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COMMUNICATIONS

Published on Web 12/01/2006

The Total Synthesis of Tubulysin D

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The tubulysins, first isolated by Höfle from myxobacterial culture broths,[1] are potential anticancer agents having exceptionally potent cell growth inhibitory activity that exceeds the epothilones, vinblastine, and taxol by a factor of 20- to 1000-fold. While the biosynthesis,[2] mechanism,[3] and anticancer activity[4] of the tubulysins have intensively been investigated, and despite a number of active synthetic efforts,[5] none of the tubulysins containing the essential *N*,*O*-acetal has yet yielded to total synthesis.[6] Here we report the first total synthesis of tubulysin D (**1**) through the development and application of *tert*-butanesulfinamide methods. An efficient scheme for introducing and carrying forward the highly labile *N*,*O*-acetal functionality was essential for completing the synthesis. Tubulysin D can be dissected into four amino acid fragments: D-*N*-methyl pipecolic acid (Mep), isoleucine, tubuvaline (Tuv), and tubuphenylalanine (Tup). Tuv and Tup each incorporate two stereocenters presented in a 1,3-relationship, and both represent excellent synthesis targets for the development of *tert*-butanesulfinamide methods. The *O*-acyl *N*,*O*-acetal functionality present on the Tuv fragment constitutes a key challenge in the synthesis of **1** because this functionality, which has very rarely been observed in natural products, is reported to be quite labile to both acidic and basic reaction conditions.[7] Appropriate protecting group selection and staging of the incorporation of the *N*,*O*-acetal functionality are therefore critical to the successful synthesis of **1**.

The synthesis of Tup was accomplished in just three steps from commercially available material (Scheme 1). The key step was a SmI_2-mediated reductive coupling of methyl methacrylate and phenylacetaldimine **2**, which was prepared by condensation of (*R*)-*tert*-butanesulfinamide and phenylacetaldehyde in 84% yield.[8] The asymmetric coupling of α-substituted α,β-unsaturated carbonyl compounds and imines has not previously been reported.[9] A wide range of solvents and additives were investigated, with both H_2O and LiBr proving to be of critical importance for achieving a high yield (99%) and good selectivity (80:15:3:2). Other methacrylate derivatives, such as benzyl or *tert*-butyl esters, gave significantly lower selectivity (data not shown). Chromatography of **3** provided diastereomerically pure material in 55% yield with the relative and absolute stereochemistry established by X-ray crystallographic analysis. Heating **3** in aqueous HCl resulted in concomitant ester hydrolysis and sulfinyl cleavage to give amine hydrochloride **4** in quantitative yield.

The convergent synthesis of Tuv (Scheme 2) was accomplished by addition of the metalloenamine derived from ketimine **5** to thiazoline aldehyde **6**, which was prepared in four steps and 67% overall yield from diethoxyacetonitrile by known methods.[10] We previously reported on highly stereoselective *N*-sulfinyl metalloenamine additions to aldehydes using zinc and magnesium counterions,[11] but with aldehyde substrate **6**, the addition product **7** was obtained with very poor addition diastereoselectivities (~1:1). Gratifyingly, after evaluating a number of different counterions and solvents, high addition selectivity (92:8) could be achieved by employing the highly covalent and coordinatively unsaturated

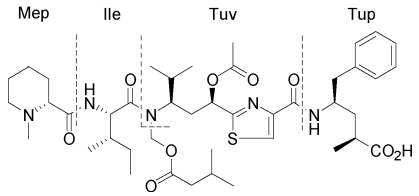
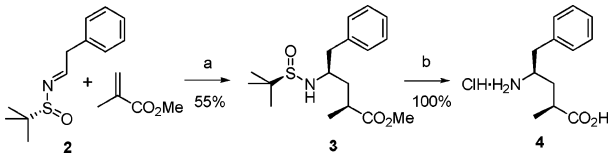


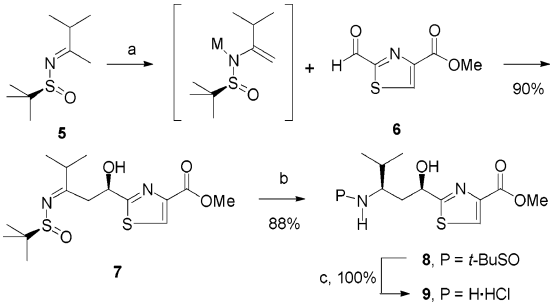
Figure 1. Tubulysin D (**1**).

Scheme 1[a]



[a] Reagents and conditions: (a) SmI_2, LiBr, H_2O, THF, −78 °C; (b) HCl, dioxane/H_2O, Δ.

Scheme 2[a]



[a] Reagents and conditions: (a) LDA, $ClTi(O\text{-}i\text{-}Pr)_3$, ether, −78 °C; (b) $NaBH_4$, $Ti(OEt)_4$, THF, −78 °C; (c) HCl, dioxane/MeOH.

titanium counterion with ether as the solvent. Under these conditions, **7** was obtained as a single diastereomer in 90% yield after chromatography. Stereoselective reduction of **7** was next accomplished using conditions that we had previously reported for the one-pot stereoselective reductive amination of ketones with *tert*-butanesulfinamide.[12] Performing the reduction at low temperature eliminated competitive reduction of the methyl ester and provided the desired 1,3-amino alcohol with 91:9 dr. After chromatography, **8** was isolated in diastereomerically pure form in 88% yield. Treatment with HCl in MeOH then provided the amine hydrochloride **9** in near quantitative yield. X-ray crystal structure analysis of the bis-*para*-bromobenzoyl adduct of **9** confirmed the predicted sense of induction for both the metalloenamine addition and imine reduction steps.

To set the stage for *N*,*O*-acetal incorporation, α-azido acid chloride **10**[13] was coupled with the Tuv intermediate **9** in 93% yield (Scheme 3). The azide masking group was selected over much more common carbamate-based amine protecting groups to enable selective introduction of the *N*,*O*-acetal on the Tuv amide nitrogen.[14] After protection of the secondary alcohol with TESOTf to provide dipeptide **12** in 98% overall yield, *N*-alkylation with chloromethyl

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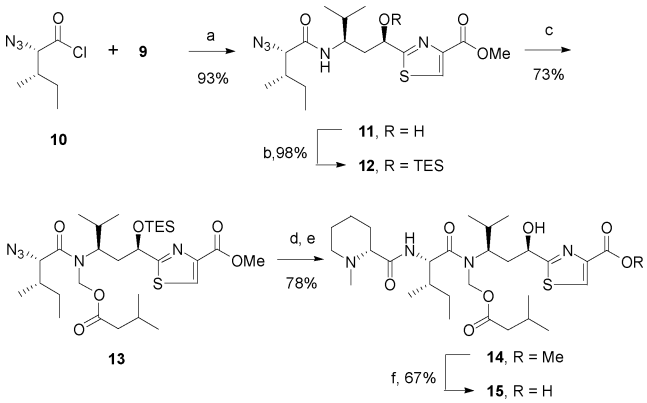
Scheme 3[a]



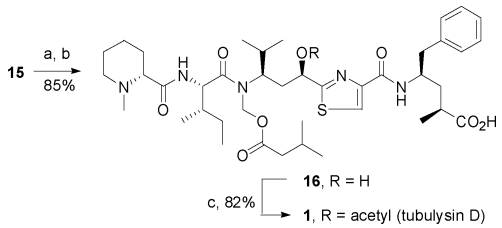
[a] Reagents and conditions: (a) *i*-Pr_2EtN, CH_2Cl_2; (b) TESOTf, lutidine, CH_2Cl_2; (c) KHMDS, THF, −45 °C, then $ClCH_2OCOCH_2CH(CH_3)_2$; (d) Mep pentafluorophenyl ester, H_2, Pd/C, EtOAc; (e) AcOH/THF/H_2O; (f) Me_3SnOH, $Cl(CH_2)_2Cl$, 60 °C.

isobutyl carbonate was next attempted. A number of bases with lithium, sodium, and potassium counterions in a number of solvents were evaluated with KHMDS in THF providing the highest yield (73%). The alkylation reaction proved to be very sensitive to sterics. For example, in investigations of alcohol protecting groups, we found that <10% alkylation occurred when the TES group was replaced by a TIPS group. The azide served as an ideal masking group not only because it prevented Ile *N*-alkylation but also because it could be reduced under neutral reaction conditions that did not result in any cleavage of the labile *N*,*O*-acetal. Pd-catalyzed hydrogenation in the presence of the pentafluorophenyl ester of Mep followed by silyl ether deprotection gave the tripeptide product **14** in 67−78% yield for the two steps. Under these conditions, undesired cyclization of the amine intermediate upon the *N*,*O*-acetal functionality was not observed. Moreover, by coupling the L-enantiomer of Mep, we further demonstrated that **14** was not contaminated with the undesired diastereomer. Selective cleavage of the methyl ester without hydrolysis of the more reactive *N*,*O*-acetal was next accomplished by employing Me_3SnOH, which Nicolaou had demonstrated to be effective for the highly selective hydrolysis of methyl esters over more hindered ester derivatives.[15] Treatment of **14** with Me_3SnOH at 60 °C for 20 h resulted in <5% cleavage of the *N*,*O*-acetal, and the desired acid **15** was obtained in 67% yield.

Incorporation of the Tup fragment was accomplished by activation of acid **15** as the pentafluorophenyl ester followed by coupling with amine hydrochloride **4** to give **16** in 85% overall yield (Scheme 4). Acetylation of **16** proceeded in 82% yield to provide **1** that was identical by all spectroscopic methods with tubulysin D isolated from natural sources.

In conclusion, the total synthesis of tubulysin D was accomplished in 13% overall yield over 16 steps for the longest linear sequence and is the first synthesis reported for any member of the tubulysin family that incorporates the essential *N*,*O*-acetal. The synthetic route should not only allow access to all of the naturally occurring tubulysin derivatives and truncated analogues but also enable the synthesis of most of the stereoisomers of tubulysin D by appropriate selection of *tert*-butanesulfinamide stereochemistry

Scheme 4[a]



[a] Reagents and conditions: (a) pentafluorophenol, DIC, CH_2Cl_2; (b) **4**, *i*-Pr_2EtN, DMF; (c) acetic anhydride, pyridine, then H_2O/dioxane.

for the synthesis of Tup and Tuv and conditions for reducing **7** (Scheme 2).[11] The synthesis and biological activity of analogues will be reported in due course.

Acknowledgment. This work was supported by the NSF (CHE-0446173). H.M.P. gratefully acknowledges a graduate fellowship from Eli Lilly, and A.W.P. acknowledges an ACS Medicinal Chemistry Fellowship sponsored by BMS. We thank Dr. Frederick J. Hollander and Dr. Allen G. Oliver of the Berkeley CHEXray facility for solving the X-ray crystal structures for the determination of the absolute configurations of **4** and **9**.

Supporting Information Available: Complete experimental details and spectral data for all compounds described (PDF, CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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JA067177Z