Natural Products

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Total Synthesis of Salinamide A: A Potent Anti-Inflammatory Bicyclic Depsipeptide**

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Salinamide A (**1**; Figure 1) is a bicyclic hexadepsipeptide that was first isolated from *Streptomyces* sp. CNB-091, which is an actinomycete found on the surface of jellyfish collected in the

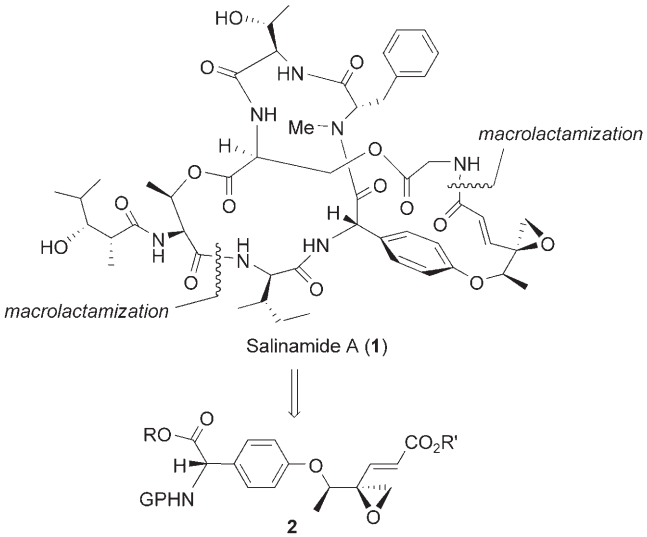
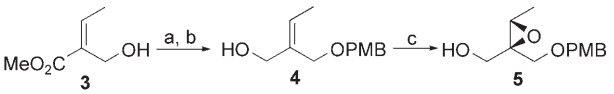


Figure 1. Structure of salinamide A and its retrosynthetic analysis.

Florida Keys.[1] It was also found in an edaphic *Streptomyces* strain (NRRL 21611) isolated from a soil sample.[2] Biological evaluations revealed that salinamide A exhibits strong inhibitory activity ($IC_{50} = 0.5\ \mu M$) against bacterial RNA polymerases without affecting human RNA polymerases;[2] it was also shown to have potent topical anti-inflammatory activity by using the phorbol ester-induced mouse ear edema assay.[1] A similarly potent anti-inflammatory activity was also observed in some members of cyclomarins[3] and halipeptins,[4] two classes of depsipeptides that were recently isolated from marine microorganisms and sponges. Considerable structural differences between these depsipeptides and clinically used anti-inflammatory drugs imply that these natural anti-inflammatory agents might have novel modes of action. A total synthesis and structure-activity relationship (SAR) studies of these depsipeptides would be helpful for fully addressing this question, thereby providing the opportunity to develop a new generation of anti-inflammatory drugs. Whereas cyclomarins[5] and halipeptins[6] have become attractive targets for the synthetic community, salinamide A has not yet been synthetically accessible. Herein, we disclose the first total synthesis of this natural product.

Upon close examination of the structure of salinamide A, which was established by X-ray analysis of its chlorohydrin derivative,[1] we retrosynthetically disconnected the α,β-unsaturated amide bond first to convert the rigid bicyclic molecule into a monocyclic hexadepsipeptide that is similar to the proposed biosynthetic intermediate.[1b,7] After additional disconnections it was evident that the molecule is comprised of several natural and unnatural amino acid residues. There are two fragments with unprecendented structural features among natural products:[2] the β-hydroxy acid unit and the complex phenylglycine-derived epoxide fragment (**2**). The high sensitivity of this epoxide fragment required careful consideration in terms of deprotection strategies that were mild and orthogonal to ring opening. Thus, the development of an effective protocol for the assembly of this moiety became our first task. We planned to employ an epoxide ring-opening strategy to create the aryl/alkyl ether bond.

The synthetic route to requisite epoxide **5** is illustrated in Scheme 1. The known alcohol (**3**)[8] was protected with a *p*-methoxybenzyl (PMB) group under acidic conditions, and then reduced with DIBAL-H to afford allyl alcohol **4**. Subjecting **4** to the Sharpless asymmetric epoxidation[9] provided **5** in 88% yield and with greater than 95% *ee*.



Scheme 1. Reagents and conditions: a) PMBO(C=NH)CCl_3, CSA, CH_2Cl_2, 0°C–RT; b) diisobutylaluminum hydride, THF, −78°C, 90% for 2 steps; c) Ti(O*i*Pr)$_4$, (+)-DIPT, *t*BuOOH, CH_2Cl_2, −20°C, 88% yield, >95% *ee*. PMB = *p*-methoxybenzyl; CSA = camphersulphonic acid; DIPT = diisopropyl tartrate.

The ring opening of **5** with a suitable phenylglycine derivative proved to be a major challenge in the course of our total synthesis. Initially, ester **6a** was utilized as a coupling partner (Scheme 2), but no reaction occurred under Lewis acid mediated conditions (Ti(O*i*Pr)$_4$).[10a] Basic conditions,[10b]

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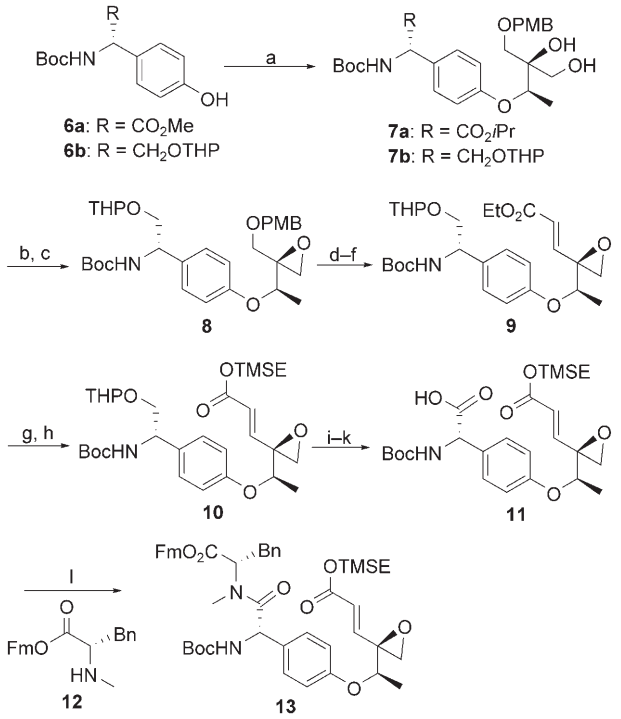
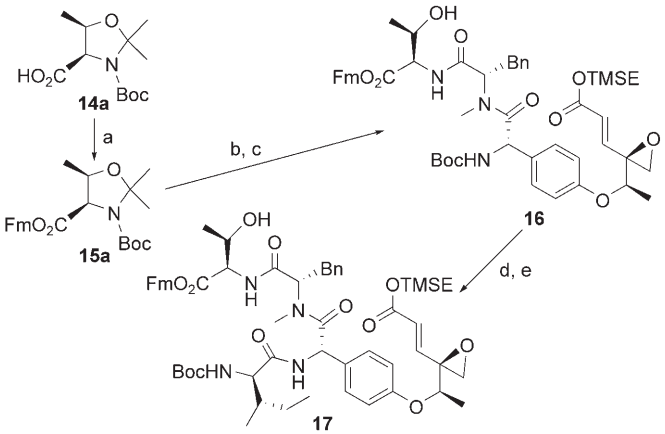
in which DMF was the solvent and either CsF, NaH, or K_2CO_3 was the base, gave rise to complex product mixtures in all cases. However, the desired coupling product **7a** could be isolated in 20% yield after refluxing a mixture of **5**, **6a**, and K_2CO_3 in *i*PrOH.[11] We were unable to improve this yield by optimizing the reaction conditions, and therefore decided to switch the coupling partner to **6b**. To our delight, ring opening proceeded smoothly to afford **7b** in 95% yield. This result was rationalized by the fact that changing the ester group to a protected hydroxymethylene group not only enhanced the substrate stability, but also increased the nucleophilicity of the phenol. Next, diol **7b** was converted into a mesylate and then into epoxide **8** in 92% overall yield. Oxidative removal of the PMB group in **8** by using DDQ and subsequent Dess–Martin oxidation of the liberated primary alcohol furnished an aldehyde; this aldehyde was then subjected to a Horner–Wadsworth–Emmons (HWE) reaction to afford α,β-unsaturated ester **9**. To facilitate cleavage of the ester in the later stages of the synthesis, the ethyl ester in **9** was converted into a trimethylsilylethyl ester to yield **10**. Finally, treatment of **10** with PPTS in methanol, oxidation of the liberated alcohol to the aldehyde, and further oxidation by using $NaClO_2$ furnished acid **11**. Compound **11**, which was activated as mixed acid anhydride, was coupled to amino ester **12** to give dipeptide **13**.[12] Any attempt to couple a more advanced di- or tripeptide to **11** resulted in very low yields or significant epimerization; a variety of other coupling reagents and conditions did not give any satisfactory results.



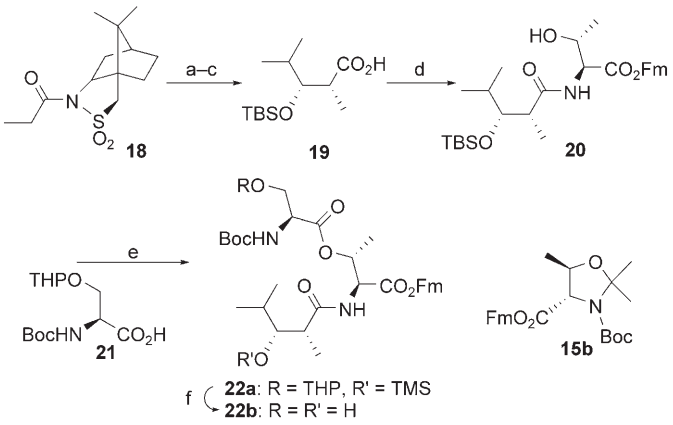
Scheme 2. Reagents and conditions: a) K_2CO_3, *i*PrOH, reflux, 20% yield for **7a**, 95% yield for **7b**; b) MsCl, Et_3N, THF, 0°C; c) K_2CO_3, DMF, 92% yield for 2 steps; d) DDQ, CH_2Cl_2, pH 7 buffer, 89%; e) Dess–Martin oxidation; f) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF, 89% yield for 2 steps; g) LiOH, H_2O, MeOH, THF; h) TMSEOH, EDC, DMAP, CH_2Cl_2, 65% yield for 2 steps; i) PPTS, MeOH, 95%; j) Dess–Martin oxidation; k) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*BuOH, H_2O; l) $ClCO_2iBu$, NMM, CH_2Cl_2, 50% yield from **10**. DDQ = 2,3-dichloro-5,6-dicyano-l,4-benzoquinone; TMSE = trimethylethyl; EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; DMAP = 4-(dimethylamino)pyridine; PPTS = pyridinium *p*-toluenesulfonate; NMM = *N*-methylmorpholine.

The peptide chain elongation of **13** is depicted in Scheme 3. Esterification of threonine derivative **14a** afforded **15a** in 88% yield and was subsequently exposed to methanolic hydrogen chloride to cleave the ketal and remove the *tert*-butoxycarbonyl (Boc) protecting group to give an amino ester. This amino ester was condensed with the deprotected acid of dipeptide **13** to provide tripeptide **16**. After treatment of **16** with formic acid, the resultant free amine was reacted with *N*-Boc-D-allo-isoleucine to produce tetrapeptide **17** in 68% yield. Notably, the unsaturated epoxide moiety was remarkably sensitive towards some deprotection conditions. Allyl ester deprotection under various conditions led to decomposition (observed in an earlier synthetic route that was later abandoned) and dilute piperidine or other secondary amines in moderately polar solvents led to competitive side reactions.

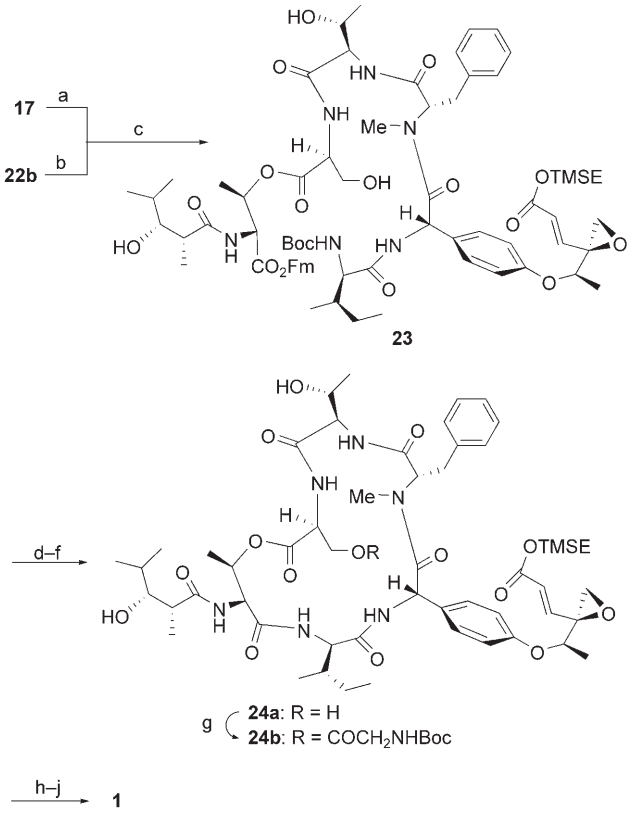


Scheme 3. Reagents and conditions: a) FmOH, MNBA, NEt_3, DMAP, 88%; b) HCl, MeOH; c) Liberated acid from **13** by treatment with Et_3N in aqueous THF, HATU, HOAt, $(iPr)_2NEt$, CH_2Cl_2, DMF, 90% yield from **13**; d) HCO_2H; e) *N*-Boc-D-allo-isoleucine, HATU, HOAt, $(iPr)_2NEt$, CH_2Cl_2, DMF, 68% yield for 2 steps. Fm = 9-fluorenylmethyl; MNBA = 2-methyl-6-nitrobenzoic acid anhydride; HATU = 2-(7-Aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOAT = 1-hydroxy-7-azabenzotriazole.

The elaboration of the desired ester fragment of salinamide A is outlined in Scheme 4. Required TBS-protected β-hydroxy acid **19** was obtained by Oppolzer's *anti*-aldol reaction by using $TiCl_4$ as a catalyst,[13] and then it was subsequently coupled to an amino ester generated from **15b** to provide amide **20**. EDC-mediated esterification of hindered secondary alcohol **20** with acid **21** produced ester **22a** at low temperatures; only subtle epimerization was detected and the epimer could be readily separated by using flash chromatography. Remarkably, **22** was both highly sensitive



Scheme 4. Reagents and conditions: a) Et_2BOTf, $(iPr)_2NEt$, then $TiCl_4$, *i*PrCHO; b) TBSOTf, 2,6-lutidine, CH_2Cl_2; c) LiOH, 30% H_2O_2, THF, 80% yield for 3 steps; d) **15b**, HCl, MeOH, then **19**, HATU, HOAt, $(iPr)_2NEt$, CH_2Cl_2, DMF, 87%; e) **21**, EDC, DMAP, CH_2Cl_2, −5 °C, 80%; f) PPTS, MeOH, 90%.



Scheme 5. Reagents and conditions: a) Et_3N, THF; b) TFA, CH_2Cl_2, c) HATU, HOAt, $(iPr)_2NEt$, CH_2Cl_2, DMF, 80% yield from **17**; d) $HNEt_2$, MeCN; e) TFA; f) HATU, HOAt, $(iPr)_2NEt$, CH_2Cl_2, DMF, 34% yield for 3 steps; g) *N*-Boc-Gly-OH, EDC, DMAP, 82% yield based on 39% recovery of **23**; h) TAS-F, DMF, 87% yield based on 47% recovery of **24b**; i) TFA, j) HATU, HOAt, $(iPr)_2NEt$, DMF, 40% yield for 2 steps. TFA = trifluoroacetic acid; TAS-F = tris(dimethylamino)sulfonium difluorotrimethylsilicate.

to base and easily saponified. Deprotection of **22** with PPTS in methanol afforded diol **22b**.

The connection of tetrapeptide **17** to ester **22b** and the completion of the synthesis are depicted in Scheme 5. Treatment of **17** with triethylamine in dry THF afforded an acid which was then coupled to the amine liberated from **22b** to provide linear peptide **23** in 80% yield. After the C terminus of **23** was carefully unmasked by the action of diethylamine in CH_3CN, cleavage of the N-terminal Boc protecting group gave a linear precursor for the first macrolactamization. Treatment of the amino acid with HATU/HOAt for 3 days provided cyclic depsipeptide **24a** in 34% yield. In this case a racemization product was not isolated and the low yield could possibly result from the degradation of the starting material as some decomposed products were identified.

To set the stage for the final macrocyclization, N-protected glycine was coupled selectively to the primary hydroxy group of the serine residue of **24a** to afford depsipeptide **24b**, completing all the required building blocks. Cautious removal of the TMSE protecting group with tris(dimethylamino)sulfonium difluorotrimethylsilicate (TAS-F)[14] furnished an acid in moderate yield without any detectable β elimination. Then, the N-terminus of the side chain was revealed to afford the final precursor for macrolactamization. According to the proposed biosynthesis[1b,7] the presence of the first macrocycle might provide a favorable conformation for the facilitating the second cyclization; our monocyclic precursor was very similar to the biosynthetic intermediate. Indeed, treatment with HATU/HOAt in DMF for 3 days did afford the rigid bicyclic target compound, salinamide A (**1**), in 40% yield (5.6 mg) over 2 steps, and all analytical data were identical to those previously reported.[1b]

In conclusion, we have achieved the first total synthesis of salinamide A (1% overall yield for 28 linear steps), which features a concise elaboration of its phenylglycine-derived epoxide fragment **2**, and the identification of two possible macrolactamization sites. This concise synthetic route could provide access to salinamide A analogues to be used to investigate the source of its anti-inflammatory properties. Additional studies in this direction are actively being pursued in our laboratory, and will be reported in due course.

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