Synthesis towards microcystins and related toxins

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A general route is described for the preparation of microcystins and nodularins, including new chemistry for the preparation of β-methyl-D-aspartic acid.

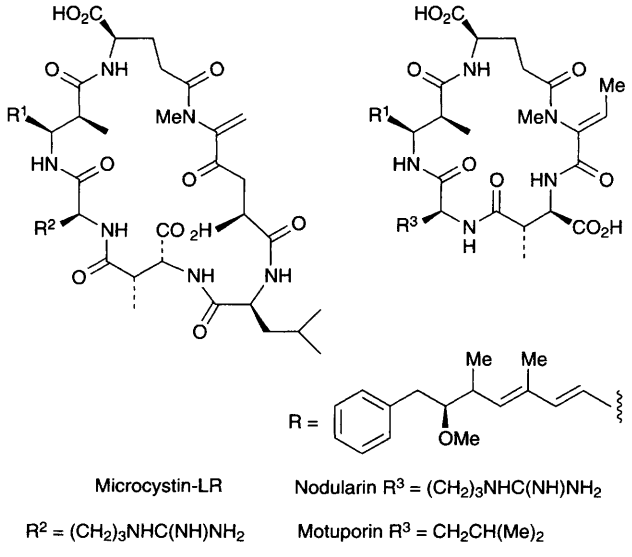
Microcystins (Fig. 1) are cyclic heptapeptides isolated from the cyanobacteria *Anabaena, Microcystis, Nostoc* and *Oscillatoria*.[1] More than 40 microcystins have been isolated and characterized. Structurally, they may be identified by the unusual amino acid Adda[2,†] together with either didehydroalanine or didehydrobutyrine, or the adduct of one of these residues with a nucleophile such as cysteins. Microcystins typically also contain either D-aspartate or α-methyl-D-aspartate, as well as D-glutamic acid, both linked *via* their side-chain carboxy groups. The conjunction of these unusual residues may be expected to result in interesting structural and chemical properties. In addition, many microcystins are potent hepatotoxins and tumour promoters.[1,3] Consequently, significant health problems are presented by water contaminated by blue–green algal blooms.[4] The toxicity of microcystins is believed to stem from their ability to inhibit protein phosphatases 1 and 2A.[5,6] Closely related to the microcystins are the pentapeptides nodularin and motuporin (Fig. 1). Nodularin was isolated from the cyanobacterium *Nodularia spumigena*,[7] but motuporin was obtained from samples of the marine sponge *Theonella swinhoei* Gray.[8] Nodularin and motuporin inhibit protein phosphatases.[8,9]

To obtain materials for examining biochemical properties and structure–function relationships within this series of compounds, we are interested in developing a versatile synthetic route that will provide access both to microcystins and nodularins with minimal alterations in the synthetic strategy. Previous work by other groups include syntheses of Adda[10,11] and β-alkylaspartates.[12] Most approaches to β-alkylaspartate have attempted to control the diastereoselectivity of aspartate alkylation. In an alternative approach, Valentekovich and Schreiber obtained β-methylaspartate from threonine using a 'double-displacement' strategy involving an intermediate aziridine. This work was published as part of a total synthesis of motuporin which represents the first synthesis of a microcystin/nodularin natural product.[11]

The general strategy that we adopted involves construction of motuporin and nodularin each from three segments and the microcystins each from four segments (Scheme 1). Using this approach, nodularin and motuporin can be prepared in a similar manner by replacing the β-methyl-D-aspartyl-L-arginine dipeptide **1** by β-methyl-D-aspartyl-L-valine **2**. Changing glutamyl-L-threonine trimethylsilylethyl ester to D-glutamyl-L-serine trimethylsilylethyl ester, and incorporation of the additional dipeptide **5**, redirects the synthesis to provide access to microcystin-LR. Synthesis of each of these fragments has been completed in preparation for modular assembly of the natural products.

Our route to *N*-Boc-Adda has been described separately.[12] Using similar chemistry, it is possible to construct β-methylaspartate methyl ester in 5 steps from (*S*)-pent-3-yn-2-ol (Scheme 2). Acylation of the alcohol using *N*-Boc-glycine, followed by reduction of the alkyne over Lindlar's catalyst provides alkene **6**. Treatment of this ester with LDA in the presence of zinc chloride promotes a highly stereoselective Claisen rearrangement,[14] and the product acid is converted to its methyl ester **7** using diazomethane. Oxidation of the alkene provides β-methyl-D-aspartate α-methyl ester **8** in 51% overall yield. Condensation of this acid with either valine allyl ester or *N*ω-MTR-arginine allyl ester is straightforward providing the required dipeptides **1** and **2** in 68 and 75% yields respectively.

In an alternative route, Scheme 3, methylation of the protected aspartate **9** using LHMDS and methyl iodide gives a difficult to separate 6:4 mixture of 3-alkylated products, as reported by previous workers.[10,12] This mixture may be converted in two steps to the α-pentafluorophenyl esters **10**,



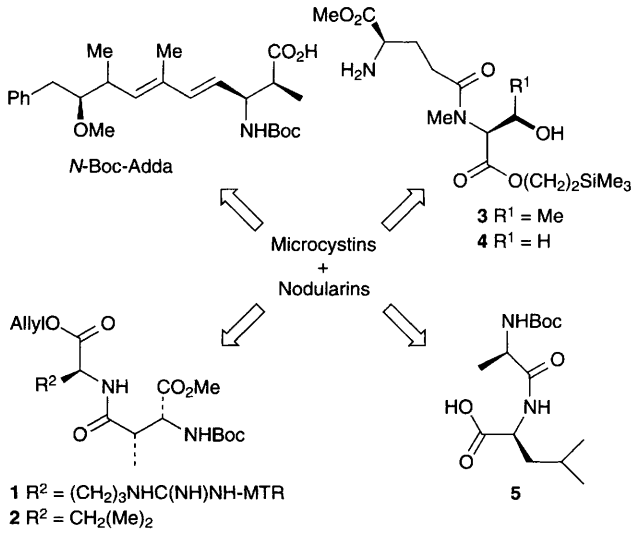
Microcystin-LR

$R^2 = (CH_2)_3NHC(NH)NH_2$

Nodularin $R^3 = (CH_2)_3NHC(NH)NH_2$

Motuporin $R^3 = CH_2CH(Me)_2$

Fig. 1 A representative microcystin and two related pentapeptides

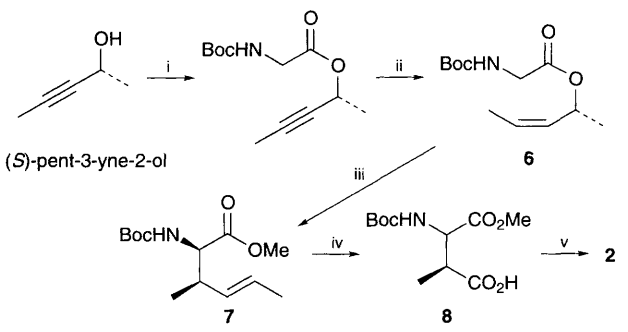


Scheme 1 Retrosynthetic analysis

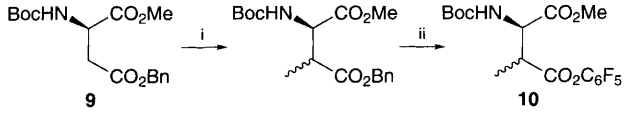
which are readily separable by column chromatography. Peptide **1** was obtained from the less polar pentafluorophenyl ester in 82% yield by coupling with *N*ω-MTR-arginine allyl ester. This approach has the advantage of brevity even though it is less stereoselective.

N-Z-D-glutamyl-L-threonine trimethylsilylethyl ester **3** was prepared starting from *N*-Boc-*O*-benzylthreonine. The protected threonine was methylated using sodium hydride and methyl iodide,[15] then converted to its trimethysilylethyl ester using DCC and deprotected to provide the secondary amine **11**, as its TFA salt (Scheme 4). *N*-Z-D-glutamic acid was converted to its α-methyl ester using the procedure of Sawayama and coworkers,[16] then coupled to the protected *N*-methyl threonine using diphenylphosphinic chloride. Simultaneous removal of the Z and benzyl protecting groups was achieved by transfer hydrogenolysis using formic acid over palladium-black in methanol. The related dipeptide *N*-Z-D-glutamyl-L-serine was prepared similarly (Scheme 4), as a precursor to didehydroalanine. The final fragment, *N*-Boc-D-alanyl-L-leucine was prepared uneventfully from *N*-*tert*-butoxycarbonyl-D-alanine and leucine benzyl ester using a DCC coupling followed by deprotection of the carboxylic acid in 73% overall yield.

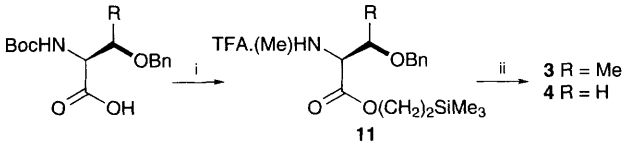
In conclusion, all the fragments required for convergent synthesis of motuporin, nodularin and microcystin-LR have been generated in preparation for exploratory studies of the proposed synthetic route. Completion of the synthesis of representative microcystin and nodularin natural products will be reported in due course.



Scheme 2 *Reagents and conditions*: i, *N*-Boc-glycine, DCC, DMAP, 95%; ii, H_2, Pd/$CaCO_3$, EtOAc, 91%; iii, (*a*) LDA, $ZnCl_2$, THF; (*b*) CH_2N_2, 72%; iv, $RuCl_3{\cdot}H_2O$, $NaIO_4$, CCl_4–MeCN–H_2O 1 : 1 : 1.5, 83%; v, isobutyl chloroformate, valine allyl ester, NMM, 68%



Scheme 3 *Reagents and conditions*: i, 2.1 equiv. LHMDS, MeI, THF, 90%; ii, (*a*) H_2, Pd/C; (*b*) C_6F_5OH, DCC, CH_2Cl_2, 90%



Scheme 4 *Reagents and conditions*: i, (*a*) NaH, MeI, THF, (R = Me, 73%; R = H, 86%); (*b*) DCC, $Me_3Si(CH_2)_2OH$, DMAP, pyridine, CH_2Cl_2, (R = Me, 65%; R = H, 67%); (*c*) TFA–CH_2Cl_2; ii, (*a*) *N*-Z-D-glutamate α-methyl ester, $Ph_2P(O)Cl$, NMM, CH_2Cl_2, (R = Me, 61%; R = H, 52%); (*b*) H_2, Pd(black), HCO_2H, MeOH, (R = Me, 95%; R = H, 60%)

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Footnote

† *Abbreviations*: Adda = (2*S*,3*S*,8*S*,9*S*,4*E*,6*E*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid; LHMDS = lithium hexamethyldisylamide; MTR = 4-methoxy-2,3,6-trimethylbenzenesulfonyl; TFA = trifluoroacetic acid.

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