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Synthesis of Macrocyclic Peptide Analogues of Proteasome Inhibitor TMC-95A

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Abstract: The synthesis of three constrained macrocyclic peptide analogues **1** of TMC-95A as potential proteasome inhibitors is described. The key step involves a Ni(0)-mediated macrocyclization of tripeptides **2** bearing halogenated aromatic side chains for the formation of the biaryl junction. In addition, an enantioselective preparation of L-7-bromotryptophan methyl ester **3** using a Corey−O'Donnell alkylation of the glycine benzophenone imine was achieved in good overall yield with very high ee (>85%) on a multigram scale.

TMC-95A and its diasteromers TMC-95B, -C, and -D, novel 17-membered macrocyclic peptides, have been recently isolated from the fermentation broth of *Apiospora montagnei Sacc.* TC1093[1] (Figure 1). They contain L-tyrosine, L-asparagine, a highly oxidized L-tryptophan, a C-terminal (*Z*)-1-propenylamine, an N-terminal 3-methyl-2-oxopentanoyl moiety, and a phenol-oxindole ring junction. Such a biaryl link is found only in the natural products neuroprotectin[2] or complestatins A and B.[3] The related phenyl−indole ring attachment encountered in natural products such as chloropeptin,[4] kistamicin,[5] complestatin,[6] SCH 212394,[7] and diazonamide[8] is more widespread.

TMC-95A was found to be a very potent, reversible, and noncovalent inhibitor of the peptidase activities (chymotrypsin-like, trypsin-like, and caspase-like) of the proteasome 20S, the last being an unprecedented mode of action.[9] The proteasome[10] is a multicatalytic protease which plays a major role in intracellular processes and its inhibition represents a promising target for drug development.[11,12]

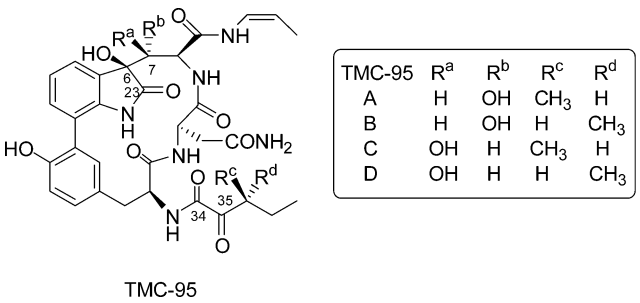

FIGURE 1. Structure of TMC-95A, -B, -C, and -D.

TMC-95A has stimulated a great interest in the scientific community mainly as the result of the importance of proteasome inhibition but also for its synthetic challenge. In fact, during the course of our work, several groups developed either routes to the unusual highly oxidized L-tryptophan fragment[13] or strategies based on macrolactamization to perform the ring closure of TMC-95A.[14] In 2002, Danishefsky et al. published the first total synthesis of TMC-95A[15,16] while Moroder et al. described the synthesis of the first analogue.[17]

As starting point of our work, we decided to focus on the synthesis of TMC-95A analogues in order to study their structure−activity relationship. Recently, a crystal structure showed that TMC-95A was bound to the trypsin-like site of proteasome by a network of five hydrogen bonds involving four centers of the TMC-95A peptide backbone and the C-23 carbonyl of the oxindole.[18] Moreover, the two hydroxyl groups at C-6 and C-7 as well as the ketoamide side chain (C-34 and C-35) do not seem to be essential for recognition. However the interactions between TMC-95A and the other active sites still remain to be established. Therefore, we based the design of TMC-

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(10) For review on proteasome, see: Groll, M.; Huber, R. Int. *J. Biochem. Cell Biol.* **2003**, *35*, 606−616 and references therein.

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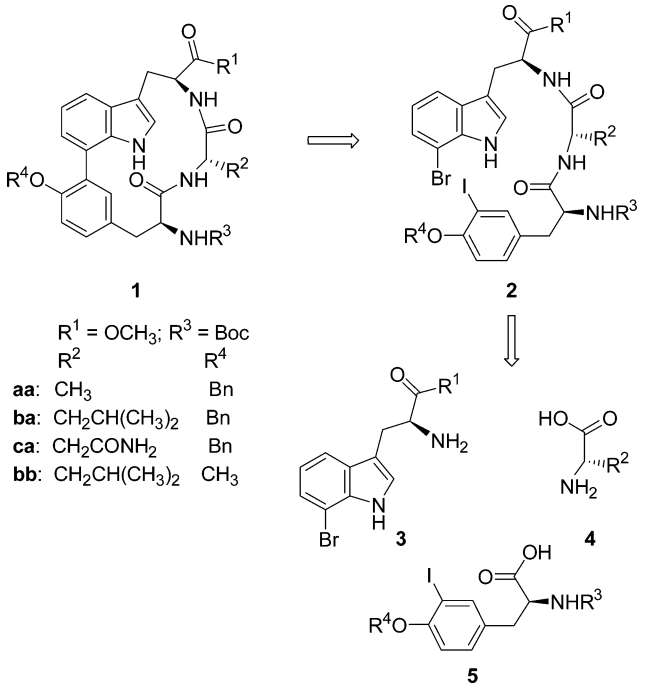
SCHEME 1. Retrosynthetic Pathway



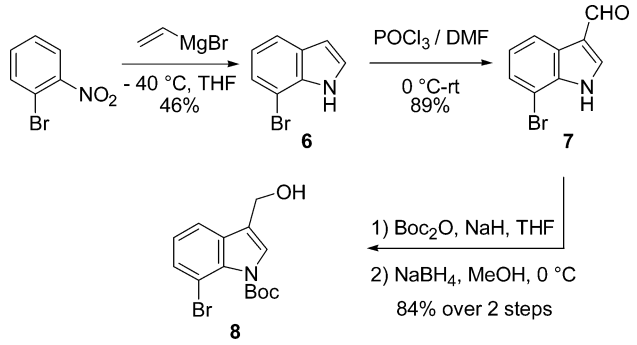
95 analogues on retaining the peptide structure but replacing the oxindole with a simple indole. We identified the general target structures **1** with two main degrees R^1 and R^2 of molecular diversity. Our retrosynthetic strategy for the macrocycles **1** is indicated in Scheme 1. It is relied on assembling the peptide backbone **2** first and then closing the ring by an intramolecular biaryl carbon−carbon reaction. This route requires three α-amino acids building blocks (**3**−**5**) as starting materials and therefore opens the possibility of generating a library of analogues for further biological assays by varying R^1, R^2, R^3, and R^4.

Here, we present our contribution to the chemistry of TMC-95s and describe the convergent synthesis of three novel macrocycles TMC-95A analogues **1** using an intramolecular biaryl coupling as the key step for performing the macrocyclization.

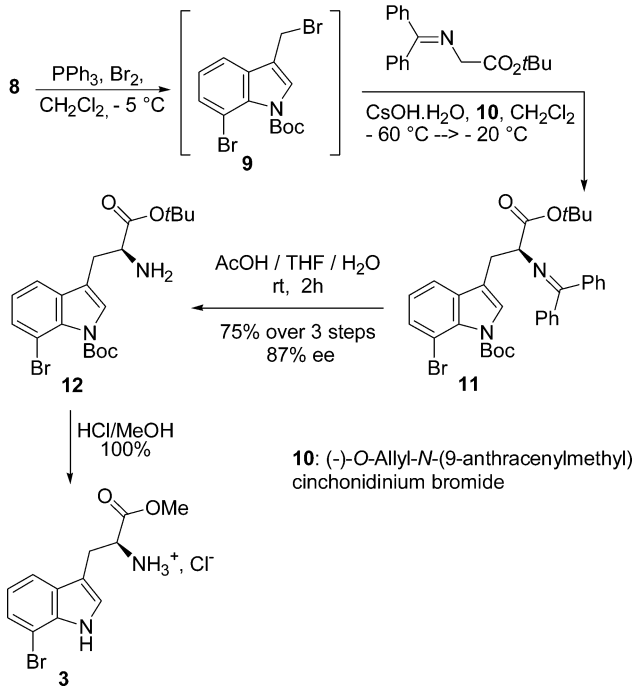
In the beginning, our efforts were focused on the enantioselective synthesis of unusual tryptophan derivative **3**. Our strategy was to form the indole nucleus of **3** prior to introduce the asymmetric carbon via an enantioselective alkylation by phase-transfer catalysis.

As depicted in Scheme 2,7-bromoindole **6** was synthesized by treatment of 1-bromo-2-nitrobenzene with vinylmagnesium bromide in 46% yield according to the Bartoli procedure.[19] Subsequent formylation under Vilsmeier−Haack conditions led to 7-bromo-3-formylindole **7** in 89% yield.[20] Following the initial protection of the indole nitrogen by a Boc group, reduction of the aldehyde function afforded alcohol **8** in 84% overall yield.

SCHEME 2. Synthesis of the Precursor 8



SCHEME 3. Synthesis of 7-Bromotryptophan Derivative 3



The protection of the indole nitrogen proved to be crucial for the stability in the subsequent steps. Bromination of the alcohol derivative **8** was performed with Br_2/PPh_3 in CH_2Cl_2 (Scheme 3). The resulting bromo compound **9** was found to be extremely unstable and was always freshly prepared just before use. According to Corey's procedure,[21] alkylation of *N*-(diphenylmethylene)-glycine *tert*-butyl ester with bromide **9** in the presence of *O*-9-allyl-*N*-(9-anthracenylmethyl)cinchonidinium bromide **10** gave the imine **11**. Then, hydrolysis of the imine function under acidic conditions (AcOH/THF/H_2O) provided the fully protected 7-bromotryptophan **12**. Starting from the alcohol **8**, the yield over three steps was excellent: 75% with an enantiomeric excess of 87%.[22] This sequence was scaled up to 5 g. The absolute configuration was established experimentally as being *S* by conversion of **12** to the known L-7-bromotrypto-

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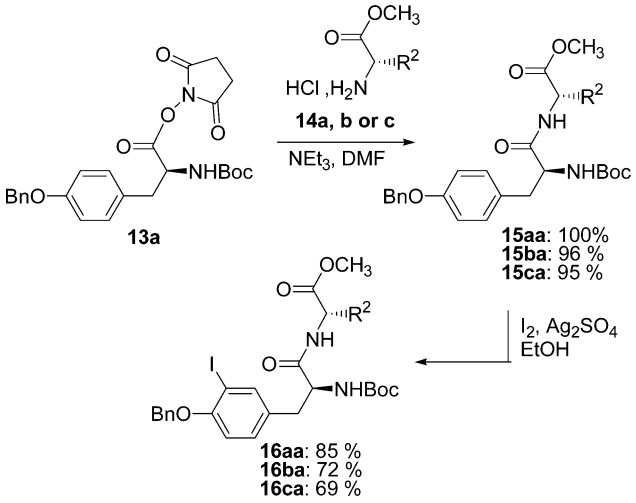
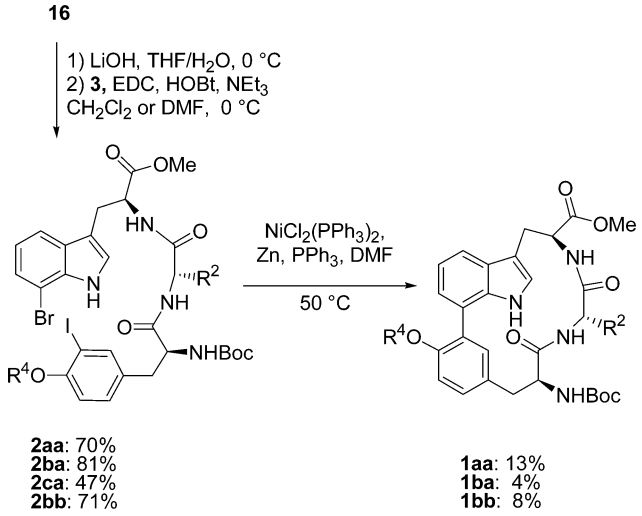
(22) Enantiopurity was determined by HPLC; see the Supporting Information.

SCHEME 4. Preparation of Dipeptides 16



SCHEME 5. Access to the Macrocycles 1



phan and comparison of its optical rotation with the literature value.[23]

HCl-saturated MeOH was then used to remove the *tert*-butyl protecting groups, and transesterification proceeded to give the amino methyl ester **3** as its hydrochloride salt quantitatively.

The second key intermediates of our convergent synthesis, dipeptides **16**, were synthesized by standard procedures of peptide chemistry[24] (Scheme 4).

Commercially available activated ester **13a**[25] was coupled to the methyl amino ester hydrochloride salts **14a**, **b**, or **c** to form the corresponding dipeptides **15aa**, **15ba**, and **15ca** in, respectively, 100%, 96%, and 95% yields. Subsequent selective iodination ortho to the benzyloxy group was achieved in good yields using I_2/Ag_2SO_4 in ethanol[26] to afford the resulting iodo compounds **16aa**, **16ba**, and **16ca**.

Quantitative saponification of esters **16** followed by coupling with protected bromotryptophan **3** in the presence of EDC/HOBt afforded the linear tripeptides **2aa**, **2ba**, **2ca**, and **2bb** in, respectively, 70%, 81%, 47%, and 71% (Scheme 5).

With the acyclic precursors **2** in hand, we next turned our attention to the formation of the 17-membered macrocycle by biaryl junction. First, the macrocyclization of **2aa** or **2ba** was attempted in a one-pot Suzuki[27] cross-coupling reaction via in situ arylboronate formation.[28] Under a set of conditions varying the source of palladium, the solvent, and the base in the presence of the pinacol ester of diboronic acid, no cyclized product could be detected although biaryl coupling under Suzuki conditions was successful with the similar 17-membered ring system of complestatin.[29] Alternatively, we investigated a nickel(0) intramolecular cross-coupling reaction to form the C−C bond between the two aromatic rings. The reaction was carried out at 50 °C using freshly prepared Ni(0).[30] The outcome of the reaction was encouraging since two desired constrained 17-membered cycles, **1aa** and **1ba**, were formed in 13% and 4% respectively. Varying the conditions (high dilution or ligands) did not improve the yields, and reduced compounds were obtained as byproducts. Application of the same conditions to the Asn-derivative **2ca** gave only a mixture of reduced linear tripeptides.[31] Because of the steric hindrance of the benzyl protecting groups,[32] we turned our attention to less bulky substituted derivatives such as the methyl-protected tripeptide **2bb**. Treatment of the linear precursor **2bb** with the zerovalent nickel complex at 50 °C in DMF afforded the TMC-95A analogue **1bb** with 8% yield. The constrained nature of the macrocycles **1** probably due to the sp^2 carbon at C-6 position can explain the low yields observed during the macrocyclization.[33] These results also reflect the difficulty of macrocyclization by biaryl coupling which had been already reported regarding similar structures.[34]

The biological activity of the macrocycles **1** and acyclic precursors **2** is currently under evaluation. Further studies on the structure−activity relationship are under investigation in order to probe the importance of the

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(31) HRMS (ESI) measurements of the mixture showed two peaks corresponding to the mono- (calcd for $[C_{37}H_{42}N_5O_8Br + Na]^+$ 786.2114, found 786.2119) and direduced (calcd for $[C_{37}H_{43}N_5O_8 + Na]^+$ 708.3009, found 708.3008) compounds.

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(33) The presence of an sp^2 carbon at the C-6 position in TMC-95 precursor or analogue prevented macrolactamization from occurring. See ref 14a and: Kaiser, M.; Milbradt, A. G.; Moroder, L. *Lett. Pept. Sci.* **2002**, *9*, 65−70.

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macrocyclic structure and the presence of the oxindole moiety in the binding properties to the proteasome.

In summary, we have synthesized three novel TMC-95A analogues using a Ni(0)-mediated ring-closure strategy. Further optimization of the key step and application to the synthesis of more TMC-95A derivatives are being actively pursued in our laboratory.

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Supporting Information Available: Experimental procedures and compound characterization data for all new compounds. Copies of ^{1}H and ^{13}C NMR spectra of compounds **7**, **12**, **3**, **2aa**, **2ba**, **2ca**, **2bb**, **1aa**, **1ba**, and **1bb**. This material is available free of charge via the Internet at http://pubs.acs.org.

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