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Concise syntheses of the abyssinones and discovery of new inhibitors of prostate cancer and MMP-2 expression

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Abstract

Hydrogen-bonding catalysis is an emerging field that facilitates rapid access to medicinally relevant enantioenriched small molecules. Here, we report the first asymmetric total syntheses of four members of the abyssinone class of natural products (I, II, III, and IV 4′-OMe) via quinine- or quinidine-derived thiourea-catalyzed intramolecular conjugate additions of β-keto ester alkylidenes. This concise strategy includes a tandem deprotection/decarboxylation final step that delivers all four natural products and their corresponding antipodes. A preliminary evaluation of all of these small molecules against a metastatic human prostate cancer cell line has identified that these compounds selectively and differentially inhibit cell growth and downregulate the expression of matrix metalloproteinase-2 (MMP-2) at non-toxic concentrations.

The abyssinones are a family of chiral, enantiomerically enriched flavanone natural products that exhibit a diverse range of biological activities, including aromatase inhibition, antimicrobial activity and antimalarial activity (see Figure 1).[1–3] Although extracts containing these small molecules have been used as traditional remedies, any investigation of their specific anti-cancer properties requires a general synthetic approach that can access them in enantiomerically enriched form. Our program geared toward the synthesis of pyran-containing natural products prompted our interest in these compounds.[4,5] *Despite their therapeutic promise, the abyssinones have not been a) synthesized with control over the absolute stereochemistry and b) evaluated for their ability to inhibit cancer progression.*[6] The unadorned structure of flavanones belies the challenge in executing a strategy that installs and maintains the configuration at the C2 position.[7–9] This stereocenter in the abyssinones is quite sensitive – mildly basic conditions promote reversible ring opening to achiral 2′-hydroxy chalcones.[10] In addition, flavanones containing electron-donating substituents (alkoxy or hydroxy) in the C4′ position are particularly susceptible to racemization.[7,11] A limited number of approaches for the stereoselective synthesis of flavanones have been developed, including Mitsunobu reactions of chiral alcohols, acylation reactions of chiral ethers, and conjugate additions of aryl substituents to chromenes.[12–16] However, these approaches are not general and would not provide the abyssinone core in a concise manner.

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Our synthetic plan is outlined in Figure 1. The key step in the strategy is the application of our asymmetric thiourea-catalyzed cyclization, which can provide controlled access to either stereoisomer of these natural products.[7,17] A Knoevenagel condensation between an appropriately protected β-keto ester and different aldehydes corresponding to each abyssinone would provide the requisite alkylidenes for our thiourea-catalyzed cyclization. Our greatest concern was maintaining the integrity of the newly formed C2 stereocenter, which would depend heavily on the identification of mild decarboxylation/deprotection conditions. We anticipated that we could maximize efficiency if the decarboxylation and unmasking of the C7 phenol were performed in a single flask as the last step of the synthesis.

The first challenge encountered in the total synthesis of the abyssinones involved accessing the alkylidene substrates for the thiourea cyclization (see Figure 2 for representative example with abyssinone I). Using the requisite aldehyde **5**, prepared from 4-hydroxybenzaldehyde,[18] we attempted the condensation with the protected β-keto ester **7** under standard Knoevenagel conditions (piperidinium acetate, Dean–Stark apparatus). These reactions produced the desired *E* alkylidene **8** in >95:5 *E*:*Z* for all aldehydes employed in the Knoevenagel condensation. [7] (We were not able to generate and isolate in quantity the *Z* alkylidene species with which to conduct cyclization studies.) Unfortunately, the reaction conditions also led to the formation of significant amounts of racemic cyclization adducts (±**9**). After surveying various conditions, we discovered that bis-morpholine aminal **6**, used directly without purification, underwent smooth Knoevenagel condensation with β-ketoester **7** (2 equiv of glacial acetic acid at 22 °C in toluene) to deliver alkylidene **8** with minimal levels of racemic cyclized compounds. As predicted, the resulting alkylidene **8** underwent the desired intramolecular conjugate addition when exposed to 10 mol % quinine-derived C6′ thiourea catalyst **I** in toluene at −25 °C.[19–20] Multiple iterations of the cyclization of alkylidene **8** consistently afforded carboxyflavanone **9** in good yield (78–80%) and with excellent enantioselectivity (90–91% ee). Importantly, the alkylidene precursors underwent smooth cyclization with excellent control over the newly formed C2 stereocenter using our chiral thiourea catalysis conditions.

The second challenge in this synthetic endeavor was the development of viable conditions that would allow for the single-flask decarboxylation of the *t*-butyl ester and MOM deprotection of 3-carboxyflavanone **9** to cleanly afford abyssinone I (**1**) in good yield and with minimal, if any, erosion in optical activity. Initially, the decarboxylation and deprotection of **9** was accomplished in a single flask utilizing $MgBr_2 \cdot OEt_2$, but the low yields for this reaction sequence (less than 25%) led us to investigate higher yielding reactions conditions. Subjecting **9** to TMSCl/NaI provided **1** in slightly higher but still disappointing yields, and with an unacceptable erosion of optical activity (see Table 1). Flavanones which contain alkoxy or hydroxy substituents in the C4′ position are susceptible to epimerization, presumably due to a stabilized benzylic cation formation (see **A**, Table 1).[7] This undesired intermediate is achiral and after aqueous workup can cyclize to afford the racemic flavanones. Various *O*-aryl-protected *t*-butyl carboxyflavanones could not be converted into **1–4** without significant epimerization or functionalization of the abyssinones containing prenyl side chains. From our initial studies with chiral flavanones, we also knew that Brønsted acid-catalyzed removal of the *t*-butyl ester epimerized the sensitive C2 center and was therefore not a viable option. Ultimately, we anticipated that exchanging the *t*-butyl ester for an allyl ester and replacing the methoxymethyl group with an allyl group might allow for mild, single-flask decarboxylation/deprotection employing palladium catalysis.

The requisite allyl-protected alkylidenes **10–13** were synthesized directly from the corresponding aldehydes using our mild bis-aminal approach.[16] These substrates set the stage for the decarboxylation and phenol deprotection to be performed in one flask to deliver

the target molecules.[21] The key asymmetric cyclizations of **10–13** were catalyzed by exposure to 10 mol % of either the quinine- or quinidine-derived thiourea **I** or **II**[19] at −25 °C in toluene (Table 2).[22] The use of morpholine in the presence of 5 mol % $Pd(PPh_3)_4$ promoted the deprotection and decarboxylation at 22 °C to afford the natural product abyssinones I, II, III and IV 4′-OMe ether cleanly and in high yields.[23] The levels of enantioenrichment for each compound from the asymmetric conjugate addition (i.e., **10–13** to **14–17**) were excellent. The quinidine-derived thiourea catalyst **II** provided each of the abyssinones (after deprotection/decarboxylation) with the naturally occurring configuration at C2, while employing the pseudo-enantiomeric thiourea **I** generated the unnatural (*R*)-abyssinones with comparable levels of stereoselectivity. These results are especially satisfying since 1) flavanones containing alkoxy or hydroxy substituents in the C4′ position are susceptible to epimerization due to stabilized benzylic cation formation and 2) flavanones can undergo reversible ring opening to form 2′-hydroxychalcones upon exposure to mild bases. The overall yields for this process (conjugate addition, allyl deprotection, decarboxylation) provided an efficient method for the construction of these flavanones, and allowed for the evaluation of our synthetic method in terms of more complex synthetic efforts.

We next sought to determine if the enantiomerically enriched abyssinone natural products and their corresponding enantiomers would elicit potentially useful, *stereodependent* biological activity. Members of the broad flavonoid family of natural products (over 5,000) have exhibited a wide variety of anti-cancer effects, acting as antioxidants, angiogenesis inhibitors and potent cytotoxic agents.24,25 In addition, several flavonoids have been shown to inhibit the activity and downregulate the expression of pro-metastatic enzymes, such as the matrix metalloproteinases (MMPs), in a variety of tumor cell lines.26–29 However, these studies have focused primarily on *achiral* isoflavones, and none of these investigations to date have been conducted on the abyssinone family of flavanone natural products.[24,30,31] Our enantioselective synthesis approach allowed for the preliminary evaluation of these compounds, and also provided *for the first time* the means to evaluate the role of stereochemistry on two important phenotypes associated with cancer progression, cell proliferation and motility.

In the United States, prostate cancer (PCa) is the second most common cause of cancer-related death in men, and mortality is caused by the development of metastatic disease.[32] In order to metastasize, PCa cells must move from the prostate gland to distant sites in the body and continue their unchecked growth. Proteases such as MMPs increase cell invasion, and thus their synthesis by cancer cells facilitates movement and metastatic behavior.33,34 MMP-2 has been shown to be a particularly important target for human PCa, since its increased expression in tissue portends metastasis.35 Many studies have demonstrated the importance of MMP-2 and its role in increasing human PCa cell invasion. Decreased MMP-2 expression leads to decreased invasion of human prostate cancer cells *in vitro* and to decreased metastasis of human prostate cancer cells in a murine model of metastasis.[30,36] As such, the MMPs, and MMP-2 in particular, represent an important and extensively studied therapeutic target. However, while some first-generation MMP inhibitors have been effective in preclinical models, there has been little success in subsequent clinical trials, predominantly due to severe systemic toxicity.[37,38]

An alternative promising strategy for counteracting the pro-invasive effect of MMP-2 is controlling the amount of this enzyme produced by tumor cells (i.e., upregulation and downregulation).36 This type of approach has been validated by studies which show that decreasing the levels of MMP-2 mRNA transcript within cancer cells leads to decreased invasive potential and metastasis. We (Bergan) have demonstrated that PCa cells treated with the isoflavone genistein exhibit decreased levels of both MMP-2 gene transcript and

protein, which leads to an overall reduction in invasive potential.30 Furthermore, studies performed on additional cancer cell lines have demonstrated that siRNA knockdown of MMP-2 leads to a reduction of both invasion and tumor-induced angiogenesis.39–41 Developing a method to downregulate MMP expression levels also has the advantage of being potentially less toxic than traditional MMP inhibitors, which suffer from a lack of specificity and generalized cell toxicity. Thus, we sought to evaluate the ability of the abyssinone natural products generated in our (Scheidt) laboratory to inhibit PCa cell growth and downregulate the expression of MMP-2, since these types of interventions could both attenuate/prevent metastasis and increase survival rates for PCa. We were also eager to evaluate the effect of stereochemistry on the ability of the abyssinones to inhibit cell proliferation and downregulate MMP-2 expression, since prior to our synthetic approach, sufficient quantities of the enantioenriched material were not available for this type of evaluation.

Our studies began by evaluating the impact of enantioenriched and racemic abyssinones (I, II, III, IV 4′-OMe) on metastatic PCa (PC3-M) cell growth. Given that enantioenriched abyssinone natural products have never been synthesized, we were particularly interested to determine if the enantiomers of each compound demonstrated differential cytotoxicity when compared to each other and also to the racemic mixture.[42] Furthermore, these cytotoxicity studies were instrumental for defining non-toxic levels of **1–4**, which guided the MMP-2 transcript expression evaluations (vide infra). Metastatic variant human PC3-M cells were treated for 3 days with 0–50 μM of racemic, (*R*)- or (*S*)-**1–4** (twelve compounds total) under conditions of exponential cell growth, then MTT assays were performed.[43] The CC_{50} values were then determined for each compound and are shown in Figure 3A. As indicated by Figure 3A, (*S*)-abyssinones III (**3**) and IV 4′-OMe (**4**) both demonstrated statistically significant levels of inhibition of PC3-M cell proliferation when compared to the (*R*) enantiomers and the racemic mixtures. In fact, both (*S*)-abyssinone III and 4′-OMe caused an approximate two-fold decrease in the proliferation of PC3-M cells, as they possessed CC_{50} values of 17 and 12 μM, respectively, compared to the CC_{50} values of the (*R*) enantiomers and racemates at 42 ((*R*)-**3**), 57 ((±)-**3**), 46 ((*R*)-**4**) and 33 μM ((±)-**4**). These results clearly indicate the key role of stereochemistry in the biological activity of these compounds, and validate our enantioselective synthetic approach for these particular natural products. Further evidence for the importance of the enantioselective synthesis of the abyssinones is also provided by the growth inhibition data for the racemic compounds (Figure 3A). Racemic abyssinone II was actually significantly *less* active than both of the enantioenriched compounds (p=0.052 and p=0.042 for comparison between racemic **2** and (*S*) and (*R*) enantiomers, respectively). A similar trend was also seen for racemic abyssinones I and III, although there was no statistical significance in these cases. This is a particularly interesting result, as it indicates that there may be some functional antagonism between the enantiomers of abyssinone II that leads to a significant reduction of activity in the racemic mixture. In fact, these findings validate our enantioselective synthesis approach for these compounds, since it is possible that the inhibitory activity of abyssinones I, II and III would have been significantly underestimated had they only been tested in their racemic form. Finally, we also performed some preliminary experiments to try to determine if the abyssinones were inhibiting cell growth via a cytotoxic (i.e., cell death) or cytostatic (i.e., cell cycle arrest) mechanism (see Figure 3B). Interestingly, when the absorbance values at 540 nm for the treated cells at 50 μM (t=72 hours) were compared with those for untreated cells at the start of the experiment (t=0 hours), (*S*)-abyssinones III and 4′-OMe both appeared to reduce the number of cells to a level at or below that of the start of the experiment. This result suggested that these compounds were acting through a cytotoxic mechanism, which was verified by cell imaging that indicated gross cell death at 72 hours (see Supplementary Information). However, all of the other compounds, including (*R*)-abyssinones III and 4′-OMe, demonstrated absorbance values that were greater than that of

the t=0 measurement, indicating that they were most likely acting through a cytostatic or combination (cytostatic and cytotoxic) mechanism. Although these experiments only provide a very preliminary look at the mechanism behind the inhibition of cell proliferation, they are also very interesting in that the enantioenriched (*S*)-abyssinones III and 4´-OMe appear to be working through a different type of mechanism that than of their corresponding enantiomers and racemates, *Therefore, all of these combined findings highlight the importance of our hydrogen-bonding catalysis approach, since this information could not have been obtained without sufficient amounts of the enantioenriched compounds for analysis*. Further, although the dose levels required for this analysis were ***not*** optimized for potency, these results are highly promising and demonstrate the urgency for, and future potential of, further studies designed to improve cytotoxicity and to determine the mechanism of action of these compounds on cell proliferation.

We next chose to evaluate the impact of (*R*)- and (*S*)-**1–4** (eight compounds total) on the levels of MMP-2 transcript, given that several members of the flavonoid family of natural products have been shown to downregulate the expression of this important pro-metastatic enzyme. Our aim was to determine not only the cytotoxicity of these compounds (i.e., their *chemotherapeutic* potential) but also to evaluate their ability to target MMP-2 synthesis and to act as *chemopreventative* agents wanted to determine if the abyssinones could selectively inhibit both the growth capable of blocking progression to a more aggressive disease state. We also and metastatic potential of PCa cells in a stereochemically-based manner. Cells were treated with each compound at 3 μM for 3 days, since this dose was not associated with cytotoxicity by the MTT assay. Importantly, we chose this non-toxic dose for our analysis to eliminate any confounding non-specific effects due to cell toxicity. After abyssinone exposure, MMP-2 transcript levels were measured by isolating RNA and performing reverse transcription then quantitative real time polymerase chain reactions (qRT-PCR). MMP-2 *transcript* levels were measured instead of MMP-2 *protein* levels because MMP-2 expression is tightly regulated at the transcriptional level, and because flavonoids have been shown to inhibit MMP-2 transcript expression.[44] Furthermore, decreased levels of MMP-2 mRNA expression have also been correlated with a lower pathological stage of prostate cancer and, by extension, an improved clinical outcome.[35,45] Therefore, the measurement of *transcript* levels provides a *direct* measure of the ability of the abyssinones to target cell-based processes responsible for regulating this clinically relevant pro-metastatic enzyme. Once the MMP-2 transcript levels were measured by qRT-PCR, they were normalized to that of the internal control gene GAPDH (glyceraldehyde-3-phosphate dehydrogenase), which was measured in both the treated and untreated cells.[46] GAPDH is expressed at constant levels across a wide array of biological systems, and is widely used for control purposes. We found that the GAPDH expression levels were the same for both the treated and untreated cells, which indicated that the effects on MMP-2 expression were ***not*** a result of non-specific downregulation of transcription.

With the MMP-2 studies *and* the cell growth studies, the abyssinone enantiomers exhibited statistically significant differential biological activity (Figure 4). In particular, for both abyssinones III and IV 4´-OMe, the *(R)* enantiomers suppressed MMP-2 expression to 65–78% of untreated control cells, respectively, at a non-toxic concentration (3 μM). Interestingly, the activity against MMP-2 expression for the unnatural *(R)*-enantiomers was significantly greater than that for the corresponding natural *(S)*- enantiomers. This unusual situation is reminiscent of reports by Boger on the surprising cytotoxicity of *ent*-roseophillin, which was found to be 2–10 fold more potent than the naturally occurring roseophillin.[47] While there are limited examples of natural products whose unnatural enantiomers demonstrate comparable biological potency (e.g., fredericamycin A,48 duocarmycin SA,49 CC-1065,50 and the cancer therapeutic mitomycin C51), we are not aware of compounds other than *ent*-roseophillin and (*R*)-abyssinones III and IV 4´-OMe that

are more active than their natural enantiomers. Also of interest was the fact that the (*S*) enantiomers were more cytotoxic than the unnatural (*R*) enantiomers (see Figure 3), while the (*R*) abyssinones were best able to downregulate the expression of MMP-2 (Figure 4). While this at first might appear to be a counterintuitive result, it is not surprising given that cancer growth and motility are *differentially regulated* processes. We have previously demonstrated this type of phenomenon with the isoflavone genistein, which is able to downregulate the expression of MMP-2 and inhibit metastasis in a murine model without causing any change in primary tumor size.[36] Therefore, not only were the abyssinones able to differentially regulate PCa cell growth and MMP-2 expression in a stereochemically-dependent manner, but they were also able to *specifically* target two independent processes important in cancer development and progression. These examples clearly demonstrate that *the construction of unnatural antipodes by stereoselective synthesis provides new avenues for potential therapeutic development*. The *in vivo* assays described above interrogate distinctly different biological functions and highlight the importance of successfully installing the stereochemical elements during the syntheses of the abyssinones. The cytotoxicity and MMP-2 studies conducted using each enantiomer show different response profiles across the abyssinones (**1–4**), with promising abilities to differentially target cell growth and metastatic potential.

In summary, the first asymmetric syntheses of abyssinones I-III and IV 4´-OMe have been accomplished using a chiral thiourea-catalyzed intramolecular conjugate addition. A tandem allyl deprotection/allyl ester decarboxylation generates the final products in a single flask while maintaining the stereochemical integrity at the C2 center. Our synthetic route delivers the natural products and the corresponding antipodes with excellent levels of enantioselectivity, thereby facilitating the evaluation of individual stereoisomers and fueling the discovery of their differential bioactivity. The key observations that the abyssinones specifically *and* differentially curtail cell growth and suppress MMP-2 expression in whole cells at non-toxic concentrations underscores the utility of our successful catalytic asymmetric synthesis of these natural products. These combined experiments fully integrate asymmetric catalysis, target synthesis application and chemical biology discovery with medicinal relevance. Continued investigations to enhance the catalytic asymmetric synthesis of the abyssinones and related compounds and to understand their biological activity against various cancer models are ongoing in our laboratories.

Methods

General catalytic asymmetric conjugate addition and tandem deprotection/decarboxylation

To a 10 mL round bottom flask was added **13** (43 mg, 81 μmol), thiourea catalyst **II** (6 mg, 8 μmol) and toluene (800 μL). The flask was purged with N_2 and the resulting solution was stored at −25 °C. Reaction progress was monitored by reverse phase high performance liquid chromatography. After 48 h toluene was concentrated *in vacuo* and the resulting residue was run though a short pad of silica gel using 10% EtOAc/hexanes as the eluent to afford (*S*)-**17** (33 mg) as a yellow oil. This oil was used without further purification. (2*S*)-allyl 7-(allyloxy)-2-(4-methoxy-3,5-bis(3-methylbut-2-enyl)phenyl)-4-oxochroman-3-carboxylate ((*S*)-**17**) (33 mg, 60 μmol), $Pd(PPh_3)_4$ (4 mg, 3 μmol), morpholine (80 μL, 900 μmol) and THF (1.2 mL) were stirred for 2 h and the solvent was concentrated *in vacuo*. Purified by column chromatography (SiO_2, 30% EtOAc/Hex) to afford (*2S*)-abyssinone IV 4´-OMe (**4**, 21 mg, 65% over two steps) as a yellow oil in 94% ee. Analytical data for **4** had identical spectral data compared to the natural material (see Supplementary information).

MTT growth inhibition assays

Three-day growth inhibition assays were performed in Falcon TC microtiter plates (Becton Dickinson, San Jose, CA) as previously described.[43] First, 800 PC3-M cells per 100 μL of cell culture media were plated into each well and were incubated for 24 hours. The synthetic abyssinones, as well as genistein (LC Laboratories, Woburn, MA; positive control), were suspended in culture media and added to the wells to give a final volume of 200 μL per well. Genistein and all of the synthetic abyssinones were suspended in DMSO (Sigma Chemical, St. Louis, MO) and stored at −20 °C prior to use. The final DMSO concentration did not exceed 0.5% in any experiment. The treated cells were then incubated for an additional 3 days, at which time MTT (dimethylthiazol-diphenyltetrazolium bromide) was added to each well (20 μL of a stock solution containing 5 mg/mL MTT in PBS) and the cells were incubated at 37 °C. Four hours later, the cells were lysed by addition of 200 μL of DMSO to each well, and the optical density at 540 nm was measured on a Bio Tek microplate reader. Assays were performed in triplicate (N=3) and repeated (N=9), and the average CC_{50} results were used for analysis.

Quantitative real-time reverse transcription PCR (qRT-PCR) for MMP-2 expression

Quantitative real-time reverse transcription and quantitative polymerase chain reaction were performed as previously described.[46] To generate the cDNA needed for PCR amplification, 1 μg of RNA was reverse transcribed with TaqMan reverse transcriptase (Applied Biosystems, Foster City, CA) using random hexamers. PCR reactions were then performed with cDNA from the equivalent of 60 ng of RNA in a reaction volume of 20 μL. Reactions were run in replicates of two using a TaqMan Universal PCR kit (Applied Biosystems) exon-spanning gene-specific sets of two primer and one probe TaqMan Gene Expression Assays for GAPDH and MMP2 (assay catalog #s: Hs99999905_m1 and Hs00234422_m1, respectively), and an Applied Biosystems 7500 Real Time Quantitative PCR System workstation. All reactions were repeated in a similar fashion at a separate time, also in replicates of two. For analysis of gene expression, the threshold cycle (Ct) for individual reactions was identified using the Applied Biosystems 7500 Real Time PCR System software. Relative gene expression was calculated using GAPDH for normalization purposes according to the $2^{-\Delta\Delta Ct}$ method. Assays were performed in duplicate (N=2) and repeated (N=3), and the average results were used for analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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42. When (*R*)-abyssinone III is exposed to cell culture media and is heated to 37 °C, there is no racemization at 9 h and 24 h as observed by HPLC (97:3 hexanes:IPA on AD-H Chiralcel column). However, (*S*)-abyssinone II does undergo loss of enantiomeric excess over a period of 24 hours under the same conditions, presumably due to the free 4′-OH phenol. Abyssinones I and IV 4′-OMe have similar structures to abyssinones III and do not have free phenols like abyssinone II. As such, these compounds are also expected to be stable to epimerization under the biological testing conditions.
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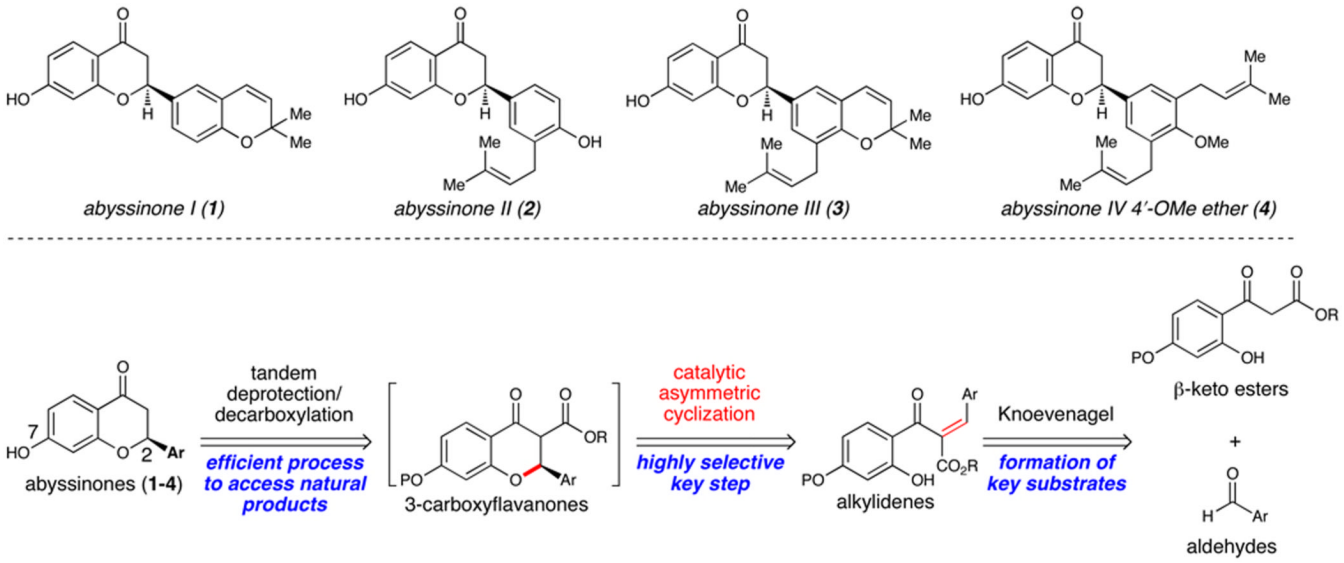


Figure 1.
Retrosynthetic Analysis of Abyssinone Natural Products

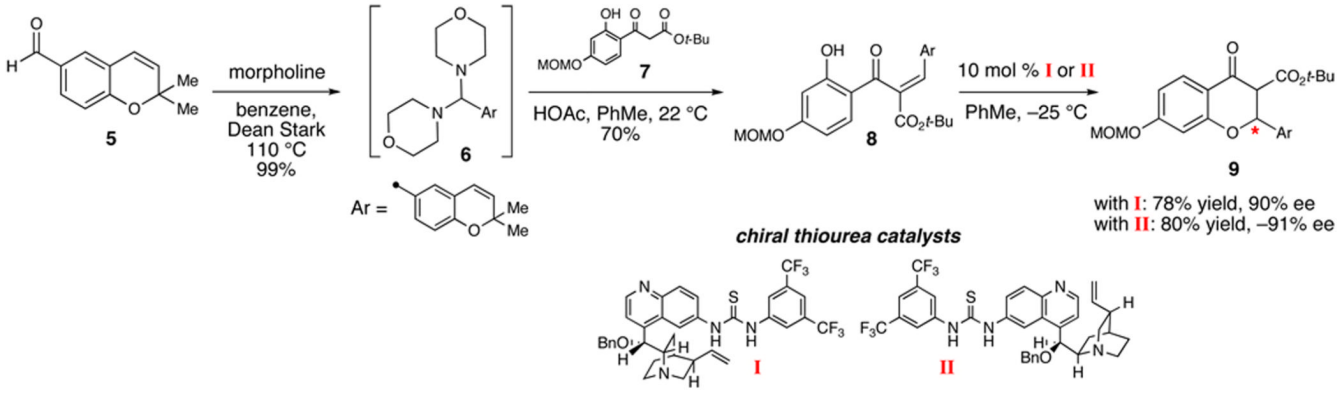


Figure 2.
Initial Route to Abyssinone I (**1**)

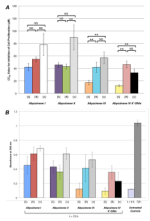


Figure 3.

A. CC_{50} values for each enantiomer and the racemic mixtures of abyssinones **1–4** against proliferation of PC3M cells. N=9 separate experiments, each performed in triplicate. Results are reported as an average CC_{50} value ± SE. A ** indicates a 2-sided t-test p value <0.05. NS indicates a 2-sided t-test p value >0.05. ***B.*** Absorbance values for MTT measurements of treated cells (t=72 hours) and untreated cells (t=0 and 72 hours). N=4 experiments, each performed in triplicate. Values are reported as the average absorbance value ± SE.

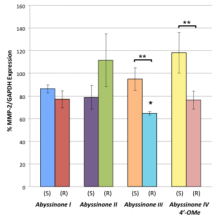
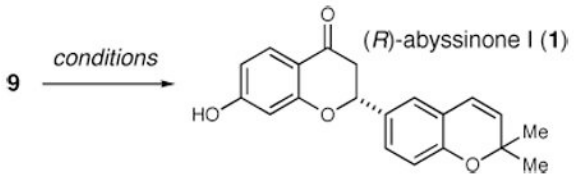


Figure 4.

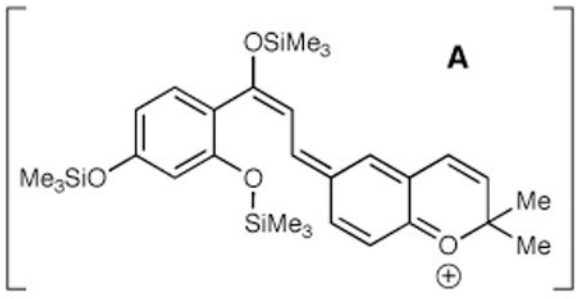
MMP-2 expression levels of abyssinone-treated PC3-M cells at 3 μM. N=3 separate experiments, each performed in duplicate. Results are reported as a percentage (mean ± SD) of the untreated control. A * denotes a two-sided t-test p value <0.05, for a comparison between each abyssinone and the untreated control. A ** denotes a one-sided t-test p value <0.05, for a comparison between enantiomers.

Table 1

First Generation Deprotection Approach



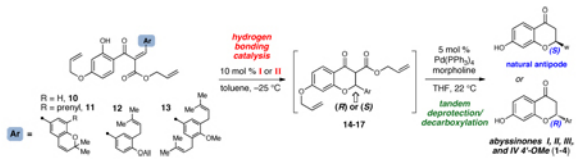
entry	conditions	ee sm/pdt (%)	yield (%)
1	6 equiv TMSCl/NaI, DTBMP, 4Å MS, CH_3CN	**90/79**	55
2	6 equiv TMSCl/NaI, DTBMP; then 1.0 M phos. buffer, CH_3CN	**89/84**	34



stabilized benzylic cation formation

Table 2

Catalytic Asymmetric Synthesis of Abyssinones



entry	thiourea catalyst	alkylidene	ee (%)[a]	yield (%)[b]		product
1	I	**10**	87	71	*(R)*-**1**	*ent*-abyssinone I
2	II	**10**	82	76	*(S)*-**1**	abyssinone I
3	I	**11**	88	61	*(R)*-**2**	*ent*-abyssinone II
4	II	**11**	89	72	*(S)*-**2**	abyssinone II
5	I	**12**	86	75	*(R)*-**3**	*ent*-abyssinone III
6	II	**12**	84	70	*(S)*-**3**	abyssinone IIII
7	I	**13**	95	65	*(R)*-**4**	*ent*-abyssinone IV 4'-OMe
8	II	**13**	94	65	*(S)*-**4**	abyssinone IV 4'-OMe

[a] Enantiomeric excess of abyssinones (**1–4**) after palladium(0) deprotection determined by HPLC analysis (Chiralcel OD-H or AD- H).

[b] Isolated yield from **10–13**.