Total Synthesis of the Novel, Immunosuppressive Agent (−)-Pateamine A from *Mycale* sp. Employing a β-Lactam-Based Macrocyclization

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Immunosuppressive natural products such as cyclosporin A, FK506, rapamycin, deoxyspergualin, and didemnin B have proven to be useful tools for dissection of cell signaling pathways.[1] Moreover, these studies have recently culminated in the synthesis of agents that exert cell and target specific responses.[2] Pateamine A (**1**) is a structurally novel and potent immunosuppressive natural product isolated from *Mycale sp.* by Munro and Blunt off the coasts of New Zealand.[3] It uniquely combines a thiazole ring and an *E,Z*-dienoate within a bis-lactone macrocycle bearing an *all-E* trienylamine side chain. This novel array of functionality makes pateamine A structurally dissimilar to other known immunosuppressants. As a first step toward elucidating the origins of its cellular effects, we now report a convergent, stereochemically flexible route to pateamine A,[4] wherein a β-lactam ring was strategically implemented to introduce the C3 amino group and to serve as an activated acyl group for macrocyclization.[5] We have previously described the synthesis of the C18−C24 fragment of pateamine A which aided in the determination of the C24 absolute stereochemistry in collaborative studies with Munro and co-workers.[6] Importantly, this assignment in conjunction with molecular modeling, extensive NMR studies, and further chemical derivitization by the New Zealand group provided a tentative stereochemical assignment of (−)-pateamine A as 3*R*, 5*S*, 10*S*, 24*S*.[7] With this information in hand, we embarked on a total synthesis that has now verified the stereochemical assignment and has enabled the synthesis of derivatives for further biological studies.

Several issues guided our retrosynthetic plan (Figure 1). These included the known lability of the C3 amino group,[3] the isomerization-prone *E,Z*-dienoate, the desire to incorporate and liberate the polar amino groups at a late stage in the synthesis, the flexibility of attaching various side chains to the macrocyclic core structure, and finally the uncertainty of the stereochemical assignment. With this last consideration in mind and due to the distance between stereocenters, we resolved to introduce the four stereocenters by reagent control. In addition, we realized that some inherent flexibility was built into the synthetic plan since

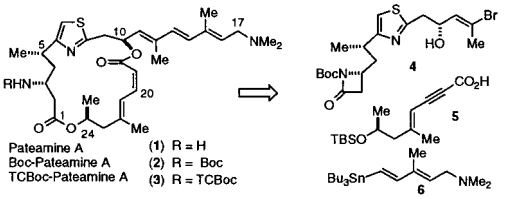
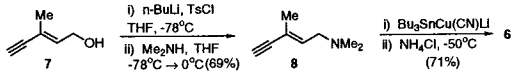


Figure 1. Retrosynthetic analysis of pateamine A and structures of Boc- and TCBoc-pateamine A.

Scheme 1



the stereochemistry at C10 could be readily retained (acylation) or inverted (Mitsunobu[8]) during the joining of β-lactam **4** and enyne acid **5**. The end-game strategy would rely upon a β-lactam-based macrocyclization to esterify the C24 alcohol and a Stille coupling to append the trienylamine side chain. Concise and efficient syntheses of β-lactam **4**, enyne acid **5**,[9] and the dienylamino stannane **6** were required at the outset.

The dienylamino stannane **6** was readily prepared in two steps from enyne alcohol **7** (Scheme 1).[10] A one-pot tosylation and displacement with dimethylamine provided the enyne amine **8**.[11] Stannylcupration of this alkyne by the method of Oehlschlager gave the desired stannane **6** as a mixture of regioisomers which could be enriched in the desired isomer (9:1).[12]

The synthesis of β-lactam **4** commenced with a Nagao acetate aldol reaction[13] between aldehyde **10**[14] and the (*S*)-valinol-derived thiazolidinone **9** (diastereomeric ratio (dr) > 19:1; Scheme 2). Following alcohol protection, aminolysis gave the amide **11** which was then converted to the corresponding thioamide with the Belleau reagent.[15] A modified Hantzsch thiazole synthesis[16] delivered thiazole ester **12**. Half-reduction of this ester followed by a Wadsworth−Emmons reaction simultaneously homologated and introduced the (*S*)-phenylglycine-derived auxiliary required for an asymmetric conjugate addition.[17] Introduction of the C5 methyl group by the method of Hruby[18] proceeded smoothly to give a mixture of methyl adducts (dr 6.4:1) from which the major diastereomer could be isolated in 77% yield. Transamidation delivered the Weinreb amide **15** with the desired stereochemistries at C5 and C10.[19] What remained was installation of the β-lactam, and to accomplish this task, we were attracted to the method of Miller involving an intramolecular Mitsunobu reaction of an aldol product.[20] In the event, half-reduction of Weinreb amide **15** to aldehyde **16** followed by a Nagao acetate aldol reaction gave the

(1) Hung, D. T.; Jamison, T. F.; Schreiber, S. L. *Chem. Biol.* **1996**, *3*, 623−639.

(2) Diver, S. T.; Schreiber, S. L. *J. Am. Chem. Soc.* **1997**, *119*, 5106−5109.

(3) Northcote, P. T.; Blunt, J. W.; Munro, M. H. G. *Tetrahedron Lett.* **1991**, *32*, 6411−6414. This initial paper on pateamine A reported antifungal and selective cytotoxic activity. Immunosuppressive activity was found by Dr. Glynn Faircloth, PharmaMar Inc., Cambridge, MA (private communication): MLR (mixed lymphocyte reaction) $IC_{50} = 2.6$ nM; LCV (lymphocyte viability assay)/MLR ratio >1000.

(4) For other synthetic studies of pateamine A, see: Critcher, D. J.; Pattenden, G. *Tetrahedron Lett.* **1996**, *37*, 9107−9110.

(5) For the use of β-lactams in intermolecular acylations, see: (a) Ojima, I. In *The Organic Chemistry of β-Lactams*; Georg, G. I., Ed.; VCH Publishers: New York, 1992; Chapter 4, pp 197−255. (b) Ojima, I. *Acc. Chem. Res* **1995**, *28*, 383−389. For an intramolecular acylhydrazine addition to a β-lactam forming a 10-membered ring, see: (c) Gardner, B.; Nakanishi, H.; Kahn, M. *Tetrahedron* **1993**, *49*, 3433−3448. For transamidations of β-lactams to medium-sized rings, see: (d) Hesse, M. *Ring Enlargement in Organic Chemistry*; VCH: New York, 1991; Chapter VI.

(6) Rzasa, R.; Romo, D.; Stirling, D. J.; Blunt, J. W.; Munro, H. M. G. *Tetrahedron Lett.* **1995**, *36*, 5307−5310.

(7) (a) Blincoe, S. N. M.Sc. Thesis, University of Canterbury, New Zealand, 1994. (b) Stirling, D. J. Ph.D. Thesis, University of Canterbury, New Zealand, 1996.

(8) Mitsunobu, O. *Synthesis* **1981**, 1−28.

(9) We have described the synthesis of enyne acid **5**,[6] although the acid used in the present study originated from a Noyori reduction of ethyl acetoacetate and was determined to be 94% enantiomeric excess by chiral GC.

(10) Alcohol **7** was generously provided by Dr. P. Weber (F. Hoffman-La Roche Ltd., Switzerland).

(11) Pisano, J. M.; Firestone, R. A. *Synth. Commun.* **1981**, *11*, 375−378.

(12) Aksela, R.; Oehlschlager, A. C. *Tetrahedron* **1991**, *47*, 1163−1167.

(13) Nagao, Y.; Hagiwara, Y.; Kumagai, T.; Ochiai, M.; Inoue, T.; Hashimoto, K.; Fujita, E. *J. Org. Chem.* **1986**, *51*, 2391−2393.

(14) Prepared by MnO_2 oxidation of the corresponding alcohol, see: Corey, E. J.; Bock, M. G.; Kozikowski, A. P.; Rao, A. V. R.; Floyd, D.; Lipshutz, B. *Tetrahedron Lett.* **1978**, *19*, 1051−1054.

(15) Lajoie, G.; Lepine, F.; Maziak, L.; Belleau, B. *Tetrahedron Lett.* **1983**, *24*, 3815−3818.

(16) Aguilar, E.; Meyers, A. I. *Tetrahedron Lett.* **1994**, *35*, 2473−2476.

(17) This type of homologation has been previously reported with the (*S*)-phenylalanine-derived auxiliary, see: Broka, C. A.; Ehrler, J. *Tetrahedron Lett.* **1991**, *32*, 5907−5910.

(18) Li, G.; Patel, D.; Hruby, V. *Tetrahedron: Asymmetry* **1993**, *4*, 2315−2318.

(19) X-ray analysis performed on an imide obtained as a byproduct during the transamidation reaction verified the C5 and C10 stereochemistry. See the Supporting Information for details.

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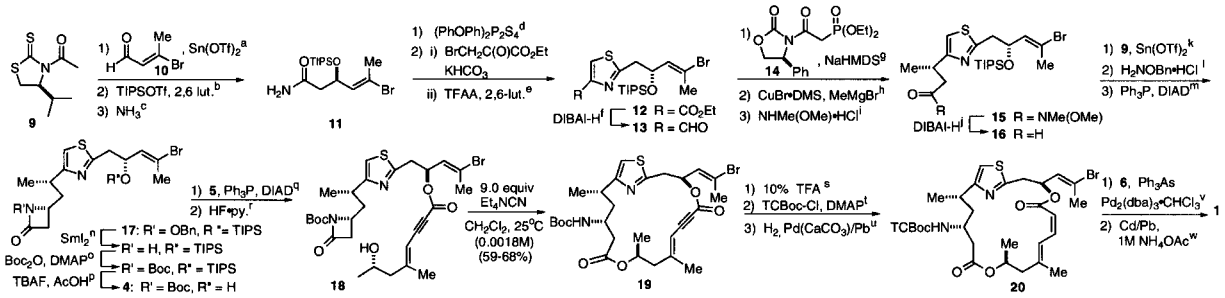
Scheme 2[a]



[a] Key: (a) *N*-ethylpiperidine, CH_2Cl_2, −40 °C (dr >19:1, 84%); (b) CH_2Cl_2, 0 °C (99%); (c) CH_2Cl_2, 0 °C (86%); (d) THF, 25 °C (82%); (e) DME, −20 → 0 °C (94%); (f) CH_2Cl_2, −90 °C (93%); (g) THF, 25 °C (90%); (h) THF/DMS (3:2), −20 °C (dr 6.4:1, 77% isolated, major diastereomer); (i) Me_3Al, CH_2Cl_2, 0 °C (82%); (j) CH_2Cl_2, −90 °C (95%); (k) *N*-ethylpiperidine, CH_2Cl_2, −40 °C (dr, >19:1, 90%); (l) Me_3Al, CH_2Cl_2, 0 °C (90%); (m) THF, 25 °C (92%); (n) THF, 0 °C (96%); (o) CH_2Cl_2 (95%); (p) THF, 0 °C (95%); (q) THF, −20 °C (86%); (r) py, THF, 25 °C (86%); (s) CH_2Cl_2, 0 °C; (t) py, 25 °C; (u) MeOH, 25 °C (3 steps, 50%); (v) 1:4 Pd(15 mol %)/ligand, THF, 25 °C (27%, 57% based on recovered **20**); (w) THF, 25 °C (80%).

desired aldol adduct in 90% yield. Conversion to the *N*-(benzyloxy)amide and intramolecular Mitsunobu reaction gave the β-lactam **17** in excellent overall yield. In preparation for the β-lactam-based macrocyclization, reduction of the N−O bond was cleanly effected with SmI_2[21] in 96% yield and the resulting β-lactam was protected as the *tert*-butyl carbamate.[22] Deprotection of the triisopropylsilyl ether afforded the final key intermediate, β-lactam **4**.

With the required fragments in hand, their coupling to provide pateamine A began by a Mitsunobu coupling between the alcohol **4** and the enyne acid **5** providing the required C10 (*S*)-stereochemistry.[23] Deprotection of the *tert*-butyldimethylsilyl ether gave alcohol **18** and set the stage for the key β-lactam-based macrocyclization. Building on conditions developed by Palomo for the intermolecular alcoholysis of β-lactams employing KCN in DMF, macrocyclization conditions were developed to avoid the use of large volumes of DMF required for high dilution.[24] Use of 9.0 equiv of CH_2Cl_2-soluble Et_4NCN cleanly promoted the β-lactam-based macrocyclization in 59−68% yield at ambient temperature. With the Boc-protected macrocycle **19** in hand, Lindlar reduction followed by Stille coupling (not shown) provided Boc-pateamine A (**2**). Although we have been unable to deprotect this compound without decomposition of the acid-sensitive pateamine A molecule,[25] this derivative allowed us to obtain tentative confirmation of the relative stereochemistry[26] and to obtain some interesting and important, preliminary biological results.[27] Pateamine A was ultimately obtained employing the trichloro-*tert*-butoxycarbamate (TCBoc) protecting group by reasoning that the mild reductive conditions recently reported by Ciufolini for the trichloroethoxy carbamate[28] would be compatible with both the triene and dienoate.[29] A three-step sequence involving acid deprotection of the Boc-macrocycle **19**, reprotection with TCBoc-Cl, and Lindlar reduction provided the TCBoc-macrocycle **20**. Stille coupling of this vinyl bromide and stannane **6** employing the conditions of Farina[30] gave TCBoc-pateamine **3** in 27% yield (57% based on recovered **20**), and deprotection using Cd/Pb couple gave (−)-pateamine A (**1**) in 80% yield. The synthetic material displayed physical and spectral properties including ^1H and ^{13}C NMR, CD, IR, and UV identical to the natural product.

In summary, a convergent synthesis has been developed for (−)-pateamine A. During the course of the synthesis, we found that N−O cleavage of a *N*-(benzyloxy)-β-lactam can be cleanly effected with SmI_2 and that Stille coupling of a vinyl bromide in the presence of an allylic or triallylic acetate can be competitive with π-allyl formation. In addition, a novel β-lactam-based macrocyclization was employed to construct the pateamine A dilactone. This synthesis confirms the stereochemistry as 3*R*, 5*S*, 10*S*, 24*S* and sets the stage for delineation of its mechanism of action by providing access to further quantities of pateamine A and designed structural derivatives.

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Supporting Information Available: Physical and spectral data for compounds **3**−**6**, **11**, **12**, **15**, and **17**−**20**, ^1H and ^{13}C NMR spectra of these intermediates, and selected procedures; spectral comparison of natural and synthetic (−)-pateamine A; and X-ray structure (Chem 3D representation) of transamidation byproduct (33 pages). See any current masthead page for ordering and Internet access instructions.

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(20) Guzzo, P. R.; Miller, M. J. *J. Org. Chem.* **1994**, *59*, 4862−4867.

(21) (a) Keck, G. E.; McHardy, S. F.; Wager, T. T. *Tetrahedron Lett.* **1995**, *41*, 7419−7422. (b) Chiara, J. L.; Destabel, C.; Gallego, P.; Marco-Contelles, J. *J. Org. Chem.* **1996**, *61*, 359−360.

(22) Although we had found in model studies that a *N*-benzyloxy group was sufficient for activation of a β-lactam toward intermolecular, nucleophilic addition of an alkoxide, we reasoned that conversion to a carbamate protecting group would enable the use of a deprotection method that would be compatible with late-stage intermediates (unpublished results of Mr. Joe M. Langehan, Texas A&M, NSF REU student). These findings will be described in detail in a full account of this work.

(23) An inversion process was required at this stage as a result of an early structural misassignment of the C10 stereocenter. (Private communication from Prof. M. H. G. Munro).

(24) Palomo, C.; Aizpurua, H. M.; Cuevas, C.; Mielgo, A.; Galarza, R. *Tetrahedron Lett.* **1995**, *36*, 9027−9030. This macrocyclization presumably proceeds by way of an acyl cyanide, and studies are underway to detect this intermediate in substrates devoid of an internal nucleophile.

(25) While we have not assigned structures to the decomposition products, it appears that the acid instability is due to the triallylic acetate portion of the molecule since deprotection of compounds devoid of this functional array (e.g., **19**) proceeded without incident.

(26) The ^1H NMR of Boc-pateamine A derived from natural material was kindly provided by Prof. Murray Munro and Prof. John Blunt (New Zealand). No additional data were available for this derivative.

(27) Prof. Jun Liu and co-workers (MIT) have found that synthetic Boc-pateamine A (**2**) exhibits only 5−10 times less potency than pateamine A in the human mixed lymphocyte reaction (IC_{50} of 10−20 nM). This result suggests a viable site of attachment for linkers required for the synthesis of hybrid molecules that will be used for putative, cellular target isolation.

(28) Dong, Q.; Anderson, C. E.; Ciufolini, M. A. *Tetrahedron Lett.* **1995**, *36*, 5681−5682. We thank Prof. Ciufolini for helpful discussions concerning these deprotection conditions.

(29) The TCBoc group was chosen over the TROC (trichloroethoxy carbamate) because we are, of course, interested in streamlining the synthesis by introduction of the TCBoc group at the outset (i.e., β-lactam **4**). The TCBoc group will ensure the desired regioselective acylation during the β-lactam-based macrocyclization.

(30) Farina, V.; Krishnan, B. *J. Am. Chem. Soc.* **1991**, *113*, 9585−9595.