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Synthesis of a TMC-95A Ketomethylene Analogue by Cyclization via Intramolecular Suzuki Coupling

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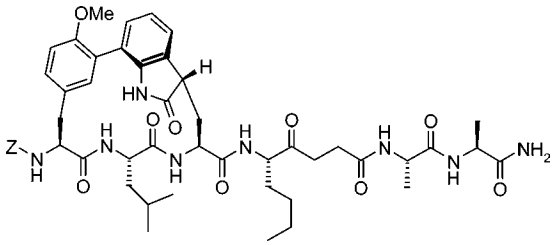
ABSTRACT



A TMC-95A analogue extended at the C-terminus with NleΨ[COCH$_2$]Gly-Ala-Ala-NH$_2$ was synthesized via side-chain cyclization of the linear precursor by a Suzuki cross-coupling reaction in solution to analyze the effect of additional P′ residues on the inhibitory potency against yeast proteasome.

Degradation of cytosolic proteins is mainly regulated by the ubiquitin−proteasome pathway[1] where upon ATP-dependent ubiquitinylation, enzymatic digestion of the unfolded polypeptide chains occurs within a multienzymatic machinery called 26S proteasome.[2] Its proteolytic core chamber is constituted by the 20S proteasome,[3] and the molecular basis of the enzymatic mechanism was derived mainly from X-ray analysis of the 20S proteasome from *T. acidophilum* or yeast complexed with synthetic[4] or natural inhibitors such as lactacystin[5] and epoxomicin.[6] More recently, a new natural product from *Apiospora montagnei* Sacc. TC1093, i.e., TMC-95A, was recognized as a potent and competitive proteasome inhibitor.[7] Crystallographic analysis of the TMC-95A/proteasome complex revealed binding of the inhibitor in a β-type extended conformation to the S-subsite of the protein and, thus, in a substrate-like mode.[8]

From this structural analysis, a simplified TMC-95A analogue was derived that was found to retain most of the inhibitory potency.[9] Since no information is available so far about the substrate-binding mode to the S′-subsite, it was compelling to investigate the use of the TMC-95A analogue

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as an anchor at the S-subsite of the proteasome. Compound **1** would bridge the TMC-95A analogue from the S-subsite with a nonscissible ketomethylene group for presentation of a C-terminal peptide extension to the S′-subsite as outlined in Figure 1.

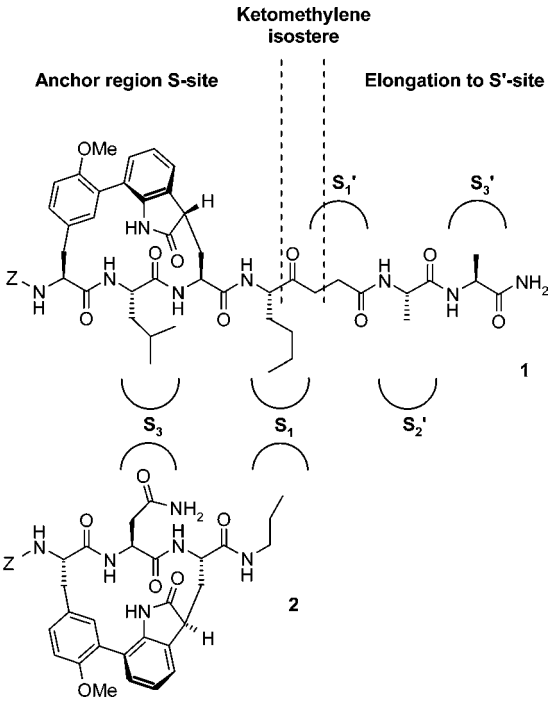


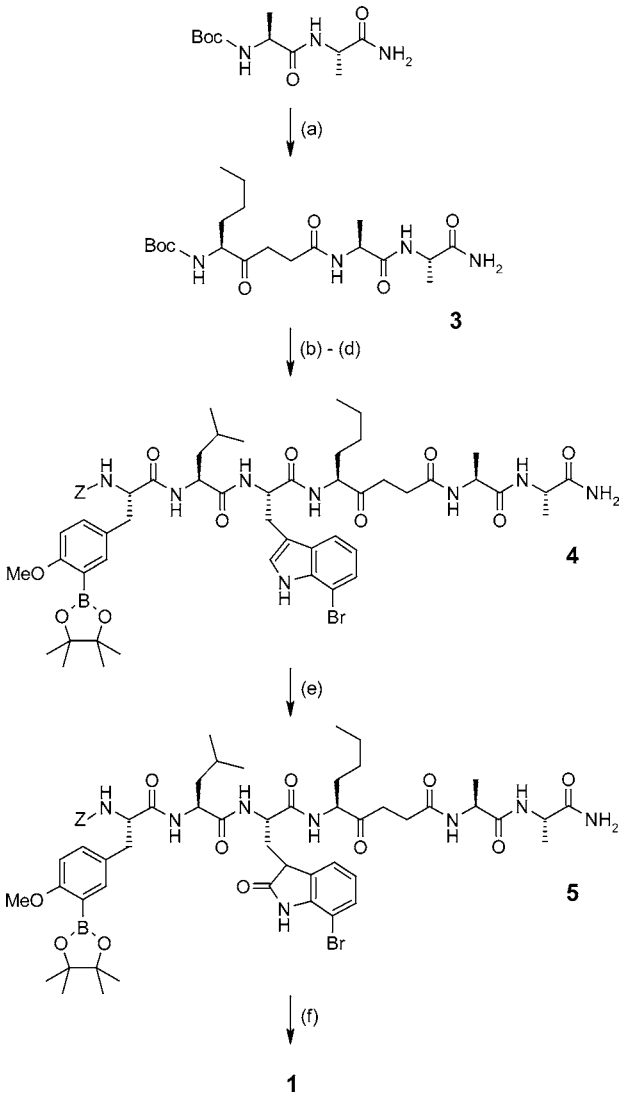
Figure 1. Structure of the target compound **1** and the previously synthesized TMC-95A analogue **2**[9] and the proposed mode of binding to the active sites of the 20S proteasome.

Our previous synthesis of the TMC-95A analogue was based on the macrolactamization of a linear precursor as the cyclization step. Similarly, all other syntheses of TMC-95A/B reported so far rely on cyclization of the biaryl-containing precursor via amide bond formation.[10] Thus, in the first instance, the synthesis of compound **1** was again attempted via the macrolactamization approach. Yields of the cyclic compound were poor and nonreproducible because of the presence of the ketomethylene moiety and purification proved to be exceedingly difficult.

The two natural products complestatin and chloropeptin contain a biaryl system very similar to that of TMC-95A/B.[11] The synthesis of related cyclic structures of complestatin and chloropeptin was achieved both by macrolactamization and side-chain cyclization via Suzuki cross-coupling.[12] We decided to use a similar side-chain cyclization to synthesize TMC-95A analogues.

Thus, in a second approach to the synthesis of compound **1**, the linear precursor **5** was prepared following the procedure illustrated in Scheme 1.

Scheme 1. Synthesis of the TMC-95A Analogue **1** Containing a Ketomethylene Moiety in the Peptide Backbone[a]



[a] Reaction conditions: (a) (i) TFA/DCM (1:1); (ii) Boc-NleΨ[$COCH_2$]Gly-OBt, DIEA, NMP. (b) (i) TFA/DCM (1:1); (ii) Boc-Trp(7-Br)-OH, PyBOP/HOBt, DIEA, NMP. (c) (i) TFA/DCM (1:1); (ii) Boc-Leu-OH, PyBOP/HOBt, DIEA, NMP. (d) (i) TFA/DCM (1:1); (ii) Boc-Tyr(3-$BO_2C_6H_{12}$,4-Me)-OH, PyBOP/HOBt, DIEA, NMP. (e) DMSO (20 equiv), glacial AcOH/concentrated HCl (4:1), rt, 4 h. (f) K_2CO_3 (3 equiv), Pd(dppf)Cl_2 (0.05 equiv), DME/H_2O (7:1), 80 °C, 5 h.

The peptide chain was built-up in a stepwise manner from H-Ala-Ala-NH_2 in solution making use of intermediate N^{α}-

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Boc protection. For this purpose, Boc-NleΨ[$COCH_2$]Gly-OH, Boc-Trp(7-Br)-OH, and the 3-boronic ester derivative of Z-Tyr(4-Me)-OH were prepared by well-established procedures (see Supporting Information). Coupling of the ketomethylene isostere to H-Ala-Ala-NH_2 was carried out with a preformed HOBt ester since standard in situ activation procedures, e.g., PyBOP/HOBt, led to side reactions and thus to low yields upon chromatographic purification. Acidic cleavage of the N^{α}-Boc group with TFA/DCM (1:1) was followed by coupling of Boc-Trp(7-Br)-OH, Boc-Leu-OH, and finally the Z-protected tyrosine-*O*-methyl 3-boronic ester derivative with PyBOP/HOBt. Our previous studies have clearly shown that cyclization to the constrained macrocyclic ring system of TMC-95A is only possible if the C3-carbon of the indole ring is sp^3-hybridized.[9,13] Therefore, oxidation of the tryptophan side-chain to the 2-oxindole ring was performed prior to cyclization. Because of the oxidatively labile boronic ester, optimal conditions were assayed on model compounds and a mixture of glacial acetic acid/concentrated hydrochloric acid (4:1) containing 20 equiv of DMSO[14] was found to be the most suitable for conversion of **4** to **5** in high yields (76%), resulting in a 1:1 mixture of C3 diastereomers. The subsequent cyclization was performed by an intramolecular Suzuki cross-coupling under standard conditions,[15] i.e., 3 equiv of K_2CO_3 as the base and Pd(dppf)-Cl_2 as the catalyst in DME/H_2O (7:1). The base-catalyzed enolization of the oxindole system provides the (*S*)-configured precursor at the C3 atom as required for the stereoselective cyclization.[9] Upon RP-HPLC of the crude product, the desired compound **1** was isolated in a 57% yield.[16]

Similarly to what has been reported for the synthesis of complestatin- and chloropeptin-related compounds,[12] the intramolecular cyclization via Suzuki coupling was found to proceed at higher rates than the bimolecular coupling required for the synthesis of the macrolactamization precursor.

The first synthesis of a TMC-95A related cyclic structure by an intramolecular Suzuki cross-coupling of the linear precursor represents a significant progress when compared to the macrolactamization approach, as it tolerates a wider spectrum of functional groups. Furthermore, it may well be suited for the solid-phase synthesis of TMC-95A related analogues.

The inhibitory potencies of compound **1** for all three proteasome activities, i.e., chymotrypsin-like (CL), trypsin-like (TL), and peptidyl-glutamyl-peptide-hydrolase (PGPH), were determined by newly optimized enzyme assays for yeast proteasome and compared to those of Ac-Leu-Leu-Nle-H and compound **2** (Table 1).

Table 1. Inhibition of Yeast Proteasome (K_i [μM]) by Compounds **1** and **2** and Ac-Leu-Leu-Nle-H

inhibitor	CL activity	TL activity	PGPH activity
compound **1**	9.1	60	≥2000
compound **2**	2.4	55	≥2000
Ac-Leu-Leu-Nle-H	1.4	364	≥2000

According to the X-ray analysis of the TMC-95A/proteasome complex,[8] the C-terminal (*Z*)-propenylamide group of the natural product acts as a P_1 residue and Asn as a P_3 residue. From modeling experiments, a replacement of these two residues by Nle and Leu, respectively, and the extension of the peptide chain via a nonscissible ketomethylene moiety with P′ residues for interaction with the S′-subsite of the enzyme were expected to improve the binding affinities, particularly for the CL active site. Therefore, rather surprising was the lower inhibitory potency of the new TMC-95A analogue **1**. A comparative X-ray analysis of the complexes of yeast proteasome with the previous TMC-95A analogue **2** and compound **1** is expected to yield the structural basis for the discrepancy with modeling experiments.

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Supporting Information Available: Synthetic procedures and spectroscopic characterization of precursors and compounds **1** and **5** and protocols for enzyme assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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