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Total Syntheses of the Histone Deacetylase Inhibitors Largazole and 2-*epi*-Largazole: Application of *N*-Heterocyclic Carbene Mediated Acylations in Complex Molecule Synthesis

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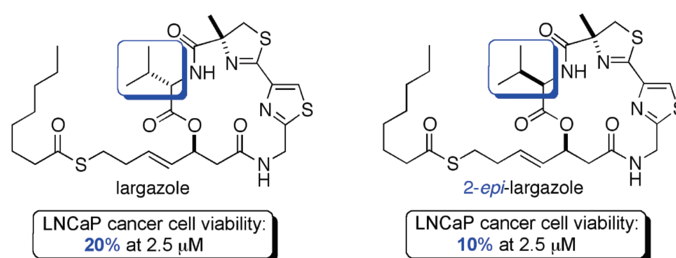

Details of the evolution of strategies toward convergent assembly of the histone deacetylase inhibiting natural product largazole exploiting γ,δ-unsaturated-α,β-epoxy-aldehydes and a thiazole-thiazoline containing ω-amino-acid are described. The initial *N*-heterocyclic carbene mediated redox amidation exploying these two types of building blocks representing largazole's structural domains of distinct biosynthetic origin directly afforded the seco-acid of largazole. This was accomplished without any protecting groups resident upon either thioester bearing epoxy-aldehyde or the tetrapeptide. However, the ineffective production of largazole via the final macrolactonization led to an alternative intramolecular esterification/macrolactamization strategy employing the established two building blocks. This provided largazole along with its C2-epimer via an unexpected inversion of the α-stereocenter at the valine residue. The biological evaluation demonstrated that both largazole and 2-*epi*-largazole led to dose-dependent increases of acetylation of histone H3, indicating their potencies as class I histone deacetylase selective inhibitiors. Enhanced p21 expression was also induced by largazole and its C2 epimer. In addition, 2-*epi*-largazole displayed more potent activity than largazole in cell viability assays against PC-3 and LNCaP prostate cancer cell lines.

Introduction

The marine natural product largazole (**1**) was isolated from a cyanobaterium collected from the waters of Key Largo, Florida, USA, and reported in early 2008.[1] The complete structural assignment of **1** was established by spectroscopic studies and the correlation of degradation fragments of **1** with known compounds. The skeleton of **1** embodies two distinct structural/biogenetic domains, the 3-hydroxy-7-octanoyl-mercaptohept-4-enoic acid and tetrapeptide-derived units, of which the thiol residues in cysteine components are condensed with the adjacent amides to generate vicinal azole rings. The potent and remarkably selective cytotoxic activities against human cancer cell lines that most dramatically distinguishes largazole from clinically employed anticancer agents has spurred many recent total synthesis efforts, with Hong and Luesch's cooperative efforts yielding the first reported.[2] The widespread supposition that largazole's primary mechanism of action involves inhibition of histone deacetylases (HDACs) is well supported by both natural occurring compounds and synthetic analogues.[1,2a,2d] Thus, largazole and its analogues may join the family of HDAC inhibitory clinical candidates, including suberoylanilide hydroxamic (SAHA),[3] (*S*)-(+)-*N*-hydroxy-4-(3-methyl-2-phenyl-butyrylamino)-benzamide

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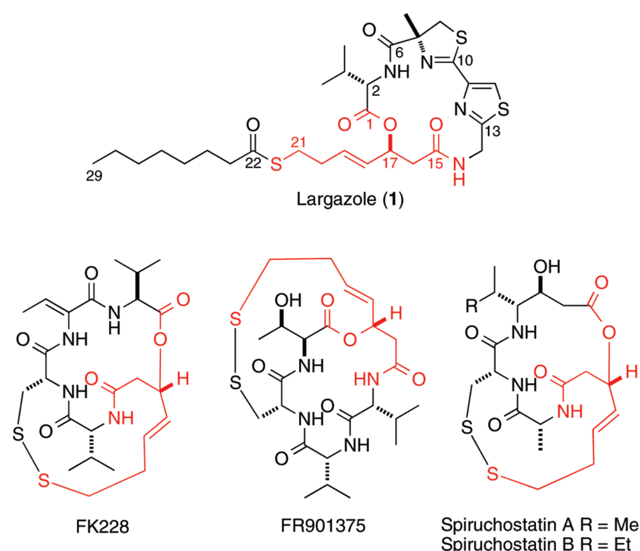
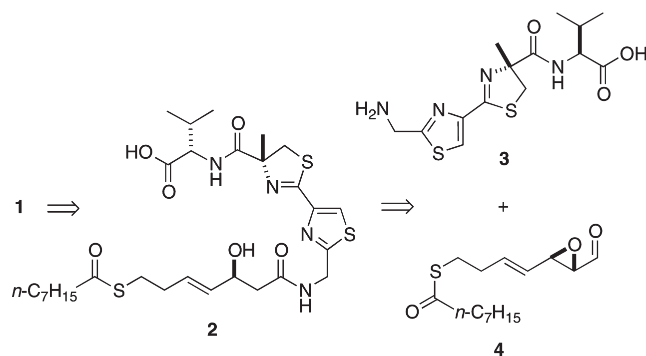


FIGURE 1. Strucutures of largazole and structurally related cyclic depsipeptides containing imbedded variants of the common 3-acyl-7-mercapto-hept-4-enoic acid moiety.

(AR42),[4] entinostat (SNDX-275, MS-275),[5] and (*R*)-trichostatin A.[6]

We were attracted by the combination of the simple yet novel architectural features embodied in **1** that conveyed highly selective cytotoxic activity. Rather than targeting one of several obvious synthetic approaches toward **1**, however, we were intrigued by the potential to assemble this remarkable natural product via a total synthesis strategy that would also provide a general entry to cyclic depsipeptides bearing the β-acylated amide structural motif. The latter include the antiproliferative natural products FK228, FR901375, and spiruchostatins, all of which contain the 3-hydroxy-7-mercapto-hept-4-enoic acid unit (Figure 1).[7] Largazole, as well as the related cyclic depsipeptide natural products may be considered to be prodrugs with regard to HDAC inhibiton. The inhibitory effects of FK228, FR901375, and spiruchostatins on HDACs presumably involve reductive cleavage of their disulfide bonds to liberate free thiol residues, which coordinate to the Zn^{2+} ions at the active site of HDACs.[3f,g] Similarly, hydrolytic cleavage of the octanoyl residue in the side chain of largazole by cellular lipases and/or esterases is a likely prerequisite for the cytotoxicity of largazole, as suggested by several studies.[2a,d] In particular, Williams et al. have shown that the termial C21 thiol analogue of **1** has HDAC inhibitory activities that are much more potent than those of the thiol ester **1**.[2d]

SCHEME 1. Original Retrosynthetic Plan for 1



Results and Discussion

NHC Mediated Amidation−Lactonization Total Synthesis Plan. Our initial total synthesis plan toward **1** involved obvious disconnection at the lactone to generate seco-acid **2** (Scheme 1). Less generally obvious was the recognition that a direct redox-amidation of the α,β-epoxy-aldehyde **4**, embodying the ketide derived domain of **1**, with an *unprotected* tetrapeptide derivative **3** representing the polypeptide region of largazole might be effected via the mediation of an *N*-heterocyclic carbene (NHC). The expected amide product **2** would retain the epoxide oxygen as a stereodefined β-hydroxyl group that would be engaged in the macrolactonization with the residual carboxylic acid (**2** → **1**). Bode and Rovis had demonstrated the utility of NHC-catalyzed amidations with α-substituted aldehydes and simple amines.[8] We were intrigued to test whether this approach could be amenable to the generation of β-hydroxy amides from *unprotected* amino acids or polypeptides, such as **3**, *without the necessity to protect the carboxylic acid moiety*. This represents an evolutionary departure from the entrenched traditional synthesis strategy of orthogonal protection/deprotection of C- vs N-termini for N-terminal peptide chain elaboration.

The aminoacid **3** would be obtained simply by a simultaneous release of both the *N*- and *C*-termini by cleavage of the *tert*-butyl ester and the *tert*-butoxycarbonyl protecting groups of the precursor **5** (Scheme 2). The initially pursued plan toward **5** involved a tandem Staudinger reduction-intramolecular *aza*-Wittig process[9] from bis-thioester **6** to affect bis-thiazoline ring closure, followed by oxidative aromatization of the

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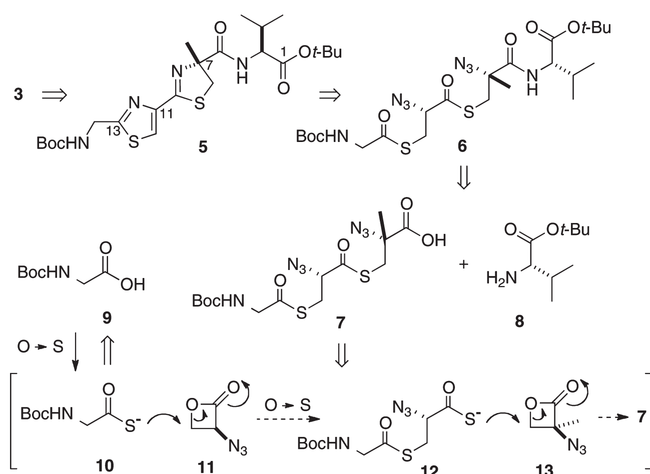
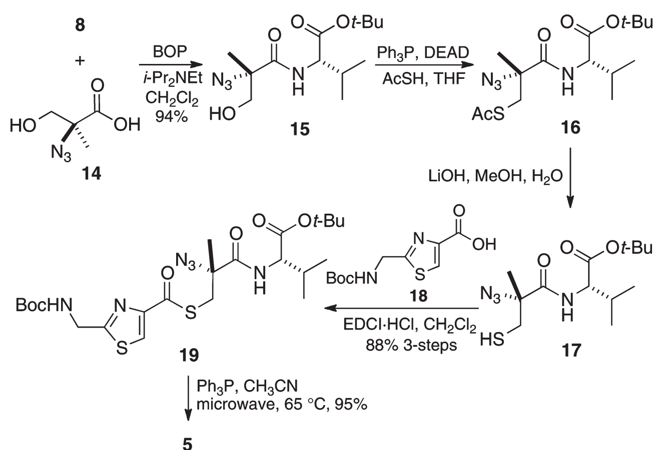
SCHEME 2. Initial Iterative β-Lactone-Opening/Staudinger Reduction/*aza*-Wittig Strategy for the Assembly of 3



SCHEME 3. Convergent Assembly of the Tetrapeptide Derivated Domain 5



C11−C13 2,4-disubstituted thiazoline (Scheme 2). Because thiol acids are presumably compatible with azide functionalities at or below ambient temperature, intermediate **6** was targeted to arise linearly from the *N*-terminal to *C*-terminal direction by iterative opening of α-azido-β-lactones (Scheme 2). This would involve sequential nucleophilic attack at the β-positions of the lactams with thiol acids derived from carboxylic acids. Specifically, the thiol acid derivative **10** of *N*-protected glycine **9** was expected to open α-azido-β-lactone **11**. A final amide coupling would complete the generation of **6**.

Thus, the implementation of this initially designed approach toward **3** required access to each of the building blocks valine *tert*-butyl ester **8**, glycine thio-*S*-acid anion **10**, α-azido-β-lactone **11**, and α-azido-α-methyl-β-lactone **13**. Whereas **8** and **10** were readily derived from commercially available amino acids, obtention of α-azido-β-lactones **11** and **13** required some additional effort. The hydrolytically labile **11** was obtained after screening many lactonization conditions via an intermolecular Mitsunobu reaction[10] in approximately 60% yield from α-azido-serine proccurred via diazo transfer of from TfN_3 to L-serine.[11] The more stable α-azido-α-methyl-β-lactone **13** was alternatively furnished in quantitative yield by treatment of the known α-methyl-α-azidoacid[12] with stoichiomitric PyAOP[13] and Et_3N. The planned deployment of these building blocks toward the assembly of **7** was immediately thwarted, however, as attempted openings of lactone **11** with *N*-Boc-glycine-thio-*S*-acid **10** were thoroughly unsuccessful. The thio-*S*-acid **12** generated *in situ* from a retro-Michael reaction of β-acylmercaptocyanide by the Fukuyama procedure[14] also failed to open the lactone **13**.

The protected form of **3**, polypeptide derivative **5**, was ultimately generated in a convergent fashion beginning with azido-acid **14** and valine ester **8** (Scheme 3). This involved efficient amide formation between **8** and **14** to yield primary alcohol **15**, and a subsequent two-step replacement of the hydroxyl group with a thiol. Thioesterification between **17** and the cysteine and glycine-derived thiazole **18**[15] provided azido-thioester **19** in excellent yield. Subsequent thiazoline formation was affected in a similarly efficient fashion by simply treating **19** with triphenylphosphine in acetonitrile under microwave irradiation to afford the complete polypeptide-derived domain of **1** in the terminally diprotected form of **3**.[9]

The ketide partner for the final convergent coupling in the form of epoxy-aldehyde **4** was initially targeted from known alcohol **20**[16] (Scheme 4). Thioesterification of **20** gave octanoylthiolide dienol **21**. However, mono-epoxidation of **21** under Shapless conditions[17] failed to afford the desired epoxyalcohol **22**. The thioester suffered cleavage during the reaction, and both the liberated thiol and isopropanol from the epoxidation catalyst formation participated in opening of the epoxide to give a complex mixture of undesired products. Alternatively, **4** was accessed from known sulfone-alcohol **23** containing a tetrazole-sulfone moiety via thioester **24** generated under Mitsunobu conditions (Scheme 4).[18] Julia-Kocienski olefination[19] exploiting **24** and the sensitive α,β-epoxy-aldehyde **25**[20] gave a 3.3: 1.0 mixture of alkenes (*E*,*Z*)-**26**, which were separated at the stage of primary alcohols **22** (45% yield, two steps). Oxidation then provided the unstable epoxy-aldehyde **4** (87%).

The next task was the key joining of oligopeptide derivative **3** and expoxy-aldehyde **4** via the projected NHC-mediated

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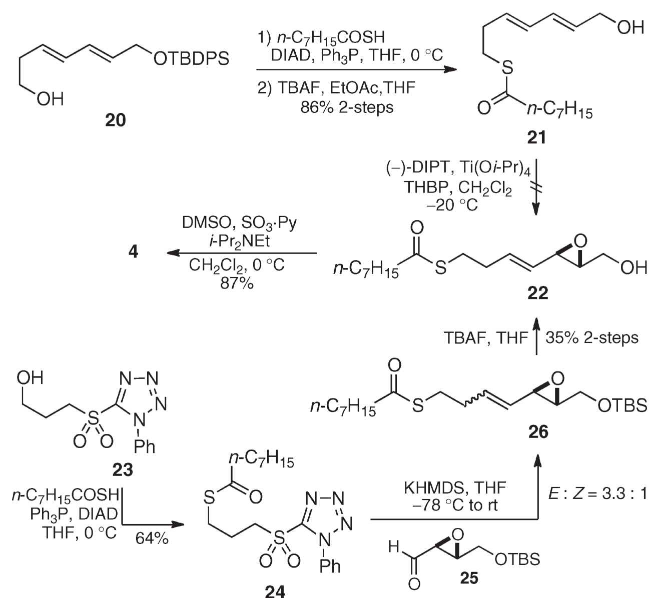
amidation. Both terminal *N*-Boc and *tert*-butyl ester protecting groups of **5** were simultaneously removed using trifluoroacetic acid to afford amino acid **3** as its ammonium trifluoroacetate salt (Scheme 5).

The marriage of amino acid **3** and epoxy-aldehyde **4** mediated by stoichiometric NHC and excess of Hünig's base resulted in 45% isolated yield of the anticipated β-hydroxy amide **2**, the seco-acid of largazole. Accompanying the formation of **2** were dehydration products (e.g., diene **vii**, Scheme 5) detected by mass spectrometric analyses. First, when NHC **28** was added to the epoxy-aldehyde **4** in the absence of the amino acid coupling partner **3**, a dienoic acid that may have resulted from the hydrolysis of **v** (Scheme 5) was detected by HRMS analysis. At this stage, the epoxy-aledehyde was no longer observed. Second, when the amino acid **3** was added, a species corresponding in mass to the corresponding diene amide **vii** was observed. Neither type of dehydration product was isolated or further characterized.

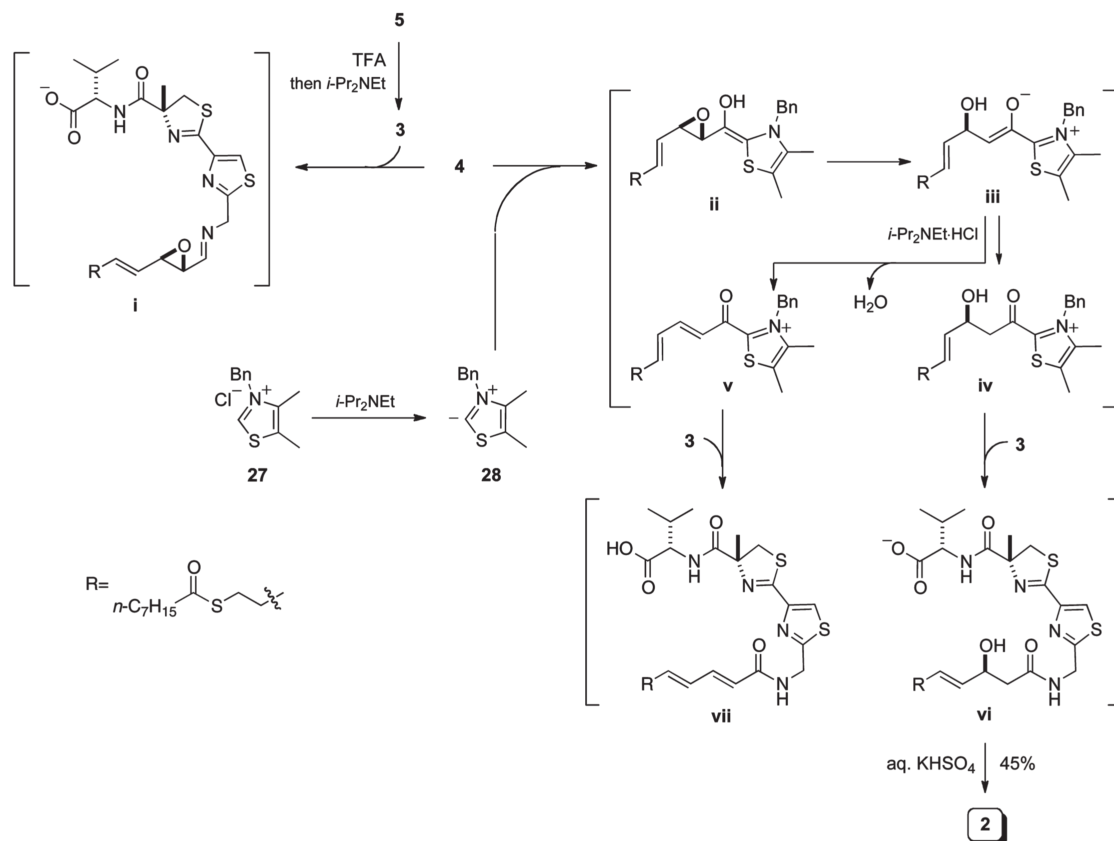
SCHEME 4. Synthesis of the Thioester Containing Epoxy-aldehyde 4



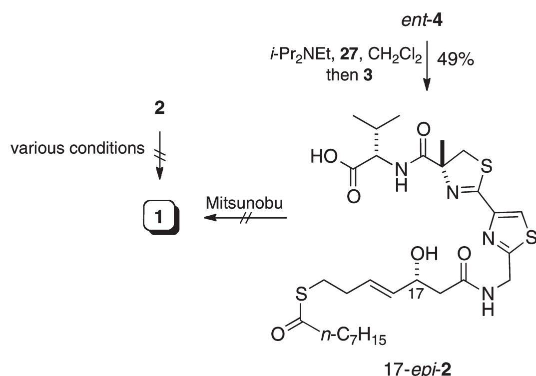
Similar amidation attempts using catalytic amounts of **28** failed to provide satisfactory results due to rapid competitive imine (**i**) formation between the aldehyde **4** and the amino terminus of **3**. Thus, a sequenced combining of aldeyde, thiazolium salt **28**, and Hünig's base operationally preceded the addition of the free amino acid. The addition of imidazole as reported by Rovis and Bode did not provide any improvement in this system. The use of an alternative NHC reagent[4b] was also examined, but little perturbation of the results was obtained.

The general mechanism for the NHC-mediated amidation proposed by Bode involves an internal redox wherein the NHC-aldehyde adduct derived from aldehyde **4** and *in situ* generated NHC undergoes tautomerization to **ii**, which represents an oxidation of the initial aldehyde carbon (Scheme 5).[9] Opening of the epoxide utilizing electron density resident on the nitrogen leads to β-hydroxy enolate **iii** via a subsequent proton transfer. The protonation of **iii** provides

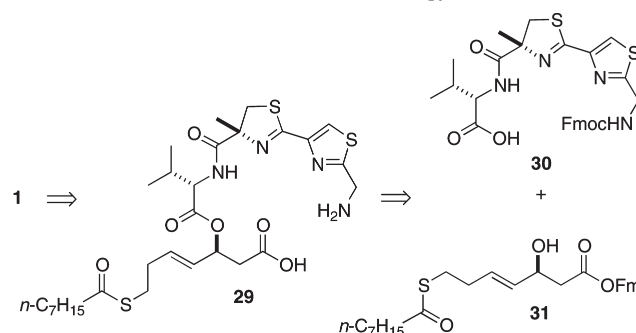
SCHEME 5. NHC-Mediated Amidation between Amio Acid 3 and Epoxy-aldehyde 4



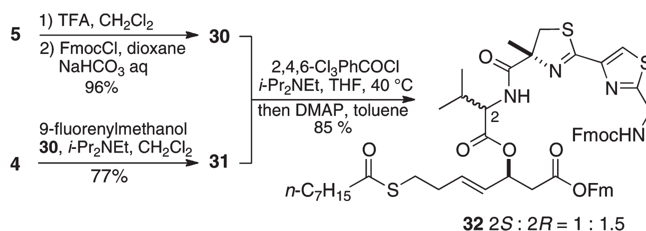
SCHEME 6. Unsuccessful Macrolactonization of Seco-acid 2 and Its C17-Alcohol Epimer



SCHEME 7. Macrolactamization Strategy toward 1



SCHEME 8. Intermolecular C1-Esterification



the β-hydroxy carbonyl **iv**, which represents the activated acyl donor that is captured by the free amino group of **3** to yield the amide **2** after acidifying.

The C17 alcohol epimer of **2** was also obtained in comparable yield via this stoichiometric NHC-mediated amidation process using the enantiomer of α,β-epoxy-aldehydes **4** (Scheme 6). This method could be extended to the use of *unprotected amino acids* to result in β-hydroxy-amides that are a common structural element among several potently biologically active cyclic depsipeptides.[7] Although synthetically useful, there are some complicating structural features of epoxy-aldehyde **4** that may contribute to the moderate yield leading to **2**. These include the presence of the γ-δ-alkene and the thioester in **4**. The former was likely to have contributed to a net dehydration to generate an α-β, γ-δ diene. For example, enolate **iv** could partition between the desired β-hydroxy carbonyl **iv** and the β-elimination product conjugated diene **v** (Scheme 5). The eliminated hydroxide may contribute to the generation of side products arising from hydrolysis of the acyl thiazolium intermediates. An empirical set of observations is that NHC-mediated amidations of **3** with thioester-aldehyde **4** were considerably lower-yielding than similar conjugations using an expoxy-aldehyde bearing a terminal silyloxy group in place of the thioester. Thus, the presence of an electron-withdrawing C21-thioester was demonstrated to be detrimental to achieving better chemical yields of the desired amidation products.

Exhaustive attempts to lactonize **2** or its C17-hydroxyl epimer via Keck,[21] Yamaguchi,[22] Mukaiyama,[23] or Mitsunobu[10] protocols and variants thereof[24] were unsuccessful (Scheme 6). There was uncertainty as to what extent the conformational ring strain and steric hindrance about the valine carboxyl residue in the intended macrocyclic precursor or the steric demand of the side chain was deterring the lactonization of **2** and its C17 hydroxyl epimer under each of the lactonization attempts. Indeed, there is no evidence among the now substantial body of published literature[2] regarding any success in attempts to convert the seco-acid of largazole or any of its side-chain analogues directly into lactones. Consequently, modifications of the original total synthesis plan were necessitated.

Total Synthesis via Macrolactamization. The revised strategy toward **1** involved closure of the largazole cyclic depsipeptide via macrolactamization of an ester-linked polypeptide/polyketide intermediate (e.g., **29**, Scheme 7). In contrast to our original synthetic plan, the valine ester would be formed prior to the polyketide-derived amide. This approach had been proven to be successful among many of the previously reported total syntheses of **1**. However, our revised strategy still takes advantage of NHC-mediated acylations, but not in the penultimate step in the original protecting group free end-game strategy. Thus, the two key domains of **1** were reinvented as the complementary synthetic coupling partners of valine-derived acid **30** and fully elaborated polyketide domain alcohol **31**. Each of these was designed to incorporate parallel 9-fluorenylmethanol-containing protecting groups on the ketide acid and peptide amino termini to facilitate simultaneous deprotection and subsequent lactamization.

The complementary polypeptide-derived amino-protected carboxylic acid **30** was generated from **5** (Scheme 2) by simple and efficient Fmoc-derivitation (Scheme 8). The requisite protected carboxylate **31** was readily obtained from α,β-epoxy-aldehyde **4** (Scheme 1) via NHC-mediated esterification with 9-fluorenylmethanol (Scheme 8). Again, mass spectroscopic analysis indicated the presence of a dehydration product accompanying the generation of **31**. The union of **30** and **31** was achieved by Yamguchi esterification to provide ester **32**, unexpectedly as 1:1.5 mixture of (2*S*) and (2*R*) valine epimers, respectively. Attempted esterification of **30** using EDCI·HCl was unsuccessful.

We hypothesize that the epimerization observed at the valine α-stereogenic center leading to (2*R*)-**32** may occur via the diketopiperazine intermediate **ix** (Scheme 9) generated in the process of the Yamaguchi esterification of **30** and **31**. Either the mixed anhydride **viii** derived from acid **30** and trichlorobenzoyl chloride or its DMAP-derivative **ix** may be

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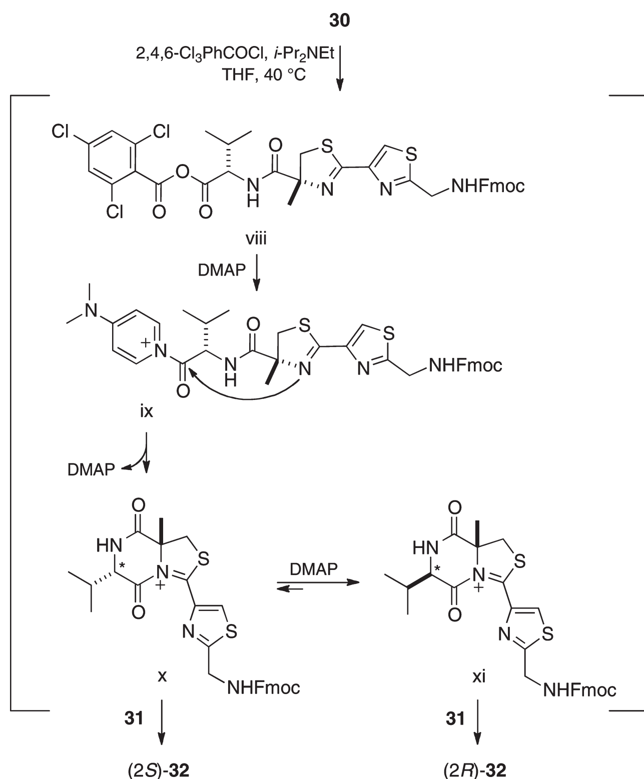
SCHEME 9. Thiazoline Facilitated C2-Epimerization

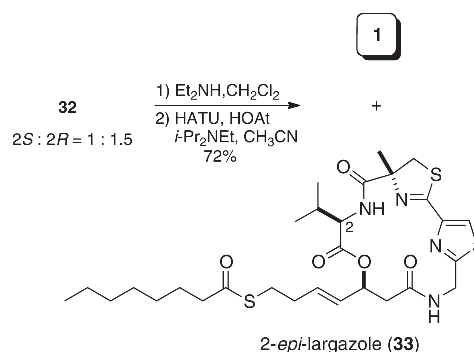


susceptible to diketopiperazine formation via involvement of the thiazoline nitrogen immediately leading to **x**. This initially formed structure **x** places the sterically demanding isopropyl group in an axial orientation on the newly formed six-membered ring. In the presence of basic DMAP, enolization−protonation may at least partially equilibrate the valine α-stereogenic center to favor the equatorially disposed valine isopropyl group in **xi**. Interception of the two epimeric diketopiperazine ions by the secondary alcohol of **31** would lead to (2*S*)-**32** and (2*R*)-**32** from **x** and **xi**, respectively.

Other conditions such as Mukaiyama-type reaction gave less epimerization at the C2 position [(2*S*)-**32**:(2*R*)-**32** ≈ 1.2:1] but also rendered much lower combined yield (< 20%) of the ester epimers. To diminish the potential epimerizing effect of the thiazoline moiety in the esterification reaction, we also explored the possibility of closing the thiazoline ring after formation of the intermolecular ester linkage between alcohol **31** and a *N*-Fmoc azido-acid derived from **19** (Scheme 3). The latter was simply obtained by protecting group manipulation of diprotected thioester **19**. However, it was found to be recalcitrant toward activation and esterification with **31** under various conditions.

The mixture of (2*S*)-**32** and (2*R*)-**32** was submitted, without separation, to the final two steps of deprotection and macrolactamization to generate a mixture of largazole and 2-*epi*-largazole (**33**)[25] in a 1:1.5 ratio, respectively, and 72% combined yield. These final products were then separated

SCHEME 10. Completion of 1 and Its C2-Epimer 33



chromatographically and subjected to biological evaluation (Scheme 10).

Although the original synthetic strategy of setting up a final macolactonization via NHC-mediated amidation between highly functionalized epoxy aldehyde **4** and *fully unprotected* tetrapeptide **3** to generate largazole seco-acid **2** was successful, the subsequent universal failure to effect macrolactonization derailed this approach toward **1**. Nonetheless, this general strategy is likely to be applicable to the delivery of related cyclic depsipeptides (Figure 1). Moreover, it demonstrates the utility of NHC-mediated chemoselective *N*-acylation of a fully unprotected amino acid/polypeptide. The simultaneous delivery of **1** and its C2 epimer **33** proved to be fortuitous, as subsequent parallel biological evaluation of each revealed unanticipated differential levels of responses.

Biological Evaluation. Synthetic largazole (**1**) and 2-*epi*-largazole (**33**) were subjected to several assays to determine their affects upon cancer cell viability and biomarker protein expression levels. Several reports comparing the HDAC inhibitory activity of largzole and an array of its analogues and known HDAC inhibitors against a panel of HDAC enzymes, as well as cell-based assays have emerged recently.[2a−d,i,j,25,26] Williams et al. showed that the termial C21 thiol analogue of **1** (**34**, Figure 2) had ca. 25- to 400-fold lower IC_{50} values than **1** with HDAC1, 2, 3, and 6, whereas **1** had consistently superior inhibitory activity over **34** against an array of melanoma cell lines.[2d] A comparison of terminal thiols **34** and its C2 epimer **35** showed the latter to be approximately 25-fold less active in inhibiting class I HDACs (HDAC1−3).[25] Upon this backdrop, it was of interest to survey the viability of prostate cancer cell lines PC-3 and LNCaP and the protein levels of acetyl-α-tubulin, α-tubulin, acetyl-H3, H3, p21, and β-actin in LNCaP cells, upon exposure to synthetic **1**, its C2-epimer **33**, and known HDAC inhibitors SAHA[3] and AR42.[4]

The MTT assay for cell viability was performed according to previously reported methods.[27] After seeding at a density of 2×10^4 cells/cm^2 for 24 h, the log phase growth of PC-3 and LNCaP cells was exposed to the indicated concentrations of agents in 10% FBS-supplemented medium for 72 h. The cells were then subject to MTT assay analysis as described

(25) Compound **51** was previously reported and assayed to have potent HDAC IC_{50} values of 30 nM (against HDAC1), 82 nM (HDAC2), 84 nM (HDAC3), and a significantly weaker inhibition of HDAC6 with IC_{50} of 0.68 μM. Bowers, A. A.; West, N.; Newkirk, T. L.; Troutman-Youngman, A. E.; Schreiber, S. L.; Wiest, O.; Bradner, J. E.; Williams, R. M. *Org. Lett.* **2009**, *11*, 1301–1304.

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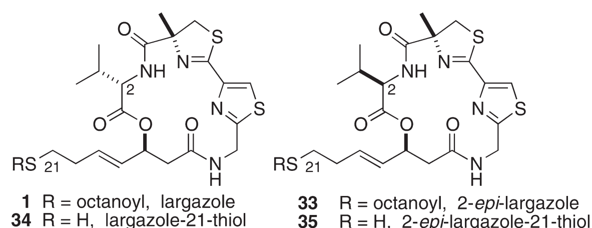

FIGURE 2. Structures of largazole, C21 thiols, and C2 epimers.

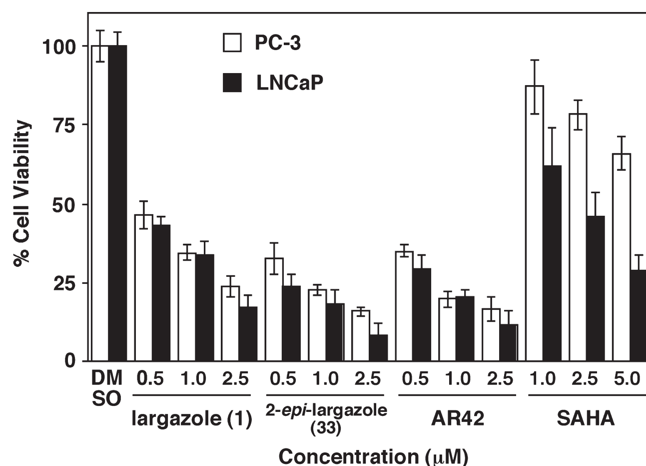

FIGURE 3. Effects of largazole, 2-*epi*-largazole, AR42, and SAHA on the viability of prostate cancer cell lines PC-3 and LNCaP ($n = 6$).

in the Supporting Information. The data depicted in Figure 3 indicate that the effects of **1** and **33**, as well as AR42 and SAHA, inhibit the growth of prostate cancer cell lines PC-3 and LNCaP. The *in vitro* data show that the agents **1**, **33**, and AR42 suppress more than 50% cell viability at 500 nM concentration, and the effects were on the order of 2-*epi*-largazole > AR42 > largazole > SAHA in both cell lines, although LNCaP is more sensitive than PC-3 to SAHA, which is consistent with literature reports.[28]

Western blotting for the levels of the proteins acetyl-α-tubulin, α-tubulin, acetyl-H3, H3, p21, and β-actin was performed as previously reported.[29] LNCaP cells were seeded at a density of 2×10^4 cells/cm^2 for 24 h and then exposed to **1**, **33**, AR42, and SAHA for 48 h for determination of differential protein levels as described in the Supporting Information. The Western blotting data (Figure 4) showed that all four agents led to dose-dependent increases of acetylation of histone H3, indicating their potencies as HDAC inhibitiors after exposure to these agents. In particular, **1** and **33** showed no changes of acetyl-α-tubulin after exposure to the indicated agents and concentrations, but treatment with AR42 and, to a lesser extent, SAHA significantly increased the acetylated α-tubulin levels. The high levels of α-tubulin acetylation is a biomarker for inhibition of class II HDAC6.[30] Consistently, these agents all led to

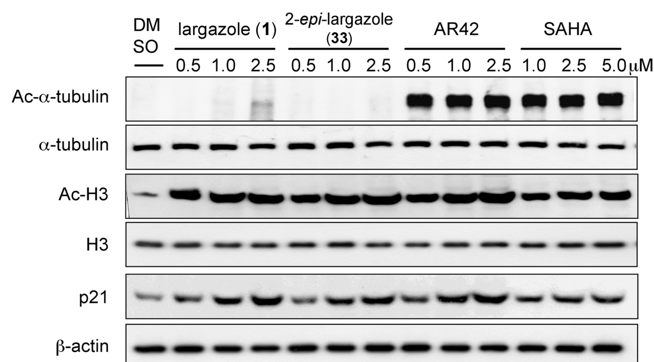

FIGURE 4. Effects of largazole, 2-*epi*-largazole, AR42, and SAHA on the protein levels of acetyl-α-tubulin, α-tubulin, acetyl-H3, H3, p21, and β-actin.

dose-dependent upregulation of p21, a frequently silenced tumor-suppressor gene whose expression in cancers could be re-expressed upon stimulation with HDAC inhibitors.[31]

The effect of 2-*epi*-largazole (**33**) is more potent than that of the parent compound largazole (**1**), in terms of inhibition of the viability of prostate cancer cells LNCaP and PC-3. Both largazole and its C2 epimer showed robust increases in levels of acetyl-H3 and upregulated the expression of the tumor suppressor gene p21. However, both largazole and 2-*epi*-largazole showed little change in the level of acetyl-α-tubulin, which is regulated by class II HDACs,[31] confirming their potential as class-selective HDAC inhibitors.

Conclusion

The cyclic depsipeptide largazole was targeted for total synthesis via a protecting group free end game strategy reliant upon an NHC-mediated amidation of an α,β-epoxy-aldehyde with a tetrapeptide. The anticipated amidation proceeded to give the desired β-hyoxy-amide, thus demonstrating the viability of this process for *acylation of an unprotected amino acid*. Although subsequent attempts to secure **1** via a final macrolactonization failed, this general strategy may be applicable to the assembly of other members of the general class of biologically active cyclic depsipeptides (e.g., Figure 1). As a consequence of activation of the *C*-terminal valine residue for esterification in an alternative synthetic sequence, the α-center of valine suffered an unanticipated epimerization to ultimately deliver the C2-epimer **33** along with largazole. Biological assays provided several new insights. First, despite the previously reported ca. 25-fold lower inhibitory activity of **33** versus **1** against HDACs 1–3,[25] **33** displayed more potent activity than **1**, AR42, or SAHA in cell viability assays against PC-3 and LNCaP prostate cancer cell lines. Second, in LNCaP cells, both **1** and **33** enhanced histone 3 acetylation levels but dramatically reduced acetyl-α-tubulin, consistent with the recently reported enzymatic profiling of **1** and related analogues as class I-selective HDAC inhibitors.[2d,25,26] Finally, **1** and **33** were shown to be at least as effective as AR42 and SAHA in inducing dose-dependent expression of the tumor suppressor gene product p21. Taken together, this study provides a novel synthetic strategy toward β-acyloxyl lactam

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cyclodepsipeptides, which in the case of **1** and **33** were demonstrated to display additional hallmarks of class I selective HDAC inhibitors. Moreover, **33** was shown to have better activity than **1** against the human prostrate cell lines PC-3 and LNCaP.

Experimental Section[32]

***O-t*-Bu-Val-α-Methylazidoserine Amide (15).** To a solution of acid **14**[33] (173 mg, 1.19 mmol) in CH_2Cl_2 (9 mL) were added *i*-Pr_2NEt (230 μL, 1.31 mmol) and benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP, 553 mg, 1.25 mmol). The mixture was stirred for 2 min before a solution of L-valine *tert*-butyl ester **9** (217 mg, 1.25 mmol) in CH_2Cl_2 (1 mL) was added. The reaction mixture was stirred for 12 h and then concentrated by rotary evaporation. The residue was purified by silica gel column chromatography (hexanes−ethyl acetate, 4:1) to give **15** (336 mg, 1.12 mmol, 94%) as a colorless oil: R_f 0.17 (hexanes−ethyl acetate, 4:1); ^{1}H NMR (500 MHz) δ 7.04 (br d, *J* = 8.0 Hz, 1H), 4.41 (dd, *J* = 9.0, 4.5 Hz, 1H), 3.89 (d, *J* = 11.5 Hz, 1H), 3.81 (d, *J* = 11.5 Hz, 1H), 2.73 (br s, 1H), 2.21 (m, 1H), 1.15 (s, 3H), 1.50 (s, 9H), 0.96 (d, *J* = 7.0 Hz, 3H), 0.94 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (100 MHz) δ 171.0, 170.5, 82.4, 67.5, 67.5, 57.8, 31.3, 28.0, 19.1, 18.9, 17.5; $[\alpha]^{22}_D$ + 11.9 (*c* 1.68, $CHCl_3$); IR (neat) 3414, 2970, 2876, 2119, 1732, 1666, 1524, 1460, 1370, 1261, 1155, 1063 cm^{-1}; HRMS calcd for $C_{13}H_{24}N_4O_4$ $[M + Na]^+$ 323.1695, found 323.1693.

Azido-thiazole (19). To a solution of Ph_3P (423 mg, 1.6 mmol) in THF (3 mL) at 0 °C was added DIAD (0.33 mL, 1.6 mmol). After 10 min, a solution of **15** (162 mg, 538 μmol) in THF (2 mL) was added. After an additional 15 min, thioacetic acid (115 μL, 1.6 mmol) was added and the reaction mixture was allowed to slowly warm to rt with stirring over 2 h. Diethyl ether and pH 7 aqueous phosphate buffer (ca. 10 mL each) were added, the organic phase was separated, and the aqueous phase was extracted with diethyl ether. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The residue was kept at 4 °C for 10 h before being suspended in diethyl ether. The resulting white precipitate was collected by filtration and washed with diethyl ether. The filtrate was concentrated and the residue was suspended in hexanes. The mixture was filtered and the solids were washed with hexanes. The filtrate was concentrated and the residue was dissolved in methanol (5 mL). A solution of $LiOH\cdot H_2O$ (40 mg, 1.0 mmol) in water (1 mL) was added. After stirring for 10 min, an aqueous pH 7 phosphate buffer was added. The mixture was extracted with diethyl ether, and the extracts were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (hexanes−ethyl acetate, 4:1) to give **17** (193 mg, with ca. 8% of an inseparable impurity) as a yellow oil. To a solution of **17** (80 mg, ∼250 μmol) and **18**[34] (50 mg, 0.19 mmol) in CH_2Cl_2 (2 mL) were added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI·HCl, 55 mg, 0.29 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (DMAP, 6 mg, 0.05 mmol). After stirring for 20 min, the mixture was concentrated then diluted with diethyl ether and saturated aqueous NH_4Cl. The mixture was extracted with diethyl ether, the combined extracts were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (hexanes−ethyl acetate, 7:3) to give **19** (103 mg, 186 μmol, 96%) as a pale yellow oil: R_f 0.21 (hexanes−ethyl acetate, 7:3); ^{1}H NMR (500 MHz) δ 8.09 (s, 1H), 7.03 (d, *J* = 9.0 Hz, 1H), 5.36 (br s, 1H), 4.64 (d, *J* = 5.5, 2H), 4.38 (dd, *J* = 8.5, 4.5 Hz, 1H), 3.63 (d, *J* = 14.0 Hz, 1H), 3.58 (d, *J* = 13.5 Hz, 1H), 2.21 (m, 1H), 1.71 (s, 3H), 1.48 (app s, 18H), 0.97 (d, *J* = 7.0 Hz, 3H), 0.95 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz) δ 184.2, 170.4, 170.1, 151.9, 124.2, 82.1, 67.1, 57.8, 36.3, 31.3, 28.3, 28.0, 22.0, 18.9, 17.6; $[\alpha]^{22}_D$ +46.3 (*c* 8.6, $CHCl_3$); IR (neat) 3360, 2974, 2932, 2120, 1723, 1681, 1514, 1368, 1275, 1161 cm^{-1}; HRMS calcd for $C_{23}H_{36}N_6O_6S_2$ $[M + Na]^+$ 579.2035, found 579.2024.

(*S*)-*tert*-Butyl 2-((*R*)-2′-(((*tert*-butoxycarbonyl)amino)methyl)-4-methyl-4,5-dihydro-[2,4′-bithiazole]-4-carboxamido)-3-methylbutanoate (5). A mixture of azido-thiazole 19 (86 mg, 0.16 mmol), Ph_3P (203 mg, 770 μmol), and CH_3CN (20 mL) was placed in a capped 35 mL CEM microwave tube. The tube was flushed with argon and irradiated in the microwave synthesizer at 65 °C (monitored by external surface sensor) for 9 h. The mixture was then concentrated and purified by silica gel column chromatography (hexanes−ethyl acetate, 4:1) to give **5** (75 mg, 0.15 mmol, 95%) as a clear yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.17 (d, *J* = 9.0 Hz, 1H), 5.59 (br s, 1H), 4.61 (d, *J* = 4.5, 2H), 4.36 (dd, *J* = 8.5, 4.5 Hz, 1H), 3.75 (d, *J* = 11.5 Hz, 1H), 3.31 (d, *J* = 11.5, 1H), 2.11 (m, 1H), 1.57 (s, 3H), 1.44 (s, 9H), 1.43 (s, 9H), 0.85 (d, *J* = 7.0 Hz, 3H), 0.82 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.4, 170.5, 163.2, 148.7, 121.2, 85.1, 81.8, 57.4, 41.5, 31.2, 28.3, 28.0, 24.7, 18.9, 17.6; $[\alpha]^{22}_D$ −33.4 (*c* 3.89, $CHCl_3$); IR (neat) 3382, 1722, 1674, 1606, 1514, 1368, 1277, 1252, 1163, 1029 cm^{-1}; HRMS calcd for $C_{23}H_{36}N_4O_5S_2$ $[M + Na]^+$ 535.2025, found 535.2022.

Dienol (21). To a solution of triphenylphosphine (Ph_3P, 183 mg, 0.70 mmol) in THF (3 mL) at 0 °C was added diisopropyl azodicarboxylate (DIAD, 145 μL, 2.37 mmol). After the mixture stirred for 5 min, a solution of alcohol **20**[35] (205 mg, 559 μmol) in THF (1 mL) was added. After an additional 5 min, a solution of thiooctanoic *S*-acid[36] (112 mg, 0.70 mmol) in THF (1 mL) was added. The mixture was kept at 0 °C for 30 min before saturated aqueous NH_4Cl was added. The mixture was extracted with diethyl ether, and the combined extracts were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexanes−ethyl acetate, 10:1) to give the thioester (254 mg, 499 μmol, 89%). To a solution of this thioester in THF (0.9 mL) and ethyl acetate (0.1 mL) was added a solution of TBAF (1.0 mL, 1 M in THF). The resulting solution was stirred for 2 h before a saturated aqueous solution of NH_4Cl and diethyl ether was added. The organic phase was separated, and the aqueous phase was extracted with diethyl ether. The combined extracts were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by chromatography (hexanes−ethyl acetate, 5:1) to give dienol **21** (132 mg, 487 μmol, 97%) as a colorless oil: R_f 0.24 (hexanes−ethyl acetate, 5:1); ^{1}H NMR (500 MHz) 6.22 (dd, *J* = 15.0, 10.5 Hz, 1H), 6.10 (dd, *J* = 15.0, 10.5 Hz, 1H), 5.77 (dt, *J* = 15.5, 5.0 Hz, 1H), 5.65 (dt, *J* = 15.5, 7.0 Hz, 1H), 4.17 (d, *J* = 5.5 Hz, 2H), 2.93 (t, *J* = 7.5 Hz, 2H), 2.54 (t, *J* = 7.5 Hz, 2H), 2.36 (dt, *J* = 7.5, 7.0 Hz, 2H), 1.65 (m, 2H), 1.30−1.26 (m, 8H), 0.88 (m, 3H); ^{13}C NMR (125 MHz) δ 199.5, 132.1, 132.3, 131.3, 130.8, 63.4, 44.2, 32.7, 31.6, 29.7, 28.9, 28.3, 25.7, 22.6, 14.0; IR (neat) 3359, 3017, 2956, 2926, 2855, 1693, 1462, 1093 cm^{-1}; HRMS calcd for $C_{15}H_{26}O_2S$ $[M + Na]^+$ 293.1546, found 293.1544.

Sulfone (24). To a 0 °C solution of triphenylphosphine (Ph_3P, 622 mg, 2.37 mmol) in THF (14 mL) was added diisopropyl azodicarboxylate (DIAD, 0.49 mL, 2.37 mmol). After the mixture stirred for 5 min, a solution of alcohol **23**[37] (562 mg,

(32) See the Supporting Information for general experimental details.

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2.09 mmol) in THF (3 mL) was added. After 5 min, a solution of thiooctanoic *S*-acid (380 mg, 2.37 mmol) in THF (3 mL) was added. The resulting mixture was kept at 0 °C for 10 min before saturated aqueous NH_4Cl was added. The mixture was extracted with diethyl ether, and the combined extracts were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash silica gel column chromatography (hexanes−ethyl acetate, 5:1) to give sulfone **24** (550 mg, 1.34 mmol, 64%) as an oil: R_f 0.43 (hexanes−ethyl acetate, 4:1); ^{1}H NMR (500 MHz) 7.70 (m, 2H), 7.63−7.59 (m, 3H), 3.81 (m, 2H), 3.06 (t, J = 7.0 Hz, 2H), 2.57 (t, J = 7.5 Hz, 2H), 2.27 (m, 2H), 1.66 (m, 2H), 1.31−1.21 (m, 8H), 0.88 (m, 3H); ^{13}C NMR (125 MHz) δ 198.8, 153.3, 133.0, 131.5, 129.8, 125.2, 125.1, 54.7, 44.2, 31.6, 28.9, 26.8, 25.6, 22.8, 22.6,14.1; IR (neat) 3059, 2912, 2852, 1698, 1498, 1404, 1343, 1294, 1157, 1049 cm^{-1}; HRMS calcd for $C_{18}H_{26}N_4O_3S_2$ [M + Na]$^+$ 433.1344, found 433.1346.

Epoxy-alcohol (22). To a −78 °C solution of sulfone **24** (320 mg, 0.78 mmol) and expoxy-aldehyde **25**[38] (202 mg, 0.94 mmol) in THF (7 mL) was added a solution of potassium bis(trimethylsilyl)amide (KHMDS, 1.7 mL of a 0.5 M soln in toluene). After stirring at −78 °C for 1 h, the reaction mixture was slowly warmed to rt over 2 h and stirred for another 3 h. A pH 7 aqueous phosphate buffer was then added. The mixture was extracted with diethyl ether, and the combined extracts were dried, filtered, and concentrated. The residue was purified by silica gel column chromatography to give a mixture of (*E*)-**26** and (*Z*)-**26** (*E*:*Z* = 3.3:1.0, by ^{1}H NMR spectroscopic analysis). To a solution of this mixture in THF (3 mL) and ethyl acetate (0.1 mL) was added a solution of tetra-*n*-butylammonium fluoride (TBAF, 0.4 mL of a 1 M soln in THF) at 0 °C. After 30 min, pH 7 aqueous phosphate buffer was added. The resultant mixture was extracted with diethyl ether, and the combined extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexanes−ethyl acetate, 9:1 to 4:1) to provide (*E*)-**22** (77 mg, 0.27 mmol, 35%) and (*Z*)-**22** (24 mg, 0.08 mmol, 10%). Analytical data for (*E*)-**22**: R_f 0.14 (hexanes−ethyl acetate, 4:1); ^{1}H NMR (500 MHz) δ 5.93 (dt, J = 15.5, 7.0 Hz, 1H), 5.31 (J = 15.5, 8.0 Hz, 1H), 3.97 (dd, J = 12.5, 2.0 Hz, 1H), 3.70 (dd, J = 12.5, 4.0 Hz, 1H), 3.40 (dd, J = 8.0, 2.0 Hz, 1H), 3.09 (m, 1H), 2.93 (t, J = 7.5 Hz, 2H), 2.54 (t, J = 7.5 Hz, 2H), 2.36 (dt, J = 7.5, 6.5 Hz, 2H), 1.82 (bs, 1H), 1.66 (m, 2H), 1.31−1.21 (m, 8H), 0.88 (m, 3H); ^{13}C NMR (125 MHz) δ 199.4, 134.5, 128.5, 61.2, 59.9, 55.4, 44.2, 32.4, 31.6, 29.7, 28.9, 27.9, 25.7, 22.6, 14.1; $[\alpha]^{22}_D$ +20.4 (*c* 0.79, $CHCl_3$); IR (neat) 3410, 2927, 2856, 1691, 1459, 1410, 1123, 1085, 1040 cm^{-1}; HRMS calcd for $C_{15}H_{26}O_3S$ [M + Na]$^+$ 309.1500, found 309.1491.

Epoxy-aldehyde (4). To a stirred solution of epoxy-alcohol (*E*)-**22** (87 mg, 0.31 mmol) in CH_2Cl_2 (0.7 mL), DMSO (120 μL, 1.7 mmol), and *i*-Pr_2NEt (311 μL, 1.8 mmol) at 0 °C was added sulfur trioxide−pyridine complex ($SO_3\cdot$Py, 97 mg, 0.61 mmol). After 1 h, an aqueous pH 7 phosphate buffer was added. The resultant mixture was extracted with diethyl ether, dried, filtered, concentrated, and purified quickly by silica gel column chromatography (hexanes−ethyl acetate−*i*-Pr_2NEt, 95:5:0.5) to give unstable epoxy-aldehyde **4** (75 mg, 0.27 mmol, 87%), which was used immediately for the next reaction. Analytical data for compound **4**: ^{1}H NMR (500 MHz) δ 9.07 (d, J = 6.0 Hz, 1H), 6.02 (dt, J = 15.5, 7.0 Hz, 1H), 5.27 (J = 15.5, 8.0 Hz, 1H), 3.63 (dd, J = 8.0, 2.0 Hz, 1H), 3.30 (dd, J = 6.0, 2.0 Hz, 1H), 2.94 (t, J = 7.0 Hz, 2H), 2.55 (t, J = 7.0 Hz, 2H), 2.39 (dt, J = 7.0, 7.0 Hz, 1H), 1.66 (m, 2H), 1.31−1.27 (m, 8H), 0.88 (m, 3H); HRMS calcd for $C_{15}H_{24}O_3S$ [M + Na]$^+$ 307.1344, found 307.1348.

Largazole-seco-acid (2). To a rt solution of thiazoline **5** (30.0 mg, 58.5 μmol) in CH_2Cl_2 (2.0 mL) was added trifluoroacetic acid (TFA, 2.0 mL). After the mixture stirred for 1 h, the solvent was removed under a flow of nitrogen, and the residue was dried under high vacuum for 1 h. The residue was redissolved in CH_2Cl_2 (1.0 mL) and *i*-Pr_2NEt (80 μL, 0.23 mmol), and the mixture was again concentrated and dried under high vacuum for 30 min to give **3** as its *i*-Pr_2NEt ammonium carboxylate salt. To a solution of thiazolium salt **27**[8a,b] (20.0 mg, 83.4 μmol) in CH_2Cl_2 (0.6 mL) in a vial was added *i*-Pr_2NEt (40 μL, 0.12 mmol). The mixture was agitated manually for 1 min and then added to neat aldehyde **4** (24.0 mg, 84.4 μmol) along with 0.3 mL of CH_2Cl_2 used to rinse the vial. After being agitated manually for 1 min, the mixture was added to the crude aminoacid along with 0.3 mL of CH_2Cl_2 used to rinse the vial. The resulting mixture was stirred for 30 min before aqueous $KHSO_4$ (1 M) was added until the aqueous phase attained pH 1. The mixture was extracted with chloroform, and the combined chloroform extract was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate−isopropanol−water, 1:0:0 to 10:1:0 to 9:1:0.5) to give **2** (16.8 mg, 26.2 μmol, 45%) as a pale yellow oil: ^{1}H NMR (500 MHz, CD_3OD) δ 8.23 (s, 1H), 5.68 (dt, J = 15.5, 6.5 Hz, 1H), 5.58 (dd, J = 15.5, 6.5 Hz, 1H), 4.72 (d, J = 16.5 Hz, 1H), 4.65 (d, J = 16.0 Hz, 1H), 4.47 (ddd, J = 7.5, 6.5, 6.0 Hz, 1H), 4.31 (d, J = 5.5, 1H), 3.76 (d, J = 11.5 Hz, 1H), 3.38 (d, J = 11.5 Hz, 1H), 2.88 (t, J = 7.5 Hz, 2H), 2.55 (t, J = 7.5 Hz, 2H), 2.50 (dd, J = 14.0, 7.5 Hz, 1H), 2.43 (dd, J = 14.0, 6.0 Hz, 1H), 2.27 (m, 2H), 2.18 (m, 1H), 1.63 (m, 2H), 1.60 (s, 3H), 1.33−1.28 (m, 8H), 0.90 (d, J = 6.0 Hz, 3H), 0.89 (m, 3H), 0.85 (d, J = 7.0 Hz, 3H); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.60 (br s, 1H), 7.33 (d, J = 9.0, 1H), 5.67 (dt, J = 15.0, 7.0 Hz, 1H), 5.56 (dd, J = 15.5, 6.5 Hz, 1H), 4.81 (dd, J = 16.0, 5.5, Hz, 1H), 4.71 (dd, J = 16.0, 5.5 Hz,1H), 4.52 (m, 1H), 4.47 (m, 1H), 3.79 (d, J = 11.5 Hz, 1H), 3.39 (d, J = 11.5 Hz, 1H), 2.87 (t, J = 7.5 Hz, 2H), 2.53 (t, J = 7.5 Hz, 2H), 2.48 (m, 2H), 2.25 (dt, J = 14.0, 7.0 Hz, 2H), 2.20 (m, 1H), 1.64 (m, 2H), 1.60 (s, 3H), 1.29−1.26 (m, 8H), 0.91 (d, J = 6.5 Hz, 3H), 0.89−0.85 (m, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 199.7, 172.3, 169.8, 163.7, 148.1, 141.3, 133.4, 128.9, 122.1, 84.8, 68.9, 43.5, 43.4, 41.0, 40.3, 32.0, 31.4, 29.3, 29.0, 28.6, 28.5, 27.7, 25.4, 23.5, 22.2, 18.6, 16.8, 13.0; IR (neat) 3390, 3108, 2956, 2921, 2852, 1682, 1651, 1599, 1538, 1504, 1454, 1415, 1257, 1180, 1039 cm^{-1}; $[\alpha]^{22}_D$ −20 (*c* 0.13, MeOH); HRMS calcd for $C_{29}H_{44}N_4O_6S_3$ [M + Na]$^+$ 663.2315, found 663.2319.

Largazole-17-*epi*-seco-acid (17-*epi*-2). To a rt solution of thiazoline **5** (14.0 mg, 27.3 μmol) in CH_2Cl_2 (1.0 mL) was added TFA (1.0 mL). After the mixture stirred for 1 h, the solvent was removed under a stream of nitrogen, and the residue was concentrated further under high vacuum for 1 h. The residue was redissolved in CH_2Cl_2 (1.0 mL) and *i*-Pr_2NEt (80 μL, 0.23 mmol) and again concentrated and dried under high vacuum for 30 min to give the amino acid as its *i*-Pr_2NEt ammonium carboxylate salt. To a solution of thiazolium salt **27** (7.9 mg, 0.33 mmol) in CH_2Cl_2 (0.3 mL) in a vial was added *i*-Pr_2NEt (40 μL, 0.12 mmol). The mixture was agitated manually for 1 min and then added to a solution of aldehyde *ent*-**4** (9.3 mg, 0.33 mmol). After the resulting mixture was agitated manually for an additional 1 min, the mixture was added to the crude amino acid along with additional CH_2Cl_2 (150 μL) used to rinse. The mixture was stirred for 30 min before aqueous $KHSO_4$ (1 M) was added to achieve pH 1. The mixture was extracted with chloroform, and the combined extracts were dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate−isopropanol−water, 1:0:0 to 10:1:0 to 9:1:0.5) to give 17-*epi*-**2** (8.6 mg, 0.13 mmol, 49%) as a pale yellow oil: ^{1}H NMR (500 MHz, CD_3OD) δ 8.26 (s, 1H), 5.70 (dt, J = 15.0, 6.5 Hz, 1H), 5.59 (dd, J = 15.5,

(38) Hayashi, Y.; Shoji, M.; Mukaiyama, T.; Gotoh, H.; Yamaguchi, S.; Nakata, M.; Kakeya, H.; Osada, H. *J. Org. Chem.* **2005**, *70*, 5643–5654.

6.5 Hz, 1H), 4.72 (d, J = 16.0 Hz, 1H), 4.69 (d, J = 16.0 Hz, 1H), 4.47 (ddd, J = 7.5, 6.5, 6.0 Hz, 1H), 4.31 (d, J = 4.5 Hz, 1H), 3.76 (d, J = 11.5 Hz, 1H), 3.39 (d, J = 11.5 Hz, 1H), 2.90 (t, J = 7.5 Hz, 2H), 2.56 (t, J = 7.5 Hz, 2H), 2.51 (dd, J = 14.0, 7.5 Hz, 1H), 2.46 (dd, J = 14.0, 6.0 Hz, 1H), 2.26 (m, 2H), 2.16 (m, 1H), 1.63 (m, 2H), 1.60 (s, 3H), 1.31−1.29 (m, 8H), 0.91−0.88 (m, 6H), 0.84 (d, J = 7.0 Hz, 3H); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.55 (br s, 1H), 7.32 (d, J = 9.0, 1H), 5.67 (dt, J = 15.5, 7.0 Hz, 1H), 5.56 (dd, J = 15.5, 6.0 Hz, 1H), 4.79 (dd, J = 16.0, 6.0 Hz, 1H), 4.71 (dd, J = 16.0, 5.5 Hz, 1H), 4.51 (m, 1H), 4.47 (m, 1H), 3.79 (d, J = 11.5 Hz, 1H), 3.41 (d, J = 11.5 Hz, 1H), 2.87 (t, J = 7.5 Hz, 2H), 2.53 (t, J = 7.5 Hz, 2H), 2.50 (m, 1H), 2.45 (dd, J = 15.0, 8.5 Hz, 1H), 2.28 (m, 2H), 2.20 (m, 1H), 1.63 (dt, J = 14.0, 7.0 Hz, 2H), 1.59 (s, 3H), 1.30−1.25 (m, 8H), 0.92 (d, J = 7.0 Hz, 3H), 0.89−0.86 (m, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 199.7, 175.0, 172.3, 169.9, 163.7, 148.1, 133.4, 128.9, 122.3, 84.8, 68.9, 43.5, 43.4, 40.9, 40.3, 32.0, 31.4, 31.0, 29.3, 28.6, 28.5, 27.7, 25.4, 23.6, 22.2, 18.5, 16.7, 13.0; $[\alpha]^{22}_D$ −24 (c 0.16, MeOH); IR (neat) 3381, 3082, 2957, 2927, 2856, 1671, 1652, 1606, 1538, 1514, 1461, 1408, 1184, 1045 cm^{-1}; HRMS calcd for $C_{29}H_{44}N_4O_6S_3$ [M + Na]$^+$ 663.2315, found 663.2322.

Fmoc-Amino Acid (30). Thiazoline **5** (18.3 mg, 35.7 μmol) was dissolved in CH_2Cl_2/TFA (1:1 v/v, 2 mL) and stirred at rt for 2 h. The solvent was removed, and the residue was dried under high vacuum for 1 h. Saturated aqueous $NaHCO_3$ (0.4 mL) was added followed by 9-fluorenylmethyl chloroformate (FmocCl, 9.2 mg, 0.43 mmol) in 1,4-dioxane (0.4 mL). After vigorous stirring for 1 h, 1 M aqueous $KHSO_4$ was added until pH 2 was attained. The mixture was then extracted with ethyl acetate, and the combined extracts were dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (chloroform−methanol, 1:0 to 20:1) to yield **30** (19.8 mg, 34.3 μmol, 96%) as a colorless oil: R_f 0.14 (HPTLC, $CHCl_3$−MeOH, 10:1); ^{1}H NMR (500 MHz, CD_3OD) δ 8.18 (s, 1H), 8.02 (br t, J = 5.5 Hz, 1H), 7.81 (d, J = 7.5 Hz, 2H), 7.67 (d, J = 7.5 Hz, 2H), 7.62 (d, J = 8.5 Hz, 2H), 7.40 (t, J = 7.5 Hz, 2H), 7.31 (d, J = 7.5 Hz, 2H), 4.60 (s, 2H), 4.45 (d, J = 6.5 Hz, 2H), 4.38 (m, 1H), 4.24 (t, J = 6.5 Hz, 1H), 3.78 (d, J = 11.5 Hz, 1H), 3.39 (d, J = 11.5 Hz, 1H), 2.11 (m, 1H), 1.61 (s, 3H), 0.92 (d, J = 7.0 Hz, 3H), 0.88 (d, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 175.4, 175.4, 172.9, 172.9, 171.7, 163.8, 157.4, 148.3, 143.8, 141.3, 127.4, 126.8, 124.8, 122.3, 119.6, 84.7, 66.7, 57.4, 57.3, 48.5, 30.5, 23.6, 18.2, 16.7; $[\alpha]^{22}_D$ −36.1 (c 0.90, MeOH); IR (neat) 3385, 2967, 1726, 1661, 1520, 1450, 1252, 1194, 1143, 1041 cm^{-1}; HRMS calcd for $C_{29}H_{30}N_4O_5S_2$ [M + Na]$^+$ 601.1555, found 601.1562.

***β*-Hydroxy-fluorenylmethanol Ester (31).** To a suspension of thiazolium salt **27** (49 mg, 0.21 mmol) in CH_2Cl_2 (0.2 mL) was added i-Pr_2NEt (71 μL, 0.21 mmol). The mixture was agitated manually for 1 min and then added to a solution of aldehyde **4** (58 mg, 21 mmol) and 9-fluorenylmethanol (159 mg, 810 μmol) in CH_2Cl_2 (0.5 mL). The mixture was stirred for 30 min before saturated aqueous NH_4Cl was added. The mixture was extracted with diethyl ether, and the combined extracts were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexanes−ethyl acetate, 9:1) to give **31** (76 mg, 0.16 mmol, 77%) as pale yellow oil: R_f 0.29 (hexanes−ethyl acetate, 4:1); ^{1}H NMR (500 MHz) δ 7.81 (d, J = 7.5 Hz, 2H), 7.62 (d, J = 7.5 Hz, 2H), 7.42 (m, 2H), 7.34 (m, 2H), 5.71 (dt, J = 15.5, 6.5 Hz, 1H), 5.54 (dd, J = 15.5, 6.5 Hz, 2H), 4.47 (m, 3H), 4.14 (t, J = 6.0 Hz, 1H), 2.92 (t, J = 7.5 Hz, 2H), 2.61 (d, J = 1.5 Hz, 1H), 2.59 (d, J = 9.0 Hz, 1H), 2.55 (t, J = 7.5 Hz, 2H), 2.31 (q, J = 7.0 Hz, 2H), 1.67 (m, 2H), 1.33−1.19 (m, 8H), 0.89 (m, 3H); ^{13}C NMR (125 MHz) δ 199.5, 172.0, 143.7, 143.6, 141.3, 132.7, 129.7, 127.9, 127.2, 125.0, 120.1, 68.5, 66.5, 46.8, 44.2, 41.6, 32.2, 31.6, 29.7, 28.9, 28.2, 25.7, 22.6, 14.1; $[\alpha]^{22}_D$ −3.49 (c 1.58, $CHCl_3$); IR (neat) 3461, 2924, 2853, 1735, 1689, 1450, 1272, 1168 cm^{-1}; HRMS calcd for $C_{29}H_{36}O_4S$ [M + Na]$^+$ 503.2232, found 503.2237.

Ester (32). A solution of 2,4,6-trichlorobenzoyl chloride (1.9 μL, 12 μmol) and i-Pr_2NEt (2.2 μL, 12 μmol) in THF (120 μL) was added to acid **30** (7.2 mg, 12 μmol). The mixture was stirred at 40 °C for 5 h before being concentrated under vacuum. A solution of β-hydroxy-ester **31** (12.0 mg, 24.9 μmol) in toluene (120 μL) was added to the residue followed by the addition of DMAP (1.5 mg, 12 μmol). The mixture was stirred for 16 h before a saturated aqueous solution of NH_4Cl was added. The mixture was extracted with ethyl acetate, the extract was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexanes−ethyl acetate, 4:1 to 3:2) to give **32** (11.0 mg, 10.6 μmol, 85%) as mixture of C2-epimers (2R:2S = 1.5:1): HRMS calcd for $C_{58}H_{64}N_4O_8S_3$ [M + Na]$^+$ 1063.3784, found 1063.3789.

Largazole (1) and 2-*epi*-Largazole (33). To a rt solution of **32** (7.0 mg, 6.7 μmol) in CH_2Cl_2 (0.5 mL), was added diethylamine (0.25 mL). After stirring for 3 h, the volatiles were removed under vacuum, and the residue was redissolved in CH_2Cl_2 (0.5 mL) and i-Pr_2NEt (0.1 mL). After the resulting solution stirred for 10 min, the solvent was removed under vacuum, and the residue was azotropically dried twice with toluene (2 × 2 mL). The residue was dissolved in CH_3CN (7 mL), and 2-(1H-7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate (HATU, 5.1 mg, 13 μmol), 1- hydroxy-7-azabenzotriazole (HOAt, 1.8 mg, 13 μmol), and i-Pr_2NEt (4.6 μL, 27 μmol) were added. The resulting solution was stirred for 12 h before being concentrated under vacuum. The residue was purified by silica gel column chromatography to give a mixture of **1** and **33** (3.0 mg, 4.8 μmol, 72%). This mixture was separated by preparative TLC (hexanes−ethyl acetate−methanol, 10:10:1) to give **1** (1.2 mg, 1.9 μmol) and **33** (1.8 mg, 2.9 μmol) as colorless oils. Analytical data for largazole (**1**): ^{1}H NMR (500 MHz) δ 7.76 (s, 1H), 7.18 (d, J = 9.5 Hz, 1H), 6.44 (dd, J = 9.5, 3.0 Hz, 1H), 5.83 (dt, J = 15.5, 6.5 Hz, 1H), 5.67 (app t, J = 7.0 Hz, 1H), 5.53 (dd, J = 16.0, 7.0 Hz, 1H), 5.30 (dd, J = 17.5, 9.5 Hz, 1H), 4.62 (dd, J = 9.5, 3.5 Hz, 1H), 4.29 (dd, J = 17.5, 3.0 Hz, 1H), 4.06 (d, J = 11.5 Hz, 1H), 3.29 (d, J = 11.5 Hz, 1H), 2.91 (t, J = 7.0 Hz, 2H), 2.86 (dd, J = 16.5, 10.0 Hz, 1H), 2.70 (dd, J = 16.0, 2.5 Hz, 1H), 2.53 (t, J = 7.5 Hz, 2H), 2.32 (br q, J = 7.5 Hz, 2H), 2.11 (m, 1H), 1.87 (s, 3H), 1.65 (m, 2H), 1.29−1.27 (m, 8H), 0.88 (m, 3H), 0.70 (d, J = 6.5 Hz, 3H), 0.53 (d, J = 6.5 Hz, 3H); ^{13}C NMR (125 MHz) δ 199.4, 173.6, 169.4, 168.9, 167.9, 164.6, 147.5, 132.7, 128.4, 124.2, 84.5, 72.0, 57.8, 44.2, 43.4, 41.1, 40.5, 34.2, 32.3, 31.6, 28.9, 28.9, 27.9, 25.7, 24.2, 22.6, 18.9, 16.7, 14.1; $[\alpha]^{22}_D$ +21 (c 0.10, MeOH); IR (neat) 3370, 3085, 2926, 2854, 1738, 1682, 1552, 1504, 1259, 1100, 1029 cm^{-1}; HRMS calcd for $C_{29}H_{42}N_4O_5S_3$ [M + Na]$^+$ 645.2210, found 645.2201. Analytical data for 2-*epi*-largazole (**33**): ^{1}H NMR (500 MHz) δ 7.68 (s, 1H), 7.21 (d, J = 8.0 Hz, 1H), 6.37 (dd, J = 7.5, 5.0 Hz, 1H), 5.89 (dt, J = 15.5, 6.5 Hz, 1H), 5.80 (app t, J = 9.0 Hz, 1H), 5.43 (dd, J = 15.5, 8.5 Hz, 1H), 5.08 (d, J = 17.0, 8.0 Hz, 1H), 4.28 (d, J = 11.0 Hz, 1H), 4.28 (dd, J = 17.0, 2.0 Hz, 1H), 4.23 (dd, J = 16.5, 5.0 Hz, 1H), 3.20 (d, J = 11.0 Hz, 1H), 2.88 (dt, J = 7.5, 2.0 Hz, 2H), 2.82 (dd, J = 16.5, 10.5 Hz, 1H), 2.58 (d, J = 16.5 Hz, 1H), 2.53 (t, J = 7.5 Hz, 2H), 2.29 (br q, J = 7.0 Hz, 2H), 2.13 (m, 1H), 1.80 (s, 3H), 1.64 (m, 2H), 1.30−1.23 (m, 8H), 0.97 (d, J = 7.0 Hz, 3H), 0.89 (d, J = 6.5 Hz, 3H), 0.88 (m, 3H); ^{13}C NMR (125 MHz) δ 199.4, 173.6, 169.4, 167.7, 167.6, 162.2, 147.7, 134.6, 128.5, 124.2, 85.0, 72.5, 59.7, 44.2, 41.6, 40.8, 40.3, 32.2, 32.2, 31.6, 28.9, 28.9, 27.8, 26.6, 25.6, 22.6, 18.8, 18.0, 14.1; $[\alpha]^{22}_D$ +43 (c 0.16, $CDCl_3$); IR (neat) 3342, 3076, 2925, 2857, 1738, 1682, 1552, 1503, 1259, 1107, 1034 cm^{-1}; HRMS calcd for $C_{29}H_{42}N_4O_5S_3$ [M + Na]$^+$ 645.2210, found 645.2201.

Materials and Methods for Biological Assays. The MTT assay for cell viability was performed according to previous reported

methods.[27] In brief, cells in each well after exposure to the indicated agents and concentrations were added with MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide] agent and incubated at 37 °C for 2 h. The supernatants were then removed, the reduced MTT dye was dissolved in DMSO, and the absorbance was determined at 570 nm.

Western blotting for the protein levels of each indicated biomarkers were performed as previously reported.[29] In brief, after exposure to the indicated agents and concentrations, LNCaP cells were collected by scrape. The cells were then centrifuged, and the cell pellets were washed with PBS once and lysed on ice for 30 min with a RIPA lysis buffer supplemented with Sigmafast protease inhibitor mixture. After centrifugation at 15,200*g* for 10 min, the supernatants were collected, and 1 μL of the lysates were used for protein concentration determination. Samples (25 μg) from each lysate were subjected to SDS-PAGE analyses and were then transferred to a PVDF membrane with a semidry system. Followed by blocking with 5% nonfat milk in a TBS buffer supplemented with 0.1% Tween 20 (TBST), the membranes were incubated with each specific antibody diluted in TBST at 4 °C at a concentration of 1:1000 (p21) and 1:2000 (acetyl-α-tubulin, α-tubulin, acetyl-H3, H3, and β-actin) for 12 h to overnight. After 5 washes with TBST (10 min each), the membranes were incubated with the horseradish peroxidase (HRP)-conjugated anti-rabbit and anti-mouse secondary antibodies at the concentration of 1:2000 for 1 h at room temperature. After 5 washes with TBST (each 10 min), the membranes were incubated with ECL reagents mixture at room temperature for 2 min and were subjected to chemluminescence detection by film exposure.

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Supporting Information Available: Copies of proton and carbon NMR spectra for new synthetic compounds and general experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.