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A Concise Formal Total Synthesis of TMC-95A/B Proteasome Inhibitors

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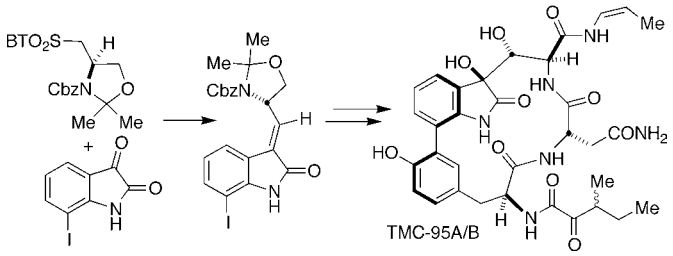
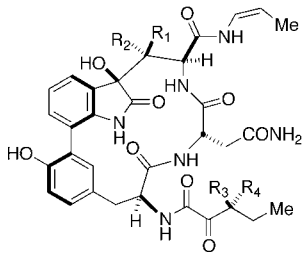
ABSTRACT



A formal total synthesis of proteasome inhibitors TMC-95A/B is described. The synthesis features a stereoselective modified Julia olefination and a diastereoselective dihydroxylation to construct the highly oxidized tryptophan residue.

TMC-95 A−D (**1**−**4**, Figure 1) are potent proteasome inhibitors isolated from the fermentation broth of *Apiospora*



TMC-95	R_1	R_2	R_3	R_4
A (**1**)	H	OH	Me	H
B (**2**)	H	OH	H	Me
C (**3**)	OH	H	Me	H
D (**4**)	OH	H	H	Me

Figure 1. Structures of TMC-95 A−D.

montagnei Sacc. TC 1093, derived from soil samples.[1] These natural products are unique cyclic peptides containing L-tyrosine, L-asparagine, a highly oxidized L-tryptophan, (*Z*)-1-propenylamine, and 3-methyl-2-oxopentanoic acid units. It has been demonstrated that these compounds are biologically active against chymotrypsin-like, trypsin-like, and peptidylglutamyl-peptide hydrolyzing proteases.[1b] Proteasome inhibitors have received considerable attention recently due to the role they play in intracellular processes such as cell progression, antigen presentation, and cytokine-stimulated signal transduction. In addition, proteasome inhibitors are proving to be valuable tools for probing the function of the proteasome in cells.[2]

The great interest emerging in the field of proteasome inhibition, the considerable biological activity, and the distinctive structures of the TMC-95 class of natural products have provided motivation to contemplate a total synthesis of these compounds. Recently, it has been determined that TMC-95A displays noncovalent and reversible inhibition of the proteasome, a mode of action not observed until recently with other inhibitors.[3] With this in mind, our goal was to establish a concise and convergent total synthesis that would be ammenable to the preparation of a variety of analogues that could exploit TMC-95s mode of action. Immediately following the publication of the structures of these novel

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cyclic peptide natural products, significant synthetic activity in this field commenced.[4]

In this paper, we describe a stereocontrolled approach to the core macrocycle of TMC-95A/B. Although the pioneering work of Danishefsky, Hirama, and Ma proved to be an invaluable resource in our synthesis, we felt that the number of synthetic steps[4c] and the lack of stereocontrol in the construction of the oxidized tryptophan moiety had to be addressed.[4a,b,e,f] Our approach is concise and provides a stereocontrolled route to the dihydroxylated oxindole fragment. Also, we have been able to intercept a late-stage intermediate in the Danishefsky synthesis and this report thus constitutes a formal total synthesis of TMC-95A/B.

When contemplating the total synthesis of TMC-95A/B, we felt that these natural products could ultimately be prepared from a macrocylic peptide such as **5** (Figure 2).

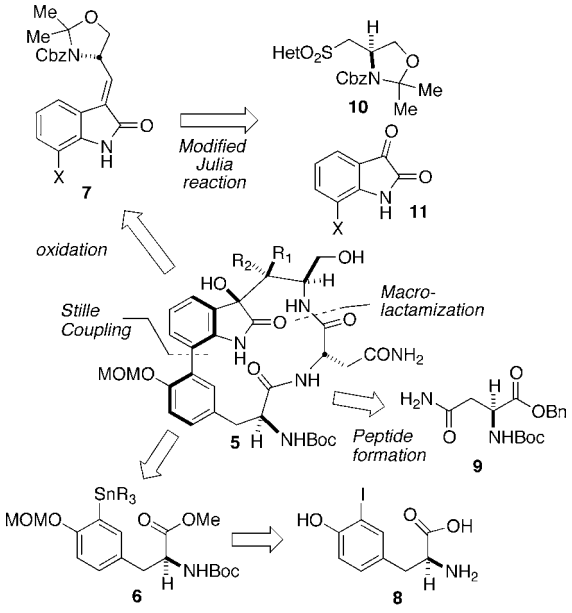
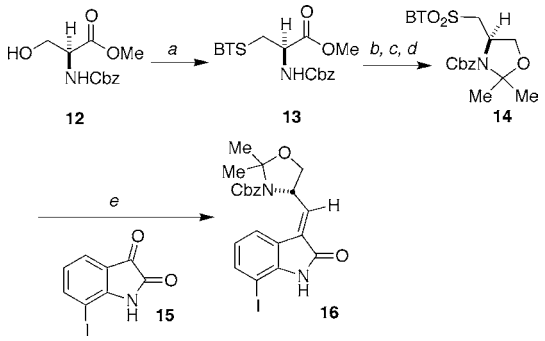


Figure 2. Retrosynthetic Analysis.

Stille coupling[5] of aryl stannane **6** with tryptophan moiety **7** followed by oxidation to the diol, peptide formation with asparagine derivative **9**, deprotection, and macrolactamization was anticipated to furnish the requisite macrocycle **5**. We envisioned that aryl stannane **6** would be derived from commercially available 3-iodotyrosine **8**. Modified Julia[6] olefination of a heteroaromatic sulfone **10** and readily available 7-substituted isatin[7] **11** would in turn furnish oxindolene **7**.

Synthesis of the highly oxidized tryptophan moiety began with treatment of readily available *N*-Cbz-serine methyl ester **12** under Mitsunobu[8] conditions with 2-mercaptobenzathiazole (BTSH), DIAD, and PPh_3 to furnish *S*-heteroaromatic cysteine derivative **13** (Scheme 1). Completion of the

Scheme 1[a]



[a] Reaction conditions: (a) BTSH, DIAD, PPh_3, THF, rt, 89%; (b) $CaCl_2$, $NaBH_4$, THF, 0 °C, and then **13**, 95%; (c) 2,2-dimethoxypropane, *p*-TsOH, CH_2Cl_2, rt; (d) $Mo_7O_{24}(NH_4)_6{\cdot}4H_2O$, H_2O_2, EtOH, 77%, two steps; (e) LiHMDS, DMF, DMPU, 0 °C, 79%, *E*:*Z* = 5:1.

modified Julia coupling partner was accomplished by (1) reduction of the methyl ester with $Ca(BH_4)_2$, (2) blocking of the carbamate nitrogen and the primary alcohol as the acetonide with DMP and *p*-toluenesulfonic acid, and (3) oxidation[9] of the thioether to sulfone **14**.

Next, our efforts were focused on optimizing the modified Julia coupling reaction between sulfone **14** and 7-iodoisatin **15**. It was determined that conditions similar to those reported by Jacobsen[10] and co-workers gave the best selectivity in the modified Julia olefination, furnishing the desired oxindolene **16** in 79% yield. By increasing the reaction temperature to 0 °C, we were able to increase the selectivity to 5:1 (*E*:*Z*), yielding the thermodynamically favored product.

With alkene **16** and aryl stannane **6**[4d] in hand, attempts were made at constructing the biaryl moiety of the TMC-95 proteasome inhibitors under the Stille conditions developed earlier in our laboratory.[4d] Despite extensive experimentation, we found that numerous combinations of Pd-catalyst and ligand gave unsatisfactory yields of biaryl product **18**

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(6) (a) Blakemore, P. R.; Cole, W. J.; Kocienski, P. J.; Morley, A. *Synlett* **1998**, 28. (b) Baudin, J. B.; Hareau, G.; Julia, S. A.; Ruel, O. *Tetrahedron Lett.* **1991**, *32*, 1175. (c) Julia, M.; Paris, J.-M. *Tetrahedron Lett.* **1973**, *14*, 4833.

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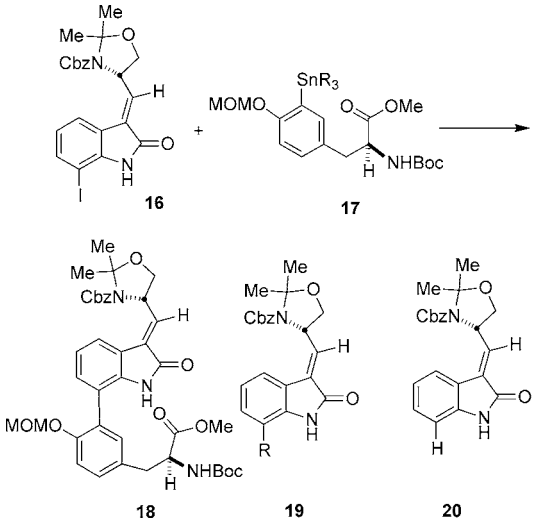
(8) Mitsunobu, O. *Synthesis* **1981**, 1.

(9) Schultz, H. S.; Freyermuth, H. B.; Buc, S. R. *J. Org. Chem.* **1963**, *28*, 1140.

(10) Liu, P.; Jacobsen, E. N. *J. Am. Chem. Soc.* **2001**, *123*, 10772.

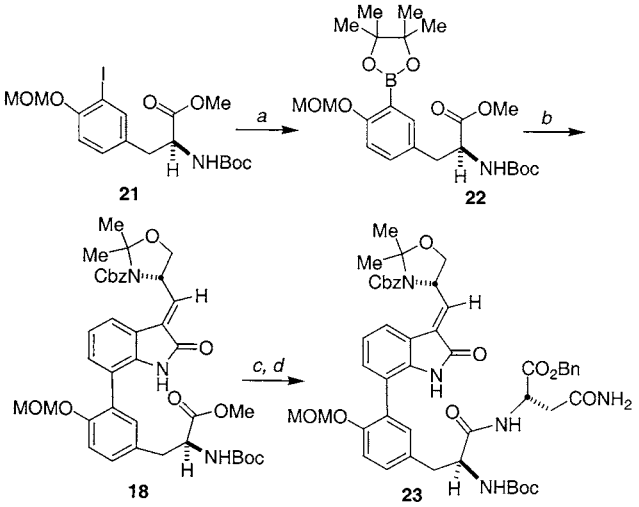
Scheme 2



(Scheme 2). The best isolated yield of coupled product **18** was ~20%, which was routinely accompanied by side-products resulting from alkyl group transfer from the stannane (**19**) and reductive removal of the iodine atom (**20**). Due to the fact that the Stille coupling gave undesired side products and insufficient yields, we decided that the Suzuki[4a,b,11] coupling was the next logical choice for constructing the biaryl bond.

Treatment of tyrosine derivative **21**[4d] with bis(pinacolato)-diboron, $Pd(dppf)Cl_2$, and KOAc in DMSO via the Miyaura protocol[12] gave boronic ester **22** (Scheme 3). Treatment of boronic ester **22** under Suzuki conditions with aryl iodide **16** and K_2CO_3 in refluxing aqueous DME catalyzed by $Pd(dppf)Cl_2$ gave the desired biaryl product **18** in 90% yield.

Scheme 3[a]

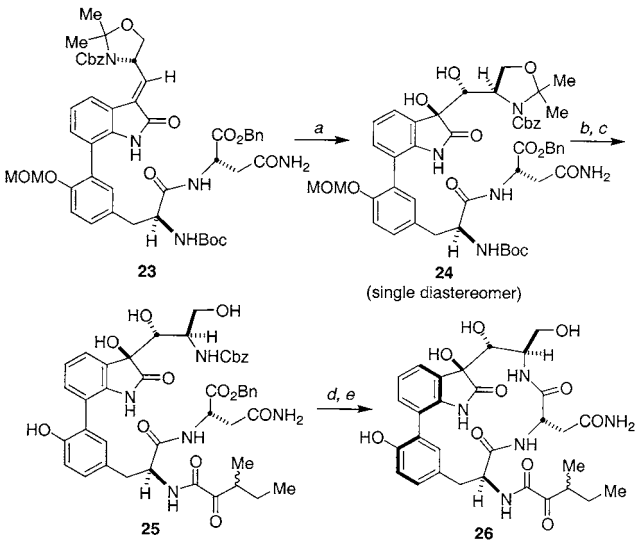


[a] Reaction conditions: (a) bis(pinacolato)diboron, KOAc, Pd(dppf)Cl_2, DMSO, 80 °C, 4 h, (80%); (b) **16**, K_2CO_3, $Pd(dppf)Cl_2$, aqueous DME, 90%; (c) LiOH, THF, H_2O, 0 °C; (d) H_2N−Asn−OBn, HOAt, EDCI, NMM, CH_2Cl_2, 0 °C, 4 h, 98%, two steps.

Incorporation of the asparagine residue was readily accomplished first via saponification of methyl ester **18.** The resulting carboxylic acid was coupled to NH_2−Asn−OBn ester[13] mediated by HOAt and EDCI in CH_2Cl_2 to give the pseudotripeptide **23** (98% yield from **18**).

Treatment of alkene **23** with OsO_4 in aqueous pyridine afforded diol **24** as a single diastereomer in 87% yield (Scheme 4). Treatment of this substance with a 1:1 mixture

Scheme 4[a]



[a] Reaction conditions: (a) OsO_4, py., H_2O, 0 °C, then $NaHSO_3$, THF, MeOH, (87%); (b) TFA, H_2O, 1:1; (c) 3-methyl-2-oxopentanoic acid, HOAt, EDCI, THF, 98%, two steps; (d) Pd black, H_2, EtOH; (e) EDCI, HOAt, CH_2Cl_2, DMF (1:1), 1 μM, 49%, two steps.

of trifluoroacetic acid−water resulted in the liberation of the acid-labile protecting groups. Coupling of the resultant free amine salt with *d,l*-3-methyl-2-oxo-pentanoic acid gave ketoamide **25** as a mixture of inseparable diastereomers. Since it is known that the ketoamide residue is labile to epimerization,[4] no attempt was made to effect the coupling of either (*R*)- or (*S*)-3-methyl-2-oxo-pentanoic acid because the same diastereomeric mixture would result.

Hydrogenolysis of the benzyloxy carbamate and the benzyl ester residues of **25** produced the requisite amino acid substrate for macrocyclization. Subjecting this substance to EDCI and HOAt afforded the TMC-95 macrocyclic core structure **26** in 49% overall yield from **25**. It was previously known[4a,b] that macrocyclization would only provide the desired atropisomer; therefore, this was of no synthetic concern. The structure of this substance was secured by conversion into a late-stage intermediate reported by Lin and Danishefsky.[4a]

The completion of the formal synthesis was accomplished by treatment of macrocyclic tetraol **26** with TESOTf and

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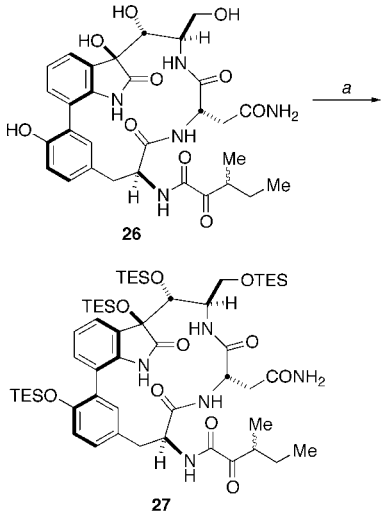
Scheme 5[a]



[a] Reaction conditions: (a) TESOTf, 2,6-lutidine, CH_2Cl_2, DMF, from 0 °C to rt, 12 h, ~40%.

2,6-lutidine to afford **27** in approximately 40% isolated yield (Scheme 5). The ^{1}H NMR spectral characteristics of this substance exactly matched those of the ^{1}H NMR spectrum kindly provided to us by Professor Danishefsky (see Supporting Information).[4a]

In summary, we have effectively applied a stereoselective modified Julia olefination reaction, followed by a diastereoselective dihydroxylation and macrocyclization with limited use of protecting group chemistry as key transformations in a concise formal total synthesis of the TMC-95 A/B proteasome inhibitors. It is felt that an efficient total synthesis of TMC-95A/B can be accomplished via elaboration of the unprotected macrocycle **26**. These efforts along with studies focused on preparing novel TMC-95 analogues are currently under investigation in our laboratories.

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Supporting Information Available: Complete spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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