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STUDIES RELATED TO THE ABSOLUTE CONFIGURATION OF CYCLOCINAMIDE A: TOTAL SYNTHESIS OF 4(*R*),11(*R*)-CYCLOCINAMIDE A

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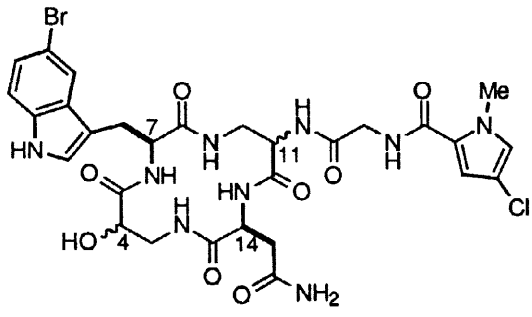
Abstract: Coupling of the fluorenylmethyl ester **3** (R = Fm) of (*S*)-5-bromotryptophan with N_α-BOC-(*S*)-asn-O-benzoyl-(*R*)-ise **9** gave rise to tripeptide **10**. Cleavage of the BOC group in **10** followed by coupling with N_α-BOC-N_β-Fmoc-(*R*)-2,3-diamino-propanoic acid afforded tetrapeptide **11** which was transformed into cyclic peptide **12**. Cleavage of the BOC in **12** followed by coupling with the glycine derived side chain **14** gave rise, after removal of the benzoyl group to 4(*R*),11(*R*)-cyclocinamide A (**2**) which was not identical to natural cyclocinamide A (**1**), suggesting that **1** possesses the 4(*S*),7(*S*),11(*S*),14(*S*) configuration.

Cyclocinamide A (**1**) is a novel cytotoxic hexapeptide isolated from the methanolic extracts of the marine sponge *Psammocinia* sps. (Thorectidae) which is found in the Milne Bay region of Papua New Guinea.[1] Cyclocinamide A contains a number of unique structural features including a 14-membered tetrapeptide core, along with a dipeptide side chain terminating in an *N*-methylchloropyrrole. While the constituency and connectivity of cyclocinamide A have been determined through extensive NMR experimentation, the absolute stereochemistry of the cyclic peptide at C(4) and C(11) remains undefined. The scarcity of peptidic material from the sponge extracts has precluded further evaluation of the absolute configuration at C(4) and C(11) and has also prevented *in vivo* evaluation.[2] In an effort to determine the absolute stereochemistry at C(4) and C(11) of cyclocinamide A, a synthesis of 4(*R*),11(*R*)-cyclocinamide A (**2**) was undertaken. We detail below a total synthesis of **2**.



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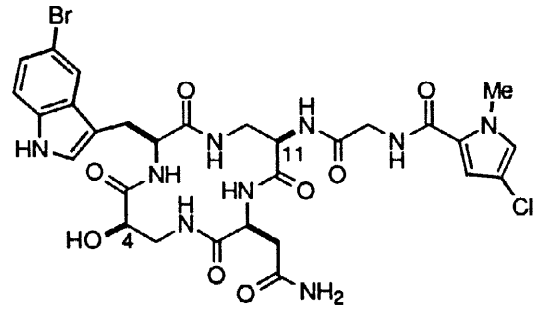

2

Assembly of the tetrapeptide core of **2** first required synthesis of the unnatural amino acid residues present in **2**. The fluorenylmethyl ester **3** (R = Fm) of 5-bromo-*N*-methoxycarbonyl-(*S*)-tryptophan was prepared from the

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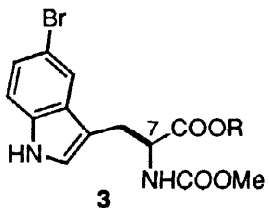
corresponding methyl ester **3** (R = Me)[3] *via* a two-step sequence. Hydrolysis [1.0 N NaOH, MeOH] of the methyl ester followed by esterification[4] with 9-fluorenylmethanol [DCC (*N,N'*-dicyclohexylcarbodiimide), CH_2Cl_2] provided fluorenylmethyl ester **3** (R = Fm) in 98% overall yield. The synthesis of *N*-BOC-*O*-benzoyl-(*R*)-isoserine **4** *via* a six-step sequence originated with (*R*)-malic acid employing the protocol of Milewska and Polonski[5] developed for the conversion of (*S*)-malic acid into (*S*)-isoserine. (*R*)-Malic acid was converted [$(CH_2O)_x$, $CHCl_3$, TsOH, reflux] in quantitative yield into dioxolone **5** which was subsequently transformed *via* a Curtius reaction [(a) $SOCl_2$, reflux; (b) NaN_3, acetone, H_2O, -20 °C; (c) *t*-BuOH, reflux] into the *N*-BOC-dioxolone **6** in 41% overall yield. Cleavage [1.0 N NaOH, MeOH] of the dioxolone followed by benzoylation [BzCl, pyr, 0 °C] provided (71%) the (*R*)-isoserine fragment **4**. The fully protected, activated (*S*)-asparagine residue **7** is commercially available.[6] The (*R*)-2,3-diaminopropanoic acid fragment **8** was prepared from N_α-BOC-(*R*)-asparagine *via* a Hoffman rearrangement.[7] Thus, treatment of N_α-BOC-(*R*)-asparagine with iodosobenzene diacetate in *n*-propanol:methyl acetate:water (8:5:1) [0 °C (1h) → 50 °C (2h)] and subsequent exposure of the resulting amine to *N*-(9-fluorenylmethoxycarbonyloxy)succinimide[8] in aqueous acetone afforded the fully protected (*R*)-2,3-diaminopropanoic acid moiety **8** in 75% overall yield.



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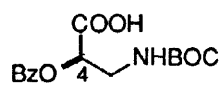

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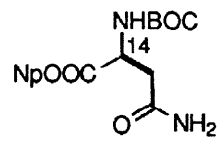

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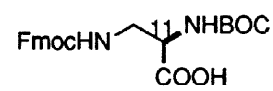

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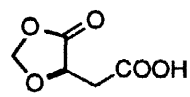

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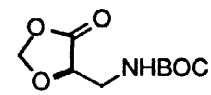
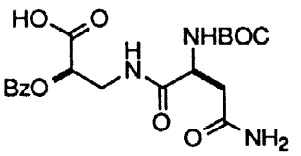


6

With the requisite amino acid fragments in hand, assembly of the linear tetrapeptide **11** was undertaken. It was anticipated that tetrapeptide **11**, needed for construction of the tetracyclic peptidic core **12** of 4(*R*),11(*R*)-cyclocinamide A (**2**), could be assembled by conventional BOP [(1*H*-benzotriazol-1-yloxy)tris(dimethylamino)-phosphonium hexafluorophosphate] promoted coupling protocols.[9] In an effort to minimize protecting group manipulations, the *O*-benzoyl-(*R*)-isoserine, derived from fragment **4**, was coupled with the commercially available (*S*)-asparagine moiety **7**.[6] Thus, the *N*-BOC protected (*R*)-isoserine **4** was deprotected [30% TFA in CH_2Cl_2] and the resulting crude trifluoroacetate salt was treated [HOBt (1-hydroxybenzotriazole), DIEA (*N,N*-diisopropylethylamine), DMF] with the activated 4-nitrophenyl ester **7** of N_α-BOC-(*S*)-asparagine giving rise to dipeptide **9** as a single diastereomer in 65% overall yield. Removal [TMSI, $CHCl_3$, reflux] of the methyl carbamate protecting group in **3** provided the corresponding fluorenylmethyl ester of (*S*)-5-bromotryptophan which, upon coupling [BOP, HOBt, DIEA, DMF] with dipeptide **9**, afforded tripeptide **10** in 57% overall yield. Cleavage [30% TFA in CH_2Cl_2] of the *N*-BOC group in tripeptide **10** gave rise to the corresponding trifluoroacetate salt which was dissolved in DMF and coupled [BOP, HOBT, DIEA] with the differentially protected (*R*)-2,3-diaminopropanoic acid **8** giving rise to tetrapeptide **11** in 76% yield.



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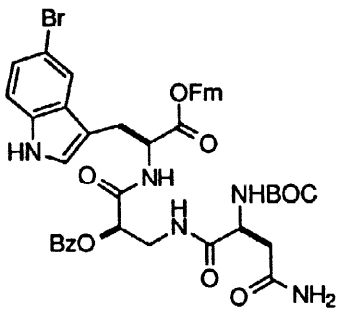

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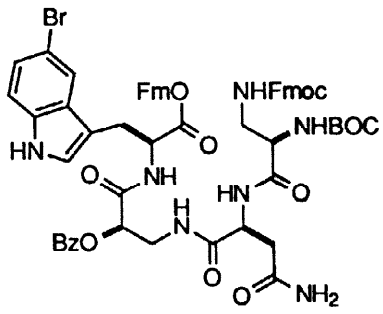
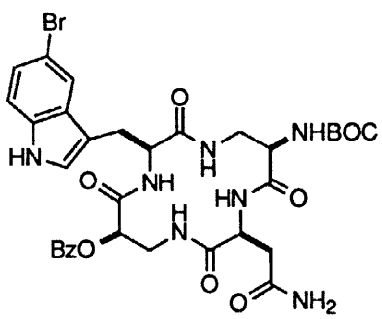


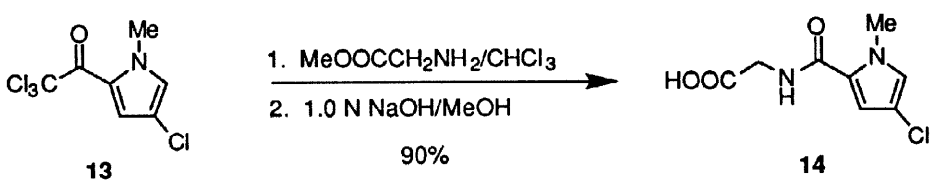
11

With the linear tetrapeptide **11** in hand, the ring closure reaction to form the core of 4(*R*),11(*R*)-cyclocinamide A (**2**) was examined. Simultaneous deprotection [20% TEA in CH_2Cl_2, DMF, 2h] of the termini of tetrapeptide **11** provided, after evaporation of the volatile components *in vacuo*, a solid residue which, upon dissolution in DMF and treatment with *N,N*-diisopropylethylamine and pentafluorophenyldiphenylphosphinate,[10] afforded in 62% overall yield the cyclized tetrapeptide core **12** of **2**.



12

Prior to completing the total synthesis of **2**, a synthesis of the glycine derived fragment **14** needed to be developed. This was accomplished *via* a two-step protocol. Condensation of methyl glycinate with 1-methyl-2-trichloroacetyl-4-chloropyrrole (**13**)[11] and subsequent hydrolysis of the methyl ester provided dipeptide fragment **14** in 90% overall yield.



13 14

Completion of the total synthesis of 4(*R*),11(*R*)-cyclocinamide A (**2**) necessitated cleavage of the BOC group on the nitrogen at C(11) in **12** followed by coupling with the dipeptide side chain **14**. Thus, cleavage (30% TFA in CH_2Cl_2) of the BOC group on the cyclic tetrapeptide core **12** provided the crude trifluoroacetate salt which was dissolved in *N,N*-dimethylformamide and coupled [BOP, HOBT, DIEA] with dipeptide **14**. Deprotection of the C(4) hydroxyl group in the coupled material employing 1% sodium hydroxide in methanol gave rise to 4(*R*),11(*R*)-cyclocinamide A (**2**) in 41% overall yield. A comparison of the ^1H NMR spectrum (500 MHz, MeOH-

d_4) of **2** with that of natural cyclocinamide A provided by Professor Crews revealed that the spectra were similar, but not identical. Biogenetic considerations would suggest that the absolute configuration at C(4) and C(11) are both *S*. Efforts are underway to prepare the remaining diastereomers [4(*S*),7(*S*),11(*S*),14(*S*); 4(*S*),7(*S*),11(*R*),14(*S*) and 4(*R*),7(*S*),11(*S*),14(*S*)] of cyclocinamide A.

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