tumor line.
[d] Previously reported results.[17] Hemi-phorboxazole congeners (+)-**60** and (+)-**63** were insoluble in DMSO.

The phorboxazole analogues were also assayed for antifungal activity against a series of *C. albicans* strains (Table 2). As noted earlier,[1] and confirmed here, (+)-phorboxazole A (**1**) exhibits potent antifungal activity against wild-type *C. albicans* (ATCC 14503)

Table 2
Antifungal activity of phorboxazole analogues in microbroth dilution assay (MIC, μg/mL) against pathogenic *Candida* strains.[a] NCCLS[28]

Compound	*C. albicans* (MIC, μg/mL)		
	ATCC 14503	UCD-FR1[b]	96–489 [b]
(+)-Phorboxazole A (**1**)[c]	1.0	2.0	1.0
(+)-Hemi-phorboxazole A (**43**)[c]	>64	>64	>64
(+)-**49a** [c]	16	16	>64
(+)-**44**	2	8	4
(+)-**48**	>64	>64	>64
(−)-**51a**	>64	>64	>64
(−)-**51b**	>64	>64	>64
(−)-**51c**	>64	>64	>64
(+)-**46a**	>64	>64	>64
(+)-**46b**	>64	>64	>64
(−)-**50b**	>64	>64	>64
(−)-**50c**	>64	>64	>64
(−)-**49b**	4	8	32
(+)-**61**	4	8	32
(+)-**62**	8	16	32

[a] NCCLS standard.
[b] For details of strains, see Ref 27.
[c] Previously reported results.[17]

with a minimum inhibitory concentration (MIC) of 1.0 μg/mL. Phorboxazole A [(+)-**1**] is also active against the fluconazole-resistant strains UCD-FR1 and 96–489[27] with MIC of 2.0 and 1.0 μg/mL, respectively. Congener (+)-**44** was the second most active, with MIC ranging from 2 to 8 μg/mL for the three strains.

Remarkably, (−)-**49b**, (+)-**61** and (+)-**62**, geometrical and ring-substitution variants of **1** that attenuated or abolished *in vitro* activity against HCT-116 tumor cells, retained potent antifungal activity against the wild-type and UCD-FR1 strains, but not the fluconazole-resistant patient isolate, 96–489. The observed rescue of antifungal activity in the truncated analogues (−)-**49b**, (+)-**61** and (+)-**62** suggests a subtle segregation of mammalian cytotoxicity and anti-*Candida* activity in phorboxazoles and a possible dual mode of action, at least within this limited panel of *Candida* strains and analogues.

3. Summary

In summary, we have prepared a new highly potent phorboxazole congener [(+)-**44**], which incorporates a cyclic acetal for the **C**-ring tetrahydropyran of the natural product in combination with a potency-enhancing terminal vinyl chloride side chain, and displays an IC_{50} value of 2.25 ng/mL against HCT-116 (colon) cells. In addition, this congener exhibits good antifungal activity against three *C. albicans* strains. Interestingly, substitution of the C(33) hemi-ketal of this compound with the corresponding mixed methyl ketal results in significant loss of cytotoxicity and abolition of antifungal activity.

We also have achieved the synthesis of the related natural product (+)-hemi-phorboxazole A (**43**), as well as a series of related analogues. While (+)-hemi-phorboxazole A (**43**) proved inactive, against two tumor cell lines (HCT-116 and SK-BR-3) and *C. albicans*, one of the hemi-phorboxazole analogues [cf. (−)-**50a**], comprising a bis-ring replacement within the phorboxazole macrolide, displayed modest cytotoxicity, while three related analogues [(−)-**49b**, (+)-**61** and (+)-**62**] demonstrate rescue of antifungal activity with MICs comparable to (+)-**44**, but less than (+)-**1**.

4. Experimental section

4.1. Phorboxazole analogue (+)-44

To a solution of (+)-**47** (2.7 mg, 2.38 μmol) in acetonitrile/water (20:1, 0.3 mL) was added lithium tetrafluoroborate (1.0 M solution in CH_3CN, 40 μL). The mixture was stirred at 50 °C for 45 min before it was cooled to rt, quenched with saturated sodium bicarbonate solution (1 mL) and extracted with EtOAc (3×1 mL). The combined organic layers were dried over sodium sulfate and concentrated to dryness (2.5 mg, 2.23 μmol). To a solution of this crude residue in THF (1 mL) was added TBAF (1.0 M in THF, 33 μL) at 0 °C and the resultant solution was stirred for 4 h. The reaction was quenched with saturated sodium bicarbonate solution (1 mL) and extracted with EtOAc (3×1 mL). The combined organic layers were dried over sodium sulfate and concentrated to dryness. The crude residue was purified by preparative TLC to provide (+)-**44** (1.9 mg, 84% over two steps): $[\alpha]_D^{20}$ +36.4 (*c* 0.15, $CHCl_3$); IR (neat): 3426 (br, w), 2924 (s), 1716 (s), 1376 (s), 1091 (s) cm^{-1}; ^1H NMR (500 MHz, C_6D_6) δ 7.53 (s, 1H), 7.03 (s, 1H), 6.91 (ddd, *J*=16.1, 10.1, 6.2 Hz, 1H), 6.21 (s, 1H), 6.12 (d, *J*=15.8 Hz, 1H), 6.10 (d, *J*=15.8 Hz, 1H), 5.97 (ddd, *J*=13.3, 7.6, 7.6 Hz, 1H), 5.82 (dd, *J*=11.2, 2.2 Hz, 1H), 5.74 (d, *J*=13.3 Hz, 1H), 5.54 (d, *J*=8.4 Hz, 1H), 5.50–5.46 (m, 2H), 5.39 (dd, *J*=15.6, 7.6 Hz, 1H), 5.38 (s, 1H), 5.20 (s, 1H), 4.80 (s, 1H), 4.62 (dd, *J*=11.2, 4.4 Hz, 1H), 4.37–4.33 (m, 2H), 4.09–4.06 (m, 1H), 3.99–3.91(m, 1H), 3.83 (dd, *J*=11.2, 4.4 Hz, 1H), 3.80–3.77 (m, 1H), 3.51–3.48 (m, 1H), 3.41–3.34 (m, 3H), 3.08 (s, 3H), 3.04 (s, 3H), 2.84 (d, *J*=15.3 Hz, 2H), 2.71 (d, *J*=15.5 Hz, 2H), 2.48–2.34 (m, 4H), 2.29 (dd, *J*=12.2, 3.7 Hz, 1H), 2.14–2.09 (m, 1H), 2.06 (s, 3H), 2.07–1.95 (m, 5H), 1.94 (s, 3H), 1.63 (s, 3H), 1.55–1.45 (m, 3H), 1.34–1.25 (m, 3H), 1.05 (d, *J*=6.9 Hz, 3H), 0.86 (d, *J*=6.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.5, 161.8, 160.9, 145.6, 142.9, 141.0, 139.1, 138.2, 137.6, 137.2, 136.3, 135.1, 134.6, 131.9, 130.6, 129.5, 128.9, 127.9, 121.5, 119.8, 119.5, 118.8, 110.5, 97.9, 97.3, 89.7, 81.9, 80.3, 79.0, 73.8, 73.7, 73.3, 71.4, 69.3, 67.2, 56.4, 55.7, 41.8, 41.5, 40.2, 40.2, 38.0, 37.9, 34.9, 33.9, 33.2, 32.5, 32.4, 31.1, 14.6, 13.8, 6.5; high resolution mass spectrum (ES^+) *m*/*z* 987.4397 [$(M+Na)^+$; calcd for $C_{52}H_{69}ClN_2NaO_{13}$: 987.4386].

4.2. Phorboxazole analogue (+)-48

To a solution of (+)-**47** (3.0 mg, 2.64 μmol) in THF (0.8 mL) was added TBAF (1.0 M in THF, 0.05 mL) at 0 °C. The resultant solution was stirred for 30 min, quenched with saturated sodium bicarbonate solution (1 mL) and extracted with EtOAc (3×1 mL). The combined organic layers were dried over sodium sulfate and concentrated to dryness. The residue was purified by flash chromatography on silica gel (50% EtOAc in hexanes to 100% EtOAc) to afford (+)-**48** (2.6 mg, 99%): $[\alpha]_D^{20}$ +6.5 (*c* 0.15, $CHCl_3$); IR (neat): 3419 (br, w), 2926 (s), 1717 (s), 1459 (m), 1376 (s), 1193 (m), 1150 (m), 1090 (s) cm^{-1}; ^1H NMR (500 MHz, C_6D_6) δ: 7.54 (s, 1H), 7.11 (s, 1H), 6.90 (ddd, *J*=16.2, 9.7, 6.8 Hz, 1H), 6.36 (s, 1H), 6.16 (d, *J*=15.7 Hz, 1H), 6.08 (d, *J*=15.8 Hz, 1H), 5.97 (ddd, *J*=13.3, 7.6, 7.6 Hz, 1H), 5.82 (dd, *J*=11.2, 2.3 Hz, 1H), 5.74 (d, *J*=13.3 Hz, 1H), 5.60 (d, *J*=8.7 Hz, 1H), 5.46 (ddd, *J*=10.7, 10.7, 2.9 Hz, 1H), 5.39 (dd, *J*=15.6, 7.6 Hz, 1H), 5.38 (s, 1H), 5.21 (s, 1H), 4.80 (s, 1H), 4.62 (dd, *J*=11.2, 4.4 Hz, 1H), 4.39–4.35 (m, 2H), 4.10–4.06 (m, 1H), 4.00–3.92 (m, 1H), 3.82 (dd, *J*=11.5, 4.3 Hz, 1H), 3.62–3.56 (m, 1H), 3.52–3.47 (m, 2H), 3.43 (d, *J*=10.1 Hz, 1H), 3.40–3.33 (m, 3H), 3.18 (s, 3H), 3.04 (s, 3H), 3.03 (s, 3H), 2.90 (d, *J*=14.7 Hz, 1H), 2.84 (d, *J*=12.1 Hz, 1H), 2.69–2.62 (m, 2H), 2.54–2.47 (m, 1H), 2.46–2.34 (m, 4H), 2.14–1.94 (partially observed, m, 6H), 2.06 (s, 3H), 1.89–1.85 (m, 1H), 1.66–1.59 (partially observed, m, 1H), 1.64 (s, 3H), 1.56–1.45 (m, 2H), 1.33–1.26 (m, 2H), 1.05 (d, *J*=6.9 Hz, 3H), 0.76 (d, *J*=6.5 Hz, 3H), 0.73 (br s, 1H); ^{13}C NMR C NMR (125 MHz, C_6D_6) δ: 165.8, 161.8, 159.9, 145.5, 142.9, 141.3, 139.3, 138.4, 137.3, 137.0, 136.8, 135.1, 134.6, 131.9, 130.6, 129.6, 127.9, 121.5, 119.8, 119.5, 119.4, 110.5, 100.7, 97.9, 89.9, 81.8, 80.3, 78.9, 73.9, 73.8, 73.7, 73.6, 71.6, 69.3, 67.2, 56.4, 55.7, 48.3, 41.8, 40.3, 40.2, 37.9, 37.9, 36.2, 34.9, 33.7, 33.2, 32.5, 32.4, 31.0, 14.6, 13.8, 13.7, 6.5; high resolution mass spectrum (ES+) *m*/*z* 1001.4536 [$(M+Na)^+$; calcd for $C_{53}H_{71}ClN_2NaO_{13}$: 1001.4542].

4.3. Acetal analogue (−)-49b

Vinyl iodide (+)-**46b** (9.6 mg, 14.4 μmol), copper iodide (0.9 mg, 4.72 μmol), tetrakis(triphenylphosphine)palladium(0) (6.0 mg, 5.19 μmol) and tri-*n*-butyltincyanide (5.7 mg, 18.03 μmol) were placed in a sealed tube and anhydrous benzene (0.8 mL) added. The reaction was heated at 90 °C for 3 h. After cooling to rt, silica gel chromatography (20–35% EtOAc in hexanes) afforded (−)-**49b** as a colorless solid (7.8 mg, 96%): $[\alpha]_D^{21}$ −2.2 (*c* 0.28, C_6D_6); IR (neat): 2923 (m), 2853 (m), 2218 (w), 1719 (m), 1655 (m) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ: 7.57 (s, 1H), 6.88 (ddd, 1H, *J*=15.5, 8.6, 5.7 Hz), 6.65–6.59 (m, 1H), 6.27 (d, 1H, *J*=16.2 Hz), 5.86 (d, 1H, *J*=15.5 Hz), 5.49 (s, 1H), 5.32 (s, 1H), 4.83 (s, 1H), 4.84–4.81 (m, 1H), 4.78 (s, 1H), 4.23 (dd, 1H, *J*=11.9, 4.6 Hz), 4.10–4.06 (m, 1H), 3.76 (dt, 1H, *J*=12.2, 1.6 Hz), 3.79–3.75 (m, 1H), 3.65–3.62 (m, 2H), 3.57 (d, 1H, *J*=10.3 Hz), 2.62–2.57 (m, 1H), 2.46–2.38 (m, 2H), 2.32 (dd, 1H, *J*=13.1, 3.1 Hz), 2.27–2.20 (m, 1H), 2.09 (s, 3H), 2.02–1.98 (m, 2H), 1.95–1.90 (m, 4H), 1.80–1.72 (m, 1H), 1.40 (d, 1H, *J*=14.0 Hz), 1.34–1.30 (m, 1H), 0.93 (d, 3H, *J*=6.6 Hz), 0.76 (d, 3H, *J*=6.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$, cryoprobe) δ: 167.0, 161.4, 161.1, 147.3, 141.4, 139.8, 135.7, 134.8, 122.8, 119.1, 116.4, 111.6, 99.1, 95.1, 86.6, 77.8, 73.3, 70.8, 69.0, 66.9, 41.1, 40.4, 38.7, 37.4, 34.4, 32.4, 31.8, 29.9, 16.6, 13.0, 6.1, 1.2; high resolution mass spectrum (ES^+) *m*/*z* 565.2909 [$(M+H)^+$; calcd for $C_{32}H_{41}N_2O_7$: 565.2914].

4.4. Phenyl analogues (−)-50b and (−)-50c

Vinyl iodide **51b**/**51c** (6.7 mg, 9.93 μmol, 4:1 mixture of isomers), copper iodide (1.1 mg, 5.78 μmol), tetrakis(triphenylphosphine) palladium(0) (3.9 mg, 3.37 μmol) and tri-*n*-butyltincyanide (4.7 mg, 14.87 μmol) were placed in a sealed tube and anhydrous benzene (0.7 mL) added. The reaction was heated at 80 °C for 3.5 h. After cooling to rt, silica gel chromatography (20% EtOAc in hexanes) followed by preparative HPLC (CH_3CN/water gradient) afforded (−)-**50b** as a colorless amorphous solid (4.1 mg, 71%); (−)-**50c** was also isolated as a colorless amorphous solid (1.1 mg, 19%). (−)-**50b**: $[\alpha]_D^{21}$ −12.4 (*c* 0.2, $CDCl_3$); IR (neat): 2956 (m), 2920 (m), 2849 (m), 2352 (w), 1581 (m) cm^{-1}; ^1H NMR (500 MHz, C_6D_6) *δ*: 8.08 (s, 1H), 7.25−7.14 (m, 3H), 7.04−7.03 (m, 1H), 6.40 (d, 1H, *J*=16.1 Hz), 6.04 (d, 1H, *J*=15.6 Hz), 5.67 (ddd, 1H, *J*=15.6, 9.2, 4.8 Hz), 5.35 (s, 1H), 4.99 (dd, 1H, *J*=11.1, 4.6 Hz), 4.76 (s, 1H), 4.65 (s, 2H), 4.24−4.21 (m, 1H), 3.91 (dd, 1H, *J*=11.9, 4.9 Hz), 3.51 (dt, 1H, *J*=12.0, 2.4 Hz), 3.33−3.21 (m, 3H), 3.03 (d, 1H, *J*=10.1 Hz), 2.46−2.43 (m, 1H), 2.23−2.15 (m, 2H), 2.11−2.04 (m, 1H), 1.93−1.87 (m, 2H), 1.78−1.72 (m, 4H), 1.74 (s, 3H), 1.68−1.61 (m, 3H), 0.82 (d, 3H, *J*=6.8 Hz), 0.78 (d, 1H, *J*=12.9 Hz), 0.60 (d, 3H, *J*=6.6 Hz); ^{13}C NMR (125 MHz, C_6D_6) *δ*: 167.0, 160.7, 147.7, 142.7, 140.4, 139.3, 133.9, 127.9, 126.8, 126.2, 124.0, 116.6, 111.4, 102.4, 99.3, 86.3, 78.1, 77.5, 74.4, 71.0, 69.5, 67.4, 41.4, 40.3, 38.5, 37.7, 36.0, 35.5, 33.4, 32.4, 16.2, 13.4, 6.0; high resolution mass spectrum (ES^+) *m*/*z* 574.3185 [$(M+H)^+$; calcd for $C_{35}H_{44}O_6N$: 574.3169]. (−)-**50c**: $[\alpha]_D^{21}$ −19.3 (*c* 0.18, $CDCl_3$); IR (neat): 2958 (m), 2922 (m), 2853 (m), 2222 (w), 1659 (m) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) *δ*: 7.40 (s, 1H), 7.30−7.22 (m, 2H), 7.17−7.15 (m, 1H), 6.98 (dt, 1H, *J*=15.8, 8.4 Hz), 6.64 (d, 1H, *J*=11.7 Hz), 5.82 (d, 1H, *J*=15.8 Hz), 5.51 (dt, 1H, *J*=11.7, 4.1 Hz), 5.48 (s, 1H), 5.29 (s, 1H), 4.84 (s, 1H), 4.78 (s, 1H), 4.68 (dd, 1H, *J*=11.0, 4.9 Hz), 4.20 (dd, 1H, *J*=10.9, 5.2 Hz), 3.94−3.87 (m, 2H), 3.76−3.70 (m, 1H), 3.62−3.58 (m, 1H), 3.52−3.46 (m, 2H), 2.88−2.81 (m, 1H), 2.48−2.46 (m, 1H), 2.40−2.32 (m, 2H), 2.26−2.17 (m, 3H), 2.02 (s, 3H), 2.03−1.73 (m, 6H), 1.42 (d, 1H, *J*=12.3 Hz), 0.69 (d, 3H, *J*=6.5 Hz), 0.28 (d, 3H, *J*=6.8 Hz); ^{13}C NMR (125 Hz, $CDCl_3$, cryoprobe) *δ*: 165.8, 161.4, 146.5, 141.5, 139.4, 137.9, 132.6, 129.2, 127.7, 127.0, 126.8, 125.3, 123.0, 116.5, 111.7, 101.9, 98.9, 86.1, 78.3, 74.3, 70.7, 70.0, 67.1, 41.0, 40.7, 38.9, 37.3, 33.8, 32.2, 22.9, 16.6, 14.4, 13.2, 5.8; high resolution mass spectrum (ES^+) *m*/*z* 574.3149 [$(M+H)^+$; calcd for $C_{35}H_{44}NO_6$: 574.3169].

4.5. Furan analogue (+)-60

Vinyl iodide (−)-**51a** (5.3 mg, 7.86 μmol), copper iodide (1.1 mg, 5.78 μmol), tetrakis(triphenylphosphine)palladium(0) (3.6 mg, 3.11 μmol) and 2-(tributylstannyl)furan (3 μL, 9.52 μmol) were placed in a sealed tube and anhydrous benzene (0.5 mL) added. The reaction was heated at 80 °C for 3 h. After cooling to rt, silica gel chromatography (20% EtOAc in hexanes) afforded (+)-**60** as a colorless solid (4.3 mg, 90%): $[\alpha]_D^{17}$ +3.6 (*c* 0.28, C_6D_6); IR (neat): 2922 (m), 2851 (m), 1719 (s), 1642 (w) cm^{-1}; ^1H NMR (500 MHz, C_6D_6) *δ*: 7.72 (s, 1H), 7.64 (d, 1H, *J*=7.8 Hz), 7.10−7.08 (m, 2H), 7.04 (br s, 1H), 6.44 (d, 1H, *J*=15.9 Hz), 6.33 (s, 1H), 6.14 (s, 2H), 5.97 (dt, 1H, *J*=15.9, 7.8 Hz), 5.88 (d, 1H, *J*=11.4 Hz), 5.62 (dt, 1H, *J*=11.4, 4.6 Hz), 5.33 (s, 1H), 4.84 (s, 1H), 4.78 (dd, 1H, *J*=11.3, 4.1 Hz), 4.75 (s, 1H), 4.23−4.19 (m, 1H), 4.00−3.94 (m, 2H), 3.67−3.60 (m, 1H), 3.54−3.48 (m, 2H), 3.45−3.42 (m, 1H), 3.43 (d, 1H, *J*=9.8 Hz), 2.61−2.59 (m, 1H), 2.53−2.43 (m, 4H), 2.27 (dd, 1H, *J*=12.9, 4.6 Hz), 2.09 (s, 3H), 2.07−2.04 (m, 1H), 1.99−1.87 (m, 4H), 1.66−1.63 (m, 1H), 1.60−1.55 (m, 1H), 1.09 (d, 3H, *J*=6.7 Hz), 0.79 (d, 3H, *J*=6.7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 166.0, 152.9, 143.9, 141.8, 141.7, 138.9, 137.8, 134.7, 133.4, 128.7, 127.5, 125.4, 124.3, 123.3, 121.9, 118.4, 111.4, 110.5, 109.7, 100.7, 89.6, 79.8, 78.0, 74.2, 73.4, 68.4, 67.2, 41.4, 39.9, 37.5, 35.2, 33.0, 32.3, 31.9, 31.3, 14.0, 13.6, 6.6; high resolution mass spectrum (ES^+) *m*/*z* 637.3158 [$(M+Na)^+$; calcd for $C_{38}H_{46}O_7Na$: 637.3141].

4.6. Oxazole analogue (+)-61

Vinyl iodide (−)-**51a** (6.1 mg, 9.04 μmol), copper iodide (1.1 mg, 5.78 μmol), tetrakis(triphenylphosphine)palladium(0) (3.9 mg, 3.37 μmol) and 2-(tributylstannyl)oxazole (3 μL, 14.3 μmol) were placed in a sealed tube and anhydrous benzene (0.6 mL) added. The reaction was heated at 80 °C for 3 h. After cooling to rt, silica gel chromatography (20% EtOAc in hexanes) afforded (+)-**61** as a colorless solid (4.2 mg, 76%): $[\alpha]_D^{18}$ +21.6 (*c* 0.40, $CDCl_3$); IR (neat): 2956 (w), 2923 (m), 2851 (m), 1719 (m) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) *δ*: 7.61 (s, 1H), 7.42 (d, 1H, *J*=8.5 Hz), 7.32−7.23 (m, 3H), 7.18 (s, 1H), 6.47 (d, 1H, *J*=15.9 Hz), 6.35 (s, 1H), 6.02−5.96 (m, 2H), 5.92 (d, 1H, *J*=11.5 Hz), 5.49 (s, 1H), 4.85 (s, 1H), 4.70 (s, 1H), 4.62 (dd, 1H, *J*=11.1, 4.6 Hz), 4.32 (dd, 1H, *J*=12.3, 4.5 Hz), 4.18−4.16 (m, 1H), 4.05−4.01 (m, 1H), 4.00−3.94 (m, 2H), 3.64−3.60 (m, 2H), 3.30−3.23 (m, 1H), 2.51−2.42 (m, 4H), 2.39−2.37 (m, 1H), 2.26 (s, 3H), 2.11 (d, 1H, *J*=13.8 Hz), 2.07−1.87 (m, 5H), 1.64−1.59 (m, 1H), 1.50 (d, 1H, *J*=13.1 Hz), 1.00 (d, 3H, *J*=6.9 Hz), 0.80 (d, 3H, *J*=6.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 165.9, 161.3, 145.2, 144.1, 141.9, 138.9, 137.8, 137.7, 133.5, 128.7, 128.3, 127.5, 125.2, 124.3, 123.3, 121.9, 115.2, 110.5, 100.7, 89.0, 79.6, 78.2, 74.2, 73.4, 68.4, 67.2, 41.4, 39.9, 37.5, 35.1, 33.0, 32.4, 31.9, 31.3, 14.5, 13.4, 6.5; high resolution mass spectrum (ES^+) *m*/*z* 616.3271 [$(M+H)^+$; calcd for $C_{37}H_{46}NO_7$: 616.3274].

4.7. Thiophene analogue (+)-62

Vinyl iodide (−)-**51a** (3.6 mg, 5.33 μmol), copper iodide (0.6 mg, 3.15 μmol), tetrakis(triphenylphosphine)palladium(0) (2.2 mg, 1.90 μmol) and 2-(tributylstannyl)thiophene (3 μL, 9.44 μmol) were placed in a sealed tube and anhydrous benzene (0.4 mL) added. The reaction was heated at 80 °C for 3.5 h. After cooling to rt, silica gel chromatography (20% EtOAc in hexanes) afforded (+)-**62** as colorless solid (2.6 mg, 76%): $[\alpha]_D^{17}$ +11.0 (*c* 0.26, $CDCl_3$); IR (neat): 2923 (m), 2849 (m), 1715 (m) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) ä: 7.55−7.52 (m, 1H), 7.42−7.38 (m, 2H), 7.32−7.23 (m, 2H), 7.03−7.02 (m, 2H), 6.63 (s, 1H), 6.47 (d, 1H, *J*=16.0 Hz), 6.02−5.96 (m, 2H), 5.93 (d, 1H, *J*=11.3 Hz), 5.49 (s, 1H), 4.85 (s, 1H), 4.71 (s, 1H), 4.62 (dd, 1H, *J*=11.5, 4.5 Hz), 4.32 (dd, 1H, *J*=11.5, 4.9 Hz), 4.18−4.14 (m, 1H), 4.05−4.01 (m, 1H), 4.00−3.93 (m, 2H), 3.63−3.60 (m, 2H), 3.31−3.24 (m, 1H), 2.52−2.42 (m, 4H), 2.38−2.36 (m, 1H), 2.10 (d, 1H, *J*=13.5 Hz), 2.04 (s, 3H), 2.02−1.89 (m, 5H), 1.64−1.57 (m, 1H), 1.50 (d, 1H, *J*=13.4 Hz), 1.00 (d, 3H, *J*=6.9 Hz), 0.79 (d, 3H, *J*=6.6 Hz); ^{13}C NMR C NMR (125 MHz, $CDCl_3$) *δ*: 166.0, 143.9, 141.8, 140.5, 138.9, 137.8, 134.2, 133.4, 128.7, 127.8, 127.5, 127.0, 125.5, 125.4, 124.3, 123.3, 123.0, 121.9, 110.5, 100.7, 89.9, 79.8, 78.0, 74.2, 73.4, 68.4, 67.2, 41.4, 39.9, 37.5, 35.2, 33.0, 32.4, 31.9, 31.3, 14.1, 13.7, 6.6; high resolution mass spectrum (ES^+) *m*/*z* 631.3107 [$(M+H)^+$; calcd for $C_{38}H_{47}O_6S$: 631.3093].

4.8. Thiazole analogue (+)-63

Vinyl iodide (−)-**51a** (4.6 mg, 6.82 μmol), copper iodide (0.9 mg, 4.72 μmol), tetrakis(triphenylphosphine)palladium(0) (3.5 mg, 3.03 μmol) and 2-tributylstannylthiazole (3 μL, 9.54 μmol) were placed in a sealed tube and anhydrous benzene (0.5 mL) added. The reaction was heated at 80 °C for 3 h. After cooling to rt, silica gel chromatography (20% EtOAc in hexanes) afforded (+)-**63** as a colorless solid (3.2 mg, 73%): $[\alpha]_D^{16}$ +15.4 (*c* 0.32, $CDCl_3$); IR (neat): 2921 (m), 2853 (m), 1723 (m) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) *δ*: 7.85 (d, 1H, *J*=3.2 Hz), 7.42 (d, 1H, *J*=7.7 Hz), 7.33−7.23 (m, 4H), 6.79 (s, 1H), 6.47 (d, 1H, *J*=16.2 Hz), 6.02−5.96 (m, 2H), 5.92 (d, 1H, *J*=11.0 Hz), 5.49 (s, 1H), 4.85 (s, 1H), 4.71 (s, 1H), 4.63 (dd, 1H, *J*=11.5, 4.4 Hz), 4.32 (dd, 1H, *J*=11.1, 4.4 Hz), 4.19−4.15 (m, 1H), 4.06−4.01 (m, 1H), 4.00−3.94 (m, 2H), 3.67 (d, 1H, *J*=10.4 Hz), 3.66−3.62 (m, 1H), 3.31−3.23 (m, 1H), 2.52−2.38 (m, 5H), 2.20 (s, 3H), 2.12−2.87 (m, 6H), 1.64−1.59 (m, 1H), 1.50 (d, 1H, *J*=13.3 Hz), 1.00 (d, 3H, *J*=6.9 Hz), 0.81 (d, 3H, *J*=6.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 165.8, 164.8, 145.4,

144.0, 143.1, 141.8, 141.5, 138.8, 133.4, 128.6, 127.4, 125.1, 124.2, 123.2, 122.9, 121.8, 119.0, 110.4, 100.6, 89.3, 79.5, 78.0, 74.1, 73.3, 68.3, 67.1, 41.3, 39.8, 37.4, 35.0, 32.9, 32.4, 31.8, 31.2, 14.8, 13.5, 6.5; high resolution mass spectrum (ES^+) m/z 632.3058 [$(M+H)^+$; calcd for $C_{37}H_{46}NO_6S$: 632.3046].

4.9. Biological assays

Cytotoxicity assays were conducted with cultured HCT-116 cells (ATCC CCL-247) grown in fetal calf serum under CO_2-air, and measurement of cell growth using MTS endpoint as previously described.[25] Antifungal assays were carried out using the following cell lines, cultured in RMPI media, and measurement of cell density by OD (λ=600 nm) after 48 h using a microplate reader, as previously described:[27] *C. albicans* (ATCC 14503), and two fluconazole-resistant strains, cultured *C. albicans* UCD-FR1 and a patient isolate, 96–489.

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Supplementary data

Experimental procedures for compounds (+)-**48**, (−)-**51b** and (−)-**51c**, and NMR spectral data for all new compounds are available in Supplementary data. Supplementary data related to this article can be found online at doi:10.1016/j.tet.2010.12.043.

References and notes

1. (a) Searle, P. A.; Molinski, T. F. *J. Am. Chem. Soc.* **1995**, *117*, 8126; (b) Searle, P. A.; Molinski, T. F.; Brzezinski, L. J.; Leahy, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 9422; (c) Molinski, T. F. *Tetrahedron Lett.* **1996**, *37*, 7879.
2. (a) Forsyth, C. J.; Ahmed, F.; Cink, R. D.; Lee, C. S. *J. Am. Chem. Soc.* **1998**, *120*, 5597; (b) Evans, D. A.; Fitch, D. M.; Smith, T. E.; Cee, V. J. *J. Am. Chem. Soc.* **2000**, *122*, 10033; (c) Smith, A. B., III; Verhoest, P. R.; Minbiole, K. P.; Schelhaas, M. *J. Am. Chem. Soc.* **2001**, *123*, 4834; (d) Gonzalez, M. A.; Pattenden, G. *Angew. Chem., Int. Ed.* **2003**, *42*, 1255; (e) Williams, D. R.; Kiryanov, A. A.; Emde, U.; Clark, M. P.; Berliner, M. A.; Reeves, J. T. *Angew. Chem., Int. Ed.* **2003**, *42*, 1258; (f) Li, D.-R.; Zhang, D.-H.; Sun, C.-Y.; Zhang, J.-W.; Yang, L.; Chen, J.; Liu, B.; Su, C.; Zhou, W.-S.; Lin, G.-Q. *Chem.—Eur. J.* **2006**, *12*, 1185; (g) White, J. D.; Lee, T. H.; Kuntiyong, P. *Org. Lett.* **2006**, *8*, 6043; (h) Lucas, B. S.; Gopalsamuthiram, V.; Burke, S. D. *Angew. Chem., Int. Ed.* **2007**, *46*, 769 and references therein.
3. For additional publications towards the total synthesis of the phorboxazoles see: (a) Vitale, J. P.; Wolckenhauer, S. A.; Do, N. M.; Rychnovsky, S. D. *Org. Lett.* **2005**, *7*, 3255; (b) Yadav, J. S.; Prakash, S. J.; Gangadhar, Y. *Tetrahedron: Asymmetry* **2005**, *16*, 2722; (c) Paterson, I.; Steven, A. *Org. Biomol. Chem.* **2004**, *2*, 3026; (d) Schaus, J. V.; Panek, J. S. *Org. Lett.* **2000**, *2*, 469; (e) Wolbers, P.; Hoffman, H. M. R. *Tetrahedron* **1999**, *55*, 1905 and references therein.
4. Smith, A. B., III; Fox, R. J.; Razler, T. M. *Acc. Chem. Res.* **2008**, *41*, 675.
5. Petasis, N. A.; Bzowej, E. I. *J. Am. Chem. Soc.* **1990**, *112*, 6392.
6. Tebbe, F. N.; Parshall, G. W.; Reddy, G. S. *J. Am. Chem. Soc.* **1978**, *100*, 3611.
7. Smith, A. B., III; Safonov, I. G.; Corbett, R. M. *J. Am. Chem. Soc.* **2001**, *123*, 12426.
8. (a) Smith, A. B., III; Safonov, I. G. *Org. Lett.* **2002**, *4*, 635; (b) Smith, A. B., III; Safonov, I. G.; Corbett, R. M. *J. Am. Chem. Soc.* **2002**, *124*, 11102.
9. Smith, A. B., III; Sfouggatakis, C.; Risatti, C. A.; Sperry, J. B.; Zhu, W.; Doughty, V. A.; Tomioka, T.; Gotchev, D. B.; Bennett, C. S.; Sakamoto, S.; Atasoylu, O.; Shirakami, S.; Bauer, D.; Takeuchi, M.; Koyanagi, J.; Sakamoto, Y. *Tetrahedron* **2009**, *65*, 6489.
10. (a) Smith, A. B., III; Mesaros, E. F.; Meyer, E. A. *J. Am. Chem. Soc.* **2005**, *127*, 6948; (b) Smith, A. B., III; Mesaros, E. F.; Meyer, E. A. *J. Am. Chem. Soc.* **2006**, *128*, 5292.
11. Smith, A. B., III; Simov, V. *Org. Lett.* **2006**, *8*, 3315.
12. Smith, A. B., III; Basu, K.; Bosanac, T. *J. Am. Chem. Soc.* **2007**, *129*, 14872.
13. De Lima, C.; Julia, M.; Verpeaux, J.-N. *Synlett* **1992**, 133.
14. (a) Smith, A. B., III; Razler, T. M.; Ciavarri, J. P.; Hirose, T.; Ishikawa, T. *Org. Lett.* **2005**, *7*, 4399; (b) Smith, A. B., III; Razler, T. M.; Ciavarri, J. P.; Hirose, T.; Ishikawa, T.; Meis, R. M. *J. Org. Chem.* **2008**, *73*, 1192.
15. (a) Smith, A. B., III; Razler, T. M.; Pettit, G. R.; Chapuis, J.-C. *Org. Lett.* **2005**, *7*, 4403; (b) Smith, A. B., III; Razler, T. M.; Meis, R. M.; Pettit, G. R. *Org. Lett.* **2006**, *8*, 797; (c) Smith, A. B., III; Razler, T. M.; Meis, R. M.; Pettit, G. R. *J. Org. Chem.* **2008**, *73*, 1201.
16. Wender, P. A.; Baryza, J. L.; Bennett, C. E.; Bi, F. C.; Brenner, S. E.; Clarke, M. O.; Horan, J. C.; Kan, C.; Lacote, E.; Lippa, B.; Nell, P. G.; Turner, T. M. *J. Am. Chem. Soc.* **2002**, *124*, 13648.
17. Smith, A. B., III; Liu, Z.; Hogan, A.-M. L.; Dalisay, D. S.; Molinski, T. F. *Org. Lett.* **2009**, *11*, 3766.
18. Dalisay, D. S.; Molinski, T. F. *Org. Lett.* **2009**, *11*, 1967.
19. (a) MacMillan, J. B.; Xiong-Zhou, G.; Skepper, C. K.; Molinski, T. F. *J. Org. Chem.* **2008**, *73*, 3699; (b) Skepper, C. K.; MacMillan, J. B.; Zhou, G.-X.; Masuno, M. N.; Molinski, T. F. *J. Am. Chem. Soc.* **2007**, *129*, 4150; (c) Dalisay, D. S.; Molinski, T. F. *J. Nat. Prod.* **2009**, *72*, 739.
20. Dalisay, D. S.; Morinaka, B. I.; Skepper, C. K.; Molinski, T. F. *J. Am. Chem. Soc.* **2009**, *131*, 7552.
21. Stille, J. K. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 508.
22. For related transformations see: (a) Duffey, M. O.; LeTiran, A.; Morken, J. P. *J. Am. Chem. Soc.* **2003**, *125*, 1458; (b) Nicolaou, K. C.; Pratt, B. A.; Arseniyadis, S.; Wartmann, M.; O'Brate, A.; Giannakakou, P. *ChemMedChem* **2006**, *1*, 41.
23. Wittig, G.; Schöllkopf, U. *Chem. Ber.* **1954**, *87*, 1318.
24. Still, W. C.; Gennari, C. *Tetrahedron Lett.* **1983**, *24*, 4405.
25. MTS: 3-4,5-dimethylthiazol-2-yl)-5-3-carboxymethoxyphenyl)-2-4-sulfophenyl)-2*H*-tetrazolium inner salt), Promega CellTiter 96® Aqueous cell proliferation assay, Technical Bulletin No. 169.
26. (a) Uckun, F. M.; Forsyth, C. J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1181; (b) Uckun, F. M.; Narla, R. K.; Forsyth, C.; Lee, C.-S.; Ahmed, F.; Cink, R. D. U.S. Patent No. 6,797,721 B2, 2004.
27. Mulder, R. J.; Shafer, C. M.; Dalisay, D. S.; Molinski, T. F. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2928.
28. National Committee for Clinical Laboratory Standards *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeast*, 2nd ed.; Approved standard M27–A2. National Committee for Clinical Laboratory Standards: Wayne, PA, 2002.