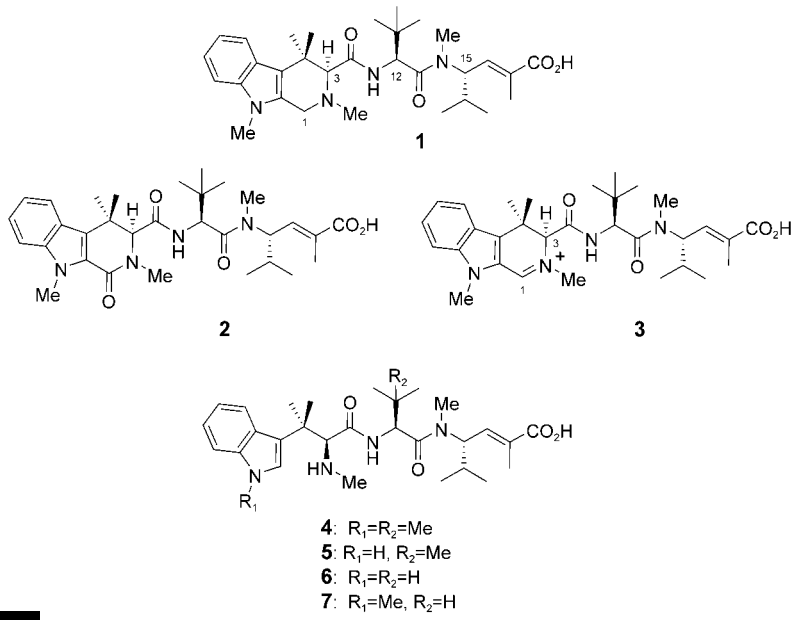

Figure 1. Milnamides A, C, and D (**1**–**3**), hemiasterlin (**4**), and hemiasterlins A–C (**5**–**7**). Numbering is taken from Reference [1a].

Natural Product Synthesis

Enantioselective Total Synthesis of (+)-Milnamide A and Evidence of Its Autoxidation to (+)-Milnamide D**

*Chaomin Liu, Makoto N. Masuno, John B. MacMillan, and Tadeusz F. Molinski**

Milnamides A,[1a] C,[1b] and D[1c] (**1**–**3**), (−)-hemiasterlin (**4**),[2a–d] and hemiasterlins A–C (**5**–**7**)[2b,d] (Figure 1) comprise a small family of exceptionally cytotoxic tripeptides that were isolated from marine sponges *Auletta* sp., *Hemiasterella minor, Cymbastella* sp. and *Siphonochalina* sp. Compounds **1**–**7** are powerful antimitotics that disrupt microtubule assembly during cell division and inhibit growth of cultured tumor cells (e.g. **4**, $IC_{50} < 1$ nM, MCF-7 breast tumor cells).[4] Hemiasterlin is a more potent cytotoxin in vitro than paclitaxel (Taxol) and is comparable to dolastatin 10.[2c] As a consequence of this exceptional activity, a synthetic analogue of **4**, SPA110, has entered advanced phase I clinical trials for the treatment of solid tumors.[4] X-ray crystallographic analyses confirmed the relative stereochemistries of **2** and (−)-**4**, and by the total synthesis of (−)-**4**, the *S* configurations at each α-amino acid residue were verified.[3,4] Very recently, the X-ray crystal structure of **2** was reported,[1c] however, the absolute stereochemical configurations of **1**–**3** were not defined. Comparison of the ^{1}H NMR spectroscopic data of **1**–**3** suggests that the differences from (−)-**4** are located solely within the region of the heterocyclic amino acid. Because the highly methylated β-carboline amino acid **8**, which is found in **1**–**3**, has no precedent in other natural products, the preparation of both 3*R* and 3*S* (β-carboline numbering) stereoisomers of **1** would inform stereochemical determinations of the milnamide family. Herein, we report the first enantioselective total syntheses of (3*S*)-**1** and (3*R*)-**1** that employs an expedient synthesis of the requisite **8** through an oxazoline–dihydrooxazinone rearrangement. The synthesis confirms the absolute configuration of natural (+)-**1** as (3*S*,12*S*,15*S*) and demonstrates the first use of the aforementioned rearrangement in natural peptide synthesis. Furthermore, we show that **1** undergoes facile autoxidation to milnamide D (**3**) which thus correlates the absolute configurations of both natural product peptides and suggests a nonbiogenic origin for the iminium salt **3**.

Retrosynthetic analysis of **1** (Scheme 1) suggests disconnections to a pivotal intermediate, namely the β-carboline amino acid (**8**), and the known dipeptide **9**.[3a] The precursor **10** to the heterocyclic amino acid arises from aromatic electrophilic substitution of *N*-methylindole (**11**) by an epoxide **12**, which itself is obtained by a Darzen's-type condensation of (4*R*)-2-chloromethyl-4-phenyloxazoline and acetone.

The synthesis of **1** is outlined in Scheme 2. The pivotal step relies upon our SeO_2-promoted oxidative rearrangement of 2-alkyl- or 2-(arylmethyl)oxazolines to 3,5-disubstituted dihydro-2*H*-oxazinones.[5] The required oxazinone **10** was synthesized in two steps as follows. *N*-methylindole and the epoxide **12**[6] were coupled under Lewis acid conditions ($SnCl_4$, CH_2Cl_2, −78 °C, 56 %)[7] to provide the requisite oxazoline

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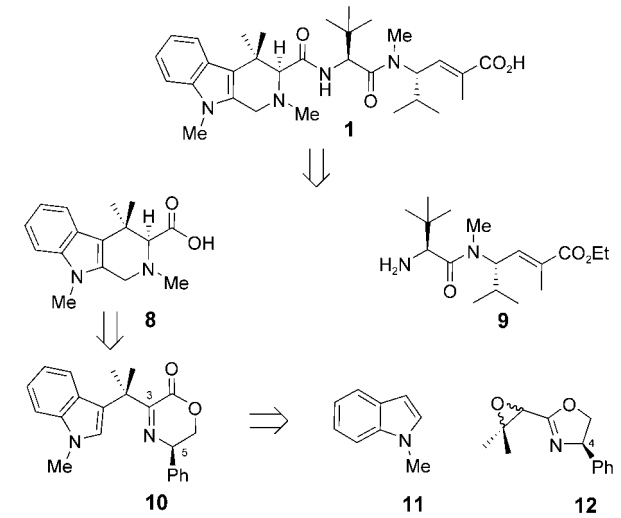
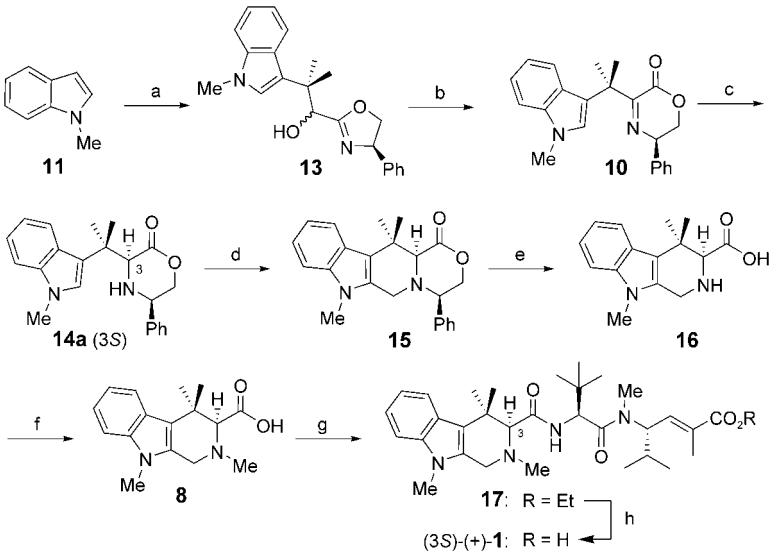


Scheme 1. Retrosynthetic analysis of **1**.



Scheme 2. Synthesis of (+)-milnamide A (**1**): a) **12**,[6] $SnCl_4$, CH_2Cl_2, −78 °C (56 %); b) SeO_2, $CHCl_3$, reflux (90 %); c) PtO_2, H_2, MeOH, room temperature (90 %); d) paraformaldehyde, $MgSO_4$, benzene, 75 °C (100 %); e) $Pd(OH)_2/C$, H_2, 4 atm, TFA, H_2O room temperature (89 %); f) paraformaldehyde, Pd/C (10 %), H_2, MeOH, 50 °C (78 %); g) **9**,[3a] pivaloyl chloride, iPr_2EtN, THF, 0 °C (10 %); h) LiOH, MeOH, H_2O, degassed, room temperature (92 %). TFA = trifluoroacetic acid.

13 as a mixture of epimers.[8] Attempted oxidative rearrangement of **13** to the required 5,6-dihydro-2*H*-1,4-oxazin-3-one **10** with SeO_2 under standard conditions (dioxane, reflux) was disappointing (≤ 23 % yield), probably owing to decomposition of the indole ring.[9] A survey of varying conditions (Table 1) identified that SeO_2 in $CHCl_3$ or ethyl acetate at reflux provided rapid reactions and the highest yields. Accordingly, oxazoline **13** was smoothly converted (SeO_2, $CHCl_3$, reflux) into **10** in 90 % yield.

Control of the correct configuration at the C3 center in **8** was anticipated from the diastereofacial hydrogenation of the C=N bond directed by the Ph substituent at the C5 center of the dihydrooxazinone **10**. The synthesis of either enantiomer of **8** is possible by the choice of the configuration of the

Table 1: Oxidative rearrangement of **13** to **10** with SeO_2 (2.6 equiv).

Entry	Solvent	*T* [°C]	*t* [h]	Yield of **13** [%]
1	dioxane	100	2	–[a]
2	dioxane	30	72	87
3	dioxane	40	72	23
4	CH_2Cl_2	40	72	53
5	EtOAc	77	2[b]	90
6	$CHCl_3$	61	4	90

[a] Trace. [b] Stirred at 24 °C for an additional 14 h.

phenylglycinol that is used in the preparation of the oxazoline **12**. Thus, reduction of (5*R*)-**10** or (5*S*)-**10** would provide (3*S*)-**1** or (3*R*)-**1**, respectively. After experimentation under different conditions (Table 2), we found that **10** underwent hydrogenation (PtO_2, H_2, 4 atm, MeOH, RT, 90 %) to give **14** with excellent diastereoselectivity (≈ 70:1). The major isomer, **14a**, which was separated from the mixture by column chromatography (silica gel), was shown to have the expected *cis* configuration by NOE studies; irradiation of the H3 center ($CDCl_3$, $\delta = 4.39$, s) resulted in an NOE enhancement of the benzylic proton H5 (oxazinone numbering). Condensation of **14a** with $H_2C{=}O$ under optimized Pictet–Spengler[10] conditions (paraformaldehyde, $MgSO_4$, benzene, 75 °C) gave **15** in quantitative yield. Removal of the chiral auxiliary from **15** by hydrogenolysis–hydrolysis ($Pd(OH)_2/C$, H_2, 4 atm, TFA/H_2O, 89 %),[11] followed by reductive methylation (paraformaldehyde, Pd/C, H_2, MeOH, 50 °C, 78 %)[12] provided the key amino acid **8**.

The amide coupling of **8** and **9**[3a] was far more troublesome than expected. Attempted amide bond formation with a variety of coupling reagents (dicyclohexyl carbodiimide (DCC), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) hydrochloride (EDC), *O*-(7-azabenzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU), bromotripyrrolidinophosphonium hexafluorophosphate (PyBrOP)) all failed to produce the expected tripeptide. Clearly, the highly hindered molecule **8** precluded the formation of the amide bond with the sterically crowded *tert*-leucyl amino terminus of **8** under conditions that were similar to those employed by Andersen et al in the synthesis of **4** from the less hindered (2*S*)-*N*-Boc-*N*,*N*′*C*,*C*-tetramethyltryptophan (52 %).[3a] Eventually, amide coupling was possible under mixed anhydride conditions (pivaloyl chloride, iPr_2EtN, THF, 0 °C, 10 %) to give the ethyl ester **17**.[13] Saponification of **17** (LiOH, MeOH, H_2O, degassed, N_2,

Table 2: Hydrogenation of **10** in the presence of PtO_2 (20 mol %).

Entry	Solvent	P_{H_2} [atm]	*t* [h]	d.r.[b] (**14a**:**14b**)	Yield of **14a**,**b** [%]
1	CH_2Cl_2	2	48	17:1	90
2	EtOAc	4	52	39:1	96
3	CH_3OH	4	72	72:1	90[a]

[a] Isolated yield of **14a**. [b] From integration of ^1H NMR spectra.

RT, 92%) completed the synthesis of (3*S*)-**1**. Its counterpart (3*R*)-**1**[14] was synthesized by the same route starting with the (4*S*)-oxazoline **12**. Synthetic (3*S*)-**1** was identical to natural (+)-**1** from ^{1}H NMR and circular dichroism (CD; see Figure 2) spectroscopic analysis, ESI-MS measurements, and LC–MS retention times.[15] However, the CD spectrum of epi-milnamide A ((3*R*)-**1**) differed significantly from that of natural (+)-**1**.

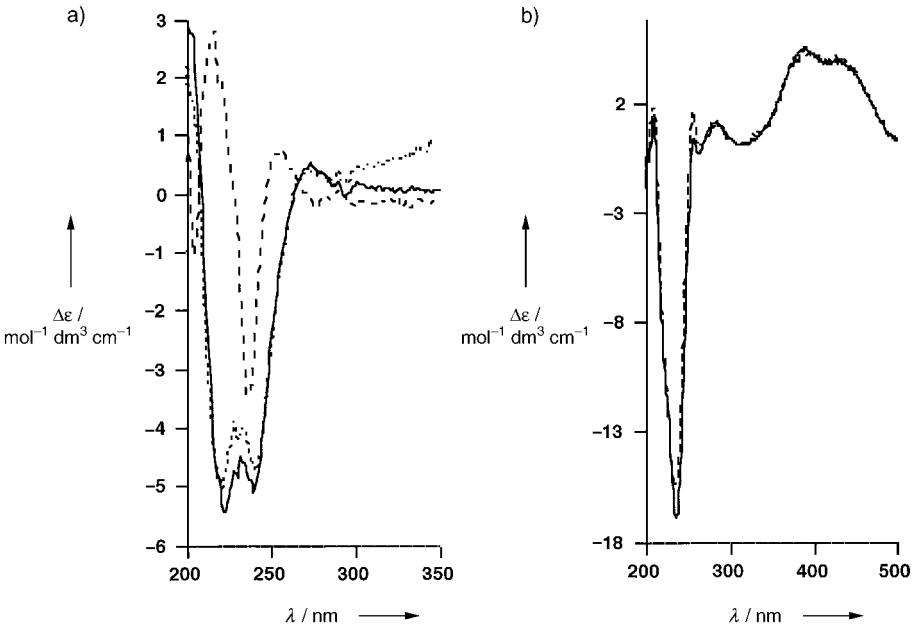


Figure 2. CD spectra of milnamides: a) synthetic milnamide A [(3*S*)-**1**] (——), synthetic epi-milnamide A [(3*R*)-**1**] (– – –), and natural milnamide A [(3*S*)-**1**] (·····); b) synthetic milnamide D [(3*S*)-**3**] (– – –) and natural (+)-milnamide D [(3*S*)-**3**] (——).

Over time, **1** slowly autoxidized to **3** upon standing in solvent. Both (3*S*)-**1** and (3*R*)-**1** were oxidized at similar rates, but the apparent rate of autoxidation was greatly accelerated when samples were stored in aged $CDCl_3$ or $CHCl_3$.[16] As the analyses of this autoxidation product **3** were identical to those of natural milnamide D by CD (Figure 2) and ^{1}H NMR spectroscopy and LCMS, we conclude that the latter also has the 3*S* configuration. Given the ease of this oxidation, it is plausible that **3**, which is obtained from natural sources, originates from the autoxidation of **1** during its isolation–purification process, although we cannot exclude **3** as a "true" natural product or an intermediate precursor in the biosynthesis of **1**.

The key feature of this synthesis of (+)-milnamide A (**1**) is the high-yielding preparation of the highly methylated β-carboline amino acid **8**, which is made possible through the facile oxidative rearrangement of oxazoline **12** to the corresponding substituted dihydrooxazinone. This rearrangement reaction was exploited for the first time in natural product synthesis for the preparation of amino acid **8** and should find application in the synthesis of other marine-derived peptides that containing rare *tert*-alkyl amino acids.[17]

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Keywords: carboline · enantioselectivity · natural products · peptides · total synthesis

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[8] All new compounds gave satisfactory HRMS and ^{1}H and ^{13}C NMR spectroscopic data. The resulting 1:1 epimeric mixture of **13** was inconsequential as both epimers were converted into **10** in the subsequent reaction.

[9] We assume from the slower rate of the SeO_2 oxidation reaction under the original conditions (reference [5]; anhydrous 1,4-dioxane, reflux) that oxidative degradation of the indole ring is competitive with the oxazoline→oxazinone rearrangement.

[10] Addition of stoichiometric or catalytic quantities of Brønsted acid (*p*-toluenesulfonic acid or CF_3COOH) to the reaction led to decomposition of the substrate.

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[13] Ester **17** was purified by HPLC (C_{18} column; gradient, CH_3CN/H_2O with HCOOH (0.01%)). A by-product, *N*-pivaloyl-**9**, which was observed during the coupling reaction, attests to the presence of the highly congested carboxy group in the mixed pivalic anhydride of **8**.

[14] Significant differences between the (3*R*)-**1** epimer and (3*S*)-(+)-**1** were seen in both the ^{1}H NMR and the CD spectra (see Experimental and Supporting Information).

[15] CD, UV, LCMS, and ^{1}H NMR spectroscopic data for synthetic (3*S*)-**1** and natural (+)-**1** were identical. The HPLC retention time (co-injection) were also identical.
[16] The ethyl ester, **17**, was also sensitive to autoxidation. Handling of NMR samples of **1** in 100% $CDCl_3$ from freshly opened ampoules did not induce autoxidation. Traces of chlorine or phosgene, which are present in aged $CHCl_3$, are possible initiators of the autoxidation of **1**. The iminium salt **3** is easily reduced back to **1** ($NaBH_4$, MeOH, 100%).
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