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## Total Synthesis of Nominal Cyclocinamide B and Investigation into the Identity of the Cyclocinamides

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### Abstract

The total synthesis of nominal cyclocinamide B, a cyclic peptide marine natural product, is reported together with an isomer of nominal cyclocinamide A. Initial attempts at the synthesis of the title compounds by inclusion of a turn inducer failed. However, direct synthesis succeeded in formation of the 14-membered cyclic peptide structure. Comparison of the data from all synthetic cyclocinamide A and B compounds with those of the natural products leads to the conclusion that the two natural products possess the same relative stereochemistry and that the true structures have not been defined.

### Keywords

cyclic peptide;  $\beta$ -amino acids; peptide synthesis; natural product synthesis

Macrocycles, defined as containing a ring of at least 12 atoms, make up about 3% of the more than 100,000 known natural product secondary metabolites.<sup>1</sup> Many of these compounds enjoy potent biological activity derived from built-in structural pre-organization that may or may not possess absolute rigidity. Yet few macrocyclic compounds have been developed into pharmaceutical agents, perhaps due to the larger molecular weight and increased heteroatom presence that constitute deviations from the ‘rule of five’.<sup>2</sup> Cyclic peptides exist as a subset of natural macrocycles. The most celebrated cyclic peptide may well be the immune repressor cyclosporine, an undecapeptide that presents multiple hydrogen bonding units yet is nonetheless membrane permeable and highly biologically active.

Nearly two decades have passed since Crews and coworkers reported the 2D and partial 3D structure of cyclocinamide A (**1a**, Fig. 1) and claimed solid tumor activity for the unusual

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**Supporting Information:** General experimental, tables of <sup>1</sup>H and <sup>13</sup>C NMR data for synthetic cyclocinamide A, cyclocinamide B, **1a**, **1b**, **1c** and **2** (Tables S1-S5), graphical representation of NMR data comparisons (Figures S1 and S2) together with copies of <sup>1</sup>H and <sup>13</sup>C NMR spectra for all new compounds.

14-membered ring hexapeptide structure.<sup>3</sup> Some years later cyclocinamide B (**2**) was reported<sup>4</sup> with an almost identical 2D structure but alternate absolute stereochemistry to **1a**. A proposal for the complete structure of cyclocinamide A appeared in 2008<sup>5</sup> with the assignment of the absolute stereochemistry of the two  $\beta$ -amino acid residues. Synthetic work to define the absolute structure of cyclocinamide A (**1a**) has appeared<sup>6,7</sup> but, to date, the identity of this cyclic peptide has not been verified. No synthetic work has been reported for **2**.

Recently we completed the total synthesis of nominal cyclocinamide A **1a** and its 11*R* isomer **1b**.<sup>8</sup> Neither compound provided data supporting identity with the natural product. Given these results, the preparation of nominal cyclocinamide B **2** was undertaken. Herein we present our synthetic work, which also resulted in the production of the enantiomer of an additional stereoisomer of cyclocinamide A (*ent*-**1c**), together with a full examination of all data from our synthetic efforts on these 14-membered cyclic peptides.

Our published synthesis of nominal and 11*R*-cyclocinamide A arose from three dipeptide units, and the parallel effort toward **2** required residues **3-5** (Scheme 1). Key to success in our previous synthesis was the use of a (cyclo)Asn derivative similar to **3** as a turn inducer, allowing the macrocyclization event of the all *S* and 11*R* isomers at the C1-N2 bond to proceed in good yield. The synthesis of **3** arises from *R*-asparagine and *S*-diaminopropionic acid, and closely follows the published procedure (eq. 1).<sup>8</sup> Similarly, fragment **4** arises from *R*-5-bromotryptophan and *S*-isoserine and is obtained through standard amide bond formation to afford **6**, followed by ester hydrolysis (eq. 2). Finally, achiral **5** is prepared from *N*-methylpyrrole and glycine methyl ester (eq. 3).

Unfortunately, a fateful decision was made to employ pivalaldehyde for preparation of **3** rather than the aromatic aldehyde used successfully in the synthesis of **1a** and **1b**. Both the aromatic and aliphatic aldehyde-derived heterocycles had seen extensive utilization in early (cyclo)Asn studies in this laboratory with equivalent success.<sup>9-11</sup> However, in the present case synthetic intermediates containing dipeptide **3** proved almost uniformly unstable to storage and handling. For example, linear hexapeptide **9** was prepared by removing the –Troc protection on **3** to afford **7** and appending the glycine-pyrrole side chain **5** to give **8** (Scheme 2). After –Fmoc removal, coupling to **4** afforded the desired branched hexapeptide **9**, with only the final cyclization and protection group removal remaining. However, compound **9** proved highly unstable and did not succumb to cyclization.

Recognition that the core ring of **2** is enantiomeric with **1c**, which was previously synthesized by Grieco via a linear tetrapeptide that did not employ a turn inducer,<sup>6</sup> led to the synthesis of both nominal cyclocinamide B **2** and *ent*-**1c** outlined in Scheme 3, adapted from Grieco's approach. Nitrogen protection was removed from **6** under standard conditions to afford free amine **10**, which was coupled to *R*-Fmoc-Asn(Tr)-OH to afford **11** in good yield, provided the reaction time was kept short. Overnight coupling of **10** with the asparagine residue resulted in formation of a compound analogous to **11** but bearing a nitrile in place of the trityl-protected asparagine side chain amide. Although the formation of a nitrile from the side chain amide functionality of an asparagine residue is well precedented,<sup>12</sup> we are unaware of any former instance of a similar one-pot deprotection/dehydration

transformation. Tripeptide **11** was *N*-deprotected and coupled with diaminopropionic acid derivative **12** over 48h to give linear tetrapeptide **13** in 52% yield for the two steps. As opposed to the previous coupling, no nitrile formation occurred over the 2 day reaction nor was any nitrile detected following any of the other coupling reactions in the sequence, some of which extended to 4 days. Removal of the –Fmoc and methyl ester protection groups proceeds only in this order; no product is isolated if the ester is deprotected first. Furthermore, production of the cyclization precursor was accompanied with some undesired loss of isoserine hydroxyl protection. Fortunately, the cyclization precursor was quite stable, in stark contrast to compound **9** in Scheme 2.

Ring formation, which again was accompanied by minor adventitious loss of C4 protection, afforded a combined 79% yield of cyclic material after extensive experimentation to define DEPBT<sup>13</sup> as the optimal coupling reagent. Subsequent to separation, C4 protected material **14** was treated with dilute TFA/Et<sub>3</sub>SiH to remove the –Boc group. Coupling with dipeptide fragment **5** was sluggish, requiring 4 days to provide a mixture of **15** and **16**. Curiously, the formerly labile –OTBDPS group became robust upon macrocycle formation; **15** required two steps (TFA, then NaOH) for complete deprotection to desired final product **2**. Compound **16** required only base treatment to yield nominal cyclocinamide B. Finally, *ent-1c* was produced via a parallel series of reactions on **14**, with –Boc removal and coupling to fragment **17**<sup>8</sup> to afford **18**. Removal of the trityl and –OTBDPS groups, the latter with TBAF, gave the target compound. NMR data obtained from the synthetic samples<sup>14</sup> **1a**, **1b**, **2** and *ent-1c* are given in Tables S1-S4,<sup>15</sup> respectively, and support the connectivity represented in Fig. 1. Our working hypothesis was that the macrocycle sp<sup>3</sup> proton and carbon chemical shifts would be least sensitive to small impurities, as compared to the hydrogen bonding amide units, while maintaining high sensitivity to changes in absolute stereochemistry. This hypothesis could be tested with the synthetic samples. The spectra of *ent-1c* and **2**, which differ only in the presence of the substituent at distal C36 (H vs. Cl), should be nearly identical in the macrocycle, whereas the spectra of **1a** and **1b**, identical structures except for the absolute stereochemistry at C11, should display distinct differences in the core.

Table 1 gives the data for both comparisons, and bear out the discussion above. Substantial differences exist between the <sup>13</sup>C resonances at C11 when comparing **1a** and **1b** (3.9 ppm), and a correspondingly large difference (0.41 ppm) is seen in the <sup>1</sup>H resonances between the C10 positions of these two compounds. Conversely, *ent-1c* and **2** have very similar spectra when comparing the macrocycle sp<sup>3</sup> resonances, with carbon differences at or below 0.4 ppm and proton differences at or below 0.08 ppm. These latter numbers, particularly the <sup>13</sup>C data, can act as a threshold for further comparison between spectra. Reinforcement of this analysis is seen in the nearly identical optical rotation values, in both sign and magnitude, of *ent-1c* ([α]<sub>D</sub> = –12.5) and **2** ([α]<sub>D</sub> = –15.9) recorded in MeOH.

The bar graph in Figure S1 gives a comparison of the <sup>1</sup>H NMR data for the core sp<sup>3</sup> protons of natural cyclocinamide A<sup>16</sup> (CC-A, blue) and synthetic cyclocinamide B (**2**, red) to natural cyclocinamide B (CC-B), and *ent-1c* to natural CC-A (green). Figure S2 gives the analogous data derived from the <sup>13</sup>C spectra of the compounds. It should be noted that the chemical shifts used in these figures for natural CC-B result from an adjustment of the <sup>1</sup>H and <sup>13</sup>C

spectra references to 2.50 ppm and 39.52 ppm, respectively, as these were the references used in this laboratory for all synthetic samples.

It is apparent from these figures that the two natural samples, once they are set to the same reference peaks, are within the threshold set by the comparison of *ent-1c* and **2** for compounds that have the same array of stereocenters. With the exception of the C10 <sup>13</sup>C value, which shows a 0.5 ppm difference, all <sup>1</sup>H and <sup>13</sup>C δ values reside within the threshold limits. The carbon spectra are particularly close, with three of the six sp<sup>3</sup> centers identical. The relative stereochemical identity of CC-A and CC-B is an attractive conclusion, suggesting that these two natural products arise from the same biosynthetic machinery. The reported optical rotations bear the same sign but differ in magnitude: [α]<sub>D</sub> = 9.6 for natural CC-B and [α]<sub>D</sub> = 29 for the initial isolation of CC-A.

Comparison of synthetic CC-B (**2**) to its natural counterpart affords a less clear-cut conclusion. The proton comparison in Figure S1 shows two centers with differences greater than the 0.08 ppm benchmark, and several centers in the carbon comparison fall beyond the 0.4 ppm limit. Furthermore, the sign of the optical rotation for the synthetic **2** (*vide supra*) is opposite that of the natural material. Likewise, the chemical shifts of the *ent-1c* and natural CC-A cores, while nicely aligned in the proton comparison, deviate significantly in the C4 and C7 carbon comparison.

In conclusion, spectral data reexamination supports the notion of identical relative stereochemistry among the four chiral centers of cyclocinamide A and B. However, there exists no compelling data to suggest that either of these cyclic peptide natural products has been prepared in the laboratory. Further studies to determine the true 3D structures of cyclocinamide A and B are continuing and will be disclosed in due course.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

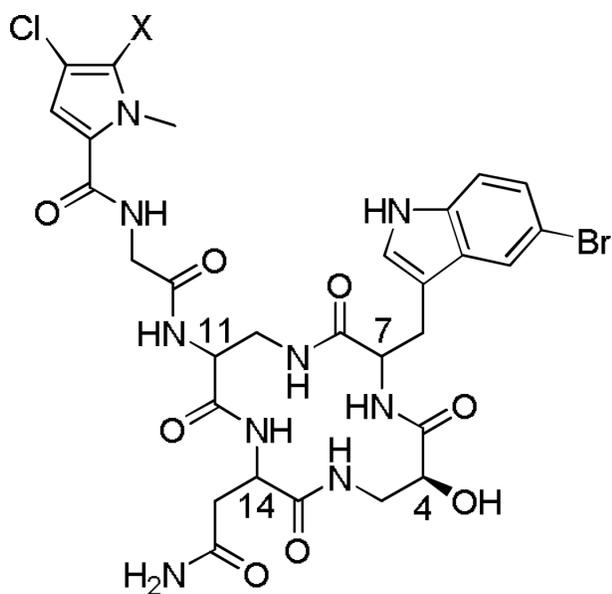
## Acknowledgements

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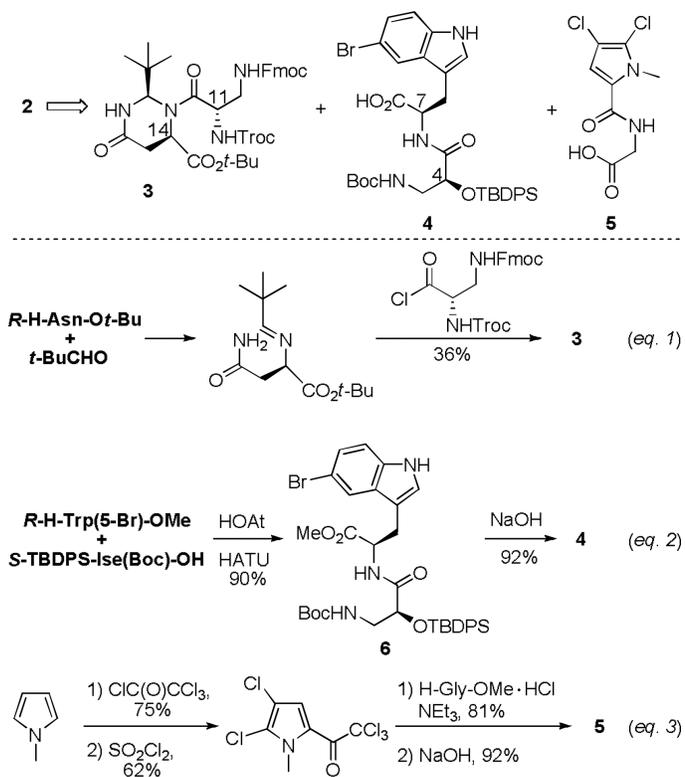
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14. Unfortunately, synthetic samples of cyclocinamide A diastereomers prepared in other laboratories are not available and NMR spectra were taken in MeOH- $d_4$ , making comparison difficult. No optical rotation data were reported for these CC-A isomers. Spectra of natural CC-A and CC-B, along with all spectra of synthetic samples prepared in this laboratory, were taken in DMSO- $d_6$ .
15. See Supporting Information for Tables.
16. See Table S5 for  $^1\text{H}$  and  $^{13}\text{C}$  resonances of the two natural products.

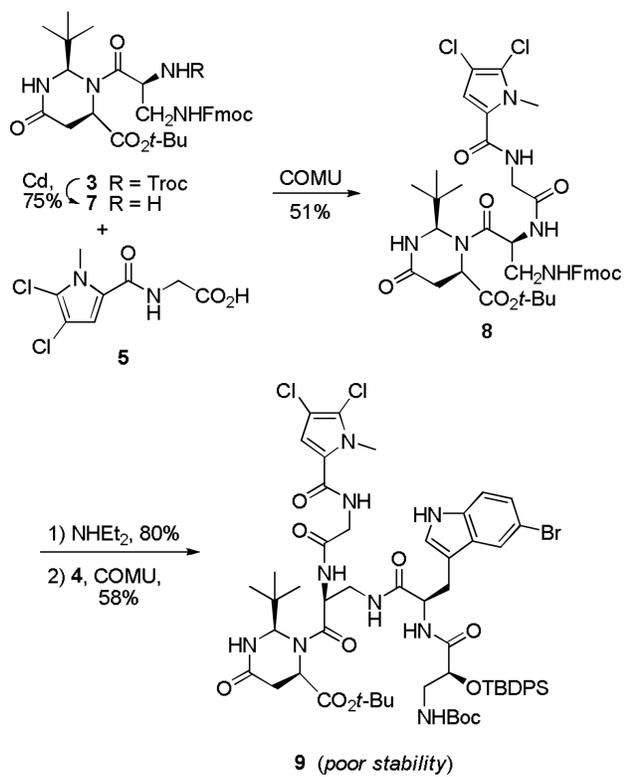


- 1a** (4*S*, 7*S*, 11*S*, 14*S*, X = H)  
**1b** (4*S*, 7*S*, 11*R*, 14*S*, X = H)  
**ent-1c** (4*S*, 7*R*, 11*S*, 14*R*, X = H)  
**2** (4*S*, 7*R*, 11*S*, 14*R*, X = Cl)

**Figure 1.**  
Cyclocinamide structures



**Scheme 1.**  
Initial retrosynthesis and preparation of dipeptides



**Scheme 2.**  
Failed attempt at synthesis of **2**.



Table 1

Comparison of Cyclic Peptide  $sp^3$   $^1H$  and  $^{13}C$  resonances

Atom number	<i>ent-1c</i>		2		$\delta$ $^1H$	$\delta$ $^{13}C$	1a		1b		$\delta$ $^1H$	$\delta$ $^{13}C$
	$^1H$	$^{13}C$	$^1H$	$^{13}C$	( <i>ent-1c</i> )-(2)	( <i>ent-1c</i> )-(2)	$^1H$	$^{13}C$	$^1H$	$^{13}C$	(1a)-(1b)	(1a)-(1b)
3 (CH <sub>2</sub> )	3.37	42.9	3.29	43.2	0.08	-0.3	2.81	42.4	2.85	42.3	-0.04	0.1
	3.56		3.51		0.05		3.59		3.56		0.03	
4* (C-H,OH)	4.06	70.8	4.03	70.8	0.03	0	3.75	69.2	3.87	69.1	-0.12	0.1
7* (C-H,R)	4.64	52.1	4.61	52.4	0.03	-0.3	4.43	53.1	4.47	53.2	-0.04	-0.1
10 (CH <sub>2</sub> )	3.29	40.5	3.36	40.4	-0.07	0.1	2.81	40.3	3.22	40.4	-0.41	-0.1
	3.42		**				3.45		3.56		-0.11	
11* (C-H,N)	4.34	54.3	4.34	54.0	0	0.3	4.17	51.6	4.19	55.5	-0.02	-3.9
14* (C-H,R)	4.55	49.7	4.54	50.1	0.01	-0.4	4.52	49.0	4.58	49.0	-0.06	0