the helix was dissolved in sodium acetate buffer (10 mM, pH 4) and added dropwise to a solution of the template in acetate buffer (pH 7.5). The condensation reaction was complete after 18 h, as monitored by analytical HPLC.

In the next step, the serine residue in the ε position of the lysine residue of the template was oxidized to the aldehyde (fivefold excess of $NaIO_4$, 5 min), and the peptide purified by RP-HPLC (56%). Subsequently, the Fmoc protecting group of the aminooxy group at the C-terminal lysine residue of the helix was removed with 20% piperidine in DMF. After precipitation with diethyl ether, the crude peptide was dissolved in 10 mM acetate buffer (pH 4); this resulted in immediate formation of the second oxime bond (>90%). The LIF1 was purified by RP-HPLC and characterized by ESI-MS (m/z: 3788).

Optical absorption spectra of the Co^{II} peptide complex were recorded on a Beckmann spectrophotometer. Prior to complex formation, the internal disulfide bond of LIF1 was reduced with 250 mM dithiothreitol (DTT) at room temperature for 10 h. After lyophilization, LIF1 (150 μM) was suspended in Tris·HCl buffer (pH 7; degassed with helium) and 150 μM $CoCl_2$ added. The addition of an equimolar amount of $ZnCl_2$ to this solution readily displaces Co^{II} from the complex.

CD spectra were recorded on a Jobin Yvon Marck VI circular dichrometer in quartz cells (path length 0.1 cm). Prior to recording the spectra the internal disulfide bond in LIF1 was reduced as described above, LIF1 was taken up in Tris·HCl, and spectra were recorded before and after addition of equimolar amounts of $ZnCl_2$.

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Studies in the Total Synthesis of Himastatin: A Revision of the Stereochemical Assignment**

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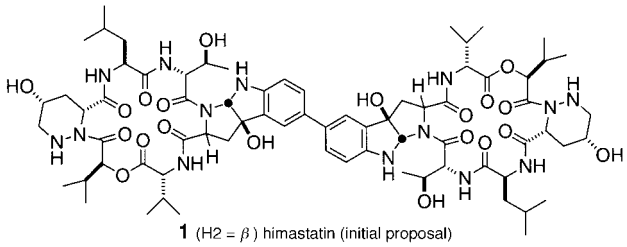
Dedicated to Professor E. J. Corey

The quest for new antibiotic and antitumor agents prompted scientists at Bristol Myers Squibb to investigate an actinomycete strain (ATCC 53653) from the state of Himachal Pradesh in India. In doing so, they encountered a new compound of formula $C_{72}H_{104}N_{14}O_2$, which they named himastatin.[1] After extensive optimization with the strain, himastatin could be obtained in scales adequate for sustaining chemical and biological investigation. While himastatin has not been developed to the point of clinical trials, its activity against gram-positive microorganisms and a variety of tumor probe systems is impressive. Based on spectroscopic investigations augmented by modest degradative studies, **1** was



1 (H2 = β) himastatin (initial proposal)
20 (H2 = α) himastatin (revised proposal)

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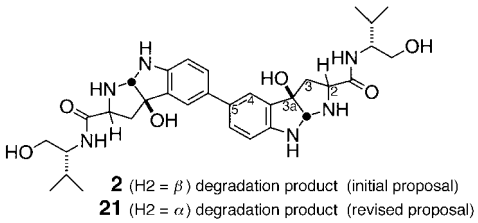
advanced as the structure of himastatin. A cleavage product of himastatin retaining only a D-valinol moiety from the peptidal domain was accordingly formulated as **2**.

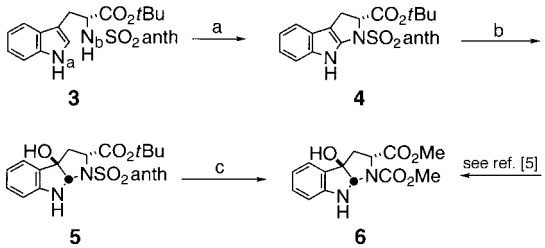


2 (H2 = β) degradation product (initial proposal)
21 (H2 = α) degradation product (revised proposal)

Our interest in himastatin first arose from the intrinsic challenge it posed to chemical synthesis. Solutions to problems of this scope often carry with them learning opportunities which transcend the confines of the particular problem under inquiry. Moreover, we had a focusing biological goal in mind. We hoped to evaluate representative "monomers" (see compound **17**) to ascertain whether the "dimeric" motif is constructed by the microorganism in response to an identifiable biological advantage.

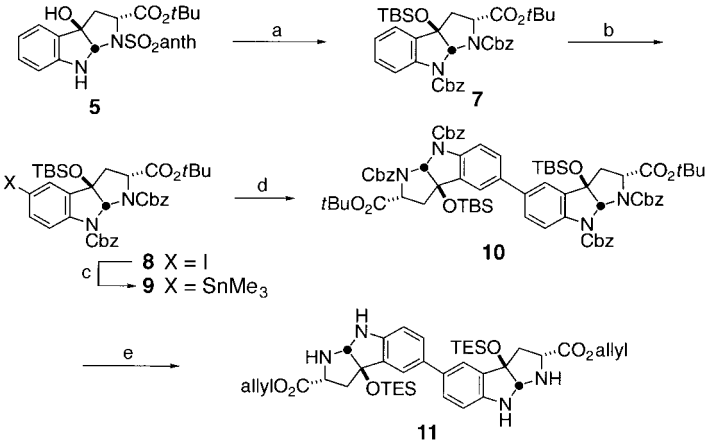
One of the many provocative structural features of **1** is the 2,3,3a,8a-hexahydropyrrolo[2,3-*b*]indole moiety bearing a potentially eliminable hydroxyl group in position 3a. Even if attention is confined to the system bearing a *cis* fusion of the five-membered rings, two possibilities for the relationship of the tryptophan carboxy equivalent at C2 to the junction present themselves. Given the structure **1** proposed for himastatin, we focused on the subtype with an *anti* relationship of this carboxy center.

The possibility, in principle, of oxidative cycloaromatization of various tryptophan derivatives (see compound **4**) had been known since the pioneering work of Witkop et al.[2] For the purpose of our synthesis, the use of an N_b-anthracenosulfonyl protecting group on the *tert*-butyl ester of tryptophan (**3**, Scheme 1)[3] proved to be of immense advantage.[4] Conversion of **3** into **4** was readily achieved, as was the conversion of **4** into **5**. For purposes of additional verification, the transformation of **5** to **6** was also accomplished. The latter correlates with a compound previously assigned to be in the *anti-cis* series.[5]



Scheme 1. Synthesis of **6**. a) NBS, TEA, CH_2Cl_2, 80%; b) 1. DMDO, CH_2Cl_2, $-78\,^{\circ}C$; 2. $NaBH_4$, MeOH, 75% (2 steps); c) 1. TFA; 2. CH_2N_2; 3. Al(Hg); 4. $ClCO_2Me$, pyridine, 50% (4 steps). anth = anthracen-9-yl, DMDO = 3,3-dimethyldioxirane, NBS = *N*-bromosuccinimide.

The use of a Stille coupling[6] was envisioned for joining the two indolyl moieties through a carbon–carbon bond. In practice compound **5** lent itself to conversion into **7** through a three-step sequence (Scheme 2). With the particular set of



Scheme 2. Synthesis of **11**. a) 1. Al(Hg), THF; 2. CbzCl, pyridine, CH_2Cl_2, 80%; 3. TBSCl, DBU, CH_3CN, $50\,^{\circ}C$, 90%; b) ICl, CH_2Cl_2, 2,6-di-*tert*-butylpyridine, 90%; c) Sn_2Me_6, THF, [$Pd(PPh_3)_4$], $60\,^{\circ}C$, 85%; d) [Pd_2dba_3], $AsPh_3$, **8**, $45\,^{\circ}C$, DMF, 50–70%; e) 1. TBAF; 2. TESCl, TEA; 3. H_2, Pd/C, EtOAc; 4. FMOC-HOSu, pyridine; 5. TESOTf, 2,6-lutidine; 6. allyl alcohol, EDCI, DMAP; 7. piperidine, CH_3CN, 30% (7 steps). Cbz = benzyloxycarbonyl, dba = 1,5-dibenzylideneacetone, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, EDCI = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, FMOC = (9*H*-fluoren-9-ylmethoxy)carbonyl, HOSu = *N*-hydroxysuccinimide, TBAF = tetrabutylammonium fluoride, TBS = *tert*-butyldimethylsilyl, TES = triethylsilyl, Tf = trifluoromethylsulfonyl.

protecting groups chosen, iodination at C_5 of the indoline occurred with ICl (→**8**). The stannyl indole **9** was prepared from **8**. Palladium-mediated coupling of **8** and **9** gave rise to **10**. Following extensive trial and error probes, it was found that the arrangement of protecting groups in **11**, which was synthesized as shown, would allow us to advance toward **1** with viable prospects for eventual deprotection. We were attracted to the possibility of twofold leveraging of the subsequent interpolation by conducting it simultaneously in both sectors of the dimer.

In a parallel series of investigations, the ester tripeptide **12**, bearing a free carboxyl group in the terminal D-threonine residue, was synthesized (Scheme 3).[7] Coupling of **11** with **12** under the conditions shown gave rise to **13** after reprotection.[8] Cleavage of the carboxyl and amino protecting groups led to **14**, the substrate for the critical macroloactamization. Cyclization smoothly led to **15**, which on deprotection afforded fully synthetic **1**.

At this point it was clear that the ^1H NMR spectrum of **1** synthesized in our laboratory did not correspond with that recorded for naturally derived himastatin.[1c] Particularly striking were the two upfield signals at $\delta = 0.55$ and 0.35 for **1** corresponding to the valine isopropyl group. No such upfield resonances had been observed in the ^1H NMR spectrum of himastatin.

The strategy used to reach dimer **1** was applied to synthesize monomeric **17** (Scheme 4). For this goal, we utilized **16**, which is readily derived from **5**, and the previously described **12**. The synthesis proceeded in much the same manner as for **1**. Once again, the ^1H NMR spectrum of **17** contained upfield peaks at $\delta = 0.55$ and 0.35 having no counterpart in that of himastatin.[9] That this discrepancy was rooted in the pyrrolindoline sector of the molecule was shown when we obtained compounds **18** and **19**. The recorded chemical shifts of the

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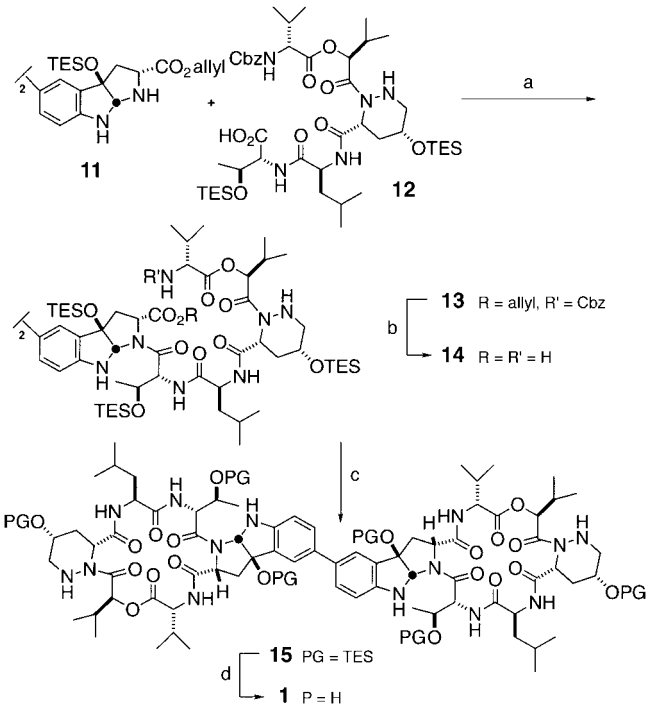
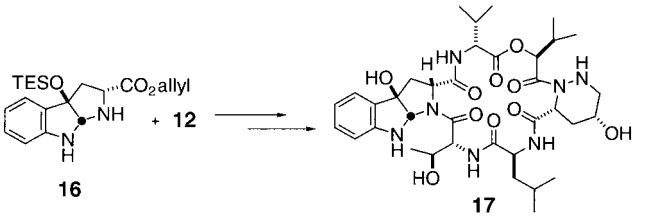
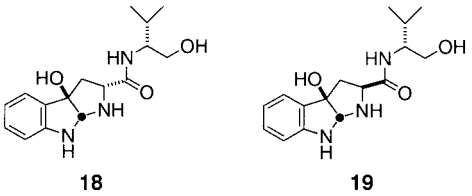


Scheme 3. Synthesis of "*epi-himastatin*" (**1**). a) 1. HATU, HOAt, collidine, CH_2Cl_2, $-10\,^{\circ}C \rightarrow RT$, 65%; 2. TESOTf, 2,6-lutidine, CH_2Cl_2, 63%; b) 1. $[Pd(PPh_3)_4]$, $PhSiH_3$, THF; 2. H_2, Pd/C, EtOAc, 40%; c) HOAt, HATU, $i Pr_2NEt$, DMF; d) TBAF, THF, HOAc, 25% (from **14**). HATU = *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethaneaminium hexafluorophosphate, HOAt = 1-hydroxy-7-aza-benzotriazole.



Scheme 4. Synthesis of the "*epi-himastatin*" monomer (**17**).

isopropyl methyl groups of the synthetic *syn-cis* monomer **19** ($\delta = 0.92$ and 0.88)[7] were in much closer harmony with the data ($\delta = 0.94$ and 0.89)[1c] of the degradation product of himastatin (hitherto formulated as **2**) than were those of the synthetic *anti-cis* structure **18** ($\delta = 0.78$ and 0.62).



Therefore, we postulated that the relationship of the *cis* junction and the C_2-carboxamido group in naturally occurring himastatin is *syn* rather than *anti*, as previously proposed. Given the chiroptical data accumulated in the Bristol Myers Squibbs investigation,[1c] we further favored a revision from a D-tryptophan matrix to the L-tryptophan series. Thus, the absolute stereochemistry of the pyrrolindoline junction remains unchanged from the previous assignment. However, the stereochemical assignment of the tricyclic carboxamido centers are reversed (*S*,*S* rather than *R*,*R*). The structure of himastatin is thus assigned as **20**, and that of its valinol degradation product as **21**. In the newly proposed structure of himastatin, the components in the depsipeptide domain are presented in alternating D and L configurations.[10] These various proposals were validated when a total synthesis of himastatin was realized.[11]

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[3] Prepared from D-tryptophan: 1) $anthSO_2Cl$, TEA, THF, H_2O; 2) *N*,*N*′-diisopropyl-*O*-*tert*-butylisourea, CH_2Cl_2 (65% yield overall).

[4] a) The anthracene sulfonamide was readily cleaved with Al(Hg) at room temperature. b) The use of the *tert*-butyl ester afforded a much higher *anti*:*syn* stereoselectivity (>15:1) than the corresponding methyl ester (3:1).

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[7] Compound **12** was synthesized similarly to **20**, which is described in ref. [11].

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[9] The ^1H NMR spectra of synthetic **1** and **17** are virtually identical upfield of 6.0 ppm.

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Total Synthesis of Himastatin: Confirmation of the Revised Stereostructure**

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Dedicated to Professor E. J. Corey

In the preceding communication[1] we have provided the background of the himastatin problem and the findings that necessitated a revision in the assignment of the configuration

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