A concise, total synthesis of the TMC-95A/B proteasome inhibitors

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A concise, total synthesis of the proteasome inhibitors TMC-95A/B has been accomplished. The synthesis features the use of an L-serine-derived E-selective modified Julia olefination reaction to ultimately control the stereochemical outcome of the highly oxidized tryptophan fragment. Additionally, the limited use of protecting groups at a late stage of the total synthesis allowed for its completion in an efficient manner.

Julia olefination | Suzuki biaryl coupling | Stille coupling

The ubiquitin-proteasome pathway is an ATP-dependent pathway discovered >20 years ago and is the major proteolytic pathway in the cytosol and nucleus of all eukaryotic cells (ref. 1 and references within). Initial studies focused on understanding the importance of this pathway in the regulation of cellular processes and benefited from biological studies in extracts of mammalian cells and genetic studies in yeasts (2). It was not until the development or isolation of cell-permeable proteasome inhibitors that the physiological roles of the proteasome were understood. These findings have shown that the proteasome catalyzes the degradation of the majority of mammalian proteins, both short- and long-lived (3, 4). The proteosomal degradation of a large variety of cellular proteins is vital to many of the intracellular processes such as cell-cycle progression, apoptosis, inflammation, immune surveillance, selective removal of misfolded or damaged proteins, and the regulation of metabolic pathways (ref. 1 and references within). Therefore, specific proteasome inhibitors are of great interest not only for use as a tool for understanding the ubiquitin-proteasome pathway but also as potential drug candidates.

In early 2000, Kohno and coworkers (5) reported the isolation of novel cyclic tripeptides TMC-95A–D (1–4) (Fig. 1). TMC-95A–D are potent proteasome inhibitors isolated from the fermentation broth of Apiospora montagnei Sacc. TC 1093, derived from soil samples. These natural products are unique cyclic peptides containing L-tyrosine, L-asparagine, and a highly oxidized L-tryptophan fragment. Additionally, the limited use of protecting groups at a late stage of the total synthesis allowed for its completion in an efficient manner.

![Fig. 1. Structures of TMC-95A-D.](image)

Materials and Methods

General Procedures. Unless otherwise noted, materials were obtained from commercial sources and used without purification. All reactions requiring anhydrous conditions were performed under a positive pressure of argon by using flame-dried glassware that was cooled under dry argon. Tetrahydrofuran (THF), dimethylformamide (DMF), and toluene were degassed with argon and passed through a solvent-purification system (J. C. Meyer, Glass Contour, Laguna Beach, CA) containing alumina or molecular sieves. Dichloromethane was distilled from CaH2 before use. Column chromatography was performed on Merck silica gel Kieselgel 60 (230–400 mesh). Mass spectra were obtained on Fisons VG Autospec. HPLC data were obtained on a Waters 600 high-pressure liquid chromatograph. 1H NMR, 13C NMR, and nuclear Overhauser effect (NOE) experiments were recorded on a Varian 300- or 400-MHz spectrometer. Chemical shifts (δ) were given in parts per million and recorded relative to the residual solvent peak unless otherwise noted. 1H NMR were tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet), coupling constant (in hertz), and number of protons. When a signal was deemed “broad,” it was noted as such. IR spectra were recorded on a Nicolet Avatar 320 Fourier transform IR spectrometer. Optical rotations were determined with a Rudolph Research Autopol III automatic polarimeter referenced to the D-line of sodium.

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Abbreviations: THF, tetrahydrofuran; DMF, dimethylformamide; NOE, nuclear Overhauser effect; BT, benzothiazole; PT, phenyl tetrazole; LiHMDS, lithium bis(trimethylsilyl)amide; DMPU, 1,3-dimethyl-3,4,5,6-tetrahydropyrimidinone; RT, room temperature; EDCI, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; HDAI, 1-hydroxyazabenzotriazole.

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Complete experimental procedures and spectroscopic and analytical data including NMR spectra can be found in Supporting Materials and Methods, which is published as supporting information on the PNAS web site.

Results and Discussion

Synthetic Plan. Retrosynthetically, we reasoned that macrocycle 5 could be elaborated to TMC-95A/B (Fig. 3) and was derived from the building blocks 6–11. The highly oxidized tryptophan moiety was envisioned to be installed via a protected oxindole of type 6, which could ultimately come from L-serine (7) and a 7-substituted isatin 8. Stille coupling (19) was planned to form the biaryl bond linkage between the oxidized tryptophan and the bottom-half tyrosine portion, which in turn could come from commercially available 3-iodotyrosine (9) and 3-methyl-2-oxopentanoic acid sodium salt (10). Incorporation of an asparagine residue (11) and macrolactamization at the C10–N9 amide bond thus would afford macrocycle 5.

Total Synthesis of TMC-95A/B. The synthesis of TMC-95A/B began with the preparation of the highly oxidized tryptophan fragment. Initially, it was determined that treatment of 7-iodooxindole 12 [readily prepared from 7-iodoisatin via hydrazine reduction (20)] with either the Garner aldehyde 13 (21, 22) or the L-serine-derived OBO-ester aldehyde 14 (23) under condensation conditions produced oxindolenes 15 and 16 (Scheme 1). Although these results proved to be very promising, Ma and Wu (13) and later Lin and Danishefsky (14, 15) reported a very similar transformation in their syntheses of the highly oxidized tryptophan fragment. It was therefore decided that an alternate and more efficient route to the highly oxidized tryptophan fragment should be developed.

Ultimately, it was found that a modified Julia olefination (24–26) proved to be an effective method to furnish an oxindolene derivative 6. The synthesis of the highly oxidized tryptophan fragment began with treatment of readily available N-benzyloxycarbonyl-L-serine methyl ester (17) under Mitsunobu (27) conditions with either 2-mercaptobenzothiazole or 1-phenyl-1H-tetrazole-5-thiol, diisopropyl azodicarboxylate, and PPh₃ to furnish S-heteroaromatic cysteine derivatives 18 (Scheme 2). Completion of the modified Julia sulfone coupling partners was accomplished by (i) reduction of the methyl ester with Ca(BH₄)₂, (ii) blocking of the carbamate nitrogen and the primary alcohol as the acetonide with 2,2-dimethoxypropane and p-toluenesulfonic acid, and (iii) oxidation (28) of the thioether to the sulfone 19. It should be noted that both the benzothiazole (BT) and phenyl tetrazole (PT) sulfone were prepared in like manner and similar yields.

With sulfones 19 in hand, we set out to determine the optimal reaction conditions necessary to couple sulfones 19 with readily available 7-iodoisatin (29–31) 20 that would give both a high-yielding and highly diastereoselective process (Table 1). It was found that conditions similar to those reported by Liu and Jacobsen (32) gave the best selectivity in the modified Julia olefination, furnishing the desired oxindolene 21. Under the same reaction conditions, we saw that the BT sulfone gave superior selectivity over that of the corresponding phenyltetrazole derivative (Table 1, entry 2 vs. 4). We also noticed that the more thermodynamically stable E-isomer can preferentially...
be prepared with greater selectivity by increasing the reaction temperature. Ultimately the optimized reaction conditions were found to involve treating the BT sulfone 19a and 7-iodoisatin with lithium bis(trimethylsilyl)amide (LiHMDS) in DMF/1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU) (1:1) at 0°C, affording a 5:1 E/Z ratio of oxindolene 21 (Table 1, entry 6). It was also possible to separate the two isomers and then isomerize the undesired Z-isomer to the E-isomer under conditions reported by the Danishefsky group (14, 15).

With oxindolene 21 in hand, we considered numerous synthetic strategies that could be used to complete the total synthesis. Of those contemplated, two disconnections involving either C6–C7 oxidation to the diol or biaryl formation were seriously considered. Because our laboratory had previously developed a Stille coupling protocol for the preparation of a simplified TMC-95 biaryl (33) and studies have shown that the C6–C7 diol is somewhat labile (34, 35), it was decided to form the biaryl bond before installation of the C6–C7 bond.

With aryl iodide 21 and aryl stannane 22 (33) in hand, attempts were made at constructing the biaryl moiety of the TMC-95 proteasome inhibitors under the Stille conditions discussed earlier. Despite extensive experimentation, we found that numerous combinations of Pd-catalyst and ligand gave unsatisfactory yields of the biaryl product 23 (Scheme 3). The best isolated yield of coupled product 23 was ~20%, which was routinely accompanied by side products resulting from alkyl group transfer from the stannane (24) and reductive removal of the iodine atom (25). Because of the fact that the Stille coupling gave undesired side products and insufficient yields, we decided that the Suzuki (35) coupling protocol was the next logical choice for constructing the biaryl bond.

Preparation of the requisite boronic ester necessary for the Suzuki coupling began with the protection of commercially available 3-iodo-L-tyrosine 26. Subjection of 3-iodo-L-tyrosine 26 to (i) thionyl chloride in methanol, (ii) di-tert-butyl dicarbonate, and (iii) chloromethyl methyl ether and diisopropyl ethylamine afforded the fully protected tyrosine derivative 27 in near quantitative yield (Scheme 4). Conversion of the aryl iodide in 27 to the boronic ester 28 was accomplished via the Miyaura protocol (36). Treatment of boronic ester 28 under Suzuki conditions with aryl iodide 21 and K2CO3 in refluxing aqueous dimethoxyethane catalyzed by dichloro[1,1’-bis(diphenylphosphino)ferrocene]palladium smoothly installed the biaryl linkage yielding 23 in 90% yield.

Saponification of the methyl ester in 23 allowed for amide bond formation between the resulting carboxylic acid and L-asparagine benzyl ester mediated by ethyl-dimethylaminopropyl carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOAt) to yield pseudotripeptide 29 in 98% yield over the two steps (Scheme 5). Pseudotripeptide 29 constitutes the complete carbon framework for the macrocyclic core. It is significant to note that the judicious choice of protecting groups has allowed for complete removal of all protecting groups in two simple transformations. With pseudotripeptide 29 in hand, we found that this was the ideal juncture in the synthesis for the oxidation to the C6–C7 diol. Subjection of 29 to OsO4 in pyridine at 0°C allowed for the oxidation of the C6–C7 double bond with complete facial selectivity opposite to the allylic carbamate yielding diol 30 in 87% yield as a single diasteromer with the correct relative configuration.

At this stage, we decided to remove all the acid labile protecting groups, liberating the C14 amine, the C25 primary

<table>
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<th>Entry</th>
<th>Heterocycle (Het)</th>
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<th>E/Z ratio†</th>
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<tr>
<td>1</td>
<td>PT</td>
<td>THF, NaHMDS, −78°C</td>
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<td>2</td>
<td>PT</td>
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<tr>
<td>3</td>
<td>BT</td>
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<tr>
<td>4</td>
<td>BT</td>
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<tr>
<td>5</td>
<td>BT</td>
<td>DMF, DMPU, LiHMDS, −45°C</td>
<td>3:1</td>
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<td>6</td>
<td>BT</td>
<td>DMF, DMPU, LiHMDS, 0°C</td>
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*In all cases yields were at least 79%.
†E/Z ratios were determined by 1H analysis of crude product mixtures.

**Table 1. Modified Julia olefination**

NaHMDS, sodium bis(trimethylsilyl)amide.
Scheme 4. Suzuki biaryl formation. Reaction conditions: a1, SOCl₂, MeOH, RT, 18 h; a2, di-tert-butyl dicarbonate, saturated NaHCO₃, CH₂Cl₂, 0 °C → RT, −12 h; a3, chloromethyl methyl ether, diisopropyl methylamine, CH₂Cl₂, 0 °C, 3 h, 95% (three steps); b, bis(pinacolato)diboron, KOAc, dichloro[1,1′-bis(diphenylphosphino)ferrocene]palladium, DMSO, 80 °C, 4 h, 80–89%; c, K₂CO₃, dichloro[1,1′-bis(diphenylphosphino)ferrocene]palladium, aqueous dioxane/CH₂Cl₂, 0 °C, 3 h, 98% (two steps); d, EDCI, HOAt, CH₂Cl₂, DMF (1:1), RT, 1 mM (49%, two steps).

Scheme 5. Preparation of the macrocyclic core. Reaction conditions: a1, LiOH, THF, H₂O, 0 °C; a2, H₂N-Asn-OBn, HOAt, EDCI, disopropyl methylamine, CH₂Cl₂, 0 °C, 4 h (98%, two steps); b, O₃, pyridine, 0 °C, 1 h, and then saturated NaHCO₃, 87%; c, trifluoroacetic acid/H₂O (1:1), RT, 4 h; d, 3-methyl-2-oxopentanoic acid sodium salt, HOAt, EDCI, THF, 0 °C (98%, two steps); e, Pd black, H₂, MeOH, RT, 6 h; f, EDCI, HOAt, CH₂Cl₂, DMF (1:1), RT, 1 mM (49%, two steps).

Scheme 6. Selective oxidation of C25. Reaction conditions: a, SO₃-pyridine, DMSO, CH₂Cl₂ (3:1), RT, 15 min; b, NaClO₂, NaH₂PO₄, 2-methyl-2-butene, 1BuOH, H₂O, RT, 5 h.

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alcohol, and the C19 phenol with trifluoroacetic acid/H₂O (1:1) (Scheme 5). Although we realized that the four free alcohols may prove to be problematic in both the incorporation of the ketoamide and the macrocyclization, the potential payoff in terms of efficiency motivated us to explore this approach. The resulting trifluoroacetic acid-amine salt was coupled to d, 3-methyl-2-oxo-pentanoic acid sodium salt mediated by EDCI and HOAt, affording the corresponding amide 31 in ~98% yield over the two steps. The high yield in this reaction proved promising for the macrocyclization step, because there is no observable competing acylation of any of the free alcohols, and promised to obviate the need for protecting groups.

Hydrogenolysis of both the benzyl ester and the N-benzyloxy carbamate with palladium black afforded the requisite amino acid necessary for macrocyclization. The resulting amino acid was treated with EDCI and HOAt to yield the key unprotected carboxylic acid 32. Carboxylic acid 32 could be accomplished via direct elaboration of macrocycle 5 by intersecting a late-stage intermediate in the Danishefsky synthesis, it was felt that an efficient synthesis of TMC-95A/B could be accomplished via direct elaboration of macrocycle 5. To realize this objective, selective oxidation of the C25 primary alcohol in the presence of the C7 secondary alcohol would need to be achieved. In addition and, even more problematic, the oxidative cleavage of the C6–C7 diol loomed as a potential pitfall. Initially, a selective oxidation of the primary alcohol directly to the necessary carboxylic acid using a platinum-catalyzed dehydrogenation reaction (37) was examined. Unfortunately, it was found that no reaction occurred or, if base was added, complete decomposition occurred.

Other direct oxidation methods of the primary alcohol to the carboxylic acid were considered, including the 2,2,6,6-tetramethyl-1-piperidinol oxyl free radical/NaClO₂/NaOCl combination; unfortunately, no desired carboxylic acid product was observed (38). Finally, after exhaustive experimentation, it was found that a two-step protocol proved successful in obtaining the desired carboxylic acid. Thus, treating macrocycle 5 with SO₃-pyridine in DMSO-CH₂Cl₂ afforded the desired aldehyde as an inseparable complex mixture of aldehyde, plus C6 and C7 lactol isomers (Scheme 6). Fortunately, subjection of this mixture to NaClO₂ and NaH₂PO₄ in the presence of 2-methyl-2-buten to produce desired carboxylic acid 32.

With carboxylic acid 32 in hand, all that remained was the incorporation of the cis-propenyl amide. There were several avenues that we chose to investigate for this transformation. Initially, we decided to test the method developed by Stille and Becker (44), which involved a transition-metal-mediated process wherein allyl amides are converted to the corresponding enamides in which the cis-configuration predominates. Coupling of
allyl amine with the carboxylic acid 32 readily provided the corresponding allyl amide. Unfortunately, all attempts to isomerize this product proved futile.

Next, the Peterson olefination method developed by the Fürstner laboratory (ref. 45 and references therein) that utilizes hydroxzyalkyl silanes for the preparation of enamides was evaluated. The requisite strong base used in this Peterson olefination protocol was anticipated to be too harsh for the sensitive functionality present in the TMC-95 macrocyclic core. Therefore, we examined the liberation of a masked alkoxide under mild conditions as a means to trigger the desired olefination. The literature revealed that fluoride-based deprotection of a tert-butylimethylsilyl ether unleashes an alkoxide species reactive enough to suffer facile Peterson olefination to yield an enamide as reported in the synthesis of crocacin D (46). Although we were able to conduct this reaction on a very simplified substrate, we were unable to produce TMC-95A/B after subjection of the corresponding hydroxzyalkyl silyl amide with a variety of fluoride sources.

Based on the aforementioned setbacks, we evaluated the enamide preparation developed by Pansare and Vederas (47) and used by Inoue et al. (16, 17) in their synthesis of TMC-95A. Treatment of carboxylic acid 32 with 1-alk-1-ino-threonine-benzyl ester hydrochloride salt (48) mediated by EDCI and HOAt afforded the corresponding amide 33 in 49% overall yield from 5 (Scheme 7). Hydrogenolysis of the benzyl ester in 33 with palladium black under an atmosphere of hydrogen produced the resultant carboxylic acid. Subjection of this material to Mitsunobu conditions afforded TMC-95A/B in 70% yield for the two steps. The individual diastereomers TMC-95A and TMC-95B were separated by HPLC to collect analytical data on each. The synthetic samples of TMC-95A and TMC-95B and the natural materials proved identical by 1H NMR, 13C NMR, mobility on TLC, mobility on HPLC, optical rotation, and high-resolution mass spectrometry.

Conclusions

A concise and efficient total synthesis of TMC-95A/B has been accomplished. The synthesis was completed in 22 total steps with only 18 steps in the longest linear sequence. It should be noted that this is a very short and efficient total synthesis of these natural substances and is an approach to commence with L-serine instead of D-serine. Our synthesis features an E-selective modified Julia olefination to form the key oxindole. It has been found that this transformation is also a viable route to other βγ-unsaturated protected amino alcohols. The synthesis recorded here constitutes an efficient strategy that is amenable to the preparation of a variety of analogs due to being highly convergent and requiring minimal protecting group manipulations.

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