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Total Synthesis and Assignment of Stereochemistry of Raocyclamide Cyclopeptides from Cyanobacterium *Oscillatoria raoi*

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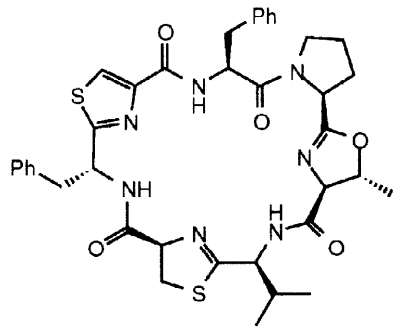
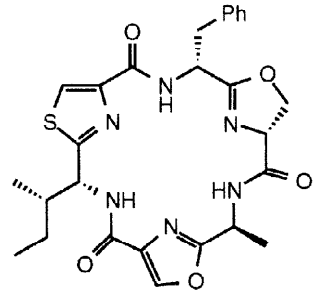
Abstract: Total synthesis demonstrates that the structures and stereochemistries of the cyclopeptides raocyclamide A and B isolated from a cyanobacterium should be altered to those shown in formulae **1** **3** and **14** respectively. © 1998 Elsevier Science Ltd. All rights reserved.

A large number of cyclopeptides have now been isolated from nature whose structures are constituted from "unnatural" *D*-amino acids and/or from unusual, highly modified, amino acids, *eg* thiazole, thiazoline, oxazole and oxazoline aminoalkyl carboxylic acids.[1] These interesting structural features raise a number of questions: *eg* Why has nature engineered the synthesis of *D*-amino acids to elaborate certain cyclopeptides? Is this feature associated with some specific folding of a peptide chain prior to macrocycle formation? Are nitrogen, sulphur and oxygen heteroatoms in the heterocyclic-based amino acid residues involved as ionophoric groups to conformationally constrain the cyclopeptide structures and their biological precursors? Is metal encapsulation and transport[2] a feature of their formation (*ie* acting as a template) and their *modus operandi* in vivo? In order to answer some of these questions we have selected the lissoclinium, *eg* lissoclinamide 4, **1**,[3] and the raocyclamide, *eg* raocyclamide A, **2**[4], families of cyclopeptides for special study. We selected these two families since their member show all the "unusual" structural features mentioned above, and their synthesis should be fairly straightforward thereby allowing the effects of modifications in the stereochemistries of their constituent amino acids on *eg* peptide folding, ease of macrocyclisation, ionophoric properties, to be evaluated. As a prelude to these detailed investigations we have investigated a total synthesis of the structure **2** proposed for raocyclamide A.



1



2

Raocyclamide A **2**, together with raocyclamide B **7**, were isolated from the cyanobacterium *Oscillatoria raoi* [4] and their structures and stereochemistries were determined by detailed nmr analysis in combination with amino acid analysis following ozonolysis and application of Marfey's HPLC method. Both molecules show hexapeptide structures formally derived from *D*-isoleucine, *D*-phenylalanine, *D*-serine, and *L*-alanine with their oxazole and thiazole moieties originating from serine and cysteine respectively.

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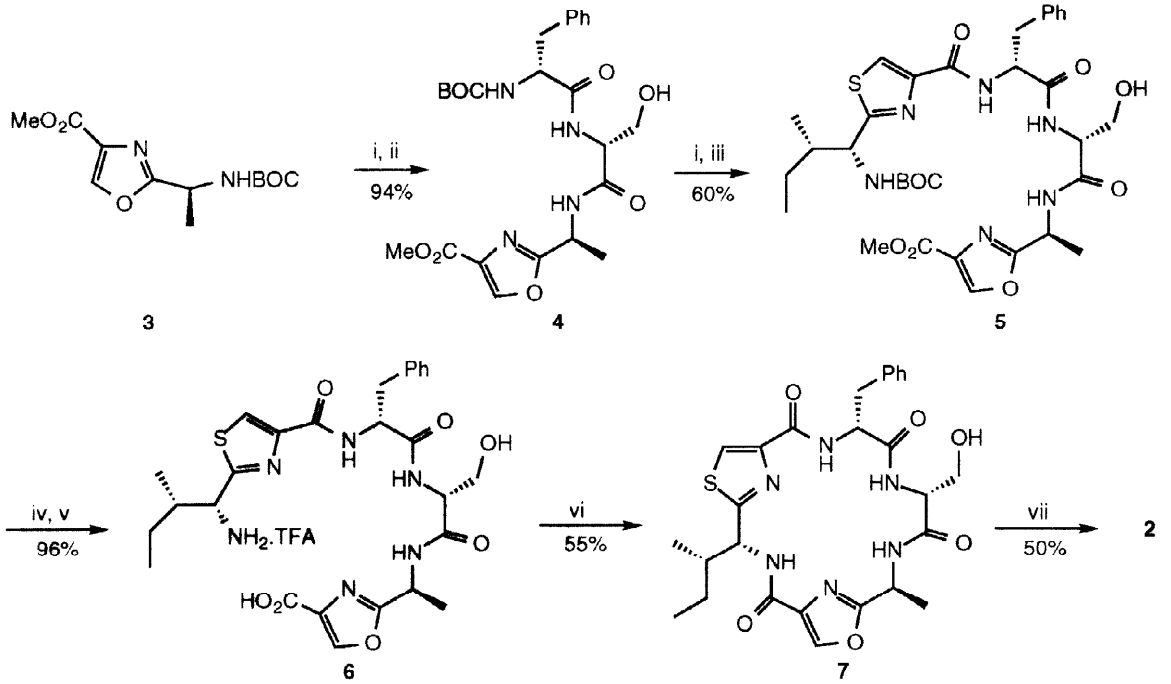
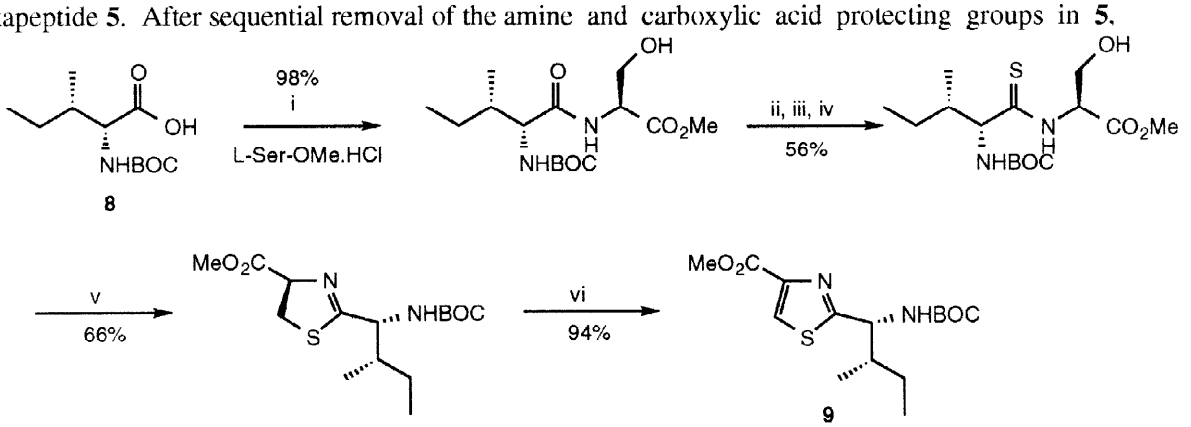
Our straightforward strategy for the synthesis of raocyclamide A **2**, proceeding *via* raocyclamide B **7**, and relied on access to the *L*-alanine derived 2,4-disubstituted oxazole **3** and the *D*-isoleucine derived 2,4-disubstituted thiazole **9**. We next planned to elaborate **3** to the tetrapeptide **4**, then fuse-on the thiazole **9** leading



Reagents: i, AcCl, MeOH, 0°, 1h; ii, Boc-D-Phe-D-Ser-OH, EDC, HOBt, Hunig's base, THF, 0° to RT, 18h; iii, acid of **9**, EDC, HOBt, THF, 0° to RT, 18h; iv, LiOH, H_2O, THF, RT, 1.5h; v, TFA:CH_2Cl_2 (1:1), RT, 1h;vi, DPPA, Hunig's base, THF, high dilution, 4d; vii, Burgess' rgt, THF, 67°, 0.5h.

Scheme 1

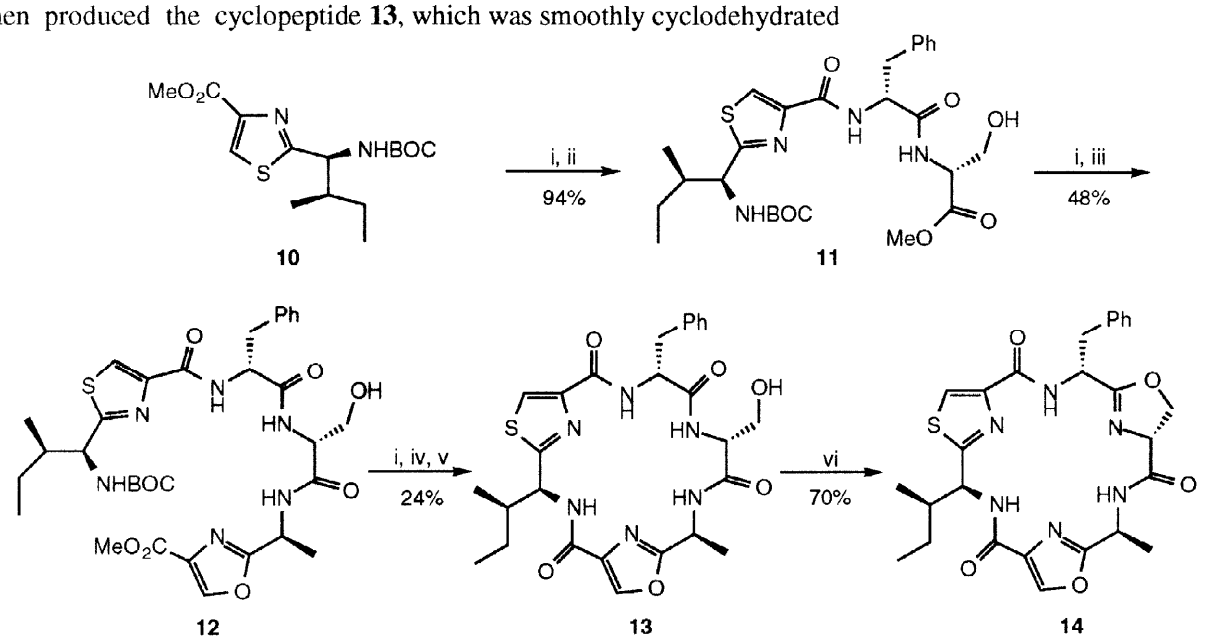
to **5**, and finally cyclise **6** to raocyclamide B **7**, en route to **2**. We and others have earlier synthesised the *L*-alanine oxazole **3**, in connection with other studies.[5] After removing the BOC protecting group in **3**, a coupling reaction between the corresponding free amine and BOC-*D*-Phe-*D*-Ser-OH then gave rise to the tetrapeptide **4** in 94% yield (Scheme 1).[6] The substituted thiazole **9** was derived from BOC-protected *D*-isoleucine **8** in six steps (overall yield 34%) as illustrated in Scheme 2.[7] Saponification of the ester group in **9**, followed by a coupling of the resulting carboxylic acid with the amine derived from **4**, next led to the hexapeptide **5**. After sequential removal of the amine and carboxylic acid protecting groups in **5**,



Reagents: i, DCC, HOBt, NMM, CH_2Cl_2, 0° to RT, 18h; ii, TBSCl, Imidazole, DMF, 0°, 18h; iii, Lawesson's rgt, PhH, 80°, 18h; iv, TBAF, THF, RT, 1.5h; v, Burgess' rgt, THF, 66°, 0.5h; vi, $BrCCl_3$, DBU, CH_2Cl_2, 0° to RT, 18h.

Scheme 2

macrocyclisation of **6** in the presence of Hünig's base and diphenylphosphoryl azide (DPPA) then produced the cyclic peptide **7**. Finally, treatment of **7** with Burgess' reagent[8] provided the structure **2** proposed for natural raocyclamide A from *O. raoi*. Unfortunately, when we compared the pmr and cmr spectra for our synthetic materials **7** and **2**,[9] with those recorded for naturally derived raocyclamides A and B, there were considerable differences. We cogitated the problem and analysed the nmr data in more detail and came to the conclusion that the natural raocyclamides A and B were most likely derived from *L*-isoleucine rather than its *D*-enantiomer. Accordingly, we synthesised the disubstituted thiazole **10** from *L*-isoleucine,[7] then elaborated **10** to **11**, and coupled the free carboxylic acid produced from **11** to the free amine derived from **3** leading to **12**. Deprotection of the ester and amino groups in **12**, followed by macrolactamisation then produced the cyclopeptide **13**, which was smoothly cyclodehydrated



Reagents: i, LiOH, H_2O, THF, RT, 1.5h; ii, HCl.NH_2-D-Phe-D-Ser-OMe, EDC, HOBt, Hunig's base, THF, 0° to RT, 18h; iii, Amine of **3**, EDC, HOBt, THF, 0° to RT, 18h; iv, TFA:CH_2Cl_2 (1:1), RT, 1h; v, DPPA, Hunig's base, THF, high dilution, 4d; vi, Burgess' rgt, THF, 66°, 0.5h.

Scheme 3

using Burgess' reagent to the oxazoline-based cyclopeptide **14**. Gratifyingly, the pmr and cmr spectra for synthetic **13** and **14**[10] were identical in every detail with those recorded for natural raocyclamides A and B respectively. Further studies are now in progress to evaluate the effects of stereochemistry of the constituent amino acids and of metal binding, on peptide folding and ease of ring closure leading to diastereomers of the raocyclamides **13** and **14**, and selected analogues.

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5. Meyers, A.I.; Tavares, F.X. *J. Org. Chem.* **1996**, *61*, 8207-8215; this oxazole was reported as an oil with an $[\alpha]_D$ -29 whereas in our hands it was a white crystalline solid with an $[\alpha]_D$ -50.
6. Satisfactory spectroscopic and mass spectrometry data were obtained for all new compounds.
7. The ethyl ester corresponding to **9** has been prepared by an alternative route. See: Hamada, H.; Shibata, M.; Sugiura, T.; Kato, S.; Shioiri, T. *J. Org. Chem.* **1987**, *52*, 1252-1255.
8. (a) Burgess, E.M.; Penton, Jr., H.R.; Taylor, E.A. *J. Org. Chem.* **1973**, *38*, 26-31. (b) Burgess, E.M.; Penton, Jr., H.R.; Taylor, E.A.; Williams, W.M. *Organic Syntheses*; Wiley: New York, 1988; Coll. Vol. VI, 1988; p 788.
9. P.m.r. data for synthetic **7**: δ_H (360 MHz, $CDCl_3$) 0.88 (3H, d, *J* 6.8, CH_3CHCH), 0.99 (3H, t, *J* 7.3, CH_3CH_2), 1.25 (1H, m, CH_3CH_2), 1.51 (3H, d, *J* 7.1, CH_3CHNH), 1.55 (1H, m, CH_3CH_2), 2.04 (1H, m, $(CH_3)CH(C_2H_5)$), 3.09 (1H, dd, *J* 7.8 13.7, CH_2Ph), 3.20 (1H, dd, *J* 5.6, 13.7, CH_2Ph), 3.45 (1H, br. s, CH_2OH), 3.67 (1H, dd, *J* 4.2, 10.9, CH_2O), 3.97 (1H, m, CH_2O), 4.43 (1H, m, $CHCH_2O$), 5.01 (1H, m, $CHCH_2Ph$), 5.23 (1H, dd, *J* 5.1, 7.7, NHCHCH), 5.33 (1H, m, $NHCHCH_3$), 7.21 (5H, m, *Ph*), 7.78 (1H, s, SCH), 7.83 (1H, d, *J* 7.2, NHCO), 7.84 (1H, d, *J* 8.0, NHCO), 8.18 (1H, s, OCH), 8.32 (2H, m, 2 x NHCO).
10. P.m.r. data for synthetic **14**: δ_H (360 MHz, $CDCl_3$) 0.95 (3H, t, *J* 7.4, CH_3CH_2), 1.06 (3H, d, *J* 6.8, CH_3CHCH), 1.20 (1H, m, CH_2CH_3), 1.40 (1H, m, CH_2CH_3), 2.04 (1H, m, $(CH_3)CH(C_2H_5)$), 3.13 (1H, dd, *J* 3.3 12.8, CH_2Ph), 3.15 (1H, dd, *J* 2.8, 12.8, CH_2Ph), 4.56 (1H, dd, *J* 8.7, 10.7, CH_2O), 4.65 (2H, m, $CHCH_2O$, $CCHCH_3$), 4.94 (1H, dd, *J* 6.3, 8.7, CH_2O), 5.27 (1H, m, $CHCH_2Ph$), 5.56 (1H, dd, *J* 3.7, 8.8, NHCHCH), 6.66 (1H, tt, *J* 1.4, 7.2, CHCHCH), 6.81 (4H, m, Ph), 7.34 (1H, d, *J* 4.6, $NHCHCH_3$), 8.04 (1H, d, *J* 8.9, $NHCHCH_2Ph$), 8.20 (1H, d, *J* 8.7, NHCHCH), 8.22 (1H, s, SCH), 8.22 (1H, s, OCH); δ_C (90.5 MHz, $CDCl_3$) 11.9 (q), 15.7 (q), 20.2 (q), 24.2 (q), 37.7 (t), 42.3 (d), 44.8 (d), 48.0 (d), 55.6 (d), 67.6 (d), 69.9 (d), 124.6 (d), 125.9 (d), 127.6 (d), 129.5 (d), 134.9 (s), 135.6 (s), 141.0 (d), 147.8 (s), 159.3 (s), 159.5 (s), 164.1 (s), 167.0 (s), 169.5 (s), 170.1 (s).