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Synthesis of Antiproliferative *Cephalotaxus* Esters and Their Evaluation against Several Human Hematopoietic and Solid Tumor Cell Lines: Uncovering Differential Susceptibilities to Multidrug Resistance

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Abstract

Deoxyharringtonine (**2**), homoharringtonine (**3**), homodeoxyharringtonine (**4**), and anhydroharringtonine (**5**) are reported to be among the most potent members of the antileukemia alkaloids isolated from the *Cephalotaxus* genus. Convergent syntheses of these four natural products are described, each involving novel synthetic methods and strategies. These syntheses enabled evaluation of several advanced natural and non-natural compounds against an array of human hematopoietic and solid tumor cells. Potent cytotoxicity was observed in several cell lines previously not challenged with these alkaloids. Variations in the structure of the ester chain within this family of alkaloids confer differing activity profiles against vincristine-resistant HL-60/RV+, signalling new avenues for molecular design of these natural products to combat multi-drug resistance.

Keywords

alkaloids; antitumor agents; multidrug resistance; total synthesis

Introduction

Alcoholic extracts of the powdered leaves and stems of *Cephalotaxus* genera yield cephalotaxine (**1**, Figure 1) as the most abundant alkaloid constituent,[1,2] whose structure was unambiguously verified by X-ray crystallographic analysis.[3–6] While cephalotaxine (**1**) accounts for approximately 50% of the mass of the crude alkaloid extract mixture, many minor constituents have also been identified. Among these are several rare C3-ester derivatives, including complex variants such as deoxyharringtonine (**2**),[7] homoharringtonine (**3**),[8] homodeoxyharringtonine (**4**),[9] and anhydroharringtonine (**5**).[10]

Early biological evaluations of these alkaloids revealed that several *Cephalotaxus* esters demonstrate acute toxicity toward various murine leukemia, murine lymphoma, and human

epidermoid carcinoma cells.[8,11] Deoxyharringtonine (**2**), homoharringtonine (**3**), and homodeoxyharringtonine (**4**) exhibit IC_{50} levels of 7.5, 17, and 56 $ng\,mL^{-1}$, respectively, against P388 leukemia cells. Likewise, anhydroharringtonine (**5**) was reported to induce 98% growth inhibition of P388 leukemia cells at 1 $\mu g\,mL^{-1}$, a level comparable to that of deoxyharringtonine (**2**).^[12] By contrast, cephalotaxine (**1**) itself was found to be biologically inactive.^[13] The cytotoxic properties of the *Cephalotaxus* esters arise from reversible inhibition of protein synthesis^[14] via induction of rapid breakdown of the polyribosome, with concomitant release of the polypeptide chain.^[15] The remarkable antileukemia activity of several *Cephalotaxus* esters spawned intense investigations into their therapeutic potential. Clinical studies were first performed in the mid-1970s in China, where the seeds of *Cephalotaxus* plants had long been used in traditional medicine. These results prompted Phase I clinical evaluation of homoharringtonine (**3**) in the US in 1981,^[16] advancing to more recent phase II studies.^[17] While difficulties in production, coupled with its hematologic toxicity and susceptibility to multidrug resistance (MDR),^[18] have hindered the development of **3**, it is still viewed as a useful drug for the treatment of chronic myeloid leukemia in combination therapy.^[17]

Cephalotaxine (**1**) has received considerable and enduring attention in the arena of total synthesis. Several elegant syntheses of **1** have been reported over the past three decades. The racemic approaches have embodied several key transformations, including Nazarov cyclization,^[19] photo-stimulated $S_{RN}1$ cyclization,^[20] Claisen rearrangement,^[21,22] oxidative ring contraction,^[23] acylnitroso Diels–Alder cycloaddition,^[24,25] transannular *N*-conjugate addition,^[26,27] intramolecular alkyne hydroamination,^[28] and reductive ring expansion of tetrahydrosioquinoline intermediates.^[29,30] Non-racemic routes have featured electrophilic aromatic substitution,^[31] Heck arylation,^[32,33] Pummerer-electrophilic aromatic substitution cascade,^[34–36] and acid catalyzed ring expansion of cyclobutanol derivatives.^[37]

On the other hand, the significance of the complex *Cephalotaxus* esters (e.g., **2–5**) extends beyond that of **1** on several levels, the most prominent being their exceedingly potent antiproliferative properties. Moreover, the scarcity of these complex ester derivatives from the natural source is far more pronounced than that of **1**; complex *Cephalotaxus* esters are typically attainable in only <0.1% of the plant dry weight. Thus, a principal goal in the work described herein was the establishment of a synthetic approach to the bioactive *Cephalotaxus* esters by a route completely distinct from previous efforts.^[38] Several key elements in the synthetic strategy include (**6**, Figure 2): 1) introduction of the nitrogen atom via Neber rearrangement; 2) construction of the benzazepine core via the strain-release rearrangement of *N*-vinyl-2-aryl aziridines; 3) assembly of the spiro-fused pyrrolidine core via 1,3-dipolar cycloaddition of azomethine ylides derived from vinylogous amides; and 4) synthesis of strained variants of advanced side chain intermediates to facilitate late-stage cephalotaxine acylation. Notably, the latter three elements had not been applied to complex natural product synthesis, yet ultimately played critical roles in the non-racemic syntheses of the *Cephalotaxus* esters **2–5**.

The success of these synthetic endeavors enabled extensive cytotoxicity evaluation of several advanced natural and non-natural compounds with an array of well established human hematopoietic and solid tumor cell lines. Potent cytotoxicity was observed in several cell lines previously not challenged with these alkaloids. Moreover, comparative cytotoxicity assays reveal the potential of synthetic structural modification of this family of alkaloids to modulate susceptibility to multi-drug resistance.

Results and Discussion

Dihydro[3]benzazepine construction via strain-release rearrangement

The first challenge addressed in the synthesis of cephalotaxine (**1**) focused on construction of its seven-membered *N*-heterocycle. Strain-release [3,3]-sigmatropic rearrangements, in which a high energy three-membered ring is incorporated into the 1,5-diene system of the substrate, have been widely used for the construction of seven-membered rings. Although the all-carbon divinyl cyclopropane rearrangement has received the most attention, the heterocyclic epoxide-, thiirane-, and aziridine-containing variants are also documented.[39] However, the aziridine-to-azepine version of this transformation[40–46] has only been sporadically used in target-directed synthesis. In this context, adaptation to the synthesis of benzazepines and heterocyclic variants thereof have focused on *N*-aryl-2-vinyl aziridines to form dihydro[1]benzazepines.[47–49]

However, the [3,3]-sigmatropic rearrangement of *N*-vinyl-2-aryl aziridines to form dihydro[3]benzazepines, such as that present in **1**, had not been reported. Thus, investigations into this reaction commenced with the synthesis of a few *N*-vinyl-2-aryl aziridines (Scheme 1) via the condensation of acetophenone derivatives **7/8/9** with hydroxylamine hydrochloride to provide the corresponding oximes (**10/11/12**) in high yields (95/95/87%, respectively).[50] Each of these oximes was exposed to LiAlH_4 and $i\text{Pr}_2\text{NH}$ at elevated temperatures to induce reductive Neber rearrangement,[51] furnishing the corresponding aziridines (**13/14/15**) in good yields (76/74/88%), and providing a series of substituted 2-aryl aziridines available for *N*-vinylation. This was most conveniently accomplished via addition-elimination with the readily available alkene electrophile 3-chloro-2-cyclopentenone (**16**), prepared in one step from the reaction of 1,3-cyclopentanedione with oxalyl chloride.[52] Condensation of the two substrates **13** and **16** with expulsion of HCl provided the vinyl aziridine **17** in moderate yield (58%). By comparison, coupling of aziridine **14** or **15** with chloroenone **16** proceeded with significantly diminished efficiency, resulting in only a 16% and 26% isolated yield of vinyl aziridines **18** and **19**, respectively.

Nevertheless, access to these three 2-aryl-*N*-vinyl aziridines **17–19** allowed for investigations into the feasibility of the ring expansion rearrangement. An optimized procedure for the thermal rearrangement of aziridine **17** involved its heating in a dilute [10 mM] solution in 1,4-dioxane at 180°C, in the presence of Cs_2CO_3 , to provide the desired dihydro[3]benzazepine **23** in low yield (30%). Importantly, variation in the aromatic substituents within the aziridine substrates was found to have a significant effect on the efficiency of the rearrangement. For example, the *p*-methoxyacetophenone-derived aziridine **18** was subjected to the same thermal rearrangement conditions, resulting in its transformation to the dihydro[3]benzazepine **24** with significantly increased efficiency (52%) compared to that of its predecessor **17**→**23**. Likewise, rearrangement of aziridine **19**, incorporating the 3,4-methylenedioxy-substituted aryl group, resulted in the formation of dihydro[3]benzazepine **25** in the most efficient example of the rearrangement thus far (68%). As expected, the rearrangement proceeded with complete regioselectivity.[53,54] Rationales for the favorable effect of electronically activating groups on the aromatic ring in the rearrangement (i.e., **18/19**→**24/25**) may arise from compression of the HOMO–LUMO gap in a concerted [3,3]-sigmatropic rearrangement. Conversely, a stepwise ionic mechanism for rearrangement might also be enhanced by initial aziridine opening to form a stabilized benzylic cation.

Although the rearrangements of aziridines **17–19** all provided the corresponding dihydro[3]benzazepine products, one exception to this trend was uncovered with the *N*-vinyl-2-arylaziridine substrate **26** (Scheme 2), derived from the conjugate addition of aziridine **15** into DMAD (57%). This substrate exhibited a clear propensity for a stepwise rearrangement pathway, as heating led exclusively to the formation of the pyrrole **27**. Its formation can be

rationalized by initial aziridine opening in **26** to form the highly reactive *p*-quinone methide zwitterion **28**, presumably due to the enhanced electron-deficient character of its *N*-vinyl substituent. Subsequent 5-*exo* cyclization by the C-nucleophile onto the benzylic position provided the dihydropyrrole **29**, which underwent facile air oxidation to provide the substituted pyrrole **27**.

Despite this final example of pyrrole formation (**27**, Scheme 2), the majority of examples of successful dihydro[3]benzazepine formation (**23–25**, Scheme 1) boded well for the synthesis of cephalotaxine (**1**). However, access to the tricyclic dihydro[3]benzazepine **25** was compromised by the low yielding condensation of aziridine **15** with β -chloroenone **16**, reflecting a trend in which π -donor substituents on the aromatic ring elicit a detrimental effect on the addition–elimination step. Further investigation of this transformation revealed that the *N*-vinylaziridine adduct **19** has an increased susceptibility to nucleophilic attack at its benzylic position, resulting in post-coupling chloride-mediated aziridine cleavage. Thus, a minor variation in the protocol to prepare dihydro[3]benzazepine **25** was implemented (Scheme 3). The addition of aziridine **15** into chloroenone **16** was conducted at elevated temperature, resulting in the isolation of benzylic chloride **30** (64%). Treatment of β -chloroamine **30** with Cs₂CO₃ in THF led to the generation of the desired dihydro[3]benzazepine **25** (68%), presumably via re-formation of the aziridine functionality in situ and subsequent rearrangement. This sequence provided a means for large scale access to dihydro[3]benzazepine **25**, facilitating investigation into the challenge of pyrrolidine construction.

Pyrrolidine construction via azomethine ylide 1,3-dipolar cycloaddition

The azomethine ylide 1,3-dipolar cycloaddition is a powerful tool for the synthesis of highly substituted pyrrolidine rings within many complex alkaloid targets.[55–59] Many methods exist for the generation of these transient 4 π -electron dipoles, both in stabilized and non-stabilized forms, wherein a common approach to the formation of the latter involves the desilylation of iminium salt intermediates. This strategy, first developed by Vedejs,[55,60] has seen use in a variety of complex molecule syntheses and has spawned a number of variants. In particular, a method of Padwa involves *N*-alkylation of vinylogous imidates with trimethylsilylmethyl electrophiles followed by desilylation.[61] Recently, we disclosed a complementary strategy to generate non-stabilized azomethine ylides from *N*-CH₂TMS substituted tertiary vinylogous amides via initial *O*-activation followed by desilylation.[62]

This method was found to be suitable for the generation of pyrrolidine structures bearing a fully substituted carbon at the α -position, a structure that directly maps onto the C5-spiro-fused pyrrolidine substructure within cephalotaxine (**1**). These efforts commenced with *N*-alkylation of dihydro[3]benzazepine **25** (Scheme 4), accomplished with TMSCH₂I to afford the tertiary vinylogous amide **31** (62%). Carbonyl *O*-activation of vinylogous amide **31** was performed by treatment with Tf₂O. This was followed by the sequential addition of DMAD as an activated dipolarophile and tetrabutylammonium triphenylsilyldifluorosilicate (TBAT)[63] as the desilylating agent. The cycloadduct **33**, incorporating the C5-spiro-fused pyrrolidine core of cephalotaxine, was isolated in 53% yield, indicating successful generation and cycloaddition of the azomethine ylide **32**.

Azomethine ylide generation from vinylogous amides via sequential *O*-sulfonylation and nucleophilic exchange

The successful synthesis of pyrrolidine **33** provided rapid access to the complete pentacyclic core of cephalotaxine. Moreover, a vinyl triflate moiety was installed at C3, the position of acyl chain attachment in the *Cephalotaxus* esters. While a number of avenues could have been pursued to use this functionality as a direct precursor for installation of the acyl side chain,

there existed the possibility of adapting this key cycloaddition step not only to pyrrolidine formation, but also for concomitant installation of the acyl chain.

Implicit in this vinylogous amide activation protocol is the initial formation of the C3-vinylogous iminium triflate **34** (Scheme 5). Vinylogous iminium triflates have been demonstrated to engage in electrophilic substitution reactions at the enol triflate carbon center. [64,65] That intermediates such as **34** are susceptible to nucleophilic attack suggested the possibility of its interception with an external nucleophile (Nu) prior to azomethine ylide formation and cycloaddition (**34**→**35**→**36**→**38**). This presented the prospect of directly introducing the *Cephalotaxus* ester side chain in the pyrrolidine-forming event. Additionally, this pursuit may find general utility in the preparation of differentially functionalized pyrrolidines from vinylogous amide precursors.

The hypothesis was evaluated with a simple model vinylogous amide **39** (Table 1), which was activated with TiF_2O . Subsequent introduction of an activated dipolarophile (DMAD), a variety of halide nucleophiles, and TBAT, led to rapid cycloaddition at 23°C. Importantly, the external halide nucleophiles were successfully incorporated into the cycloadducts **40–42** (entries 1–3), thereby validating the feasibility of this in situ nucleophilic exchange protocol for azomethine ylide cycloadditions.

The concept was further extended to that of the *Cephalotaxus* esters, involving exchange with external carboxylate nucleophiles. Activation of the dihydro[3]benzazepine-derived vinylogous amide **31** (Scheme 6a) with TiF_2O was performed to provide the corresponding transient triflyl imidate. Prior to ylide formation via desilylation, triethylammonium benzoate was introduced to generate the corresponding acylimidate, which underwent subsequent azomethine ylide formation with TBAT and cycloaddition with DMAD to provide the C3-substituted cycloadduct **43** in 35% yield. A significantly improved efficiency for this reaction was achieved with cesium benzoate as the nucleophilic species, affording the cycloadduct **43** in 64% yield. While this promising result presented a convenient method for transient nucleophilic exchange in an azomethine ylide cycloaddition, the ultimate purpose for which it was developed, that of introduction of an intact *Cephalotaxus* ester side chain in the cycloaddition event, met with no success. For example, the racemic cesium carboxylate **44** (Scheme 6b) was prepared from itaconic acid via a modification of the sequence of Weinreb, [66] and was introduced as a nucleophilic exchange reagent for the azomethine ylide cycloaddition with vinylogous amide precursor **31**. Unfortunately, *none* of the desired cycloadduct **45**, incorporating the deoxyharringtonine acyl chain, was detected in this operation, despite extensive attempts at optimization.

Asymmetric synthesis of (–)-cephalotaxine (1)—Azomethine ylide generation and cycloaddition via O-acylation of vinylogous amides

The varied difficulties encountered in the above-mentioned synthetic approach prompted an alteration in strategy. While the aziridine-rearrangement/dipolar-cycloaddition reactions (Schemes 5 and 10) remained at the heart of the synthetic plan, the goal of installing the acyl chain in an operation concomitant with azomethine ylide cycloaddition was set aside in favor of pursuing an asymmetric construction of the cephalotaxine core 1 as the initial target. Investigations on this front were initiated to determine the responsiveness of the 1,3-dipolar cycloaddition reaction to elements of relative stereochemical control in the formation of the C5-spiro ring fusion. Thus, a chiral azomethine ylide such as **46** (Scheme 7), incorporating proximal C1 and C2 substituents, was anticipated to bias facial-selective approach of the dipolarophile.[67] Such a substrate was envisioned to take the form of β -chloroenone **53** (Scheme 8), which could be prepared in non-racemic form from D-ribose.

The early incarnation of the synthesis of chloroenone **53** relied on a key olefination sequence first reported by Borchardt and coworkers,[68–70] and indeed provided initial quantities of β -chloroenone **53** for investigation.[38] However, the unpredictability of the above-mentioned olefination reaction crippled subsequent attempts at securing larger workable quantities of this intermediate. As a result, a second generation synthesis of **53** was developed (Scheme 8). The selectively protected D-ribofuranose **48**[71] was treated with triphenylphosphonium methylide to effect C1 olefination (75%). This was followed by C4 oxidation ($\text{SO}_3\cdot\text{Pyr}$) to afford enone **49** (88%). Addition of vinyl magnesium bromine to ketone **49** proceeded stereoselectively (8:1 dr) via Cram chelation control to provide the allylic alcohol **50** (93%), whose 1,6-diene functionality underwent ring closing olefin metathesis (Grubbs II) to afford the cyclopentene **51** (95%).[72] Regioselective chloroselenylation of the alkene within **51** followed by selenide oxidation and elimination afforded the chlorocyclopentene **52** (98%). Finally, silyl ether deprotection revealed a vicinal diol (99%), which underwent periodate-mediated oxidative cleavage to furnish the chiral β -chloroenone **53** (90%) in a robust and scalable synthetic sequence.

Use of β -chloroenone **53** in the synthesis of the dihydro[3]benzazepine core of cephalotaxine (**1**) involved addition-elimination with the racemic aziridine nucleophile **15** at ambient temperature (Scheme 9). This afforded a 1:1 diastereomeric mixture of the *N*-vinyl aziridine **54** (85%), interestingly with no evidence of chloride induced aziridine opening (cf. **30**, Scheme 3). Heating of a dilute solution of **54** in 1,4-dioxane led to efficient rearrangement to afford the dihydro[3]benzazepine **55** (76%).

It is worth noting that although the rearrangement precursor **54** existed as a 1:1 mixture of diastereomers, the formation of **55** proceeded in >50% yield. This implies that the C11-*R* diastereomer **54a** (Scheme 10) likely proceeded through an aziridine rupture step prior to azepine formation. For example, if the rearrangement occurred in a concerted fashion, a strain-release variant involving an internal aziridine ring would necessitate an *endo*-disposed boat-like transition state (Scheme 10), such as **56a** for the C11-*R*-diastereomer **54a**, or **56b** for the C11-*S*-diastereomer **54b**. While the concerted conversion of the C11-*S*-diastereomer **54b** to **57** via the transition state **56b** appears reasonable, direct rearrangement of the C11-*R*-diastereomer **54a** is unlikely, given the severe steric interaction between the aryl ring and the isopropylidene ketal in transition state **56a**. As a consequence, the C11-*R*-diastereomer **54a** could relieve this strain by first forming the *p*-quinone methide zwitterion **58** followed by re-closure to the C11-*S*-diastereomer **54b** prior to sigmatropic rearrangement via **56b**. Conversely, if a stepwise ionic mechanism is invoked, both aziridine diastereomers may open to the common *p*-quinone methide zwitterion **58**, followed by 7-*exo-trig* cyclization to afford the azepine **57**.

At this stage, advancement of the pentacyclic dihydro[3]-benzazepine **55** to cephalotaxine (**1**) relied on the 1,2-di-*O*-isopropylidene substituent to serve as a chiral controller in establishing the stereoselectivity of the key azomethine ylide cycloaddition. The dihydro[3] benzazepine **55** (Scheme 11) was *N*-alkylated with TMSCH_2I to afford the tertiary vinylogous amide **59**. *O*-Activation of the vinylogous amide group in **59** was then investigated with an electrophilic agent distinct from Tf_2O in order to preclude any possibility of nucleophilic exchange involving the transient iminium intermediate (see above). As a result, the highly reactive acyl electrophile, pivaloyl triflate, generated in situ by the reagent combination of pivaloyl chloride and AgOTf ,[73] proved suitable for this purpose. Subsequent desilylation with TBAT led to azomethine ylide formation (**60**) and cycloaddition with phenyl vinyl sulfone, affording the spiro-fused pyrrolidine **62** (77%) as a single constitutional stereo-isomer. This high level of stereoselectivity in the cycloaddition signals the effectiveness of the C1–C2 isopropylidene ketal as a stereodetermining element, albeit with an unanticipated result.[74]

With the formation of the putative non-stabilized azomethine ylide **60**, the phenylvinyl sulfone dipolarophile was initially thought to approach the dipole face distal to the isopropylidene ketal (i.e., **63**) in an early transition state. This would lead to the generation of a C5-*S* cycloadduct **64**, which would be appropriate for the synthesis of *ent*-(**1**) as an enantiomeric model system. However, the sole product of cycloaddition, **62**, possessed the C5-*R* configuration, verified by single crystal X-ray analysis. While this unexpected outcome provided a convenient means to access the natural enantiomer of cephalotaxine from naturally abundant D-ribose, the reason for the stereochemical outcome is unclear. Favorable bias for transition state **61** over **63** could be rationalized in a late transition state model where the nitrogen atom is significantly pyramidalized.[75] As a consequence, transition structure **61**, with α -approach of the dipolarophile, would lead to a smaller net dipole given that the developing nitrogen lone pair is oriented opposite to that of the electronegative oxygen atoms of the isopropylidene ketal. By contrast, β -approach of the dipolarophile (**63**) would lead to an enhancement of a net dipole, despite a more sterically forgiving arrangement of atoms. This dipole moment rationalization, be it in a concerted cycloaddition or a stepwise ionic mechanism, is of course predicated on a kinetically controlled reaction. Indeed, one cannot discount the possibility of thermodynamic selection via either a reversible cycloaddition process, or post-cycloaddition C5-epimerization pathways such as reversible trans-annular ring fragmentation. Unfortunately, these hypotheses could not be explored since the C5-*S* diastereomer **64** could not be detected.

The remaining sequence in the non-racemic synthesis of (–)-cephalotaxine (**1**) involved functional group manipulations of hexacyclic cycloadduct **62** (Scheme 12). Reductive desulfurization of **62** to produce pyrrolidine **65** (74%) proceeded with SmI_2 in the presence of HMPA[76,77] with 10 equiv of *t*BuOH as a proton source to avoid rupture of the pyrrolidine ring via elimination.[78] Subsequent extensive experimentation revealed that the pivaloate enol ester moiety in **65** was recalcitrant to both hydrolysis and hydrogenation.[79] As a result, reductive cleavage of the enol ester in **65** was performed with Schwartz' reagent[80,81] to provide the enol **66** (99%), which was then re-acylated with benzyl chloroformate and KHMDS to provide the enol benzyl carbonate **67** (86%). Interestingly, when Et_3N was used as base for this transformation, *N*-acylation occurred with concomitant β -elimination to afford enone **68**. Differentiation of the C1 and C2 oxygen substituents in **67** was then initiated with isopropylidene removal (99%). Regioselective derivatization of the corresponding diol proved challenging, as several attempts at regioselective silylation, acylation, and alkylation with numerous reagent combinations were unsuccessful. The only suitable derivatization protocol involved the Lewis acid catalyzed acylation procedure of Clarke and co-workers,[82–84] in which treatment of the C1,C2-diol with Boc_2O and $\text{Yb}(\text{OTf})_3$, necessarily in its polyhydrated form, led to selective C1-*O*-acylation. Subsequent C2 Oxidation using IBX furnished enone **69** (50%, from **67**), allowing for CrCl_2 -mediated reductive deoxygenation of the Boc carbonate and benzylcarbonate hydrogenolysis to provide the enol **70** (42%, 2 steps). Sequential methyl enol ether derivatization of the C2 ketone and stereoselective reduction of the C3 enol functionality with NaBH_4 [31] concluded the synthesis of (–)-cephalotaxine (**1**).[38]

Synthesis and attachment of the acyl chain of antitumor cephalotaxus esters

The bulk of the synthetic reports concerning the *Cephalotaxus* alkaloids have focused on cephalotaxine (**1**, see Figure 1). On the other hand, reports on the synthesis of natural antileukemia *Cephalotaxus* esters have been relatively scarce, likely a result of the difficulties associated with appending a fully intact acyl side chain onto the C3-OH of cephalotaxine. The challenge of such an acylation arises from extensive steric obstruction, marked by the secondary C3-hydroxyl nucleophile buried within the concave face of cephalotaxine, and exacerbated by a fully α -substituted acyl electrophile in the side chain. Indeed, the difficulty of this acylation event is highlighted in numerous semi-syntheses of the *Cephalotaxus* esters from cephalotaxine (**1**), wherein the bulk of these efforts employed a less hindered prochiral

C2'-sp² hybridized side chain derivative in the acylation event, followed by subsequent nonstereoselective functional group manipulation.[85–88] A notable exception to this strategy used an acyl chain substrate specifically appropriate for homoharringtonine in which the C1'-ester moiety was constrained as a cyclic derivative to allow for acylation with the C1'-electrophile.[89] This approach was introduced with racemic substrates and has recently evolved to non-racemic examples wherein enantio-enriched side chain substrates were prepared in >10-step sequences.[90]

Since the most pressing late-stage challenge in the synthesis of the *Cephalotaxus* esters is the efficient attachment of hindered acyl chain derivatives, an approach was explored whereby novel bond angle strain elements were imparted to these substrates to enable their use in high yielding acylations of cephalotaxine (**1**). This strategy initially led to the facile synthesis deoxyharringtonine (**2**), and subsequently to other members of this alkaloid class, namely anydroharringtonine (**5**), homoharringtonine (**3**), and homodeoxyharringtonine (**4**) (i.e., see Figure 1).

The initial steps in the synthesis of several *Cephalotaxus* acyl chains involved the application of the Seebach concept of “self-reproduction of chirality,”[91] an approach that has shown promise in the preparation of chiral non-racemic α -alkylmalates.[92,93] Beginning with (*R*)-malic acid (**71**, Scheme 13) as a readily available chiral starting material, its C1' carboxylic acid and C2' hydroxyl were tethered by a *tert*-butyl acetal upon treatment with 2,2-dimethylpropanal and TMSOTf. Only a single diastereomer of the acetal **72** was observed (82%), after which double deprotonation was induced with excess LHMDs. Although the formation of dilithium carboxylate-enolate **73** resulted in destruction of the C2' stereocenter, its stereochemical information was preserved in the chiral acetal carbon bearing the *tert*-butyl group. This sterically demanding substituent forced enolate alkylation with prenyl bromide from the distal face, thereby securing the C2'-*R* configuration in **74** (66%). Transesterification of **74** with NaOBn removed the acetal to afford benzyl ester **75** (88%) as a single enantiomer.

In an effort to facilitate the esterification of cephalotaxine (**1**), the strategy of constraining both the C2'-hydroxyl and the C1'-carboxylic acid in **75** into a β -lactone functionality such as **76** appeared attractive. The strain energy arising from endocyclic bond angle compression within β -lactone ring in **76** would necessarily induce exocyclic bond angle expansion, thereby relieving local steric congestion at the electrophilic C1' site. Moreover, the angle strain in a four-membered ring imparts higher hybrid orbital s-character in the exocyclic bonds, an effect that could result in increased C1' electrophilicity through induction. In addition, the increased p-character of the *endocyclic* bonds within the β -lactone may also aid in stabilizing the formation of C1'-acylium like intermediates in activated ester derivatives of **76** through vicinal π -delocalization. Despite these potential advantages, however, the strain associated with the β -lactone moiety in **76** could also serve to be a liability, as undesired ring expansion reaction manifolds may ensue upon C1'-ester activation.

Nevertheless, these aspects were investigated by the treatment of hydroxy acid **75** with 2,4,6-Cl₃C₆H₂COCl[94] to afford the corresponding β -lactone, which was subsequently treated with H₂ and Pd to reduce both the alkene and the benzyl ester to afford the carboxylic acid **76** (50%, 2 steps). Fortunately, activation of the acid **76** as the Yamaguchi mixed anhydride allowed for efficient acylation of cephalotaxine to form the ester **79** (81%, 23°C, <1 min) without compromising the integrity of the β -lactone. Subsequent methanolysis of the β -lactone provided (–)-deoxyharringtonine (**2**, 76%), whose spectral data was identical to that of the natural product. To get a better measure of the beneficial effects of the β -lactone moiety in the acylation step, an analogous *acyclic* acyl electrophile **78** was prepared, beginning with trimethylsilyldiazomethane treatment of hydroxy acid **75** to afford the methyl ester **77** (>99%). Acetylation of the C2' hydroxyl group in **77** followed by benzyl ester hydrolysis and alkene

hydrogenation provided the carboxylic acid **78** (74%, 2 steps), which was devoid of the ring strain elements present in the β -lactone **76**. Attempts at cephalotaxine acylation with **78** under otherwise identical conditions led to only trace quantities of protected deoxyharringtonine. Furthermore, heating of the reaction for several hours was also unsuccessful, signaling the critical beneficial effect of the β -lactone moiety in **76** in the synthesis of the bioactive cephalotaxus esters.

The successful synthesis of deoxyharringtonine (**2**) also allowed for rapid access to the antileukemia alkaloid anhydroharringtonine (**5**) through interception of the chiral hydroxy diester **77** (Scheme 14), previously prepared in the acylation studies toward **2** (see Scheme 13). This substrate was subjected to intramolecular alkene alkoxymercuration and reduction (Scheme 14) to furnish the corresponding tetrahydrofuran (77%). Subsequent benzyl ester hydrogenolysis provided the acylation precursor **80** (99%). Although the strain imparted by the tetrahydrofuran ring in **80** is significantly less than that of β -lactone **76** in the synthesis of **2**, the use of **80** in the acylation of cephalotaxine produced (–)-anhydroharringtonine (**5**) in excellent yield (99%, 23°C, 1 h), yet with a significantly extended reaction time (i.e., 1 h for **80** as opposed to <1 min for **76**). This effort furnished two natural product cephalotaxus esters (**2** and **5**), as well as a host of non-natural synthetic intermediates for expansive antitumor evaluation.

Antiproliferative activity of deoxyharringtonine (**2**), β -lactone **79**, and anhydroharringtonine (**5**)

The completion of the synthesis of **2** and **5** permitted, for the first time, an expanded evaluation of their in vitro cytotoxicity. Following the early screening of the cephalotaxus esters against murine P388 and L1210 cell lines,[95] many of the cytotoxic evaluations focused on leukemia and lymphoma, with comparatively fewer reports on activity profiles against solid tumor cell lines.[17] As a result, deoxyharringtonine (**2**), anhydroharringtonine (**5**), and the β -lactone intermediate **79** (generated in the synthesis of **2**, Scheme 13) were evaluated against a variety of human hematopoietic and solid tumor cell lines (Table 2).[96,97] These include HL-60 (acute promyelocytic leukemia), HL-60/RV+ (a P-glycoprotein over-expressing multidrug resistant HL-60 variant which was selected by continuous exposure to the vinca alkaloid vincristine), JURKAT (T cell leukemia), ALL3 (acute lymphoblastic leukemia recently isolated from a patient treated at MSKCC and characterized as Philadelphia chromosome positive), NCEB1 (Mantle cell lymphoma), JEKO (B cell lymphoma), MOLT-3 (acute lymphoblastic T-cell), SKNLP (neuroblastoma), Y79 (retinoblastoma), PC9 (adenocarcinoma), H1650 (adenocarcinoma), H1975 (adenocarcinoma), H2030 (adenocarcinoma), H3255 (adenocarcinoma), TC71 (Ewing3s sarcoma), HTB-15 (glioblastoma), A431 (epithelial carcinoma), HeLa (cervical adenocarcinoma), and WD0082 (well-differentiated liposarcoma).

Several general features are evident in the cytotoxicity data accumulated in the initial screening campaigns (Table 2). As expected, evaluation of deoxyharringtonine (**2**) revealed exceedingly potent cytotoxic activity against all of the hematopoietic cell lines tested (HL-60, HL-60/RV+, JURKAT, ALL3, NCEB1, JEKO, MOLT-3); moreover, the alkaloid exhibited similarly high activity against most of the solid tumor cell lines tested (SKNLP, PC9, H1650, H1975, H2030, H3255, A431, HeLa, TC71, HTB-15, WD0082). Interestingly, the late-stage β -lactone variant **79** (see also Scheme 13) exhibited significant cytotoxicity, yet at attenuated levels compared to the parent alkaloid **2**, revealing the likely necessity of a hydroxyl group or an H-bond donor functionality at the C2'-position. Surprisingly, the cytotoxicity profile of anhydroharringtonine (**5**) revealed fairly poor antitumor activity. While an early report noted comparable cytotoxic activity of anhydroharringtonine (**5**) to that of deoxyharringtonine (**2**) against murine P388,[12] the present result indicates that the activity of **5** is generally several

orders of magnitude lower in human HL-60 tumor cells. This unimpressive potency level of **5** thus effectively disqualifies it as a potential therapeutic agent despite previous cytotoxicity data, and is consistent with the proposed need for a 2'-hydroxy group in the acyl chain to confer adequate activity (see above).

Synthesis of additional *Cephalotaxus* ester natural products and variants to probe susceptibility to multidrug resistant cancer

The development of vincristine-resistance in cancer cells, such as HL-60/RV+ (Table 2) is believed to arise from classic multidrug resistance (MDR). This involves the overexpression of ATP-dependent efflux pumps, such as P-glycoprotein (Pgp) and multidrug resistance-associated protein (MRP), leading to expulsion of natural product hydrophobic drugs (e.g., *vinca* alkaloids, anthracyclines, actinomycin-D, paclitaxel) from the transformed cell.[98] Previous reports have noted that the activity of homoharringtonine (**3**), the cephalotaxus ester currently being evaluated in clinical trials, is also compromised in MDR human leukemia cells. [18] Remarkably, the susceptibility of MDR cancer cells to different *Cephalotaxus* esters has not been systematically probed. Prevention of MDR would significantly improve therapeutic response to this family of chemotherapeutics and extend their use in the clinic. One possible way to achieve this would be to develop anticancer agents that are not substrates for these ATP-dependent transporters, thus overcoming their efflux from cells.

In examining variations in potencies of deoxyharringtonine (**2**) against this extensive panel of cell lines (Table 2), it is worth noting that its activity against vincristine-resistant HL-60/RV+ cells (IC₅₀ 0.22 μ M), relative to its non-resistant counterpart HL-60 (IC₅₀ 0.02 μ M), shows only a $\approx$ 10-fold decrease in potency. This trend is also reflected in the β -lactone derivative **79** (albeit with lower absolute cytotoxicity levels). This rather low observed 10-fold resistance index spawned an interest in probing potential molecular design criteria that may offset MDR susceptibility in this class of alkaloids. Fortunately, our current synthetic approach to deoxyharringtonine (**2**) permits the rapid and versatile attachment of sterically demanding acyl chains onto the cephalotaxine core. Thus, the synthetic strategy to deoxyharringtonine (**2**) was further extended to the construction of two additional antileukemia *Cephalotaxus* ester natural products, namely homoharringtonine (**3**), and homodeoxyharringtonine (**4**), all reported to be potent antileukemia alkaloids.

The syntheses of homoharringtonine **3** and homodeoxyharringtonine **4** involved a common early sequence (Scheme 15) beginning with the *R*-malic acid derived acetal **72**, which underwent double deprotonation and diastereoselective enolate alkylation with allyl bromide (59%).[91] Following NaOBn-mediated transesterification of the resultant acetal-ester to afford the *R*- α -hydroxy benzyl ester **81** (85%), β -lactone formation was accomplished via the Yamaguchi mixed anhydride to provide the strained intermediate **82** (67%). Subsequent alkene cross metathesis (Grubbs II) with excess alkene **83**[99,100] provided disubstituted alkene **85** (61%) along with the dimeric bis(lactone) **84** (22%) as an equilibrium mixture. Although the direct conversion of **82** to **85** was moderate, the recovered dimer **84** could be re-equilibrated under olefin metathesis conditions with excess **83** to accumulate additional quantities of **85**. Following selective transfer hydrosilylation of **85**, the resultant acid **86** (85%) was employed in a highly efficient cephalotaxine acylation to prepare the corresponding ester (97%), whose β -lactone was then subjected to methanolysis to furnish **87** (79%). This intermediate was then diverged to both of the natural products homoharringtonine (**3**) and deoxyhomoharringtonine (**4**). When the allylic benzyl ether in **87** was subjected to Pd/C-catalyzed hydrogenolysis/hydrogenation in MeOH, followed by the addition of AcOH in the latter stages of the reaction, (–)-homoharringtonine (**3**, 79%) was isolated (presumably through initial alkene hydrogenation followed by benzyl ether hydrogenolysis). On the other hand, when the Pd/C-catalyzed reduction was performed in glacial AcOH solvent at the outset, deoxygenation

preceded alkene reduction (presumably through E1 elimination of the allylic benzyl ether prior to hydrogenation) to afford (–)-homodeoxyharringtonine (**4**, 69%).

The efficient synthesis of the acyl chain precursors in the preparation of the natural product cephalotaxus esters **3** and **4** also presented the opportunity to prepare a non-natural analogue for biological evaluation with only a minor variation in the synthetic sequence. This analogue took the form of bis(demethyl)deoxyharringtonine **89** (Scheme 16), also anticipated to exhibit potent antiproliferative activity, although much simpler in structure and more easily prepared than **3** or **4**. The synthesis of *Cephalotaxus* ester **89** (Scheme 16) involved interception of the β-lactone acyl chain **82**, derived from (*R*)-malic acid in three steps (refer to Scheme 15). Following hydrogenolysis/hydrogenation of the alkenyl ester **82** (97%), the resulting carboxylic acid was activated as the Yamaguchi mixed anhydride to effect the acylation of cephalotaxine (**1**), providing the ester-β-lactone **88** (81%). Methanolysis of the β-lactone in **88** proceeded efficiently to afford the non-natural bis(demethyl)deoxyharringtonine analogue **89** (93%).

The completion of the syntheses of the natural *Cephalotaxus* esters **2–4** together with two non-natural synthetic analogues, namely benzyldehydrohomoharringtonine **87** and bis(demethyl)deoxyharringtonine **89**, permitted their comparative biological evaluation against “sensitive” and MDR tumor cell lines (Figure 3). When tested against the “sensitive” HL-60 cell line, all were found to be exceedingly potent ($IC_{50} < 0.08 \mu M$). When evaluated against the “resistant” HL60/RV+ cell line, stark differential response levels were observed within this collection of cephalotaxus esters (Figure 4). Interestingly homoharringtonine (**3**) displayed a 125-fold decrease in activity toward HL-60/RV+ relative to that of HL-60 (resistance index=125). By contrast, much lower resistance indices of 11, 3, 12, and 19 were observed with the esters **2**, **4**, **87**, and **89**, respectively, indicating that these latter natural and non-natural products are significantly less susceptible to MDR. One possible explanation for the high MDR susceptibility of homoharringtonine (**3**) is its decreased lipophilicity as a consequence of its acyl chain structure, thereby rendering it a good substrate for the efflux pumps.

The relationship of the calculated lipophilicity values ($clogP$) to the resistance indices for the highly potent cephalotaxus esters **2–4**, **87**, and **89** is presented in Figure 4, wherein compounds with $clogP$ values greater than 1.2 lead to generally low susceptibility to MDR (i.e., resistance indices=19 for the cephalotaxus esters **2**, **4**, **87**, and **89**). The exception is homoharringtonine (**3**), exhibiting a relatively low $clogP$ value (0.95, relatively more polar) to reflect an increased susceptibility to MDR (i.e., resistance index 125). Although these data were obtained on a limited set of analogues, they provide for the first time new insights into the contribution of acyl chain structure modification toward overcoming MDR for this class of compounds.

It is worth emphasizing that the only structural difference on the acyl chain between homoharringtonine (**3**) and homodeoxyharringtonine (**4**) is a hydroxyl group on the 6'-position (Figure 4). While only a minor structural perturbation, this 6'-substitution difference drastically affects the lipophilicity of the molecules, ranging from a $clogP$ value of 0.95 (polar) for **3** to a more hydrophobic compound **4** with a $clogP$ value of 2.33 (i.e., Figure 5). Importantly, with a resistance index of only 3 (as in the case with homodeoxyharringtonine **4**), both comparative cell lines can be considered as “sensitive” to the compound of interest. As a consequence, this minor structural variation from **3** to **4** has allowed for effective quelling of MDR in this cell line. Given this finding, it is thus surprising that despite its MDR liability, homoharringtonine (**3**) is employed as the favored cephalotaxus ester for advancement in the clinic, exemplified by a current phase III clinic prospective trial with **3** for use as a combination therapy for chronic myeloid leukemia.[101] One practical reason for this may lie in the increased natural abundance of homoharringtonine (**3**) relative to other cephalotaxus esters.[102] Moreover, semisynthetic sources of homoharringtonine have built on the seminal work of Kelly, wherein the 6'-oxygen

functionality is a prerequisite for efficient acyl chain attachment to cephalotaxine.[89] Notably, this semi-synthetic approach is uniquely suited for homoharringtonine (**3**). Fortunately, the synthetic strategies described herein enable unfettered access to other, more therapeutically viable cephalotaxus esters, such as **2**, **4**, **87**, and **89**, for the development of additional lines of chemotherapeutic defense against leukemia.

Resistance of vincristine-sensitive Y79 retinoblastoma to cephalotaxus esters

In the initial cytotoxicity evaluation (Table 2), it is also worth highlighting that the Y79 retinoblastoma cell line uniquely showed significant resistance to both deoxyharringtonine (**2**) and its β -lactone derivative **79**. Indeed, this selective resistance of Y79 appears to be a general phenomenon (Table 3) upon evaluation with a few of our active cytotoxic non-natural synthetic *Cephalotaxus* ester analogues, including the benzyldehydrohomoharringtonine **87**, the β -lactone ester **88**, and bis-(demethyl)deoxyharringtonine **89**. All of these compounds behaved similarly to that of deoxyharringtonine (**2**) and its β -lactone derivative **79** (cf. Table 2), exhibiting broad spectrum cytotoxicity with the exception of the Y79 cell line, to which the molecules were essentially impotent.

Though this specific lack of cytotoxicity in Y79 could also be attributed to the overexpression of multidrug resistance genes (MDR), Conway and co-workers have reported the Y79 cell line to be sensitive to vincristine with an IC_{50} value of approx 0.8 μ M.[103] Furthermore, a comparative microarray analysis of the Y79 cell line with normal retinal tissue detected upregulation of several genes typically found to be markers of stem cell like characteristics including the *mdr* gene ABCG2.[104] Based on this, we postulate that perhaps the mechanism of resistance to cephalotaxus esters by Y79 is not entirely mediated through the classical ATP-dependent efflux pumps alone but rather through an as yet unknown mechanism involving stem cell like characteristics. This is consistent with the hypothesis that the appearance of subsequent tumors in leukemias, brain tumors, breast cancer, lung cancer, as well as many other cancers, is linked to the persistence of cancer stem cells. This observation suggests that designed *Cephalotaxus* esters have the potential to serve as small molecule probes for interrogating the genetic basis of this highly resilient retinoblastoma cell line as well as potentially shedding some light on how to overcome this persistence phenomena in these dormant progenitor cancer stem cells.

Conclusion

The development, optimization, and application of novel synthetic strategies have enabled the synthesis of the potent antileukemia agents (–)-deoxyharringtonine (**2**), (–)-homoharringtonine (**3**), (–)-homodeoxyharringtonine (**4**), and (–)-anhydroharringtonine (**5**). Several advances served as key elements in the preparation of (–)-cephalotaxine (**1**) and should find general applicability in complex *N*-heterocycle synthesis. These included 1) a strain-release aziridine rearrangement of 2-aryl-*N*-vinyl aziridines for dihydro[3]benzazepine synthesis, and 2) a vinylogous amide-derived azomethine ylide cycloaddition which takes an unusual and unexpected stereochemical course. Efforts to advance these synthetic pursuits beyond that of (–)-**1** to that of the rare antineoplastic C3-*O*-ester derivatives (i.e., **2–5**) have led to an efficient non-racemic synthesis of several cephalotaxus acyl chains. Construction of strained β -lactone intermediates enabled late-stage C3-*O*-acylation of cephalotaxine, a longstanding challenge in the synthesis of sterically congested bioactive *Cephalotaxus* esters. This technology enabled cytotoxicity screening of several natural and non-natural *Cephalotaxus* esters against an expansive array of human hematopoietic and solid tumor cell lines. These evaluations were instrumental in discovering novel non-natural cephalotaxus esters with potent antitumor effects. Moreover, these efforts have uncovered the potential of specific members of this family of alkaloids to overcome resistance in MDR HL-60/RV+ tumor cells through the preparation

of acyl chain variants, uniquely made available with our acyl chain attachment approach. This presents new avenues for molecular design of these alkaloids to offset multi-drug resistance, offering new lines of chemotherapeutic defense against leukemia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Huang L, Xue Z. Alkaloids 1984;23:157–226.
2. Paudler WW, McKay J, Kerley GI. J Org Chem 1963;28:2194–2197.
3. Powell RG, Weisleder D, Smith CR, Wolff IA. Tetrahedron Lett 1969;10:4081–4084.
4. Abraham DJ, Rosenstein RD, McGandy EL. Tetrahedron Lett 1969;10:4085–4086.
5. Arora SK, Bates RB, Grady RA. J Org Chem 1974;39:1269–1271. [PubMed: 4833379]
6. Arora SK, Bates RB, Grady RA, Germain G, Declercq JP, Powell RG. J Org Chem 1976;41:551–554. [PubMed: 1245909]
7. Mikolajczak KL, Smith CR, Powell RG. Tetrahedron 1972;28:1995–2001.
8. Powell RG, Weisleder D, Smith CR Jr, Rohwedder WK. Tetrahedron Lett 1970;11:815–818. [PubMed: 5436615]
9. Takano I, Yasuda I, Nishijima M, Hitotsuyanagi Y, Takeya K, Itokawa H. J Nat Prod 1996;59:965–967.
10. Wang DZ, Ma GE, Xu RS. Acta Pharm Sin 1992;27:173–177.
11. Morita H, Arisaka M, Yoshida N, Kobayashi J. Tetrahedron 2000;56:2929–2934.
12. Wang DZ, Ma GE, Xu RS. Acta Pharm Sin 1992;27:178–184.
13. Miah MAJ, Hudlicky T, Reed JW. Alkaloids 1998;51:199–269.
14. Huang MT. Mol Pharmacol 1975;11:511–519. [PubMed: 1237080]
15. Fresno M, Jimenez A, Vazquez D. Eur J Biochem 1977;72:323–330. [PubMed: 319998]
16. Legha SS, Keating M, Picket S, Ajani JA, Ewer M, Bodey GP. Cancer Treat Rep 1984;68:1085–1091. [PubMed: 6478448]
17. Kantarjian HM, Talpaz M, Santini V, Murgu A, Cheson B, O'Brien SM. Cancer 2001;92:1591–1605. [PubMed: 11745238]
18. Benderra Z, Morjani H, Trussardi A, Manfait M. Leukemia 1998;12:1539–1544. [PubMed: 9766497]
19. Auerbach J, Weinreb SM. J Am Chem Soc 1972;94:7172. [PubMed: 5072334]
20. Semmelhack MF, Chong BP, Stauffer RD, Rogerson TD, Chong A, Jones LD. J Am Chem Soc 1975;97:2507–2516. [PubMed: 1133420]
21. Yasuda S, Yamada T, Hanaoka M. Tetrahedron Lett 1986;27:2023–2026.
22. Yasuda S, Yamamoto Y, Yoshida S, Hanaoka M. Chem Pharm Bull 1988;36:4229–4231.
23. Kuehne ME, Bornmann WG, Parsons WH, Spitzer TD, Blount JF, Zubieta J. J Org Chem 1988;53:3439–3450.
24. Burkholder TP, Fuchs PL. J Am Chem Soc 1988;110:2341–2342.
25. Burkholder TP, Fuchs PL. J Am Chem Soc 1990;112:9601–9613.
26. Lin XD, Kavash RW, Mariano PS. J Am Chem Soc 1994;116:9791–9792.
27. Lin XD, Kavash RW, Mariano PS. J Org Chem 1996;61:7335–7347. [PubMed: 11667659]

28. Koseki Y, Sato H, Watanabe Y, Nagasaka T. *Org Lett* 2002;4:885–888. [PubMed: 11893177]
29. Li W DZ, Wang YQ. *Org Lett* 2003;5:2931–2934. [PubMed: 12889911]
30. Li W DZ, Ma BC. *J Org Chem* 2005;70:3277–3280. [PubMed: 15822994]
31. Isono N, Mori M. *J Org Chem* 1995;60:115–119.
32. Tietze LF, Schirok H. *Angew Chem* 1997;109:1159–1160. *Angew Chem Int Ed Engl* 1997;36:1124–1125.
33. Tietze LF, Schirok H. *J Am Chem Soc* 1999;121:10264–10269.
34. Ishibashi H, Okano M, Tamaki H, Maruyama K, Yakura T, Ikeda M. *J Chem Soc Chem Commun* 1990:1436–1437.
35. Ikeda M, Okano M, Kosaka K, Kido M, Ishibashi H. *Chem Pharm Bull* 1993;41:276–281.
36. Ikeda M, El Bialy SAA, Hirose K, Kotake M, Sato T, Bayomi SMM, Shehata IA, Abdelal AM, Gad LM, Yakura T. *Chem Pharm Bull* 1999;47:983–987. [PubMed: 10434399]
37. Planas L, Perard-Viret J, Royer J. *J Org Chem* 2004;69:3087–3092. [PubMed: 15104447]
38. Eckelbarger JD, Wilmot JT, Gin DY. *J Am Chem Soc* 2006;128:10370–10371. [PubMed: 16895394]
39. Hudlicky T, Fan R, Reed JW, Gadamasetti KG. *Org React* 1992;41:1–133.
40. Lwowski W, Maricich TJ, Mattingly TW Jr. *J Am Chem Soc* 1963;85:1200–1202.
41. Stogryn EL, Brois SJ. *J Org Chem* 1965;30:88.
42. Pommelet JC, Chuche J. *Tetrahedron Lett* 1974;15:3897–3898.
43. Zora M. *J Org Chem* 2005;70:6018–6026. [PubMed: 16018698]
44. Hassner A, Dcosta R, McPhail AT, Butler W. *Tetrahedron Lett* 1981;22:3691–3694.
45. Viallon L, Reinaud O, Capdevielle P, Maumy M. *Tetrahedron Lett* 1995;36:4787–4790.
46. Lindstrom UM, Somfai P. *J Am Chem Soc* 1997;119:8385–8386.
47. Scheiner P. *J Org Chem* 1967;32:2628.
48. Figeys HP, Jammal R. *Tetrahedron Lett* 1980;21:2995–2998.
49. Figeys HP, Jammal R. *Tetrahedron Lett* 1981;22:637–640.
50. Harada T, Ohno T, Kobayashi S, Mukaiyama T. *Synthesis* 1991:1216–1220.
51. Tanida H, Okada T, Kotera K. *Bull Chem Soc Jpn* 1973;46:934–938.
52. Ullrich JW, Chiu FT, Tinerharding T, Mariano PS. *J Org Chem* 1984;49:220–228.
53. Dixon WT. *Tetrahedron Lett* 1968;9:189.
54. Dauksas VK, Purvanekas GV, Udrenaitis EB, Gineityte VL, Barauskaite AV. *Heterocycles* 1981;15:1395–1404.
55. Vedejs E, West FG. *Chem Rev* 1986;86:941–955.
56. Gothelf KV, Jorgensen KA. *Chem Rev* 1998;98:863–909. [PubMed: 11848917]
57. Najera C, Sansano JM. *Curr Org Chem* 2003;7:1105–1150.
58. Pearson WH, Stoy P. *Synlett* 2003:903–921.
59. Coldham I, Hufton R. *Chem Rev* 2005;105:2765–2809. [PubMed: 16011324]
60. Vedejs E, Martinez GR. *J Am Chem Soc* 1979;101:6452–6454.
61. Padwa A, Haffmanns G, Tomas M. *Tetrahedron Lett* 1983;24:4303–4306.
62. Epperson MT, Gin DY. *Angew Chem* 2002;114:1856–1858. *Angew Chem Int Ed* 2002;41:1778–1780.
63. Pilcher AS, Ammon HL, Deshong P. *J Am Chem Soc* 1995;117:5166–5167.
64. Baraznenok IL, Nenajdenko VG, Balenkova ES. *Tetrahedron* 1998;54:119–128.
65. Baraznenok IL, Nenajdenko VG, Balenkova ES. *Eur J Org Chem* 1999:937–941.
66. Auerbach J, Ipaktchi T, Weinreb SM. *Tetrahedron Lett* 1973;14:4561–4564.
67. Exhaustive investigation of azomethine ylide precursors incorporating only a C2 group led to no diastereoselectivity in the cycloaddition.
68. Ali SM, Ramesh K, Borchardt RT. *Tetrahedron Lett* 1990;31:1509–1512.
69. Blaser A, Reymond JL. *Helv Chim Acta* 1999;82:760–768.
70. Chu CK, Jin YH, Baker RO, Huggins J. *Bioorg Med Chem Lett* 2003;13:9–12. [PubMed: 12467606]
71. Yadav JS, Pamu S, Bhunia DC, Pabbaraja S. *Synlett* 2007:992–994.

72. Jin YH, Liu P, Wang JN, Baker R, Huggins J, Chu CK. J Org Chem 2003;68:9012–9018. [PubMed: 14604375]
73. Effenberger F, Eberhard JK, Maier AH. J Am Chem Soc 1996;118:12572–12579.
74. Extensive efforts were directed at the generation and use of a complex acyl triflate derived from an intact *Cephalotaxus* ester side chain for vinylogous amide activation, although with no success.
75. Ess DH, Houk KN. J Am Chem Soc 2007;129:10646–10647. [PubMed: 17685614]
76. Kunzer H, Stahnke M, Sauer G, Wiechert R. Tetrahedron Lett 1991;32:1949–1952.
77. Craig D, Jones PS, Rowlands GJ. Synlett 1997:1423–1425.
78. Notably, attempts at reductive desulfurization with a variety of traditional Raney-Ni protocols led to highly variable yields.
79. Attempts at cleavage of the C3–pivaloyl enol ester were problematic, resulting in unproductive dihydro [3]benzazepine fragmentation. Moreover, attempts at reduction of the C3–C4 enol ester, either by conjugate addition protocols or hydrogenation protocols at hydrogen pressures up to 2500 psi, led only to its decomposition.
80. Schwartz J, Labinger JA. Angew Chem 1976;88:402–409. Angew Chem Int Ed Engl 1976;15:333–340.
81. Cenac N, Zablocka M, Igau A, Majoral JP, Skowronska A. J Org Chem 1996;61:796–798. [PubMed: 11667010]
82. Clarke PA, Holton RA, Kayaleh NE. Tetrahedron Lett 2000;41:2687–2690.
83. Clarke PA, Kayaleh NE, Smith MA, Baker JR, Bird SJ, Chan C. J Org Chem 2002;67:5226–5231. [PubMed: 12126410]
84. Clarke PA, Arnold PL, Smith MA, Natrajan LS, Wilson C, Chan C. Chem Commun 2003:2588–2589.
85. Mikolajczak KL, Smith CR, Weisleder D, Kelly TR, McKenna JC, Christen Pa. Tetrahedron Lett 1974;15:283–286.
86. Mikolajczak KL, Smith CR. J Org Chem 1978;43:4762–4765.
87. Hiranuma S, Hudlicky T. Tetrahedron Lett 1982;23:3431–3434.
88. Hiranuma S, Shibata M, Hudlicky T. J Org Chem 1983;48:5321–5326.
89. Kelly TR, McNutt RW, Montury M, Tosches NP, Mikolajczak KL, Smith CR, Weisleder D. J Org Chem 1979;44:63–67.
90. Robin JP, Dhal R, Dujardin G, Girodier L, Mevellec L, Poutot S. Tetrahedron Lett 1999;40:2931–2934.
91. Seebach D, Naef R, Calderari G. Tetrahedron 1984;40:1313–1324.
92. El Bialy SAA, Braun H, Tietze LF. Eur J Org Chem 2005:2965–2972.
93. Huang PQ, Li ZY. Tetrahedron: Asymmetry 2005;16:3367–3370.
94. Inanaga J, Hirata K, Saeki H, Katsuki T, Yamaguchi M. Bull Chem Soc Jpn 1979;52:1989–1993.
95. Powell RG, Weisleder D, Smith CR. J Pharm Sci 1972;61:1227–1230. [PubMed: 5050371]
96. Antczak C, Shum D, Escobar S, Bassit B, Kim E, Seshan VE, Wu N, Yang GL, Ouerfelli O, Li YM, Scheinberg DA, Djaballah H. J Biomol Screening 2007;12:521–535.
97. Shum D, Radu C, Kim E, Cajuste M, Shao Y, Seshan VE, Djaballah H. J Enzyme Inhib Med Chem. in press
98. Gottesman MM, Fojo T, Bates SE. Nat Rev Cancer 2002;2:48–58. [PubMed: 11902585]
99. Salomon RG, Reuter JM. J Am Chem Soc 1977;99:4372–4379.
100. Chatterjee AK, Choi TL, Sanders DP, Grubbs RH. J Am Chem Soc 2003;125:11360–11370. [PubMed: 16220959]
101. Legros L, Hayette S, Nicolini FE, Raynaud S, Chabane K, Magaud JP, Cassuto JP, Michallet M. Leukemia 2007;21:2204–2206. [PubMed: 17541396]
102. Powell RG. Phytochemistry 1972;11:1467–1472.
103. Conway RM, Madigan MC, Billson FA, Penfold PL. Eur J Cancer 1998;34:1741–1748. [PubMed: 9893663]
104. Seigel GM, Hackam AS, Ganguly A, Mandell LM, Gonzalez-Fernandez F. Mol Vision 2007;13:823–832.

Figure 1.

Figure 2.

Figure 3. Comparative antitumor effects of cephalotaxus esters against sensitive (filled symbols) and vincristine-resistant (open symbols) HL-60: ●/○: 4, ▼/▽: 3, ■/□: 2, ◆/◇: 89, ▲/△: 87.

Figure 4.
Comparative antitumor effects of cephalotaxus esters against sensitive and vincristine-resistant HL-60.

Figure 5. Correlation of calculated $\log P$ values and MDR ratio for deoxyharringtonine (**2**), homoharringtonine (**3**), homodeoxyharringtonine (**4**), benzyldehydrohomoharringtonine **87**, and bis(dimethyl)deoxyharringtonine **89**.

Scheme 1.

a) $HONH_2 \cdot HCl$, $NaOH$, $EtOH$, H_2O , $60-80^\circ C$; b) iPr_2NH , $LiAlH_4$, THF , $60^\circ C$; c) Et_3N , THF , $23-60^\circ C$; d) Cs_2CO_3 , 1,4-dioxane, $100-150^\circ C$.

Scheme 2.

(a) DMAD, PhH, 23 °C, 57%; (b) Cs₂CO₃, 1,4-dioxane, 100 °C, 92%.

Scheme 3.

a) Et₃N, THF, 60 °C, 64%; b) Cs₂CO₃, 1,4-dioxane, 100 °C, 68%.

Scheme 4.

a) TMSCH_2I , NaH , THF, 50°C , 62%; b) TiF_2O ; DMAD; TBAT, CH_2Cl_2 , 23°C , 53%.

Scheme 5.

Scheme 6.

a) TiF_2O ; $\text{PhCO}_2\text{H}\cdot\text{NEt}_3$; DMAD; TBAT, CH_2Cl_2 , 23 °C, 35%; b) TiF_2O ; PhCO_2Cs ; DMAD; TBAT, CH_2Cl_2 , 23 °C, 64%; c) **31**, TiF_2O ; **44**, Cs_2CO_3 ; DMAD; TBAT, CH_2Cl_2 , 23 °C.

Scheme 7.

Scheme 8.

a) KHMDS, Ph_3PMeBr , THF, 60 °C, 75%; b) DMSO, Et_3N , $\text{SO}_3\cdot\text{Pyr}$, CH_2Cl_2 , 23 °C, 88%; c) $\text{CH}_2=\text{CHMgBr}$, THF, $-78\rightarrow 23$ °C, 93%, dr 8:1; d) Grubbs II, CH_2Cl_2 , 23 °C, 95%; e) PhSeCl , MeCN, 0 °C; *m*CPBA, Et_3N , CH_2Cl_2 , 0 $\rightarrow$ 23 °C; 98%; f) TBAF, THF, 23 °C, 99%; g) NaIO_4 , CH_2Cl_2 , H_2O , 23 °C, 90%.

Scheme 9.

a) Et₃N, THF, 23 °C, 85%; b) Cs₂CO₃, 1,4-dioxane, 100 °C, 76%.

Scheme 10.

Scheme 11.

a) CS_2CO_3 , $TMSCH_2I$, MeCN, 23 °C, 75%; b) Me_3CCOCl , AgOTf; $PhSO_2CH=CH_2$; TBAT, CH_2Cl_2 , $-45 \rightarrow 23$ °C, 77%.

Scheme 12.

a) SmI₂, HMPA, *t*BuOH, THF, -45 °C, 74%; b) Cp₂ZrHCl, THF, 40°C, 99%; c) Et₃N, CbzCl, CH₂Cl₂, 23 °C, 50%; d) KHMDS, CbzCl, THF, 0°C, 86%; e) 2N HCl, MeOH, 23°C; Boc₂O, Yb-(OTf)₃·xH₂O, CH₂Cl₂, 0 °C; f) IBX, DMSO, 23°C, 50% (2 steps); g) CrCl₂, acetone, H₂O, 23 °C; h) H₂, Pd/C, EtOAc, 23 °C, 42% (2 steps); i) HC(OMe)₃, *p*TsOH, CH₂Cl₂, 55 °C, 90%; j) NaBH₄, MeOH, -78→23 °C, 95%.

Scheme 13.

a) TMSCl , TMS_2NH , CH_2Cl_2 , 23°C ; Me_3CCHO , TMSOTf , CH_2Cl_2 , -25°C , 82%; b) LHMDS , $\text{Me}_2\text{C}=\text{CHCH}_2\text{Br}$, THF , -78°C , 66%; c) NaH , BnOH , THF , 0°C , 88%; d) $2,4,6\text{-Cl}_3\text{C}_6\text{H}_2\text{COCl}$, DMAP , CH_2Cl_2 , 23°C , 50%; e) H_2 , Pd/C , EtOAc , 23°C , 99%; f) $2,4,6\text{-Cl}_3\text{C}_6\text{H}_2\text{COCl}$, DMAP , **1**, CH_2Cl_2 , 23°C , 81%; g) NaOMe , MeOH , 23°C , 76%; h) TMSCHN_2 , 7:2 PhH/MeOH , 23°C , 100%; i) Ac_2O , DMAP , pyr , 23°C , 74%; j) Pd/C , H_2 , EtOAc , 23°C , >99%.

Scheme 14.

a) $\text{Hg}(\text{OAc})_2$, NaBH_4 , 1:1 THF: H_2O , 23 °C, 77%; (b) Pd/C , H_2 , EtOAc, 23°C, 99%; (c) 2,4,6-trichlorobenzoyl chloride, DMAP, TEA, **1**, CH_2Cl_2 , 23 °C, 99%.

Scheme 15.

a) LHMDs, allyl bromide, THF, -78°C , 59%; b) BnOH, NaH, THF, $0 \rightarrow 23^{\circ}\text{C}$, 85%; c) 2,4,6-trichlorobenzoyl chloride, DMAP, Et_3N , CH_2Cl_2 , 23°C , 67%; d) Grubbs II, 23°C , 61%; e) Pd (OAc) $_2$, Et_3SiH , Et_3N , CH_2Cl_2 , 23°C , 85%; f) 2,4,6-trichlorobenzoyl chloride, DMAP, Et_3N , **1**, CH_2Cl_2 , 23°C , 97%; g) NaOMe, MeOH, 0°C , 79%; (h) Pd/C, H_2 , MeOH then 9:1 MeOH/HOAc, 23°C , 79%; i) Pd/C, H_2 , HOAc, 23°C , 69%.

Scheme 16.

a) Pd/C, H₂, EtOAc, 23 °C, 97%; b) 2,4,6-trichlorobenzoyl chloride, DMAP, TEA, **1**, CH₂Cl₂, 23 °C, 81%; c) NaOMe, MeOH, 23°C, 93%.

Table 1Azomethine ylide generation from vinylogous amides via sequential *O*-sulfonylation and nucleophilic exchange.

Entry	Bu ₄ N ⁺ Nu ⁻	Cycloadduct	Yield [%]
1	Bu ₄ NI	40 (Nu=I)	52
2	Bu ₄ NBr	41 (Nu=Br)	45
3	Bu ₄ NCl	42 (Nu=Cl)	52

Table 2Cytotoxicity of deoxyharringtonine (**2**), β -lactone **79** and anhydroharringtonine (**5**).

Cell Line	2 IC ₅₀ [μ M]	79 IC ₅₀ [μ M]	5 IC ₅₀ [μ M]
HL-60	0.02	2.68	22.7
HL-60/RV+	0.22	21.8	>100 ^[a]
JURKAT	0.04	5.71	42.99
ALL3	<0.1 ^[b]	1.47	>100 ^[a]
NCEB1	0.07	8.62	>100 ^[a]
JEKO	0.08	10.48	>100 ^[a]
MOLT-3	0.02	2.68	26.83
SKNLP	<0.1 ^[b]	6.46	5.34
Y79	70.59	>100 ^[a]	>100 ^[a]
PC9	0.03	4.23	29.08
H1650	0.04	4.53	n.a.
H1975	0.06	8.42	n.a.
H2030	0.10	7.72	n.a.
H3255	0.08	5.55	n.a.
A431	0.06	n.a.	n.a.
HeLa	0.04	n.a.	n.a.
TC71	0.06	12	>100 ^[a]
HTB-15	0.20	52	>100 ^[a]
WD0082	0.10	5	>100 ^[a]

^[a] Highest compound concentration tested.^[b] Lowest compound concentration tested and yielding 100% cellular killing.

Table 3Cytotoxicity of compounds **87–89**.

Cell line	87 IC ₅₀ [μM]	88 IC ₅₀ [μM]	89 IC ₅₀ [μM]
HL-60	0.01	5.73	0.08
HL-60/RV+	0.19	40.30	0.80
JURKAT	0.03	12.01	0.19
ALL3	<0.01	4.24	0.16
NCEB1	0.06	39.24	0.50
JEKO	0.08	25.1	0.56
MOLT3	0.01	6.41	0.06
SKNLP	<0.01	10.04	0.11
Y79	>100	>100	>100
PC9	0.04	11.29	0.13
TC71	0.03	24	0.20
HTB-15	0.10	58	0.50
WD0082	0.05	11	0.20