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Total Synthesis of Nominal (11*S*)- and (11*R*)-Cyclocinamide A

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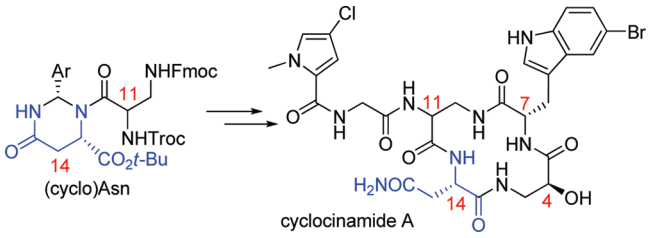
ABSTRACT



The cyclocinamides possess a unique $\beta^2\alpha\beta^2\alpha$ 14-membered tetrapeptide core. The initially reported biological data and intriguing structure, which was without full stereochemical identification, necessitated synthesis of both nominal (all-*S*) cyclocinamide A and the 11*R* isomer. The completed synthesis is highlighted by the use of a (cyclo)asparagine-containing dipeptide as a turn inducing fragment. Due to inconsistencies in analytical data between natural and synthetic samples, a re-evaluation of the natural product stereochemistry appears necessary.

Cyclic peptides represent a diverse class of unique compounds, in both structural detail and biological activity. Synthetically, the cyclization event often poses the greatest challenge to success, particularly for linear peptides with fewer than seven all-L or -*S* amino acids,[1] due to the preference for the *s*-trans conformation at each amide bond.[2]

The structure of the marine natural product cyclocinamide A (**1**, Scheme 1) appeared in 1997 and consists of a unique $\beta^2\alpha\beta^2\alpha$ 14-membered ring with an attached dipeptide chain devoid of chirality.[3] In that initial disclosure the C4 and C11 stereocenters were left undefined, while stereocenters C7 and C14 were both assigned an *S* configuration. Synthetic work on **1**, which was claimed to possess solid tumor activity, began almost immediately. Two of the possible four diastereomers were successfully prepared, but neither the 4*R*, 11*R* (**1c**,[4] cyclization at the 8−9 bond) nor the 4*R*, 11*S* (**1d**,[5] cyclization at the 1−2 bond) isomer proved identical to the natural product. In addition, attempts to form the two remaining possible stereoisomers via cyclization at the 1−2 amide bond failed.[5] In 2008 a second sample of natural **1** was obtained, leading to the proposition, via Marfey's analysis, that the absolute stereochemistry was that of all-*S* diastereomer **1a**.[6] Ireland's isolation of cyclocinamide B (**2**) added yet another (stereochemical) dimension, as this cyclic peptide was virtually identical to **1** in structure but was claimed to possess the 4*S*, 7*R*, 11*S*, 14*R* stereochemistry.[7]

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(1) Lambert, J. N.; Mitchell, J. P.; Roberts, K. D. *J. Chem. Soc., Perkin Trans. 1* **2001**, 471–484.
(2) Meutermans, W. D. F.; Bourne, G. T.; Golding, S. W.; Horton, D. A.; Campitelli, M. R.; Craik, D.; Scanlon, M.; Smythe, M. L. *Org. Lett.* **2003**, *5*, 2711–2714 and references therein.
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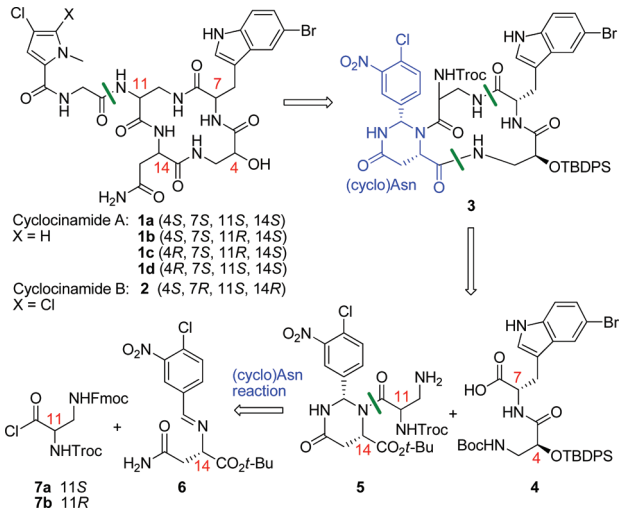
Given the biological activity, the extreme rarity of 14-membered cyclic tetrapeptides[8] with multiple stereocenters and the desire to secure the complete identity of the natural product, for which no samples remain, we undertook the preparation of the two diastereomers of **1** that have not yet been synthesized, namely **1a** and **1b**. Herein we describe our results which, when coupled with the analytical expertise of the original cyclocinamide A isolation team, lead us to the conclusion that a reexamination of the natural product stereostructure is necessary.

Although much research on α/β peptides, particularly α/β[3] peptides, has appeared in recent years,[9] cyclic α/β peptides have been little explored. Nonetheless, it seemed reasonable to assume that the cyclization of a linear precursor to **1a** would be challenging,[10] particularly given that there are no *N*-alkyl amides to help sample the *s*-cis amide conformation. The synthetic plan relied on the use of (cyclo)Asn (**3**, Scheme 1, blue), a protected asparagine residue[11] that acts as a turn inducer.[12] Our previous work with (cyclo)Asn indicated that this residue would both aid in the organic solubility of late-stage synthetic intermediates and protect the asparagine chiral center from epimerization in the key cyclization step, i.e., 1−2 amide bond formation.[13,14] Thus, the site of ring closure was dictated more by key precursor availability and less by predicted ease of cyclization.[15] Conversely, this approach would allow for another exploration of a previously unsuccessful closure site.

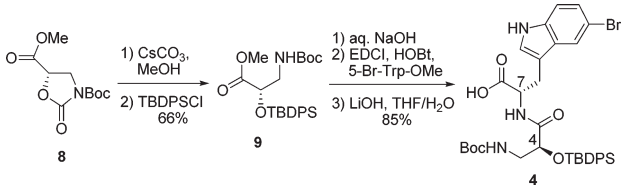
Scheme 1. Retrosynthesis of Cyclocinamides



The retrosynthesis for **1a** and **1b** is depicted in Scheme 1 and was envisioned to arise from three dipeptide segments. Addition of the glycine-pyrrole side chain would be a late-stage event, as would liberation of the asparagine residue. Thus, the proposed product of cyclization would be tetrapeptide **3**. Fission of the bonds indicated affords two suitably protected dipeptides of roughly equivalent size and complexity: isoseryl-5-bromotryptophan (Ise-5-Br-Trp) **4** and diaminopropionyl(cyclo)asparagine [Dap-(cyclo)Asn] **5**. Compound **5** can be accessed from known asparagine imine **6**[16] and the appropriate diaminopropionic acid chloride **7a** or **7b**.

Synthesis of dipeptide **4** (Scheme 2) began with known oxazolidinone **8**,[17] which underwent selective ring opening with cesium carbonate[18] to give the corresponding alcohol prior to treatment with *tert*-butyldiphenylchlorosilane to afford fully protected isoserine **9** in 66% yield over two steps. Due to protection group lability of α-silyloxy-β-amino acids,[19] the methyl ester deprotection was performed with only 1 equiv of NaOH. The resulting crude acid was immediately activated with HOAt and EDCI hydrochloride before introduction of (*S*)-5-bromotryptophan methyl ester;[20] the desired dipeptide was isolated in 85% yield. For further use the methyl ester was reacted as needed with excess lithium hydroxide to give the desired free acid dipeptide **4**, which was taken on crude to the coupling reaction with Dap-(cyclo)Asn dipeptide **5**.

Scheme 2. Synthesis of Dipeptide **4**



Syntheses of the two Dap-(cyclo)Asn dipeptide diastereomers **5a** and **5b** (Scheme 3) began with construction of a

(8) (a) Karle, I. L.; Handa, B. K.; Hassall, C. H. *Acta Crystallogr.* **1975**, *B31*, 555–560. (b) Hassall, C. H.; Sanger, D. G.; Handa, B. K. *J. Chem. Soc. C* **1971**, 2814–2818.

(9) Boersma, M. D.; Haase, H. S.; Peterson-Kaufman, K. J.; Lee, E. F.; Clarke, O. B.; Colman, P. M.; Smith, B. J.; Horne, W. S.; Fairlie, W. D.; Gellman, S. H. *J. Am. Chem. Soc.* **2012**, *134*, 315–323.

(10) (a) Skropeta, D.; Jolliffe, K.; Turner, P. *J. Org. Chem.* **2004**, *69*, 8804–8809. (b) Cyclization of an all-*S* linear tetrapeptide precursor to cyclocinamide A void of any turn-inducing element failed. See ref 5 for details.

(11) Postema and Liu experienced problems with asparagine free amide, which would suggest protection would be useful. See ref 5 for details.

(12) Konopelski, J. P.; Wei, Y.; Olmstead, M. M. *J. Org. Chem.* **1999**, *64*, 5148–5151.

(13) We have previously employed a (cyclo)Asn derivative in a "self-reproduction of chirality" approach to enantiomerically pure α-methyl-asparagine derivatives. See: Hopkins, S. A.; Ritsema, T. A.; Konopelski, J. P. *J. Org. Chem.* **1999**, *64*, 7885–7889.

(14) Epimerization at the C-terminal amino acid in the cyclization event of small peptides bearing pseudoproline turn inducers has been documented. See ref 10a.

(15) Cavelier-Frontin, F.; Pèpe, G.; Verducci, J.; Siri, D.; Jacquier, R. *J. Am. Chem. Soc.* **1992**, *114*, 8885–8890.

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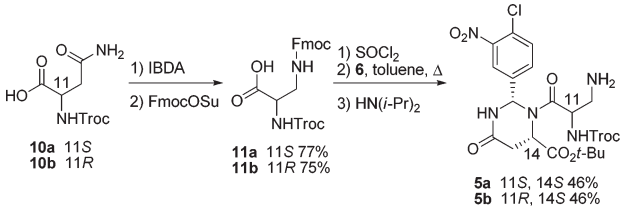
(19) Greco, M. N.; Zhong, H. M.; Maryanoff, B. E. *Tetrahedron Lett.* **1998**, *39*, 4959–4962.

(20) See Supporting Information for detailed synthesis, characterization, and full spectroscopic details.

suitably protected diaminopropionic acid fragment. Either (*S*)- or (*R*)-Troc-asparagine (**10a** or **10b**, respectively) was treated with iodobenzene diacetate (IBDA) to affect the desired Hofmann rearrangement,[21] and the resulting zwitterion was *N*-protected in excellent yield. Compounds **11** were then treated with thionyl chloride and a catalytic amount of DMF to form the acid chlorides **7**. The crude acid chlorides were used in the key (cyclo)Asn reactions; the best yields were obtained by heating in toluene at 80 °C for 6 h. The Dap-(cyclo)Asn dipeptides, isolated as mixtures of rotamers about the newly formed amide bond, were transformed to free amines **5a** and **5b** in good yields (92% and 83%, respectively) by standard conditions.

Scheme 3. Preparation of Dap-(cyclo)Asn Diastereomers **5**

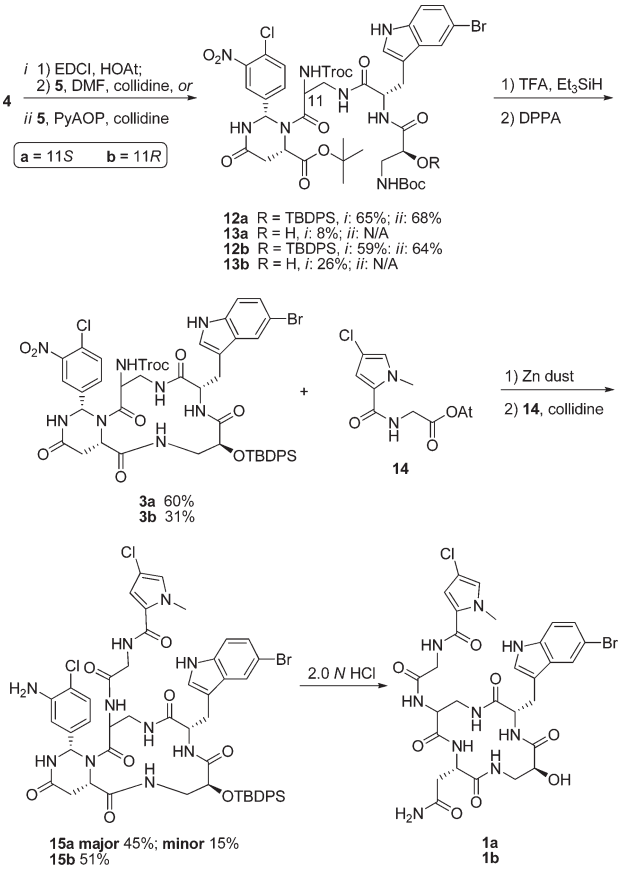


With the (cyclo)Asn turn inducer successfully incorporated, focus shifted to production of the necessary linear tetrapeptide. Dipeptide **4** was activated with HOAt and EDCI (Scheme 4) and then coupled to the Dap-(cyclo)Asn free amine (either **5a** or **5b**). Unfortunately, mixtures of desired TBDPS-protected **12** and free alcohol **13** were obtained; reproducible reprotection of **13a** to **12a** was unsuccessful. This disadvantageous deprotection was also evident when PyAOP was used in the coupling reaction. In preparation for the key cyclization step, the linear tetrapeptides were treated with trifluoroacetic acid in the presence of triethylsilane.

As expected, significant experimentation was needed to optimize the cyclization of **12a**. Ultimately, DPPA and PyAOP afforded **3a** in yields of 60% and 63%, respectively; DPPA was used preferentially due to its ease of handling and lack of phosphoramide side product. Additionally, cyclization of **12b** with DPPA gave a 31% yield of **3b**. Treatment of the cyclic tetrapeptides **3** with zinc and acetic acid resulted in Troc removal as well as aryl nitro reduction to afford the corresponding diamines, which were independently coupled to activated side chain **14**.[20] Two compounds of identical molecular weight were isolated for compound **15a** as a 3:1 separable mixture; HMBC correlations substantiated the coupling of the side chain to the Dap α-amine. Only one product was formed in the reaction toward **15b**, in 51% yield. Compounds **15a major** and **15b** were treated with 2.0 N HCl in dioxane to affect TBDPS-deprotection and (cyclo)Asn ring opening. The crude materials **1a** and **1b** were purified via RP-HPLC, and their analytical data were compared to those of the natural material.

(21) Zhang, L. H.; Kauffman, G. S.; Pesti, J. A.; Yin, J. *J. Org. Chem.* **1997**, *62*, 6918–6920.

Scheme 4. Cyclization and Completion of Syntheses



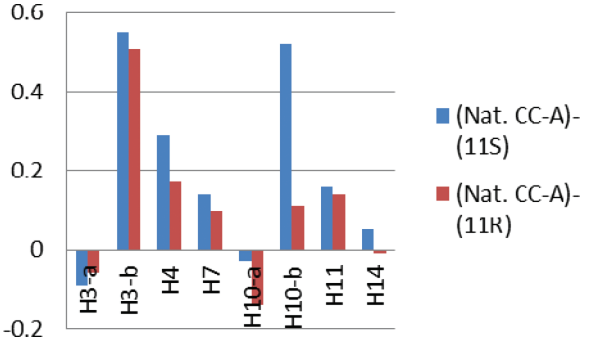


Figure 1. Comparison of ring sp^3 ^1H NMR data between synthetic 11*S*- (**1a**, blue) or 11*R*- (**1b**, red) and natural cyclocinamide A.

To verify that the all-*S* absolute stereochemistry of **1a** had been retained throughout the synthesis, approximately 3:1 mixtures of synthetic samples of both enantiomers of Ise and 5-Br-Trp were subjected to acid hydrolysis followed by reaction with 1-fluoro-2,4-dinitrophenyl-5-L-leucinamide (L-FDLA). Similar mixtures were formed from commercial samples of L- and (±)-Asp and Dap, again followed by reaction with the Marfey reagent.

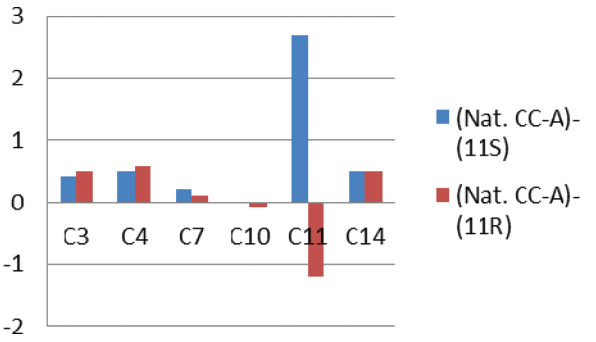


Figure 2. Comparison of ring sp^3 ^{13}C NMR data between synthetic 11*S*- (**1a**, blue) or 11*R*- (**1b**, red) and natural cyclocinamide A.

HPLC conditions were found to separate all amino acid derivatives, thus forming the standards necessary for analysis (Figure S1). Finally, hydrolysis of **1a** and treatment with L-FDLA produced a sample that contained only *S* amino acids as judged by LC/MS analysis.[20] However, the reported magnitude of the rotation of the natural product ($[\alpha]_D^{25}$ = 29 (*c* = 0.1, MeOH)) is higher than that recorded for **1a** ($[\alpha]_D^{25}$ = 14.2 (*c* = 0.1, MeOH)).

The two unique isolations of cyclocinamide A provided spectroscopically identical material. Figure 1 displays the differences in ^1H NMR data for the ring sp^3 protons between synthetic **1a** (blue) and **1b** (red) and natural cyclocinamide A (CC-A). Corresponding data from the ^{13}C spectra are given in Figure 2; tables of NMR data are given in the Supporting Information. Since stereochemistry can have a profound impact on the properties of cyclic peptides,[22] it was felt that the nonamide protons and carbons of the cyclocinamide ring would be the most diagnostic for our analysis. All spectra were taken in DMSO-d_6 and calibrated to solvent peaks at δ 2.50 for ^1H spectra and δ 39.52 for ^{13}C spectra. These spectral comparisons, in our opinion, do not support the identity of either **1a** or **1b** to the natural product.

In summary, we have successfully completed the first synthesis of the nominal structure of cyclocinamide A and the corresponding 11*R* isomer. The route relies on the turn-inducing nature of (cyclo)Asn. Cyclization reactions occur in good yield, particularly for the all-*S* isomer, without racemization. Spectral comparisons with those of the natural product do not support the validity of the proposed stereostructure. Ongoing work in our laboratory is focused on the synthesis of nominal cyclocinamide B and other isomers of cyclocinamide A. Our results on these projects will be reported in due course.

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Supporting Information Available. Experimental details, characterization data, NMR spectra and comparison tables, and Marfey analysis data of **1a**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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