Total Synthesis of Largazole – Devolution of a Novel Synthetic Strategy

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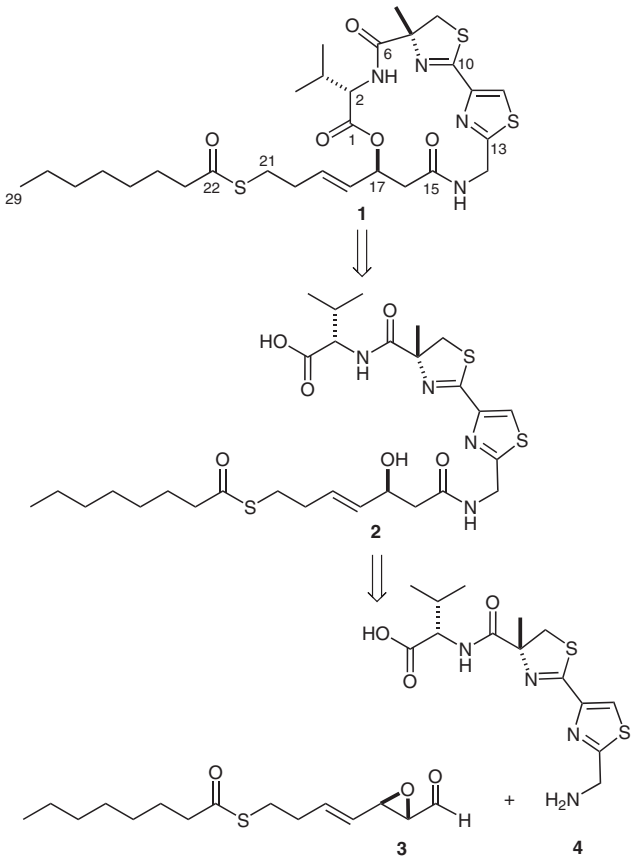
Abstract: The cyanobacterial isolate largazole embodies a compelling combination of a relatively simple, yet unique cyclic depsipeptide structure with remarkable levels of selective cytotoxicity against cancer cell lines versus nontransformed cells. The unique structure of largazole inspired a strategically novel and aggressive approach towards its expedient total synthesis. This involved an initial dissection into an epoxy aldehyde and an unprotected tetrapeptide, representing the polyketide and polypeptide domains of largazole, respectively. These fragments were successfully joined using NHC-mediated amidation, but subsequent assembly of the cyclic depsipeptide via lactonization was thwarted. A reordering of key couplings led to a successful assembly of largazole.

Key words: total synthesis, heterocycles, depsipeptide, N-heterocyclic carbene, thiazoline

The marine natural product largazole (**1**) (Scheme 1) was isolated from a cyanobacterium collected from the waters of Key Largo, Florida, USA. The structure and preliminary biological activities of this novel cyclic depsipeptide were reported in early 2008. The structure of largazole embodies two distinct structural/biogenetic domains, polyketide thioester- and polypeptide-derived units. While the structure of **1** is unprecedented, it is the potent and remarkably selective cytotoxic activities against human cancer cell lines that most dramatically distinguishes largazole from clinically employed anticancer agents.[1] This has spurred many total syntheses,[2] as well as analogues and mechanism of action studies in the past two years. The widespread supposition that largazole's primary mechanism of action involves inhibition of histone deacetylases seems to be well supported.[1,2a,d] We were also intrigued by the combination of the simple, yet novel architectural features that manifested themselves in such highly selective biological activity. Rather than targeting one of several obvious synthetic approaches towards **1**, we were intrigued by the potential to assemble this remarkable natural product via an unprecedented cyclodepsipeptide total synthesis strategy. Herein we disclose this novel strategy and its necessary devolution to achieve a novel total synthesis of **1**.

Our initial total synthesis plan incorporated one of the most obvious disconnections of opening the cyclic depsipeptide at the lactone to generate seco-acid **2** (Scheme 1). Less generally obvious was the recognition that amidation of the α,β-epoxy aldehyde **3**, embodying the full ketide-derived domain of **1**, with an *unprotected* tripeptide derivative **4**, 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-hydroxy group that would be engaged in a final macrolactonization with the residual carboxylic acid (**2**→**1**).



Scheme 1 Structure of largazole (**1**) and initial retrosynthetic plan

Bode and Rovis had demonstrated the utility of NHC-catalyzed amidations with α-substituted aldehydes and simple amines.[3] We were intrigued to test whether this approach could be amenable to the generation of β-hydroxy amides from *unprotected* amino acids, such as **4**, *without the necessity to protect the carboxylic acid moiety*.

Implementation of this synthetic plan was initiated by the synthesis of the epoxy aldehyde **3** (Scheme 2). This began with the generation of the thioester **7** containing a tetrazolyl sulfone moiety under Mitsunobu conditions. Julia

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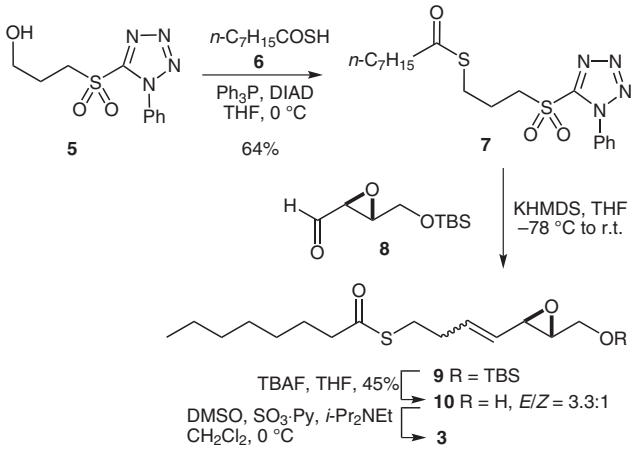
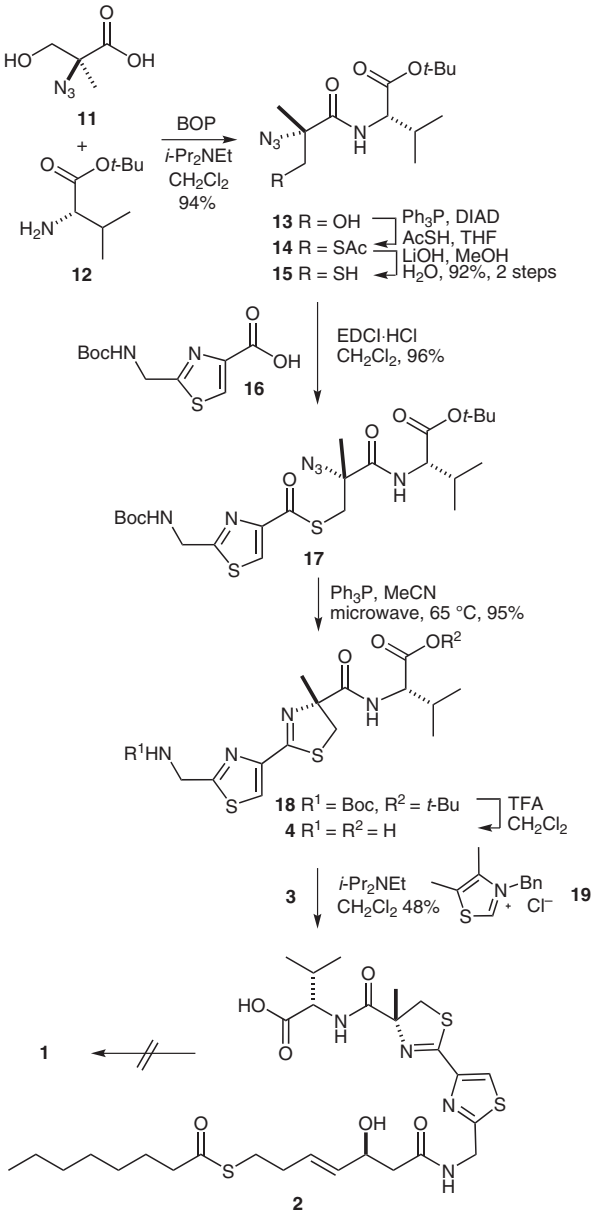
olefination using **7** and the sensitive α,β-epoxy aldehyde **8** gave a 3.3:1.0 mixture of alkenes (*E*/*Z*)-**9**, which were separated at the stage of primary alcohols **10** (45% yield, two steps). Oxidation then provided the unstable α,β-epoxy aldehyde **3** (87%).



Scheme 2 Facile synthesis of the largazole polyketide derivative **3**

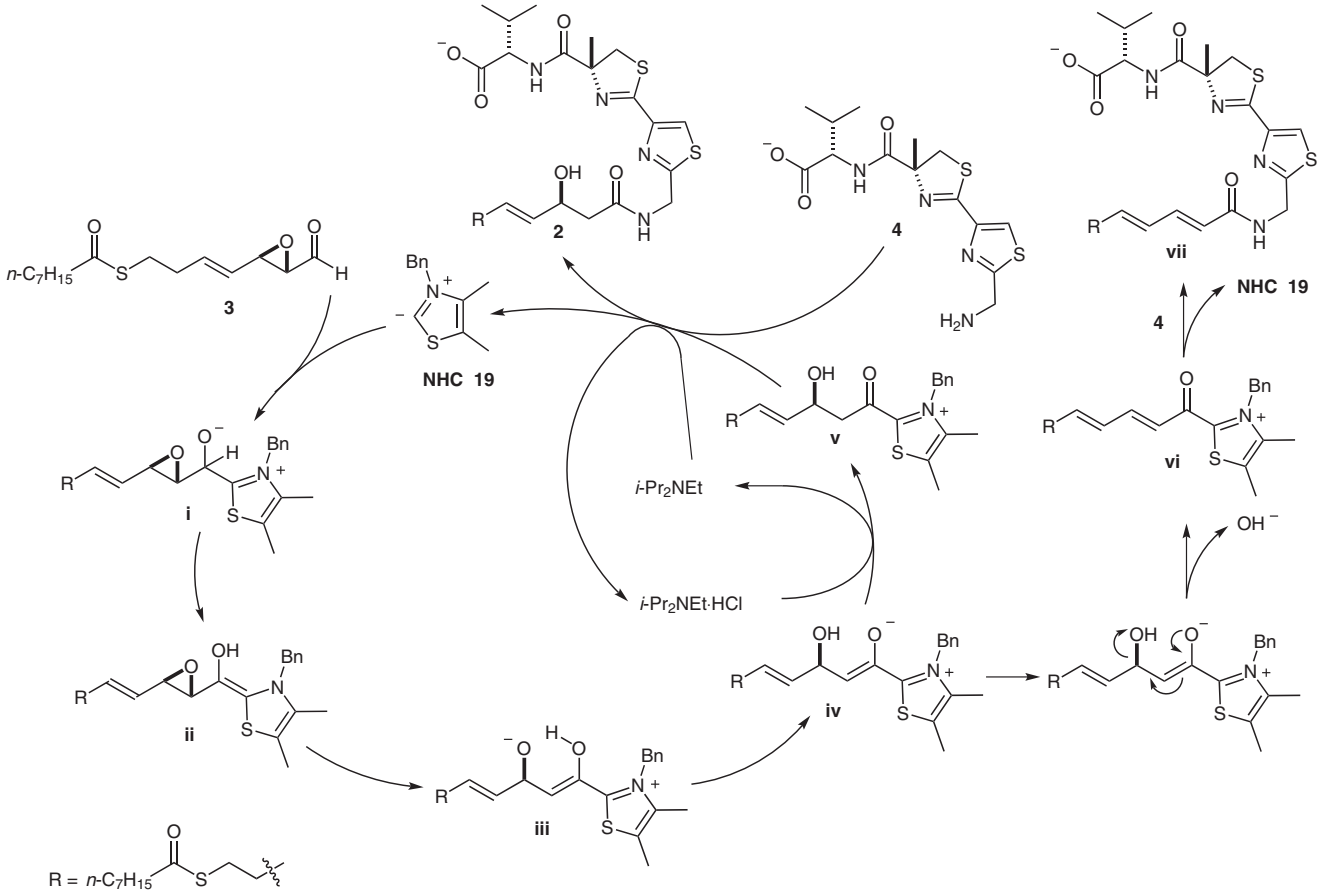
The complementary polypeptide derivative **4** was generated in an expedient fashion beginning with known azido acid **11**[4] and valine ester **12** (Scheme 3). The azide moiety was chosen to allow implementation of a late-stage Staudinger reduction/aza-Wittig process for the installation of largazole's thiazoline moiety.[5] This involved efficient amide formation between **11** and **12** to yield primary alcohol **13**, and a subsequent two-step replacement of the hydroxy group with a thiol (92% yield). Thioesterification between **15** and the cysteine and glycine-derived thiazole **16**[6] provided azido thioester **17** in excellent yield. Subsequent thiazoline formation was effected in a similarly efficient fashion simply by treatment of **17** with triphenylphosphine in acetonitrile under microwave irradiation to afford the complete polypeptide-derived domain of **1** in the terminally diprotected form of **18**.[5]

In what was anticipated to be the final deprotection step in the projected total synthesis of **1**, both the terminal *N*-Boc and *tert*-butyl ester protecting groups of **18** were simultaneously removed using trifluoroacetic acid to afford amino acid **4** as its ammonium trifluoroacetate salt (Scheme 3). The key joining of the two biogenetically distinct domains of largazole was then initiated by amide formation between **4** and epoxy aldehyde **3**. This relied upon the use of stoichiometric amounts of the NHC generated by sufficient amounts of Hünig's base (*i*-Pr_2NEt) to render the diisopropylethylammonium carboxylate salt and liberate the free amine of **4**, and to deprotonate the thiazolium salt **19**, precursor to the NHC.[3a] This resulted in 48% isolated yield of the anticipated β-hydroxy amide **2**, the seco-acid of largazole. Accompanying the formation of **2** were substantial amounts of the corresponding dehydrated diene (e.g., **vii**, Scheme 4). In spite of this complication, a stoichiometric NHC-mediated amidation procedure using α,β-epoxy aldehydes was extended to the use of an unprotected amino acid to result in a β-hydroxy amide that is a common structural element among cyclic depsipeptides.



Scheme 3 Assembly of the largazole seco-acid **2**

The general mechanism for the NHC-mediated amidation proposed by Bode involves an internal redox wherein the NHC–aldehyde adduct **i**, derived from aldehyde **3** and in situ generated NHC undergoes tautomerization to **ii**, which represents an oxidation of the initial aldehyde carbon (Scheme 4). Opening of the epoxide utilizing electron density resident on the nitrogen then establishes the β-alkoxy group in **iii**. A pair of subsequent proton transfers leads to the β-hydroxy carbonyl **v** via enolate **iv**. Intermediate **v** represents the activated acyl donor that is captured by the free amino group of **4** to yield the amide **2** and release the NHC. Similar amidation attempts using catalytic amounts of **19** failed to provide satisfactory results, due to rapid competitive imine formation between the epoxy aldehyde and the amine. Thus, a sequenced combination of



Scheme 4 Proposed mechanism for the formation of amide **2** (Bode et al.)[3a] and diene **vii** under NHC mediation

aldehyde, **19**, and Hünig's base operationally preceded the addition of the naked amino acid.

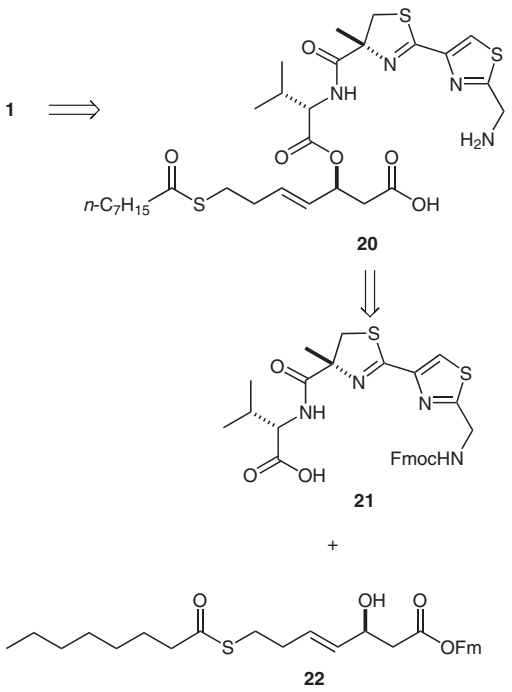
The addition of imidazole as reported by Rovis and Bode did not provide any improvement in this system. The use of a different NHC reagent[3b] was also examined, but similar results were obtained.

Although the use of an unprotected amino acid in the NHC-mediated amidation process was demonstrated to be synthetically useful, there are some complicating structural features of epoxy aldehyde **3** that may contribute to the moderate yield leading to **2**. These include the presence of the γ,δ-alkene and the thioester in **3**. 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 **v** and the β-elimination product conjugated diene **vi** (Scheme 4). An empirical set of observations is that NHC-mediated amidations of **4** with thioester–aldehyde **3** were considerably lower yielding than similar conjugations using α,β-epoxy-γ,δ-alkenyl aldehydes bearing a terminal siloxy group at C21. Thus, the presence of an electron-withdrawing C21-thioester was demonstrated to be detrimental to achieving better chemical yields of the desired amidation products.

The successful implementation of the direct convergent coupling of amino acid **4** with epoxy aldehyde **3** resulting in the largazole seco-acid, β-hydroxy amide **2**, left a final lactonization step to complete a total synthesis of **1**. At the time that this synthetic strategy was conceived and implemented, there were no empirical results to deter our expectation that largazole could be assembled via a seemingly trivial final lactonization of **2**. Such was not to be the case, however. Exhaustive attempts were made to lactonize **2** or its hydroxy epimer via Keck,[7] Yamaguchi,[8] Mukaiyama,[9] and Mitsunobu[10] protocols and variants thereof,[11] with no notable successes. Indeed, there is no evidence among the now substantial body of published literature regarding any success in attempts to convert the seco-acid of largazole directly into the lactone. Conformational ring strain in **1**, steric blockage about the valine carboxyl residue, and a propensity towards hydroxyl elimination to generate a conjugated diene may all conspire to deter lactonization of **2** and its hydroxy epimer under each of the chemical methods that have been applied.

Consequently, a modification of the original total synthesis plan was necessitated. The revised strategy involved closure of the largazole cyclic depsipeptide via lactamization of an ester-linked polypeptide/polyketide intermediate (**20**, Scheme 5). In contrast to our original synthetic plan, the valine ester would be formed prior to the polyketide-derived amide. Thus, the two key domains of **1** were reinvented as the complementary synthetic coupling partners of valine-derived acid **21** and fully elaborated polyketide domain alcohol **22**. Each of these was

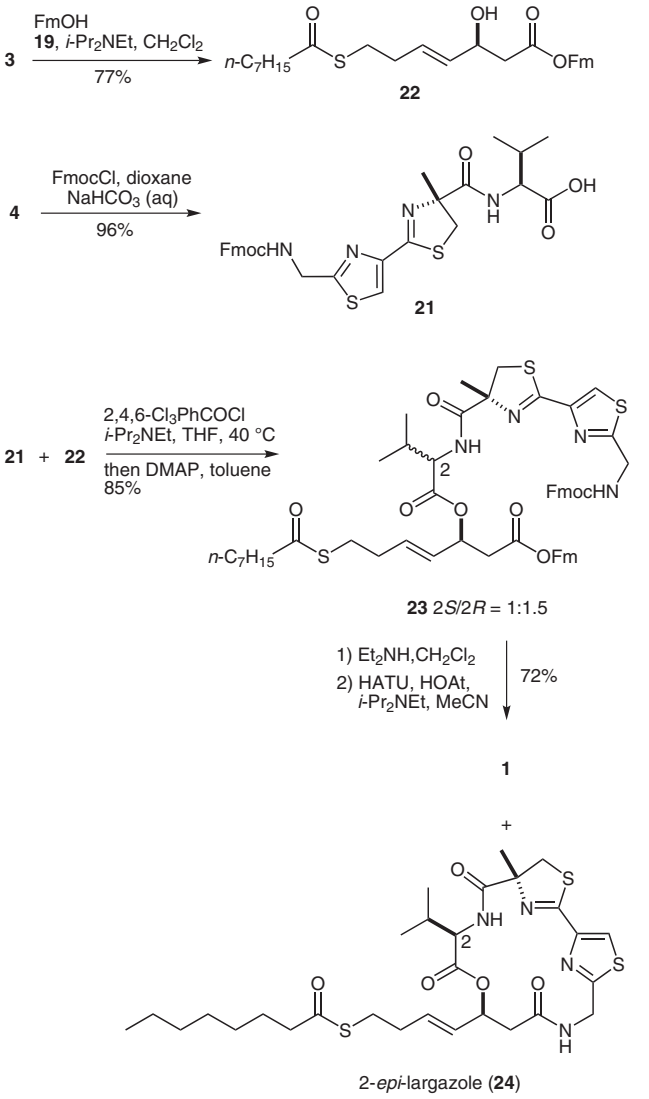
designed to incorporate parallel protecting groups containing fluoren-9-ylmethanol (FmOH) on the ketide acid and peptide amino termini to facilitate simultaneous deprotection and subsequent lactamization.



Scheme 5 Second-generation retrosynthetic dissection of **1**

The requisite protected carboxylate **22** was readily obtained from α,β-epoxy aldehyde **3** (Scheme 2) via NHC-mediated esterification with fluoren-9-ylmethanol (Scheme 6). The complementary polypeptide derived amino-protected carboxylic acid **21** was generated from **4** (Scheme 3) by simple and efficient Fmoc derivatization (Scheme 6). The union of **21** and **22** was achieved by Yamaguchi esterification to provide ester **23**, unexpectedly as a 1:1.5 mixture of 2*S*/2*R*-valine epimers, respectively. Without separation, these epimers were subjected to the final two steps of deprotection and macrolactamization, which generated a mixture of largazole (**1**) and 2-*epi*-largazole (**24**) in a 1:1.5 ratio, in 72% combined yield. These final products were then separated chromatographically.

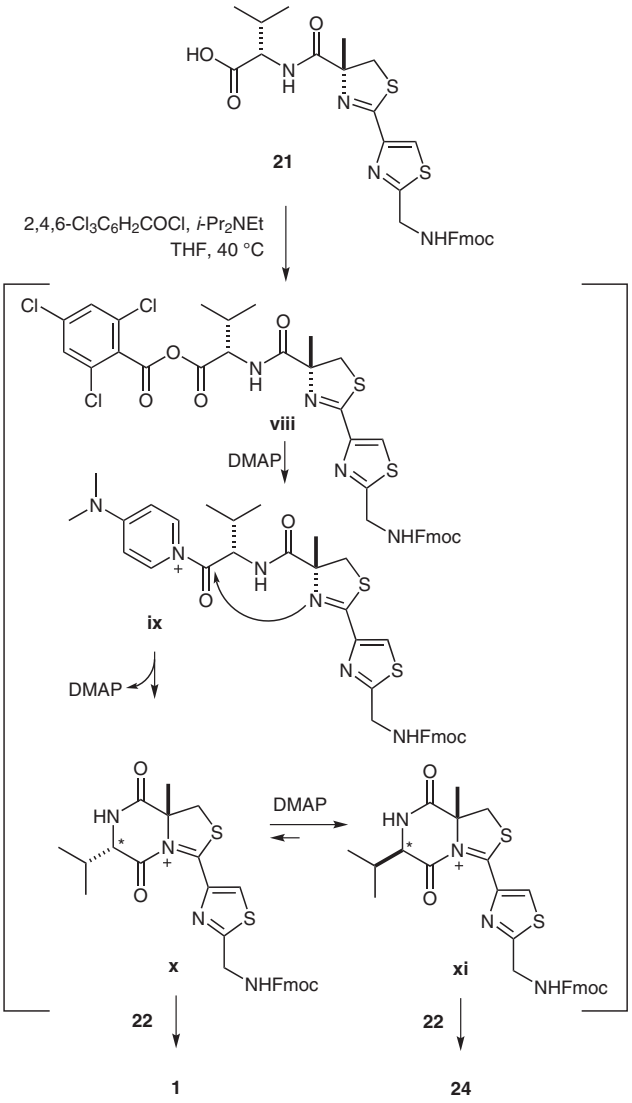
We hypothesize that the epimerization observed at the valine α-stereogenic center leading to (2*R*)-**23** may occur via the diketopiperazine intermediate **xi** (Scheme 7) generated in the process of the Yamaguchi esterification of **21** and **22**. Either the mixed anhydride **viii** derived from acid **21** and trichlorobenzoyl chloride, or its DMAP derivative **ix** may be 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 diketopiperaziridinium ions by the secondary alcohol of **22** would lead to (2*S*)-**23** and (2*R*)-**23** from **x** and **xi**, respectively.



Scheme 6 Total synthesis of largazole

In summary, a novel total synthesis strategy towards largazole (**1**) was designed to implement a late-stage NHC-mediated amidation of an *unprotected* polypeptide derivative **4** and a fully functionalized epoxy aldehyde **3**. In practice, the resultant amide emerged in modest yield as β-hydroxy amide **2**, bearing a free carboxylic acid, the seco-acid of largazole. All attempts to affect macrolactonization from hydroxy acid **2** or its hydroxy epimer were unsuccessful in our hands, as corroborated by the original total synthesis of **1**.[2] Nonetheless, the direct formation of elaborate β-hydroxy amides from epoxy aldehydes and *unprotected* amino acids using stoichiometric NHC mediation, as demonstrated here, may well be applicable to the rapid synthesis of cyclic depsipeptides bearing the β-acylated amide structural motif. Several of the naturally occurring cyclic depsipeptides that are structurally and

mechanistically related to **1** embody this specific functional group array.[12] The total synthesis of **1** was achieved by a reordering of ester and amidation events, but was complicated by the aggressive synthetic strategy of preinstalling the thiazoline moiety in the acyl donor, N-protected carboxylic acid **21**. This led to an unavoidable partial epimerization at C2.



Scheme 7 C2-Epimerization

All air-sensitive reactions were carried out under argon in oven-dried glassware using standard syringe, cannula, and septa techniques. Unless otherwise noted, all reactions were carried out at r.t. (20–25 °C). Molecular sieves (MS) were activated by heating at 120 °C for 24 h under vacuum. THF, CH_2Cl_2, and toluene were purified by the Innovative Technology, Inc. Pure-Solv solvent purification system, and MeCN and *i*-Pr_2NEt were distilled from CaH_2 under N_2. Microwave-assisted reactions were performed in sealed-tube mode on a CEM discover S-class reactor. $CDCl_3$ was neutralized by anhyd K_2CO_3. Other reagents were used as received from commercial resources. Flash column chromatography was performed on Silicycle SilicaFlash P60. Analytical TLC was performed on Silicycle glass-backed TLC extra-hard-layer 60 plates and Kieselgel silica gel 60 F_{254s} HP-TLC plates visualized by fluorescence upon 254 nm irradiation and/or staining with anisaldehyde reagent [90% EtOH (450 mL), aq H_2SO_4 (25 mL), AcOH (15 mL), anisaldehyde (25 mL)] and/or PMA reagent [phosphomolybdic acid (25 g), $Ce(SO_4)_2$ (87.5 g), H_2O (479 mL), H_2SO_4 (25 mL)]. ^1H NMR and ^{13}C NMR spectra of samples prepared in $CDCl_3$ were referenced to the residual $CHCl_3$ peaks [$\delta = 7.27$ (^1H) and $\delta = 77.0$ (^{13}C)]; 400-MHz or 500-MHz Bruker instruments were used. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter at the sodium D line (589 nm) using 3.5 i.d. × 100 mm cylindrical glass cells and were reported in concentration (*c*, g/100 mL) at 23 °C. HRMS was performed on a Bruker MicroTOF mass spectrometer.

Epoxy Alcohol 10

DIAD (0.49 mL, 2.37 mmol) was added to Ph_3P (622 mg, 2.37 mmol) in THF (14 mL) at 0 °C. After the mixture had stirred for 5 min, a soln of alcohol **5**[13] (562 mg, 2.09 mmol) in THF (3 mL) was added to it. After another 5 min, a soln of thiooctanoic *S*-acid **6**[14] (380 mg, 2.37 mmol) in THF (3 mL) was added. The reaction mixture was kept at 0 °C for 10 min before sat. aq NH_4Cl (20 mL) was added. The product was extracted with Et_2O (3 × 10 mL), dried ($MgSO_4$), concentrated, and purified by chromatography (EtOAc–hexanes, 9:1); this gave sulfone **7** as a white solid; yield: 550 mg (64%).

To a –78 °C soln of sulfone **7** (320 mg, 0.78 mmol) and epoxy aldehyde **8** (202 mg, 0.94 mmol) in THF (7 mL) was added a 0.5 M soln of KHMDS in toluene (1.7 mL, 0.85 mmol). After being stirred at –78 °C for 1 h, the reaction mixture was slowly warmed to r.t. over 2 h and stirred for another 3 h. Aq phosphate buffer (pH 7) was then added. The mixture was extracted with Et_2O (2 × 5 mL), and the combined extracts were dried ($MgSO_4$), filtered, concentrated, and purified by column chromatography (silica gel, EtOAc–hexanes, 10:1); this gave a mixture of (*E*)-**9** and (*Z*)-**9** (*E*/*Z* = 3.3:1.0, by ^1H NMR analysis).

A 1 M soln of TBAF in THF (0.4 mL, 0.4 mmol) was added to a soln of this mixture of **9** in THF (3 mL) and EtOAc (0.1 mL) at 0 °C. After 30 min, pH 7 aq phosphate buffer was added. The resultant mixture was extracted with Et_2O (3 × 3 mL), the combined extracts were dried (Na_2SO_4) and filtered, and the filtrate was concentrated and purified by column chromatography (EtOAc–hexanes, 4:1); this provided (*E*)-**10** and (*Z*)-**10**.

Yield [(*E*)-**10**]: 77 mg (35%); yield [(*Z*)-**10**]: 24 mg (10%).

Alcohol (*E*)-10

$[\alpha]_D^{22}$ +20.4 (*c* 0.79, $CHCl_3$).

IR (neat): 3410, 2927, 2856, 1691, 1459, 1410, 1123, 1085, 1040 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): $\delta = 5.93$ (dt, *J* = 15.5, 7.0 Hz, 1 H), 5.31 (dd, *J* = 15.5, 8.0 Hz, 1 H), 3.97 (dd, *J* = 12.5, 2.0 Hz, 1 H), 3.70 (dd, *J* = 12.5, 4.0 Hz, 1 H), 3.40 (dd, *J* = 8.0, 2.0 Hz, 1 H), 3.09 (m, 1 H), 2.93 (t, *J* = 7.5 Hz, 2 H), 2.54 (t, *J* = 7.5 Hz, 2 H), 2.36 (dt, *J* = 7.5, 6.5 Hz, 2 H), 1.82 (br s, 1 H), 1.66 (m, 2 H), 1.31–1.21 (m, 8 H), 0.88 (m, 3 H).

^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 199.4, 134.5, 128.5, 61.2, 59.9, 55.4, 44.2, 32.4, 31.6, 29.7, 28.9, 27.9, 25.7, 22.6, 14.1$.

HRMS (ESI): *m*/*z* calcd for $C_{15}H_{26}O_3S$ [M + Na]: 309.1500; found: 309.1491.

Azidothiazole 17

i-Pr_2NEt (230 μL, 1.31 mmol) and BOP [benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate; 553 mg, 1.25 mmol] were added to a soln of acid **11**[4] (173 mg, 1.19 mmol) in CH_2Cl_2 (9 mL). The mixture was stirred for 2 min before a soln of L-valine *tert*-butyl ester **12** (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 column chromatography (silica gel, EtOAc–hexanes, 80:20); this gave **13** as a colorless oil; yield: 336 mg (94%).

DIAD (0.33 mL, 1.6 mmol) was added to a soln of Ph_3P (423 mg, 1.6 mmol) in THF (3 mL) at 0 °C. After 10 min, a soln of **13** (162 mg, 538 μmol) in THF (2 mL) was added. After another 15 min, thioacetic acid (115 μL, 1.6 mmol) was added. The reaction mixture was slowly warmed to r.t. over 2 h. Then Et_2O (5 mL) and pH 7 aq phosphate buffer were added. The product was extracted with Et_2O (3 × 5 mL), and the mixture was dried ($MgSO_4$), filtered, and concentrated. The residue was kept at 4 °C for 10 h, before Et_2O (1 mL) was added. The resulting white precipitate was collected by filtration and washed with Et_2O. The filtrate was concentrated, and then hexanes (2 mL) were added. The resultant white precipitate was collected by filtration and washed with hexanes. The filtrate was concentrated and the residue was dissolved in MeOH (5 mL), and then H_2O (1 mL) and LiOH·H_2O (40 mg, 1.0 mmol) were added. After the mixture had stirred for 10 min, an aq pH 7 phosphate buffer was added. The product was extracted with Et_2O (3 × 5 mL), and the mixture was dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography (EtOAc–hexanes, 1:9); this gave **15** as a yellow oil; yield: 193 mg (ca. 92%), with a small amount (ca. 8%) of an inseparable impurity.

EDCI·HCl (55 mg, 290 μmol) and DMAP (6 mg, 50 μmol) were added to a soln of **15** (80 mg, ca. 250 μmol) and **16**[6] (50 mg, 193 μmol) in CH_2Cl_2 (2 mL). After stirring for 20 min, the mixture was concentrated, and then diluted with Et_2O (3 mL) and sat. aq NH_4Cl (3 mL). The product was extracted with Et_2O (3 × 3 mL), dried ($MgSO_4$), concentrated, and purified by flash chromatography (EtOAc–hexanes, 85:15); this gave **17** as a pale yellow oil; yield: 103 mg (96%).

$[\alpha]_D^{22}$ +46.3 (*c* 8.6, $CHCl_3$).

IR (neat): 3360, 2974, 2932, 2120, 1723, 1681, 1514, 1368, 1275, 1161 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 8.09 (s, 1 H), 7.03 (d, *J* = 9.0 Hz, 1 H), 5.36 (br s, 1 H), 4.64 (d, *J* = 5.5, 2 H), 4.38 (dd, *J* = 8.5, 4.5 Hz, 1 H), 3.63 (d, *J* = –14 Hz, 1 H), 3.58 (d, *J* = –13.5, 1 H), 2.21 (m, 1 H), 1.71 (s, 3 H), 1.48 (app s, 18 H), 0.97 (d, *J* = 7.0 Hz, 3 H), 0.95 (d, *J* = 7.0 Hz, 3 H).

^{13}C NMR (125 MHz, $CDCl_3$): 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.

HRMS (ESI): *m*/*z* calcd for $C_{23}H_{36}N_6O_6S_2$ [M + Na]: 579.2035; found: 579.2024.

Thiazoline 18

A mixture of azidothiazole **17** (86 mg, 0.16 mmol), Ph_3P (203 mg, 770 μmol), and MeCN (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 for 9 h. The mixture was then concentrated and purified by column chromatography (silica gel, EtOAc–hexanes, 80:20); this gave **18** as a colorless yellow oil; yield: 75 mg (95%).

$[\alpha]_D^{22}$ –33.4 (*c* 3.89, $CHCl_3$).

IR (neat): 3382, 1722, 1674, 1606, 1514, 1368, 1277, 1252, 1163, 1029 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.94 (s, 1 H), 7.17 (d, *J* = 9.0 Hz, 1 H), 5.59 (br s, 1 H), 4.61 (d, *J* = 4.5 Hz, 2 H), 4.36 (dd, *J* = 8.5, 4.5 Hz, 1 H), 3.75 (d, *J* = –11.5 Hz, 1 H), 3.31 (d, *J* = –11.5 Hz, 1 H), 2.11 (m, 1 H), 1.57 (s, 3 H), 1.44 (s, 9 H), 1.43 (s, 9 H), 0.85 (d, *J* = 7.0 Hz, 3 H), 0.82 (d, *J* = 7.0 Hz, 3 H).

^{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.

HRMS (ESI): *m*/*z* calcd for $C_{23}H_{36}N_4O_5S_2$ [M + Na]: 535.2025; found: 535.2022.

Fmoc-Protected Amino Acid 21

Thiazoline **18** (18.3 mg, 35.7 μmol) was dissolved in CH_2Cl_2–TFA (1:1, 2 mL) and stirred at r.t. for 2 h. The solvent was removed and the residue was dried under high vacuum for 1 h. Sat. aq $NaHCO_3$ (0.4 mL) was added followed by FmocCl (9.2 mg, 0.43 mmol) in 1,4-dioxane (0.4 mL). After vigorous stirring of the mixture for 1 h, 1 M aq $KHSO_4$ was added until pH 2 was achieved. The mixture was then extracted with EtOAc (4 × 1 mL), and the combined extracts were dried (Na_2SO_4), filtered, concentrated, and purified by column chromatography (silica gel, $CHCl_3$–MeOH, 1:0 to 20:1); this gave **21**; yield: 19.8 mg (96%).

$[\alpha]_D^{20}$ –36.1 (*c* 0.90, MeOH).

IR (neat): 3385, 2967, 1726, 1661, 1520, 1450, 1252, 1194, 1143, 1041 cm^{-1}.

^1H NMR (500 MHz, CD_3OD): δ = 8.18 (s, 1 H), 8.02 (br t, *J* = 5.5 Hz, 1 H), 7.81 (d, *J* = 7.5 Hz, 2 H), 7.67 (d, *J* = 7.5 Hz, 2 H), 7.62 (d, *J* = 8.5 Hz, 2 H), 7.40 (t, *J* = 7.5 Hz, 2 H), 7.31 (d, *J* = 7.5 Hz, 2 H), 4.60 (s, 2 H), 4.45 (d, *J* = 6.5 Hz, 2 H), 4.38 (m, 1 H), 4.24 (t, *J* = 6.5 Hz, 1 H), 3.78 (d, *J* = –11.5 Hz, 1 H), 3.39 (d, *J* = –11.5 Hz, 1 H), 2.11 (m, 1 H), 1.61 (s, 3 H), 0.92 (d, *J* = 7.0 Hz, 3 H), 0.88 (d, *J* = 7.0 Hz, 3 H).

^{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.

HRMS (ESI): *m*/*z* calcd for $C_{29}H_{30}N_4O_5S_2$ [M + Na]: 601.1555; found: 601.1562.

Fluorenylmethyl β-Hydroxy Ester 22

SO_3·py (97 mg, 0.61 mmol) was added to a soln of epoxy alcohol (*E*)-**10** (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. After 1 h, an aq pH 7 phosphate buffer was added. The resultant mixture was extracted with Et_2O (3 × 1 mL), dried ($MgSO_4$), filtered, concentrated, and purified quickly by column chromatography (silica gel, EtOAc–hexanes–*i*-Pr_2NEt, 95:5:0.5); this gave epoxy aldehyde **3**; yield: 75 mg (87%). To a suspension of the thiazolium salt **19** (49 mg, 205 μmol) 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 soln of aldehyde **3** (58 mg, 21 mmol) and fluoren-9-ylmethanol (159 mg, 810 μmol) in CH_2Cl_2 (0.5 mL). The mixture was stirred for 30 min before sat. aq NH_4Cl (1 mL) was added. The mixture was extracted with Et_2O (3 × 1 mL), dried ($MgSO_4$), filtered, concentrated, and purified by column chromatography (silica gel, EtOAc–hexanes, 1:9); this gave **22**; yield: 76 mg (77%).

$[\alpha]_D^{20}$ –3.49 (*c* 1.58, $CHCl_3$).

IR (neat): 3461, 2924, 2853, 1735, 1689, 1450, 1272, 1168 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.81 (d, *J* = 7.5 Hz, 2 H), 7.62 (d, *J* = 7.5 Hz, 2 H), 7.42 (m, 2 H), 7.34 (m, 2 H), 5.71 (dt, *J* = 15.5, 6.5 Hz, 1 H), 5.54 (dd, *J* = 15.5, 6.5 Hz, 2 H), 4.47 (m, 3 H), 4.14 (t, *J* = 6.0 Hz, 1 H), 2.92 (t, *J* = 7.5 Hz, 2 H), 2.61 (d, *J* = 1.5 Hz, 1 H), 2.59 (d, *J* = 9.0 Hz, 1 H), 2.55 (t, *J* = 7.5 Hz, 2 H), 2.31 (q, *J* = 7.0 Hz, 2 H), 1.67 (m, 2 H), 1.33–1.19 (m, 8 H), 0.89 (m, 3 H).

^{13}C NMR (125 MHz, $CDCl_3$): 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.

HRMS (ESI): *m*/*z* calcd for $C_{29}H_{36}O_4S$ [M + Na]: 503.2232; found: 503.2237.

Fluorenylmethyl Ester 23

A soln of 2,4,6-trichlorobenzoyl chloride (1.9 μL, 12 mmol) and *i*-Pr_2NEt (2.2 μL, 12 μmol) in THF (120 μL) was added to acid **21** (7.2 mg, 12 μmol). The mixture was stirred at 40 °C for 5 h before being concentrated under vacuum. A soln of ester **22** (12.0 mg, 24.9 μmol) in toluene (120 μL) was added to the residue, followed by DMAP (1.5 mg, 12 μmol). The mixture was stirred for 16 h before a sat. aq soln of NH_4Cl (0.5 mL) was added. The mixture was extracted with EtOAc (3 × 0.5 mL), dried (Na_2SO_4), filtered, concentrated, and purified by column chromatography (silica gel, EtOAc–hexanes, 1:4 to 2:3); this yielded **23** as a mixture of C2-epimers (2*R*/2*S* = 1.5:1); yield: 11.0 mg (85%).

HRMS (ESI): *m*/*z* calcd for $C_{58}H_{64}N_4O_8S_3$ [M + Na]: 1063.3784; found: 1063.3789.

Largazole (1) and 2-*epi*-Largazole (24)

Et_2NH (0.25 mL) was added to a soln of **23** (7.0 mg, 6.7 μmol) in CH_2Cl_2 (0.5 mL). After the mixture had stirred for 3 h, the solvent was removed under vacuum and the residue was redissolved in CH_2Cl_2 (0.5 mL) and *i*-Pr_2NEt (0.1 mL). After stirring of the resultant soln for 10 min, the solvent was removed under vacuum and the residue was azeotropically dried twice with toluene (2 × 2 mL). The residue was dissolved in MeCN (7 mL), and HATU [*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; 5.1 mg, 13 μmol], HOAt (1-hydroxy-7-azabenzotriazole; 1.8 mg, 13 μmol), and *i*-Pr_2NEt (4.6 μL, 27 μmol) were added. The reaction mixture was stirred for 12 h before being concentrated under vacuum. The residue was purified by column chromatography (EtOAc–hexanes–MeOH, 10:10:1) to give a mixture of **1** and **24**; yield: 3.0 mg (72%). This mixture was separated by preparative TLC (EtOAc–hexanes–MeOH, 10:10:1) to give **1** [yield: 1.2 mg (29%)] and **24** [yield: 1.8 mg (43%)].

Largazole (1)

$[\alpha]_D^{20}$ +21 (*c* 0.10, MeOH).

IR (neat): 3370, 3085, 2926, 2854, 1738, 1682, 1552, 1504, 1259, 1100, 1029 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.76 (s, 1 H), 7.18 (d, *J* = 9.5 Hz, 1 H), 6.44 (dd, *J* = 9.5, 3.0 Hz, 1 H), 5.83 (dt, *J* = 15.5, 6.5 Hz, 1 H), 5.67 (app t, *J* = 7.0 Hz, 1 H), 5.53 (dd, *J* = 16.0, 7.0 Hz, 1 H), 5.30 (dd, *J* = –17.5, 9.5 Hz, 1 H), 4.62 (dd, *J* = 9.5, 3.5 Hz, 1 H), 4.29 (dd, *J* = –17.5, 3.0 Hz, 1 H), 4.06 (d, *J* = –11.5 Hz, 1 H), 3.29 (d, *J* = –11.5 Hz, 1 H), 2.91 (t, *J* = 7.0 Hz, 2 H), 2.86 (dd, *J* = –16.5, 10.0 Hz, 1 H), 2.70 (dd, *J* = –16.0, 2.5 Hz, 1 H), 2.53 (t, *J* = 7.5 Hz, 2 H), 2.32 (br q, *J* = 7.5 Hz, 2 H), 2.11 (m, 1 H), 1.87 (s, 3 H), 1.65 (m, 2 H), 1.29–1.27 (m, 8 H), 0.88 (m, 3 H), 0.70 (d, *J* = 6.5 Hz, 3 H), 0.53 (d, *J* = 6.5 Hz, 3 H).

^{13}C NMR (125 MHz, $CDCl_3$): 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.

HRMS (ESI): *m*/*z* calcd for $C_{29}H_{42}N_4O_5S_3$ [M + Na]: 645.2210; found: 645.2201.

2-*epi*-Largazole (24)

$[\alpha]_D^{20}$ +43 (*c* 0.16, $CDCl_3$).

IR (neat): 3342, 3076, 2925, 2857, 1738, 1682, 1552, 1503, 1259, 1107, 1034 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.68 (s, 1 H), 7.21 (d, *J* = 8.0 Hz, 1 H), 6.37 (dd, *J* = 7.5, 5.0, Hz, 1 H), 5.89 (dt, *J* = 15.5, 6.5, Hz, 1 H), 5.80 (app t, *J* = 9.0 Hz, 1 H), 5.43 (dd, *J* = 15.5, 8.5, Hz, 1 H), 5.08 (d, *J* = –17.0, 8.0 Hz, 1 H), 4.28 (d, *J* = –11.0 Hz, 1 H), 4.28 (dd, *J* = –17.0, 2.0 Hz, 1 H), 4.23 (dd, *J* = 16.5, 5.0 Hz, 1 H), 3.20 (d, *J* = –11.0 Hz, 1 H), 2.88 (dt, *J* = 7.5, 2.0, Hz, 2 H), 2.82 (dd, *J* = –16.5, 10.5, Hz, 1 H), 2.58 (d, *J* = –16.5 Hz, 1 H), 2.53 (t, *J* = 7.5 Hz, 2 H), 2.29 (br q, *J* = 7.0 Hz, 2 H), 2.13 (m, 1 H), 1.80 (s, 3 H), 1.64 (m, 2 H), 1.30–1.23 (m, 8 H), 0.97 (d, *J* = 7.0 Hz, 3 H), 0.89 (d, *J* = 6.5 Hz, 3 H), 0.88 (m, 3 H).

^{13}C NMR (125 MHz, $CDCl_3$): 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.

HRMS (ESI): *m*/*z* calcd for $C_{29}H_{42}N_4O_5S_3$ [M + Na]: 645.2210; found: 645.2201.

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