Diastereocntrol in Radical Addition to $\beta$-Benzyloxy Hydrazones: Revised Approach to Tubuvaline and Synthesis of O-Benzyltubulysin V Benzyl Ester

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1. INTRODUCTION

Stereocntrolled Mn-Mediated Radical Additions to Chiral Hydrazones. Radical additions to imino compounds offer a useful carbon−carbon bond construction approach to chiral amine synthesis.1 Seminal efforts to generalize this reaction for intermolecular coupling led to stereocntrolled alkylborane-, zinc-, and tin-mediated additions by Naito,2 Bertrand,3 and our group.4 A significant early limitation on this chemistry restricted the scope of radicals to simple 2° and 3° alkyls, usually from reagents in large excess, due to unfavorable halogen atom transfer or competitive reduction of the radicals. Such concerns make it difficult to apply this chemistry to more complex synthetic targets, prompting our search for alternative methods to generate the radicals for this purpose. With this in mind, we initiated a long-standing effort to develop the scope of a photochemical method to initiate radical addition to imino compounds via halogen atom abstraction by $^\cdot$Mn(CO)$_5$.5 Meanwhile, the portfolio of radical generation conditions continues to widen6 and now includes a variety of photoredox catalysis methods 7 as well as very recent approaches to Mn-mediated radical chemistry that render the processes catalytic in Mn.8

Our focus has been on establishing reliable and versatile modes of stereocntrol for radical addition to imino compounds, so that this type of transformation can be applied for synthesis design even when both the radical and acceptor bear structural complexity.9,10 Chiral N-acylhydrazones11 (Figure 1a) emerged as excellent radical acceptors that afford $>95:5$ diastereomer ratios for a host of intermolecular radical additions to the C$\equiv$N bond. Together with the Mn-mediated...
conditions, this approach has been successfully applied to functionalized chiral amines (Figure 1b) such as γ-amino acids12 and α,α-disubstituted α-amino acids,13 to radical−polar crossover annihilations,14 and to a formal synthesis of quinine.15 Broad vetting showed compatibility with multifunctional radical acceptors as well as multifunctional radicals, where large excesses of either component would normally be prohibitive. This feature makes Mn-mediated radical addition to chiral N-acylhydrazones well-suited for target-oriented synthesis and for preparing unusual amino acids and other chiral amine building blocks in the context of medicinal chemistry. These considerations drew our attention to application toward synthesis of tubulysins and analogues.

**Tubulysins.** Naturally occurring peptides containing unusual amino acids offer bioactivities of potential utility in drug discovery as exemplified by dolastatin 10, plitidepsin, and didemnin B, all of which have reached Phase 2 clinical trials as cancer chemotherapeutic candidates.16 Similarly, the tubulysin family of antimitotic agents (Figure 2),17 the first examples of stereocontrolled enolate oxidation,28,29 metalloenamine aldol addition,30 thiazolyl anion addition,31 hydride reduction,32−35 hetero-Diels−Alder reactions,36 and proline-catalyzed aldol reactions.37 An interesting multicomponent coupling reaction (MCR) rapidly built both C11 and the thiazole, albeit with modest stereocontrol38,39 that was later improved via a catalytic asymmetric Passerini reaction.40 Most Tuv syntheses begin with precursors having the C13 chiral amine already established from various valine derivatives and their homologues. Asymmetric induction at C13 has been accomplished by kinetic resolution of racemicaza-Michael or Mannich adducts,43,44 hydride reduction,30 and additions of various C-nucleophiles (enolate,45 organomagnesium,46 and allylindium reagents47) to imines. A nitrene cycloaddition approach established both C11 and C13 stereogenic centers.45 The breadth of the innovative synthetic route designs directed toward Tuv over many years attest to the challenge of this unusual multifunctional amino acid as well as the continuing interest in the tubulysins as potential cancer chemotherapeutics.46

Our own efforts toward Tup and Tuv were initially published in 2004,47 highlighting the utility of radical addition to chiral N-acylhydrazones in control of the chiral amine configurations of both Tup and Tuv. Subsequent optimizations led to an efficient seven-step route to Tuv (Scheme 1).48

![Diagram of tubulysins](image.png)

Figure 2. Structures of selected tubulysins, highlighting the unusual amino acids tubuvaline (green) and tubuphenylalanine (blue).

which were reported by Höfle et al. in 2000.18 are tetrapeptides of myxobacterial origin and are composed of d-N-methylpypecolic acid (Mep), l-isoleucine (Ile), tubuvaline (Tuv), and a C-terminal γ-amino acid, either tubuphenylalanine (Tup) or tubutryosine (Tut).19 These extraordinarily active antimitotic agents rival dolastatin 10 and epothilone, with some members of the family reaching picomolar potency through a mechanism involving noncompetitive binding at the vinca domain of β-tubulin.20 Early evaluation of tubulysin A indicated selective cytotoxicity and potential antiangiogenic activity,21 although more in vivo studies indicated a limited therapeutic window.22 More recently, targeting strategies involving folate−tubulysin and antibody−tubulysin conjugates have attracted attention to address the toxicity problem.23

As a consequence of their potent antimitic effects, much effort has been devoted to chemical synthesis of tubulysins,24 especially 1−4 (Figure 2), leading to several approaches to the natural products as well as numerous analogues.25 Creative strategies for stereocontrol have appeared in preparations of Tuv and Tup,26 and the N,O-acetal functionality in certain tubulysins (e.g., 3) has also inspired new methodology.27 In the diverse slate of creative strategies to prepare Tuv, the main innovations tend to focus on stereocontrol issues (known to impact activity28) at the hydroxyl- or acetoxy-bearing center at C11, stereocontrol at the C13 chiral amine, and introduction of the thiazole. The C11 configuration has been addressed by

According to the year 2000, beginning with known alcohol 5,49 a three-step sequence to N-acylhydrazones 6 was followed by photolysis with isopropyl iodide in the presence of InCl3 and Mn3(CO)10. This key step efficiently furnished 7 with complete stereosecontrol at the C13 chiral amine (dr >98:2, minor isomer not detected). Three functional group transformations led to γ-amino acid 8. We later disclosed a high-yielding but step-intensive route to convert 8 to N-TFA tubuvaline methyl ester (10, Scheme 2) in six steps and its application in a synthesis of a tubulysin analogue with a C-terminal alcohol (11, Figure 3).48 Attempts at oxidation of the C-terminus of 11 led to complicated mixtures; a putative C-terminal aldehyde intermediate appeared to have engaged in a cyclization with the γ-amino group as a nucophile. Thus, we considered more conventional strategies that introduced the C-terminus at the carboxylate oxidation state, along with improvements to synthesis of Tuv derivative 10. Despite the excellent overall yield of the prior route to 10, undesirable step economics prompted development of two
alternative plans to access 10 (Scheme 3). First, because constructing the tubuvaline thiazole added six steps after introduction of the chiral amine, we envisioned an improved step economy if the thiazole could be tolerated in the radical acceptor such as 13 (route A). Second, we also envisioned merging our previous Mn-mediated radical addition with a less step-intensive thiazole construction that introduced sulfur via condensation of cysteine with aldehyde 14 (route B). Here, we present the results of investigating these two routes, the former of which led to greater versatility of Mn-mediated radical addition and uncovered new insights concerning 1,3-diastereoccontrol in a C=N radical acceptor and the latter of which culminated the synthesis of a dibenzyl analogue of tubulysin V (12, Figure 3).

2. RESULTS AND DISCUSSION

Mn-Mediated Radical Addition Compatibility with Aromatic Heterocycles. An improved step-economic synthesis of 10 according to route A (Scheme 3) required solving a problem that had emerged during prior synthetic studies on quinine. Preliminary studies in that venture revealed an incompatibility of the Mn-mediated radical addition with the basic nitrogen of a quinoline aromatic N-heterocycle present in the N-acylhydrazone radical acceptor. With this in mind, we anticipated that further modification of the Mn-mediated radical addition conditions would be needed in order to accommodate other N-heteroaromatics such as a thiazole.

We began this study with a screen of the effects of various aromatic heterocyclic additives on the Mn-mediated isopropyl addition to hydrazone 15 (Table 1). According to our established method, the reaction conditions include a Lewis acid (InCl₃) to activate the acceptor toward nucleophilic radical addition. The Lewis acid coordinates in bidentate fashion to N-acylhydrazones, as evidenced by spectroscopic data. Various N-heterocycles were placed into the control reaction as additives to assess their effects on conversion and yield of isopropyl adduct 16. The control reaction under the usual conditions (conditions A) without any additive gave 16 with a normal yield of 86% (Table 1, entry 1). In separate runs, the presence of four different aromatic N-heterocycles (pyridine, imidazole, benzothiazole, and thiazole 17) in equimolar quantity each diminished the yields of the control reaction to an average of 40% (entries 2–5).

We hypothesized that diminished yields in the presence of aromatic heterocycles was attributable to basic sites of aromatic heterocycles interfering with the desired bidentate binding of InCl₃. Modifying the conditions to obviate this problem, we found that increasing the Lewis acid loading to 3.5 equiv and diluting the reaction mixture to one-sixth of the usual concentration generally improved the results. The

| entry | additive  | A (%) | B (%) |
|-------|-----------|-------|-------|
| 1     | none      | 86    | 72    |
| 2     | pyridine  | 44    | 54    |
| 3     | imidazole | 29    | 61    |
| 4     | benzothiazole | 24 | 32 |
| 5     | 17        | 64    | 70    |
isolated yields of 16 in the presence of the N-heterocycles using these modifications (conditions B) increased to an average of 54% (Table 1). The hypothesis suggests that more basic N-heterocycles should interfere to a greater degree; indeed, pyridine and imidazole (entries 2 and 3) exhibited more substantial improvement with the modified conditions. Reactions were also cleaner under the modified conditions, with recovery of unreacted 15 accounting for much of the mass balance.

To apply these improved conditions to a more step-economical second-generation approach to tubuvaline, a series of N-acylhydrazone radical acceptors were required. From formyl thiazole 18 (Scheme 4), treatment with allylzinc bromide afforded homoolactic alcohol 19 (racemic). Keck allylation26 was identified as a suitable enantioselective counterpart affording 19 with er 10:1, but most of the subsequent route was initially developed with racemic 19. After O-benzylation to afford 17, reduction of the ester gave primary alcohol 20. Oxidative cleavage55 and subsequent condensation of the racemic aldehydes with enantiopure N-aminooxazolidinone 21 then furnished N-acylhydrazone 22 as a diastereomeric mixture. The primary alcohol was acylated to furnish the pivalate derivative 23. A variety of related hydrazone acceptors with various replacements of the Piv ester with other groups, such as silyl or benzyl ethers, were prepared in a similar fashion (see the Supporting Information). However, these were not as effective in subsequent radical additions.

When 23 (dr 1:1, Scheme 4) was subjected to Mn-mediated addition of isopropyl radical, there was exceptionally clean reactivity under the modified conditions (conditions B of Table 1). Although the reaction was incomplete, with 48% recovery of unreacted hydrazone (23′), it furnished 47% yield of isopropyl adduct 24 (90% yield based on conversion). Surprisingly, radical adduct 24 produced in this reaction appeared to be a single diastereomer; it gave only one set of signals in the 13C NMR spectrum, in contrast to the starting 1:1 11R/11S mixture 23 which had shown two sets of signals as expected.56 In attempting to secure complete conversion of N-acylhydrazone 23, an unexpected phenomenon was observed; resubjection of recovered 23′ to the Mn-mediated radical addition did not provide any of the desired adduct 24, and with longer reaction times, gradual decomposition was instead observed. Closer comparison of analytical data for original reactant 23 and recovered reactant 23′ showed that recovered 23′ had only one set of 13C NMR resonances. This contrasted with the two sets of 13C NMR peaks observed for reactant 23, as expected for the C11 epimer mixture. Thus, both 24 and recovered 23′ were single diastereomers; one diastereomer of 23 had produced 24 via radical addition, while the other afforded no radical adduct.

**Elaboration to Tubuvaline.** Conversion of radical adduct 24 to a protected form of tubuvaline (Scheme 4) entailed TFA installation and reductive N–N bond cleavage (SmI2), removal of the Piv ester (K2CO3, MeOH), and oxidation to the C-terminal carboxylate. The latter conversion involved telescoped oxidation and esterification, as the intermediate carboxylic acid suffered significant material loss upon attempted isolation. Thus, following oxidation of the primary alcohol by catalytic TEMPO in the presence of water, direct esterification by MeI gave the desired methyl ester. This sequence smoothly proceeded to afford N-TFA-O-benzyltubuvaline methyl ester (10) with spectroscopic properties that matched those of 10 from our previously published route.48 This confirmed the configurational assignment of (11S,13R)-24. Overall, the yield of 10 was 8.6% over 10 steps via route A (Scheme 3), a three-step improvement in step economy versus our prior approach.

**An Unexpected Kinetic Resolution Reveals 1,3-Diasterecontrol.** The isolation of 23′ and 24 as single diastereomers indicates a kinetic resolution process via double diastereoselection; the 1:1 diastereomeric mixture of 23 would present matched and mismatched stereocontrol involving the two stereocontrol elements. The lower relative rate of the mismatched case could permit isolation of one diastereomeric product 24 and recovery of unreacted 23′, also with diastereomeric enrichment. This kinetic resolution via radical addition is unprecedented, prompting further analysis.

For allylmetal addition to chiral β-alkoxylimines, the seminal studies of Yamamoto addressed double diastereoselection involving the β stereogenic center of 25 with alternate configurations of a chiral auxiliary at the imine nitrogen (Figure 4a).57 The chelated (allylMgCl) and nonchelated (allylboran) stereocontrol models discussed by Yamamoto have relevance to various other nonradical additions to β-substituted imino compounds.58,59 However, despite considerable literature searches, we have identified no previously documented cases of double diastereoselection in intermolecular radical additions to β-alkoxylimino compounds.60 In the closest precedent, Lin et al. reported a SmI2-mediated azapinacol coupling with β-alkoxysulfinimine 27 (Figure 4b) that incorporates two stereocontrol elements, but stereocontrol
was attributed to the N-sulfinyl group and no comparison was made of matched and mismatched control. Similarly, we had observed complete stereocontrol in our Mn-mediated radical addition to β-alkoxyhydrazone (Scheme 1) and related compounds, but without examining matched/mismatched cases, we lacked a basis to expect strong contributions from 1,3-diastereocontrol.

Because these closest precedents were of questionable relevance to our radical addition, we explored our 1,3-diastereocontrol further. First, to determine the C11 configuration of 24, N-acylhydrazone 23 was prepared with diastereomeric enrichment from a precursor of known configuration at C11. Asymmetric Keck allylation of formyl thiazole in the presence of (R)-BINOL afforded (R)-19 (er 10:1, Scheme 4) as judged by Mosher ester analysis, with a configuration consistent with the Keck precedent. Benzylation, carboxylate reduction, oxidative cleavage, and condensation with N-aminooxazolidinone afforded (11R)-23 by the same sequence described in Scheme 4. The 13C NMR spectrum of this compound did not match that of 23′ recovered from incomplete reactions; instead, it matched the spectrum of the 23 diastereomer that was consumed during the radical addition. Thus, the recovered 23′ was assigned the 11S configuration, and the product 24 was assigned the 11R configuration.

The configurational assignments of 23′ and 24 allow for structural hypotheses regarding modes of stereocontrol via chelated structures A and B (Scheme 5). In structure A that can reasonably be presumed to form upon mixing (11S)-23 with InCl3, both re and si faces of the C==N acceptor carbon are blocked by the chiral N-acylhydrazone and the thiazole, respectively. This is consistent with poor reactivity due to mismatched double diastereocontrol. In alternative structure B with the thiazole coordinating to InCl3, the si face of the C==N in B appears to be unhindered; thus, we favor structure A to explain the mismatched double diastereocontrol with (11S)-23.

If the hypothetical structure A effectively blocks both faces as proposed, an analogous structure bearing the C11 stereogenic center as the only stereocontrol element would also be predicted to provide control of the relative configuration in the radical addition. A simple deletion of the N-acylhydrazine stereocontrol element tested this prediction. Racemic N-acylhydrazine 31 (Scheme 6) was prepared from alkene rac-20 and achiral N-aminooxazolidinone 29 through oxidative cleavage, condensation, and esterification (i.e., the same sequence used to convert 20 to 23 in Scheme 4). Isopropyl radical addition, under the same Mn-mediated conditions of Scheme 4, gave rac-32 with excellent diastereoselectivity, with dr >95:5 as judged by 1H NMR and 13C NMR spectra. Trifluoroacetilation and
reductive cleavage of the N–N bond gave rac-33 (dr >95:5), with spectroscopic data matching the previous nonracemic sample obtained during conversion of 24 to 10 (Scheme 4). All these data confirm that auxiliary and substrate stereocontrol were mutually reinforcing in N-acylhydrazine (11R)-23, both favoring si face radical addition to the imino carbon, and that the β-alkoxy substituent is indeed a viable stereocontrol element for such radical additions. Thus, this route to Tuv yielded important new insights into 1,3-diastereocontrol and double differentiation in the context of the radical addition approach to asymmetric amine synthesis.

**Alternative Elaboration of the Tubuvaline Thiazole.** Although we had some success in our plan to improve the step efficiency by conducting radical addition in the presence of the thiazole (route A, Scheme 3), the overall chemical yield of 8.6% was less than desired. Our attention had also been drawn to a tactic from the tubulysin synthetic studies of Zanda33 and Chandrasekhar,29 in which tubuvaline assembly included cyclocondensation of an aldehyde with cysteine methyl ester followed by oxidation to forge the thiazole ring. We hypothesized that this thiazole construction could be applied as an alternative way to streamline our original preparation of the tubuvaline fragment. Thus, from alcohol 34 (Scheme 7), Swern oxidation gave the corresponding aldehyde; cysteine methyl ester hydrochloride was directly added to the Swern oxidation mixture to furnish thiazoline 35 in a one-pot operation. Oxidation with manganese dioxide then completed route B to N-TFA-O-benzyltubuvaline methyl ester (10). This material gave spectral data matching that produced from route A as well as our earlier published route.48 Importantly, the efficiency as judged by both step count and overall yield were excellent, with 45% yield over eight steps from alcohol 5.

**Modified Route to Tubulysins.** Our prior studies of tubulysin tetrapeptide assembly yielded a tetrapeptide C-terminal alcohol analog 11 (Figure 3),38 but oxidation to the corresponding carboxylic acid proved difficult. To avoid the late-stage oxidation, the tetrapeptide was assembled with the C-terminal amino acid Tup already in the carboxylate oxidation state. In hopes that an endgame debenzylation would deprotect both C11–OH and the C-terminal carboxylate concurrently, the Tup unit was introduced as a benzyl ester. This material was obtained from primary alcohol 36 (Scheme 8), available in stereochromically pure form via our previously published Mn-mediated radical addition route to Tup.48 Exchange of the N-TFA for N-Boc and oxidation with PDC proceeded via known compounds 37,28 and 38,34 and the known sequence of basic hydrolysis and benzyl esterification gave N-Boc-γ-amino ester 39.34 Removal of the Boc group with trifluoroacetic acid furnished Tup ester 40 in the free amine form, suitable for peptide assembly.

Our previously reported tetrapeptide assembly48 required modification of the sequence of couplings. Basic conditions hydrolyzed both N-TFA and methyl ester functions of Tup derivative 10 (Scheme 9); the crude material was taken up in methanolic HCl to reinstall the methyl ester, providing amino ester 41. Next, attachment of the Mep-Ile dipeptide 42 via its mixed anhydride gave tripeptide 43. Ester saponification at the C-terminus and DECP-mediated coupling with Tup benzyl ester 40 then completed the tetrapeptide assembly to furnish 12 in excellent yield.

The final step en route to tubulysin V was envisioned to be a convenient hydrogenolysis of both benzyl groups. To our dismay, all efforts to hydrogenate the di-O-benzyl tetrapeptide 12 led to destruction of this material or no reaction at all.63 After considerable experimentation, a surprisingly effective alternative was finally identified: In a model study, a mixture of 10 and stannic chloride in boiling dichloromethane cleanly removed the benzyl ether to furnish 45 (eq 1).

Exploratory experiments on debenzylation of the intact tetrapeptide 12 offer some evidence of its feasibility. Exposure of 12 to SnCl4 in refluxing CH2Cl2 indeed furnished a compound that had been twice debenzylated, as judged by high-resolution mass spectrometry (HRMS). The chemical behavior of this compound was consistent with the known reactivity of tubulysin V; it reacted with Ac2O and pyridine to give an O-acetyl derivative (tubulysin U) as judged by mass spectrometry. These results provide supporting evidence for the structure of 11-O-benzyltubulysin V benzyl ester (12), prepared in 43% overall yield via a 12-step longest linear sequence.

**3. Conclusion**

Building on prior data establishing Mn-mediated radical addition as an excellent means of stereocontrol for tubuphenylalanine (Tup) and tubuvaline (Tuv) synthesis, two alternative routes are presented toward the goal of improved efficiency in synthesis of Tuv. One of these routes
required modification of Mn-mediated radical addition conditions to accommodate a thiazole in the radical acceptor, allowing broader versatility of the radical addition approach to asymmetric amine synthesis. While this route did not meet the efficiency goals, it uncovered a case of 1,3-diastereoretrocontrol in radical addition to β-alkoximino compounds: in combination with the chiral N-acryhydrazone as a second stereocontrol element, a kinetic resolution occurred, separating two diastereomeric radical acceptors according to their differential reactivity in radical addition. The second route merged our radical addition chemistry with a rapid cyclocondensation and reactivity in radical addition. The second route allowed broader versatility of the radical addition approach to conditions to accommodate a thiazole in the radical acceptor, radical addition to 

**4. EXPERIMENTAL SECTION**

**Materials and Methods.** Reactions employed oven- or flame-dried glassware under nitrogen unless otherwise noted. Toluene, tetrahydrofuran (THF), and CH₂Cl₂ were purchased inhibitor-free, dried glassware under nitrogen unless otherwise noted. Toluene, 4. EXPERIMENTAL SECTION

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**Conditions for Compatibility of N-Heteroaromatics with Mn-Mediated Radical Addition. General Procedure A (Table 1, Footnote a).** A Schlenk flask charged with N-acryhydrazone (1 equiv), Mn-mediated radical addition cation of Mn-mediated radical addition (10 equiv) was added via syringe, followed by dimanganese decacarbonyl (1 equiv). The Schlenk flask was sealed and irradiated (broad spectrum with maximum at 300 nm, Rayonet photoreactor) for 6 h. Concentration and flash chromatography or gradient flash chromatography furnished the N-acryhydrazone 16. Preparation and characterization of hydrazide 16 have been previously reported.

**General Procedure B (Table 1, footnote b).** A Schlenk flask charged with N-acryhydrazone 15 (1 equiv), additive (1 equiv), indium chloride (3.5 equiv), and CH₂Cl₂ (0.017 M) was stirred for 30 min. Isopropyl iodide (3 equiv) was added via syringe, followed by dimanganese decacarbonyl (1 equiv). The Schlenk flask was sealed and irradiated (broad spectrum with maximum at 300 nm, Rayonet photoreactor) for 6 h. Concentration and flash chromatography or gradient flash chromatography furnished the N-acryhydrazone 16.

**Preparation and Characterization Data for New Compounds.**

Ethyl 2-(1-Hydroxybut-3-enyl)thiazole-4-carboxylate (rac-19). To a mixture of ZnCl₂ (5.90 g, 43.3 mmol) and THF (120 mL) was added allylmagnesium chloride (2 M in THF, 19.7 mL, 39.4 mmol) slowly via syringe. After being stirred for 0.5 h, this mixture was transferred via cannula into a solution of formyl thiazole 18 (7.20 g, 39.4 mmol) in THF (150 mL). After 3 h, the reaction was quenched with water (20 mL) and extracted with EtOAc. The organic phase was dried over Na₂SO₄. Concentration and gradient flash chromatography (25% EtOAc in petroleum ether to 50% EtOAc in petroleum ether) dried homohyloc alcohol rac-19 (5.27 g, 60% yield) as a pale yellow solid: mp 46.0–46.5 °C; IR (film) 3411, 2983, 1725, 1489, 1215 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 8.13 (s, 1H), 5.90–5.76 (m, 1H), 5.27–5.20 (m, 2H), 5.11 (dd, J = 7.9, 4.0 Hz, 1H), 4.42 (q, J = 7.1 Hz, 2H), 2.91–2.62 (m, 2H), 2.79–2.62 (m, 1H), 2.79–2.52 (m, 1H), 1.40 (t, J = 7.2 Hz, 3H); ¹³C{¹H} NMR (75 MHz, CDCl₃) δ 175.9, 161.5, 146.9, 132.8, 127.5, 119.7, 71.0, 61.6, 42.4, 14.4; HRMS (ESI) m/z [M + H]⁺ calcd for C₁₀H₁₂NO₃S 228.0694; found 228.0686.

Ethyl 2-(1R-Hydroxybut-3-enyl)thiazole-4-carboxylate ((R)-19). To activated 4 Å molecular sieves (23 mg) were added (R)-BINOL (6.2 mg, 0.022 mmol). CH₂Cl₂ (0.6 mL) and Ti(O-i-Pr)₄ (12 µL, 0.040 mmol) were added via syringe. After 1 h, formyl thiazole 18 (20 mg, 0.11 mmol) was added. After cooling to −78 °C, allyltributylstannane (0.10 mL, 0.32 mmol) was added via syringe. The mixture was stirred for 10 min at −78 °C and then stored in a freezer (ca. −20 °C) for 24 h. The mixture was partitioned between saturated aqueous sodium bicarbonate solution (2 mL) and EtOAc, and the organic phase was dried over Na₂SO₄. Concentration and

**Scheme 9**

**10 (R¹ = TFA)**

**11-O-Benzylthubulysin V benzyl ester (12)**

**Longest linear sequence: 12 steps, 43% yield**

**Required**
flash chromatography (17% EtOAc in hexane to 33% EtOAc in hexane to 50% EtOAc in hexane) afforded alcohol (R)-19 (22.7 mg, 93% yield) as a pale yellow solid: $\delta_{\text{CDCl}_3} = 7.06$ (1.14, CHCl$_3$).

**Configuration Assignment: Mosher Ester of (R)-19 (S1).** To a solution of (R)-19 (1 mg, 4.4 mmol) in CH$_2$Cl$_2$ (0.25 mL) was added pyridine (1 $\mu$L, 0.0124 mmol) via syringe. (S)-Mosher’s acid chloride (5 mg, 0.020 mmol) was added. After 24 h, reaction mixture was concentrated. Flash chromatography afforded Mosher’s S1 (1.6 mg, 82% yield) as a colorless oil: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.14 (s, 1H), 8.09 (s, minor diastereomer peak), 7.58–7.51 (m, 2H), 7.45–7.36 (m, 3H), 6.43 (dd, $J= 7.5, 4.7$ Hz, 1H), 5.80–5.70 (m, minor diastereomer peak), 5.70–5.60 (m, 1H), 5.19–5.11 (m, minor diastereomer peak), 5.08–5.00 (m, 2H), 4.43 (dd, $J= 14.3, 7.0$ Hz, 2H), 3.58 (s, minor diastereomer peak), 3.54 (s, 3H), 2.95–2.76 (m, 2H), 1.40 (t, minor diastereomer peak), 1.41 (t, $J= 7.1$ Hz, 3H).

**Ethyl 2-(1-(Benzoyloxy)but-3-enyl)thiazole-4-carboxylate** (S2). To a solution of homoallylic alcohol rac-19 (1.16 g, 5.12 mmol) in THF (100 mL) was added KH (30 wt % in mineral oil, 821 mg, 6.14 mmol) in one portion. After 5 min, benzyl bromide (0.73 mL, 6.14 mmol) was added via syringe. After 4 h, the reaction mixture was quenched with water (30 mL), concentrated to remove THF, and extracted with EtOAc. The organic phase was dried over Na$_2$SO$_4$. Concentration and flash chromatography (9% EtOAc in petroleum ether) afforded benzyl ether S2 (384 mg, 54% yield) as a pale yellow oil: IR (film) 3015, 2970, 2932, 2867, 1763, 1642, 1454, 1324, 1075 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.17 (s, 1H), 7.36–7.28 (m, 3H), 5.90–5.77 (m, 1H), 5.15–5.05 (m, 2H), 4.91 (dd, $J= 7.0, 5.4$ Hz, 1H), 4.58 (ABq, $\Delta \nu = 23.9$ Hz, $J= 11.6$ Hz, 2H), 4.44 (q, $q = 7.1$ Hz, 2H), 2.74–2.63 (m, 2H), 1.41 (t, $J= 7.1$ Hz, 3H); $^{13}$C{1H} NMR (75 MHz, CDCl$_3$) $\delta$ 174.8, 161.5, 147.1, 147.3, 134.0, 128.6, 128.1, 128.0 (2C), 118.5, 78.8, 72.3, 61.6, 41.4, 14.5; HRMS (ESI) m/z [M + H]$^+$ calcld for C$_{21}$H$_{22}$N$_2$O$_5$SNa 474.1463; found 474.1451.

**Ethyl 2-(1-(Benzoyloxy)but-3-enyl)thiazole-4-carboxylate (rac-20).** A solution of ester S2 (1.56 g, 4.91 mmol) in THF (98 mL) was cooled to $-78$ °C, and lithium aluminum hydride (2.0 M in THF, 4.91 mL, 9.82 mmol) was added slowly via syringe. After 1 h, the reaction was warmed to room temperature, quenched by slow addition of water (50 mL), and extracted with EtOAc. The organic phase was dried over Na$_2$SO$_4$ and concentrated. Flash chromatography (67% EtOAc in petroleum ether) afforded alcohol rac-20 (1.26 g, 93% yield) as a pale yellow oil: IR (film) 3339, 3078, 3031, 2978, 2869, 1642, 1454, 1324, 1075 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.40–7.25 (m, 5H), 7.20 (s, 1H), 5.93–5.74 (m, 1H), 5.19–5.02 (m, 2H), 4.83–4.74 (m, 3H), 4.58 (ABq, $\Delta \nu = 36.5$ Hz, $J= 11.6$ Hz, 2H), 3.36 (br s, 1H), 2.80–2.57 (m, 2H); $^{13}$C{1H} NMR (75 MHz, CDCl$_3$) $\delta$ 174.2, 156.2, 137.6, 133.2, 128.5, 128.0 (2C), 118.2, 115.3, 78.7, 72.0, 60.8, 41.4; HRMS (ESI) m/z [M + H]$^+$ calcld for C$_{21}$H$_{22}$N$_2$O$_5$SNa 474.1463; found 474.1451.

**To a solution of (S)-3-(3-(Benzyloxy)-3-4-(4-hydroxymethyl)thiazol-2-yl)-prop-2-yl-propylidenemino)-4-benzylxazolidin-2-one (22).** To a solution of alkenne rac-20 (56 mg, 0.31 mmol) in THF (21 mL) and water (21 mL) was added osmium tetroxide (2.5 wt % in tert-butyl alcohol, 0.12 mL, 0.012 mmol). After 5 min, sodium periodate (265 mg, 1.24 mmol) was added. After 5.5 h, the reaction was quenched with saturated aqueous thiosulfate solution (50 mL) and extracted with EtOAc. The organic phase was dried over Na$_2$SO$_4$, then filtered through a short column of silica gel, eluting with EtOAc. Concentration furnished the crude aldehyde, which was dissolved in CH$_2$Cl$_2$ (31 mL) along with (S)-3-4-phenylmethyl-2-oxazolidinone (21, 119 mg, 0.62 mmol). After 12.5 h, concentration and gradient flash chromatography (83% EtOAc in petroleum ether to EtOAc to 5% methanol in EtOAc) afforded 22 (106 mg, 75% yield, inseparable 1:1 mixture of diastereomers) as a colorless wax: IR (film) 3404, 3013, 1763, 1405, 1217, 1092 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.91–7.84 (m, 1H), 7.38–7.19 (m, 9H), 7.09–7.04 (m, 2H), 5.08–5.02 (m, 1H), 4.75–4.52 (m, 1H), 4.33–4.23 (m, 1H), 4.20–4.15 (m, 1H), 4.10–4.03 (m, 1H), 3.73 (br s, 1H), 3.15–2.93 (m, 3H), 2.77–2.63 (m, 1H), (diastereomer peaks were unresolved); $^{13}$C{1H} NMR (75 MHz, CDCl$_3$) $\delta$ 172.5, 156.8, 154.0, 149.6 and 149.4, 137.0, 134.9, 129.2, 128.8 and 128.42, 127.9 (2C), 127.2, 115.4, 76.8 and 76.4, 71.9 and 71.8, 65.6, 60.4, 40.04 and 39.97, 36.1, and 36.0 (some diastereomer peaks were unresolved); HRMS (ESI) m/z [M + Na]$^+$ calcld for C$_{23}$H$_{27}$N$_2$O$_5$SNa 474.1643; found 474.1451.
A solution of TEMPO (0.7 mg, 5 µmol), BAIB (30 mg, 0.092 mmol) and CH₂Cl₂ (0.6 mL) was prepared. A portion (ca. 10%) of this solution was added into alcohol S₃ (1.9 mg, 0.0046 mmol), then water (0.012 mL) was added. After 30 min, an additional portion of TEMPO (0.3 mg, 2 µmol) was added. After 20 h, the reaction mixture was partitioned between diethyl ether and saturated sodium bicarbonate aqueous solution. The aqueous phase was acidified with 2 M HCl, then extracted with EtOAc. The organic phase was dried over Na₂SO₄ and concentrated to afford the crude carboxylic acid as colorless oil. To a solution of this carboxylic acid in DMF (0.2 mL) was added potassium carbonate (0.7 mg, 5.2 µmol) and iodosmethane (0.6 mg, 3.9 µmol; measured via syringe weight before and after addition). After 21 h, flash chromatography (25% EtOAc in hexane) afforded methyl ester 10 as colorless oil (1.3 mg, 64% yield).

**Procedure via oxidation of thiazone 35.** To a solution of thiazone 35 (10 mg, 0.02 mmol) in anhydrous acetonitrile was added MnO₂ (20 mg, 0.2 mmol). After 9 h, another portion of MnO₂ (20 mg, 0.2 mmol) was added. After 12 h, the reaction mixture was filtered through Celite and concentrated. Flash chromatography (2:1 petroleum ether/ethyl acetate) afforded 10 (8 mg, 80% yield) as a pale yellow oil: [α]₂⁰owered 12.0 (c 0.26, CHCl₃); IR (film) 3583, 3315, 2962, 2924, 1722, 1710, 1180; ¹H NMR (400 MHz, CDCl₃) δ 8.21 (s, 1H), 7.48–7.29 (m, 5H), 6.63 (d, δ = 9.7 Hz, 1H), 4.89 (dd, δ = 9.7, 3.8 Hz, 1H), 4.54 (ABq, J = 10.6 Hz, 3H), 2.92–4.12 (m, 1H), 3.97 (s, 3H), 2.12–1.98 (m, 2H), 1.78 (dd, δ = 13.4, 6.7 Hz, 1H), 0.94 (d, δ = 6.8 Hz, 3H), 0.90 (d, δ = 6.8 Hz, 3H); ¹³C{¹H} NMR (100 MHz, CDCl₃) 174.8, 161.6, 157.0 (q, δ = 36 Hz), 146.9, 136.4, 128.6, 128.6, 128.4, 128.2, 115.9 (q, δ = 288 Hz), 76.3, 73.2, 52.6, 52.1, 39.2, 31.8, 18.9, 18.0; MS (ESI) m/z (rel intensity) 467 [(M + Na)⁺, 100], 445 [(M + H)⁺, 26]; HRMS (ESI) m/z [M + Na]⁺ calc. for C₁₁H₁₃F₃N₂O₅Na 467.2283; found 467.2244.

**3-(Benzyloxy)-3-(4-hydroxymethyl)thiazol-2-yl propyldienemino)oxazolidin-2-one (rac-30).** To a solution of alkenne rac-20 (170 mg, 0.62 mmol) in THF (31 mL) and water (31 mL) was added osmium tetroxide (2.5 wt % in tert-butyl alcohol, 0.06 mL, 0.006 mmol). After 5 min, sodium periodate (535 mg, 2.5 mmol) was added. After 13 h, the reaction was quenched with saturated aqueous sodium thiosulfate solution (100 mL) and extracted with EtOAc. The organic phase was dried over Na₂SO₄, and filtered through silica gel, eluting with EtOAc. Concentration furnished the crude aldehyde (180 mg), part of which (150 mg) was dissolved in CH₂Cl₂ (50 mL) along with 3-amino-2-oxazolidinone (120 mg, 1.18 mmol). After 12 h, concentration and flash chromatography (5% MeOH in CH₂Cl₂) afforded hydrazide rac-30 (98 mg, 53% yield over two steps, racemic) as a colorless wax: IR (film) 3400, 3018, 2922, 1772, 1407, 1215, 1091 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) δ 7.37–7.27 (m, 7H), 7.22 (s, 1H), 6.94 (t, δ = 5.5 Hz, 1H), 4.98 (dd, δ = 6.8, 5.5 Hz, 1H), 4.74 (s, 2H), 4.61 (ABq, Δv = 85.0 Hz, δ = 11.7 Hz, 2H), 4.44 (dd, δ′ = 80.0 Hz, 2H), 3.69–3.62 (m, 2H), 3.28 (brs s, 1H), 3.06–2.95 (m, 2H); ¹³C{¹H} NMR (150 MHz, CDCl₃) δ 172.5, 156.7, 154.6, 143.6, 137.2, 128.6, 128.19, 128.16, 115.5, 76.6, 72.0, 61.5, 60.8, 42.1, 39.4; HRMS (ESI) m/z [M + H]⁺ calc. for C₁₅H₁₄N₂O₂SNa 362.1174; found 362.1167.

**Methyl (2-(1′R,3′R)-3′-(trifluorocetamido)-1′-benzoyloxy-4′-methyl-3′-(trifluorocetamido)pent-1′-yl)-thiazol-4′-yl)carboxylate (N-TFA-11-0-benzyl-tubulidine methyl ester, 10).** Procedure via oxidation of alcohol S₃.
temperature over 3 h and then heated at 40 °C with an oil bath.
After 12 h, the mixture was cooled to room temperature and concentrated. To a solution of the residual material in diethyl ether (2.7 mL) at 0 °C were added triethylamine (0.033 mL, 0.23 mmol) and Boc anhydride (17 mg, 0.078 mmol), and the reaction was allowed to reach ambient temperature. After 12 h, the reaction was quenched with saturated aqueous NaHCO3 and partitioned between water and CH2Cl2. The organic phase was washed with brine, dried over Na2SO4, and concentrated. Flash chromatography (petroleum ether to 1:1 petroleum ether/ethyl acetate) afforded the known alcohol 37 (25 mg, 44%).

N-Methyl-o-picecolyl-L-isoleucine tert-Butyl Ester (S4). A solution of N-methyl-o-picecolic acid (100 mg, 0.55 mmol) in DMF (9.1 mL) was cooled to 0 °C followed by addition of disopropylethylamine (0.387 mL, 2.22 mmol). To this mixture was added t-isoleucine tert-butyl ester hydrochloride (56 mg, 0.35 mmol) followed by addition of diethyl cyanophosphate (DECP, 0.101 mL, 0.66 mmol). The reaction was allowed to reach ambient temperature. After 12 h, the reaction was quenched with water and partitioned between saturated aqueous NaHCO3 and EtOAc. The organic phase was washed with brine, dried over Na2SO4, and concentrated. Flash chromatography (1:1 petroleum ether/ethyl acetate to 1:4 petroleum ether/ethyl acetate) afforded S4 (144 mg, 83% yield) as a pale yellow oil: [α]24 D +70.5 (c 0.96, CHCl3); IR (film) 3388, 2965, 2936, 2791, 1736, 1727, 1677, 1502, 1147, 847 cm−1; 1H NMR (CDCl3, 400 MHz) δ 6.96 (d, J = 9.5 Hz, 1H), 4.38 (d, J = 9.2, 4.5 Hz, 1H), 2.83 (br d, J = 11.6 Hz, 1H), 2.21 (d, J = 11.2, 3.4 Hz, 1H), 1.95 (ddd, J = 12.7, 11.6, 3.1 Hz, 1H), 1.88–1.80 (m, 2H), 1.71–1.62 (m, 1H), 1.60–1.46 (m, 2H), 1.46–1.36 (m, 2H), 1.38 (s, 9H), 1.28–1.03 (m, 2H), 0.92 (t, J = 6.7 Hz, 3H), 0.91 (d, J = 6.8 Hz, 3H); 13C (1H) NMR (150 MHz, CDCl3) δ 174.0, 170.7, 81.4, 69.6, 56.1, 55.3, 44.7, 37.7, 30.6, 27.9, 25.2, 21.3, 15.6, 11.6; MS (ESI) m/z (rel intensity) 335 ([M + Na]+, 4), 313 ([M + H]+), 100; HRMS (ESI) m/z [M + H]+ calcd for C21H23N2O4S 378.1526; found 378.1514.

N-Methyl-o-picecolyl-L-isoleucine Trifluoroacetic Acid Salt (42). A solution of tert-butyl ester S4 (50 mg, 0.16 mmol) in dichloromethane (1.6 mL) was treated with trifluoroacetic acid (0.204 mL, 2.66 mmol) followed by reflux for 4 h with heat applied by oil bath. The mixture was then cooled to room temperature and concentrated to afford the carboxylic acid 42 as the TFA salt, which was used without further purification. MS (ESI) m/z (rel intensity) 257 ([M + H]+), 100.

N-Methyl-o-picecolyl-L-isoleucinyl-(O-benzyl)thiobutanoic Methyl Ester (E3). A solution of trifluoroacetyl amino ester 10 (12 mg, 0.02 mmol) in methanol–water (1 mL, 5:1) was cooled to 0 °C followed by addition of Ba(OH)2·8H2O (42 mg, 0.16 mmol). The reaction mixture was allowed to warm ambient temperature over 3 h and then maintained at 40 °C overnight with heat applied by oil bath. The mixture was then cooled to room temperature and concentrated to afford the crude amino acid: MS (ESI) m/z (rel intensity) 368 ([M + Na]+, 5), 335 ([M + H]+), 100. The crude amino acid was treated with methanolic HCl (2 mL of a stock solution prepared from 5 mL methanol and 0.71 mL acetyl chloride) at 0 °C for 10 min and then heated at reflux for 2.5 h. The reaction mixture was cooled to 0 °C.
and concentrated. The residue was taken up in toluene and concentrated to ensure azotropic removal of traces of acetic acid. Flash chromatography (2:1 petroleum ether/ethyl acetate to 9:1 dichloromethane/methanol) afforded the Tuv methyl ester 41 (9 mg, 96% yield) as a pale yellow oil; MS (ESI) m/z (rel intensity) 371 ([M + Na]+, 3), 349 ([M + H]+, 100). This material was used immediately in the coupling reaction.

To a solution of the Mep-Ile dipeptide 42 (12 mg, 0.03 mmol) in anhydrous ethyl acetate (0.212 mL) was added N-methyl morpholine (0.004 mL, 0.03 mmol). The mixture was cooled to −10 °C and isobutyl chloroformate (0.005 mL, 0.034 mmol) was added via syringe, followed by a solution of Tuv amino ester 41 (6 mg, 0.01 mmol) in anhydrous ethyl acetate (0.085 mL) via cannula. The reaction mixture was allowed to warm slowly to ambient temperature. After 12 h, the reaction mixture was partitioned between water and EtOAc. The organic phase was washed with brine and quenched with water and partitioned between saturated aqueous NaHCO3 and EtOAc. The organic phase was dried over Na2SO4. Concentration afforded tetrapeptide (6 mg, 87% yield) as a pale yellow oil; MS (ESI) m/z ([M + Na]+, 3), 349 ([M + H]+, 100). This material was used immediately for the following synthetic steps.

To a solution of the Mep-Ile dipeptide 42 (5 mg, 0.015 mmol) in DMF (0.110 mL) followed by 4-(N,N-diisopropylcarbodiimide)benzylamine (4.5 mg, 0.015 mmol) in DMF (0.01 mL) at 0 °C, 1.5 equiv 3-(Boc-amido)pent-1′-uoroacetate salt of 2,3-dimethylthiazol-4-yl-carboxylate (43) was added as a 0.05 M solution in DMF (0.005 mL, 0.0025 mmol). The mixture was cooled to −10 °C and isobutyl chloroformate (0.004 mL, 0.03 mmol) was added via syringe, followed by a solution of Mep-Ile dipeptide 42 (6 mg, 0.01 mmol) in anhydrous ethyl acetate (0.085 mL) via cannula. The reaction mixture was allowed to warm slowly to ambient temperature. After 12 h, the reaction mixture was partitioned between water and EtOAc. The organic phase was washed with brine and quenched with water and partitioned between saturated aqueous NaHCO3 and EtOAc. The organic phase was dried over Na2SO4. Concentration afforded tetrapeptide (18 mg, 35% yield) as a pale yellow oil; 1H NMR (CDCl3, 400 MHz) 175.9, 174.9, 171.0, 161.8, 146.9, 137.1, 128.6, 121.8, 115.8 (q, JZ = 25 Hz, 1H), 113.2, 108.6 (d, J = 27 Hz, 1H), 104.4 (d, J = 27 Hz, 3H); 13C{1H} NMR (100 MHz, CDCl3) 175.8, 161.8, 158.6 (q, JZ = 25 Hz, 1H), 137.1, 128.6, 121.8, 115.8 (q, JZ = 25 Hz, 1H), 104.4 (d, J = 27 Hz, 3H), 104.4 (d, J = 27 Hz, 3H); HRMS (ESI) m/z [M + H]+ calc for C35H53N5O6S 672.3795; found 672.3801.

To a solution of Tup benzyl ester (40 mg, 0.03 mmol) in CH2Cl2 (0.25 mL) was added tin(IV) chloride (0.03 mL, 0.25 mmol) at ambient temperature. The reaction mixture was stirred for 72 h and then acidi ed with TFA to pH 1–2. The mixture was partitioned between water and ethyl acetate, and the organic phase was dried over Na2SO4. Concentration afforded carboxylic acid 44 (5 mg, 75%) and 1H, 13C and 19F NMR spectra were acquired using TFA to pH 1–2. The mixture was dried over Na2SO4. Concentration afforded carboxylic acid 44 (5 mg, 75% yield) as a pale yellow oil; 1H NMR (CDCl3, 400 MHz) δ 8.02 (s, 1H), 7.66–7.28 (m, 15H), 7.03 (d, J = 8.8 Hz, 1H), 6.27–6.08 (d, J = 9.8 Hz, 1H), 5.09 (q, J = 12.3 Hz, 2H), 4.68 (dd, J = 10.6, 2.6 Hz, 1H), 4.61–4.42 (m, 3H, 4.42–4.21 (m, 1H), 4.20–4.07 (m, 2H), 3.02–2.83 (m, 3H), 2.78–2.63 (m, 1H), 2.52 (dd, J = 11.6, 3.5 Hz, 1H), 2.23 (s, 3H), 2.11–1.98 (m, 11H), 1.97–1.70 (m, 4H), 1.20 (d, J = 7.1 Hz, 3H), 0.96 (d, J = 6.7 Hz, 3H), 0.93–0.87 (m, apparent overlap of doublets, 9H), MS (ESI) m/z (rel intensity) 874 ([M + Na]+, 100), 852 ([M + H]+, 75); HRMS (ESI) m/z [M + H]+ calc for C63H84N7O7S 852.4734; found 852.4723. Due to material loss, 13C NMR data were unavailable for this compound. Treatment of 12 with SnCl4 using the procedure given for 45, furnished a yellow oil with mass spectral data consistent with 2-fold debenzylization: HRMS (ESI) m/z [M + H]+ calc for C63H84N7O7S 672.3795; found 672.3801.

**Methyl 2-((1′R,3′R)-1′-Hydroxy-4′-methyl-3′-(trifluoroacetamidopent-1)-yl)thiazol-4-yl)carboxylate (45).** To a solution of N-TFA amino ester 10 (25 mg, 0.05 mmol) in CH2Cl2 (0.25 mL) was added tin(IV) chloride (0.03 mL, 0.25 mmol) at ambient temperature. The reaction mixture was then heated at reflux for 25 h. After cooling to 0 °C the reaction was quenched by dropwise addition of sat NaHCO3 and extracted with CH2Cl2. The organic phase was washed with brine and dried over Na2SO4. Concentration and flash chromatography afforded alcohol 45 (15 mg, 83% yield) as a pale yellow oil; 1H NMR (CDCl3, 400 MHz) δ 8.15 (s, 1H), 6.46 (d, J = 9.5 Hz, 1H), 4.96 (ddd, J = 11.1, 4.8, 2.4 Hz, 1H), 4.24 (d, J = 5.0 Hz, 1H), 4.08–4.16 (m, 1H), 3.93 (s, 3H), 2.26 (ddd, J = 11.4, 11.6, 2.4 Hz, 1H), 2.02–1.83 (m, 2H), 1.00 (d, J = 7 Hz, 3H), 0.99 (d, J = 7 Hz, 3H); 13C{1H} NMR (100 MHz, CDCl3) 175.8, 161.8, 158.6 (q, JZ = 25 Hz, 1H), 146.6, 127.8, 115.8 (q, JZ = 25 Hz, 1H), 68.7, 52.6, 52.4, 40.2, 31.8, 19.2, 18.2, MS (ESI) m/z (rel intensity) 377 ([M + Na]+, 100), 355 ([M + H]+, 8).

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.1c01798.

Comparisons of tubulysin syntheses, 1H and 13C NMR spectra for new compounds, enantiomer ratio and configuration assignment of (R)-19 (PDF).

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**Notes**

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