Enantiospecific Total Synthesis of the Protein Phosphatase Inhibitor Motuporin

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Protein serine/threonine phosphatases (PSPs) play a critical role in intracellular signaling processes and are sensitive to a diverse set of natural products.[1] Examples of small molecules displaying inhibitory activity against class 1, 2A, or 2B protein phosphatases (PPs) include okadaic acid,[2] the microcystins,[3] calyculin A,[4] nodularin,[5] tautomycin,[6] and cantharidine,[7] as well as the complexes of FK506 and cyclosporin A with their cognate immunophilins.[8] Recently, enzyme assay-guided screening and fractionation of crude extracts from the marine sponge *Theonella swinhoei* Gray led to the isolation of the extremely potent PP-1 inhibitor motuporin (**1**).[9] Motuporin (Figure 1) is a cyclic pentapeptide that belongs to a family of structurally related toxins, including the microcystins and nodularin (**2**), that are characterized by the hallmark C_{20} amino acid (2*S*,3*S*,8*S*,9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid (Adda). As part of our continuing investigations into the mechanisms of signal transduction and our fascination with biologically active peptides derived from marine sponges belonging to the genus *Theonella*,[10] we now report the first total synthesis of motuporin. We designed a convergent and efficient synthesis of motuporin in which all of the target's stereocenters are derived from either common amino acids or D-mandelic acid.

Of the five residues comprising motuporin's macrocycle, only one, valine, is a common α-amino acid linked through its α-amino and α-carboxyl functionalities. The remainder of the molecule is composed of the β-amino acid Adda, the dehydroamino acid (*N*-methylamino)dehydrobutyrate (NMeΔBut), and the two *iso*-linked α-amino acids glutamate and D-*erythro*-β-methylaspartate (β-MeAsp). Although previous syntheses of protected Adda[11] and β-alkylaspartates[12] have been published, we chose to develop a novel route in which each of the three unusual amino acids in motuporin was derived from the same

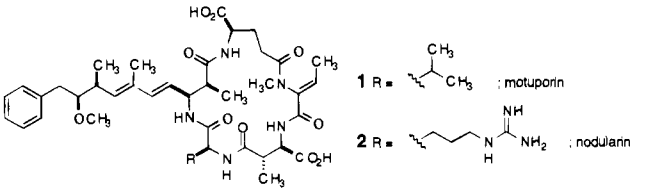

Figure 1.

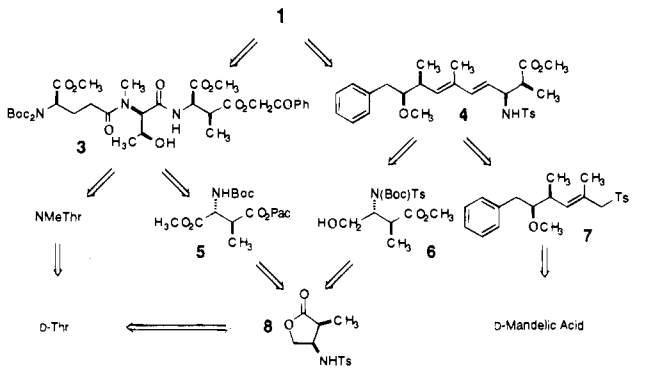

Figure 2.

chiral building block, D-threonine (Figure 2). Adda and β-MeAsp were synthesized by a "double-displacement" strategy and share a common γ-lactone intermediate **8**. The olefin functionality of the NMeΔBut residue is unmasked very late in the synthesis by a β-elimination.

Protected D-threonine was converted to γ-lactone **8** in five steps (Scheme 1) through the intermediate tosylaziridine **9**. This lactone was converted in six steps either to **10**, the backbone portion (C1–C4) of the Adda residue,[13] or to **11**, a differentially protected β-MeAsp derivative (Scheme 1). Protected β-MeAsp **11** was converted to phenacyl (Pac)-protected **5**.

The stereocenters of the Adda side chain (C5–C10) were installed through a Lewis acid promoted crotylstannane addition[14] to benzyloxymethyl-protected mandelaldehyde (Scheme 2).[15] The chain was then elaborated by standard methods[16] to give sulfone **7**. A stereoselective diene-forming coupling of the Adda fragments was achieved through a modified Julia olefination[17] to give protected Adda **4**. When the sulfone-stabilized anion of **7** was added to **10**, an unexpected *in situ* internal Boc group migration to the resulting alkoxide occurred, producing a diastereomeric mixture of β-tosyl *tert*-butoxycarbonate adducts. Treatment of the mixture with sodium naphthalene gave coupling product **4**. Only the (*E*,*E*) isomer of **4**

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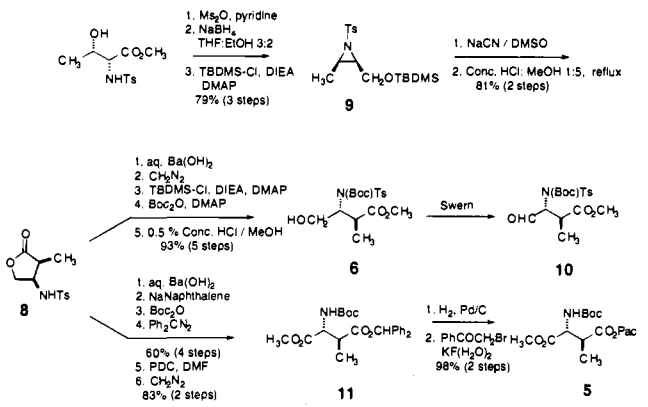
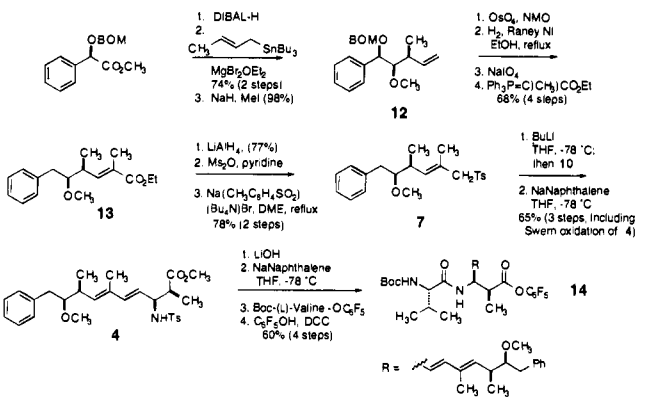
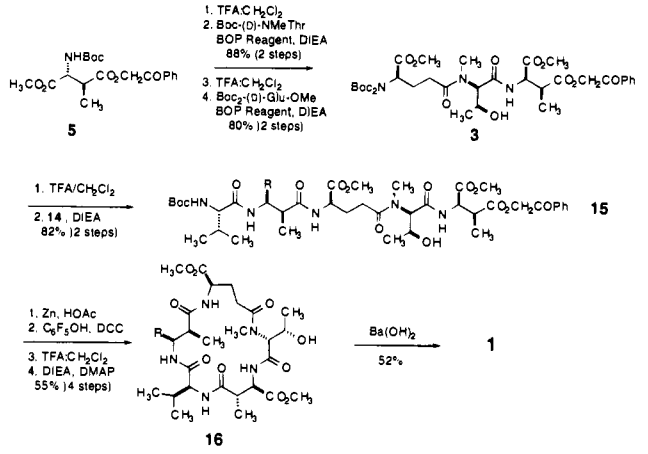
Scheme 1



Scheme 2



Scheme 3



was detected by ^{1}H NMR. Adda ester hydrolysis, amine deprotection, coupling with Boc-L-valine pentafluorophenyl ester, and Adda pentafluorophenyl ester formation produced activated ester **14**. The final acid activation step (DCC, C_6F_5-OH) in this sequence can be avoided by the use of excess Boc-valine pentaflourophenyl ester (5 molar equiv) in the previous coupling step. This modification provides **14** directly, albeit in somewhat lower yield (45%).

β-MeAsp **5** was deprotected and coupled to *N*-methyl-D-threonine ((benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent), diisopropylethylamine (DIEA)). Deprotection of the *N*-Boc group of the resulting dipeptide, followed by coupling to protected D-glutamate, gave tripeptide **3** (Scheme 3). The presence of several amino acid linkages lacking an α-amido substituent in motuporin's peptide backbone makes a convergent "fragment coupling" strategy possible without the usual complication of epimerization. Boc-amine deprotection of tripeptide **3**, followed by neutralization and treatment with the dipeptide, activated ester **14**, provided the desired protected pentapeptide **15**. Macrolactamization was achieved by a four-step process. Reductive removal of the phenacyl group was followed by pentafluorophenyl ester formation at the C-terminus. N-Terminal Boc group deprotection, followed by dilution and neutralization with excess Hünig's base, gave the desired macrocycle **16** in 55% yield. Concurrent *N*-methylthreonine dehydration and double methyl ester deprotection with aqueous $Ba(OH)_2$ produced motuporin (**1**) in 52% yield upon acidification with dilute HCl and HPLC purification. The disodium salt of motuporin was generated by base treatment ($NaHCO_3/CH_3OH$) and desalted by gel filtration chromatography on LH-20. The ^{1}H NMR spectrum of motuporin (CD_3OD, 298 K) is very dependent on pH, and it is only the spectrum of the disodium salt that matches the corresponding spectrum of an authentic natural sample.

Observations made during the late stages of the synthesis provided several notable insights into the structure of motuporin, particularly regarding the conformation of the NMeΔBut residue. Complete conversion of *N*-methyl-D-threonine to NMeΔBut under the final alkaline aqueous conditions used for methyl ester deprotection was unexpected. Evidence that the resulting olefin geometry is thermodynamically controlled was observed. Under basic conditions similar to those used in the dehydration step (saturated $NaHCO_3/CD_3OD$), solvent exchange of motuporin's NMeΔBut allylic methyl protons was observed to occur with complete retention of olefin geometry. Assuming free rotation about the $\alpha-\beta$ bond of the intermediate anion, this implied that the product olefin geometry was preferred thermodynamically. ^{1}H NMR data also indicated the importance of the dehydroamino acid functionality in forcing constraints on motuporin's solution structure. The ^{1}H NMR spectrum ($CDCl_3$, 298 K) of motuporin's dimethyl ester is reported to exhibit only one set of sharp peaks, while macrocycle **16**, differing only by the formal hydration of NMeΔBut to *N*-methyl-D-threonine, displayed two distinct sets of broad resonances (5:1; $CDCl_3$, 298 K) characteristic of slow amide-bond rotamer exchange on the ^{1}H NMR time scale. The resonances only coalesce at temperatures above 383 K (DMSO-d_6).

This first total synthesis of motuporin provides access to ample quantities of the natural product for structural and enzymological studies, as well as for chemical and cell biological investigations of the role of PSPs in the regulation of intracellular signaling pathways. Further studies on motuporin's solution conformation, inhibitory potency, and enzyme specificity are underway.

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Supporting Information Available: Complete physical and spectral data for compounds **3**–**9**, **11**–**16**, and (−)-motuporin and comparison ^{1}H NMR spectra of natural and synthetic motuporin (38 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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