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Synthesis of the Tubuvaline-Tubuphenylalanine (Tuv-Tup) Fragment of Tubulysin

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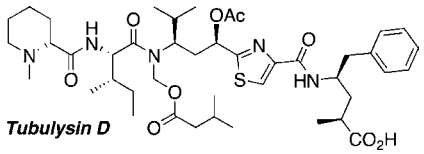
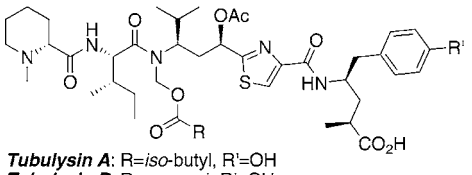
ABSTRACT



Advanced intermediates and analogues of tubulysins were prepared in a convergent strategy.

A new family of cytostatic peptides, the tubulysins, were isolated in 2000 by Sasse et al. from the myxobacterial strains *Archangium gephyra* and *Angiococcus disciformis* (Figure 1).[1] The tubulysin sequence is related to metabolites of the cyanobacterium *Lyngbya majuscula*, the dolastatins,[2] and hemiasterlin.[3] Dolastatin 10 and dolastatin 15 served as lead compounds for the development of the anticancer drug LU-103793, which has entered Phase II clinical investigations.[4] Tubulysins have also proven to be extraordinarily potent microtubule-perturbing agents. All tubulysins are highly active in mammalian cell cultures. Tubulysin D showed an average IC_{50} of 0.04 ng/mL in these growth inhibition assays, compared to 0.4 ng/mL for dolastatin 10.



Tubulysin A: R=*iso*-butyl, R'=OH
Tubulysin B: R=*n*-propyl, R'=OH
Tubulysin C: R=ethyl, R'=OH
Tubulysin D: R=*iso*-butyl, R'=H
Tubulysin E: R=*n*-propyl, R'=H
Tubulysin F: R=ethyl, R'=H

Dolastatin 10

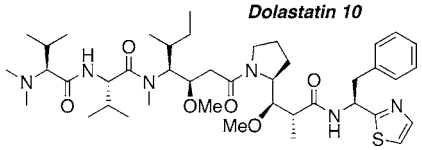


Figure 1. Structures of tubulysins and related peptide antimitotics.

Recently, Höfle's group reported the hydrolysis of tubulysin D by hydrochloric acid,[5] yielding 3-methylbutyric acid, formaldehyde, *N*-methyl-D-pipecolic acid, L-isoleucine, and the novel amino acids tubuvaline (Tuv) and tubuphenylalanine (Tup) (Figure 2).[6]

Although the tubulysins boast impressive biological activity, the lack of water-solubility of the natural products likely represents a major detriment in their development toward clinically useful anticancer agents.[7] Therefore, we intend to

[†] On sabbatical leave from Sankyo Agro Co., Ltd., Yasu, Shiga, Japan, 2002−2003.

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(6) For recent synthetic studies, see: Friestad, G. K.; Marie, J.-C.; Deveau, A. M. *Org. Lett.* **2004**, *6*, 3249.
(7) QikProp (v2.1, Schrödinger Inc.: New York, 2003) analysis of tubulysin D predicts a log *S* of −7.8 for aqueous solubility, an apparent Caco-2 permeability of 1.6 nm/s, and a total solvent-accessible surface area (SASA) of 1324 Å^2. These values are considerably outside the 95% range of drugs.

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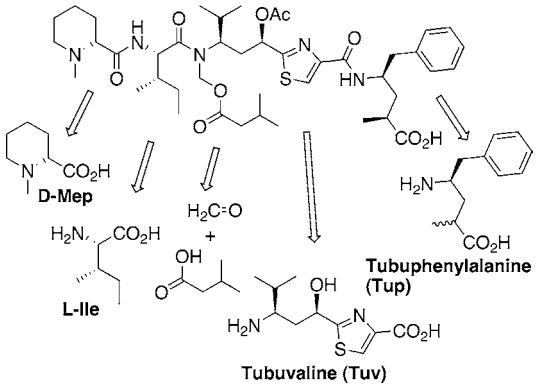


Figure 2. Acidic hydrolysis of tubulysin D.

use the natural product mainly as a lead structure for the design of a focused library of analogues with improved physicochemical properties.

On the basis of the precedence of the dolastatin congeners, we also anticipate that the replacement of the *N*-methyl pipecolate in tubulysins with an *N*,*N*-dimethylvaline group should provide compounds of comparable activity, further simplifying the construction of analogues. Our general synthetic strategy for tubulysin library synthesis is demonstrated by the preparation of oxazoline, thiazoline, oxazole, and thiazole derivatives shown in Figure 3. Segments **A** and

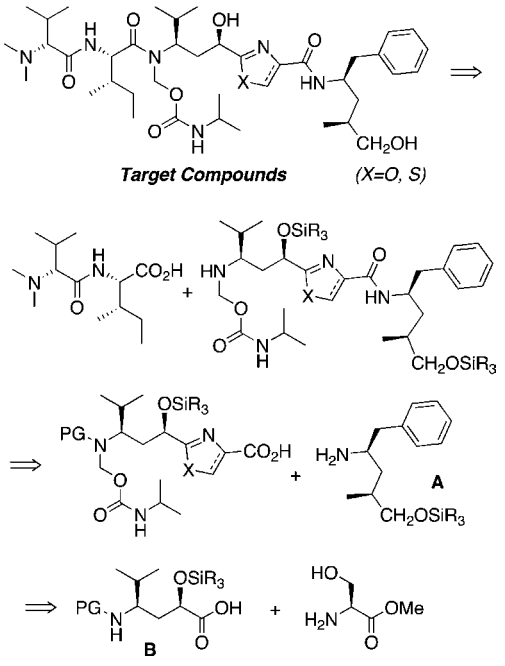
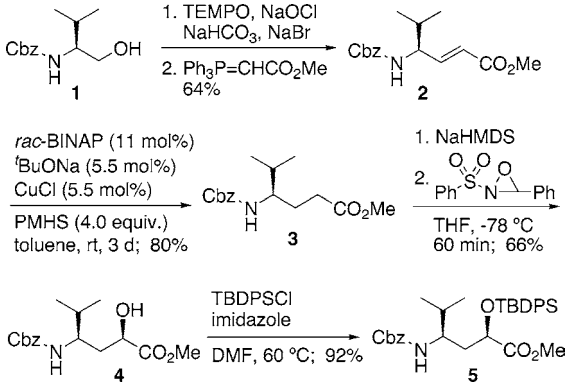


Figure 3. Retrosynthetic analysis for tubulysin D analogues.

B are prepared by diastereoselective hydroxylation or hydrogenation of the corresponding γ-amino acid derivatives.

We envisioned preparing the α-hydroxy-γ-amino acid derivative **4** by Davis oxidation[8] of γ-amino acid derivative

Scheme 1. α-Hydroxy-γ-amino Acid Synthesis



3 (Scheme 1). The α,β-unsaturated ester derivative **2** was readily obtained from *N*-Cbz-*(S)*-valinol (**1**) by TEMPO oxidation[9] followed by Wittig condensation. Enoate **2** was also prepared in moderate yield from *N*-Cbz-(*S*)-valine methyl ester by a one-pot reaction developed by the Knaus group.[10] Hydrogenation of **2** under various conditions, including 3% Pd/C in ethyl acetate,[11] did not afford the γ-amino acid derivative **3** in good yield but rather led to lactam formation. To achieve a selective reduction of the α,β-unsaturated ester without deprotecting the *N*-Cbz group, we employed the copper-catalyzed reduction conditions developed by the Buchwald group.[12] Commercially available *rac*-BINAP was used in place of the chiral ligand in our chemoselective reduction. In the presence of *rac*-BINAP, ^{t}BuONa, CuCl, and PHMS, the γ-amino acid derivative **3** was obtained in good yield (80%). This selective conjugate reduction represents a convenient method for the synthesis of various *N*-Cbz-protected γ-amino acids.

Our approach required the α-hydroxylation of **3**. The Hanessian group has reported a diastereoselective alkylation of similar γ-amino acid derivatives.[13] Presumably, a highly chelated dianionic species was involved in this conversion. Accordingly, we investigated analogous reaction conditions in the enolate hydroxylation. Treatment of **3** with NaHMDS in THF at −78 °C, followed by the achiral Davis reagent, gave α-hydroxy derivative **4**[14] as a single diastereomer in 66% yield. The use of KHMDS, LiHMDS, and LDA gave lower yields of diastereomerically pure **4** (37−50%). The corresponding *N*-Boc and *N*-trifluoroacetyl derivatives were also subjected to the hydroxylation reaction, but product yields remained low (30−50%). The diastereomeric purity of **4** was determined by ^{1}H and ^{13}C NMR analyses. Finally, the hydroxyl group was protected as the TBDPS ether **5** in good

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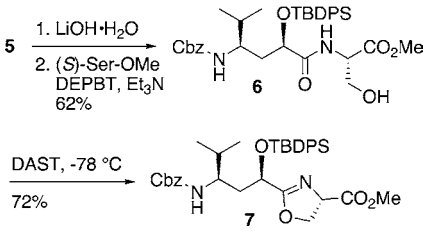
(13) Hanessian, S.; Schaum, R. *Tetrahedron Lett*. **1997**, *38*, 163.
(14) All spectral data were in good agreement with the reported data: Sutherland, A.; Willis, C. L. *J. Org. Chem*. **1998**, *63*, 7764.

yield by treatment with TBDPSCl and imidazole in DMF at 60 °C. Thus, the α-silyloxy-γ-amino acid methyl ester **5** was obtained in five steps and 31% yield from valinol **1**.

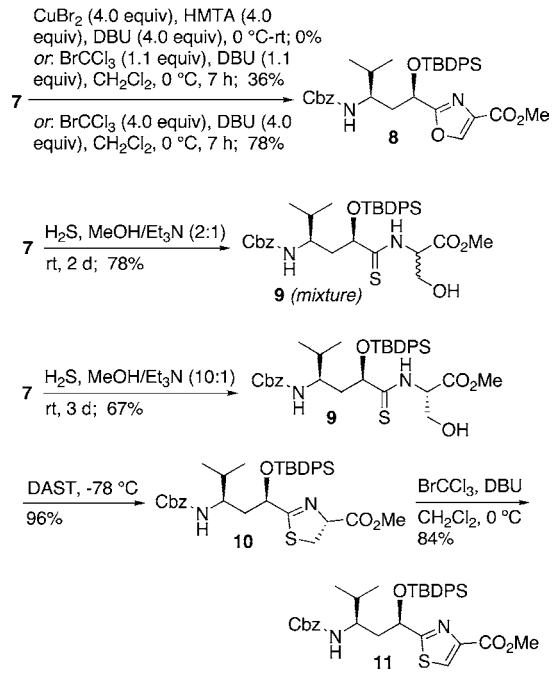
Saponification of **5** in 1 M LiOH in THF/H_2O (3:1)[15] and immediate coupling with (*S*)-serine methyl ester and Goodman reagent, DEPBT,[16] provided dipeptide **6** in 62% yield. The introduction of the oxazoline moiety was achieved by cyclodehydration with DAST[17] and led to the oxazoline derivative **7** in 72% yield (Scheme 2).

Scheme 2. Cyclodehydration of a Serine Residue Provides Oxazoline Precursor for Divergent Heterocycle Synthesis



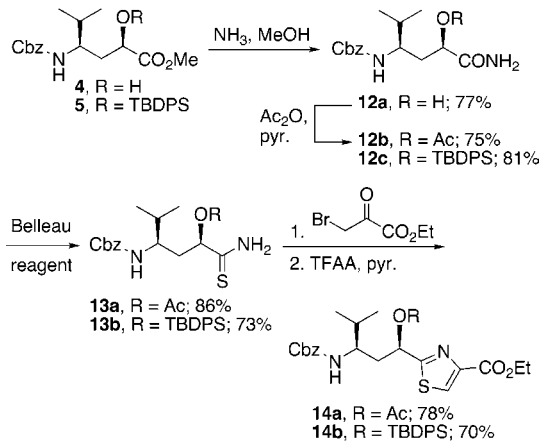
Alternatively, we explored the thiolysis of **7** to access sulfur-containing heterocyclic analogues. Exposure to saturated hydrogen sulfide solution in MeOH/Et_3N (2:1)[18] gave thioamide **9** in good yield but as a mixture of diastereomers. In contrast, thiolysis with H_2S in MeOH/Et_3N (10:1) produced **9** without epimerization after 3 d at room temperature. Cyclization of **9** with DAST at −78 °C provided thiazoline **10** in 96% yield. Subsequent dehydrogenation with $BrCCl_3$ and DBU[19] analogous to the synthesis of oxazole **8** gave thiazole **11**. Thus, four tubuvaline building blocks

Scheme 3. Conversion of Oxazoline **7** to Oxazole **8**, Thiazoline **10**, and Thiazole **11**



possessing oxazoline (**7**), oxazole (**8**), thiazoline (**10**), and thiazole (**11**) rings were readily accessible from the α-silyloxy-γ-amino acid methyl ester **5**. Although this cyclodehydration methodology was well-suited for the synthesis of tubuvaline analogues, we also sought a more direct method for the synthesis of authentic tubuvaline. Conversion of intermediates **4** and **5** to the terminal amides **12a** and **12c** proceeded smoothly with anhydrous NH_3 in MeOH (Scheme 4). Protection of **12a** as the acetate by treatment with acetic

Scheme 4. Modified Hantzsch Approach to Thiazoles **14a** and **14b**



anhydride and pyridine in CH_2Cl_2 led to **12b**, and treatment with Belleau reagent[20] generated thioamides **13a** and **13b**. No epimerization was detected by ^1H and ^{13}C NMR. With thioamides **13a** and **13b** in hand, conversion to thiazoles **14a** and **14b** was accomplished in 78% and 70% yield, respectively, by a modified Hantzsch protocol.[21] Compared to the iterative route shown in Scheme 3, this approach reduces the number of steps for the synthesis of thiazole **11** (i.e., the methyl ester of **14b**) from **5** from five to three and increases the overall yield from 24% to 41%.

For the preparation of the tubuphenylalanine building block **17**, *N*-Boc-(*S*)-phenylalaninol was oxidized to the aldehyde with catalytic TEMPO and chain-extended under Wittig conditions (Scheme 5). Several attempts to hydrogenate **15** diastereoselectively by Ru-BINAP catalysis[22] failed. Thus, saponification of **15**, hydrogenation over 10% Pd/C, and reduction of the mixed anhydride with $NaBH_4$

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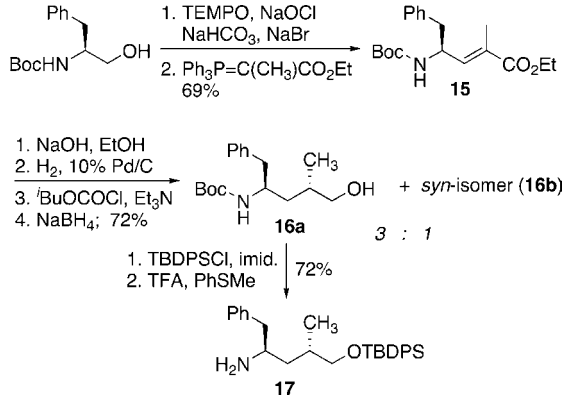
(22) Kitamura, M.; Tokunaga, M.; Noyori, R. *J. Org. Chem.* **1992**, *57*, 4053.

Scheme 5. Preparation of Tubuphenylalanine Segment

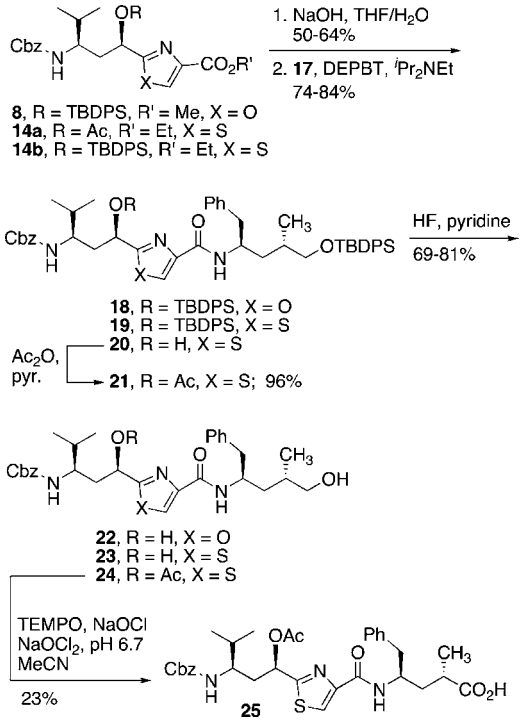


gave **16** as a mixture of diastereomers (*anti*:*syn* = 3:1). The spectral data for the minor isomer were in good agreement with an authentic sample of this isomer prepared by a known method.[13] After separation by chromatography on SiO_2, the *anti*-isomer **16a** was silylated and *N*-deprotected to give the primary amine **17**.

Completion of the synthesis of Tuv-Tup analogues **22** and **23** and the authentic Tuv-Tub subunit **25** of tubulysin D followed a parallel reaction scheme. Saponification and DEPBT coupling with **17** led to amides **18**−**20** (Scheme 6). The secondary alcohol in **20** was reacetylated in high yield. Cleavage of the silyl ethers with HF/pyridine led to the oxazole and thiazole building blocks **22** and **23**. Oxidation of thiazole acetate **24** with TEMPO and $NaOCl_2$/NaOCl[23] led to the *N*-protected Tuv-Tup segment **25**. This acid was isolated in 60% yield as a 9:1 mixture, and further separation by reverse-phase HPLC provided pure **25** in 23% yield.

In conclusion, we have developed a diastereoselective hydroxylation of γ-amino acid ester **3** with Davis reagent that provides access to interesting γ-amino-α-hydroxy acid derivatives.[24] Tubuvaline and Tuv-Tup segments possessing

Scheme 6. Segment Condensations



oxazoline (**7**), oxazole (**22**), thiazoline (**10**), and thiazole (**23**−**25**) rings were readily prepared from the key intermediates **5** and **17**. Further work toward *N*-terminal extended analogues of tubulysin D and biological evaluation of these derivatives is in progress.

Acknowledgment. This work has been supported by a grant from NIH (GM-55433).

Supporting Information Available: Experimental procedures and spectral data for all new compounds, including copies of ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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