Natural Product Synthesis

Total Synthesis of TMC-95A**

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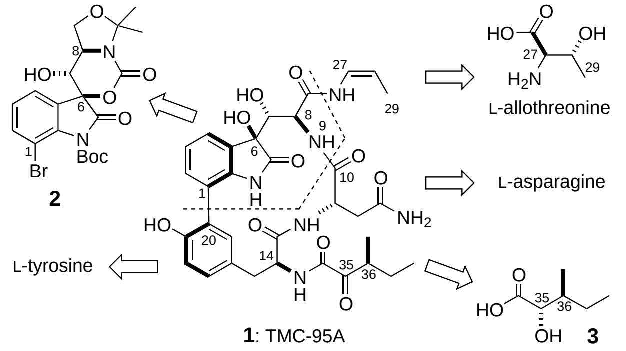
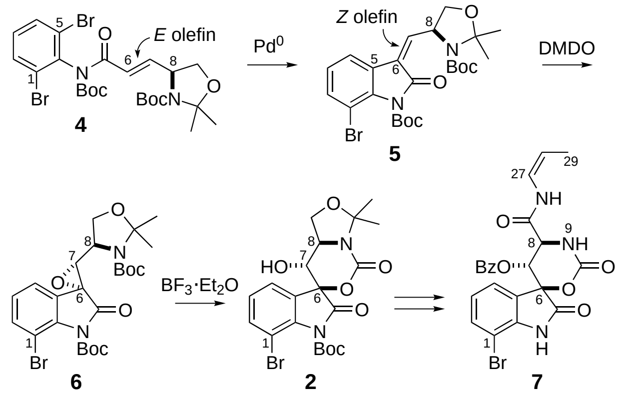
A proteasome is a multicatalytic protease that is involved in a variety of biologically important processes, including immune responses, cell-cycle control, metabolic adaptation, stress response, and cell differentiation.[1] The selective inhibition of proteasomes may therefore be a means of controlling these essential pathways.[2] TMC-95A (**1**, Scheme 1) and its C36 epimer TMC-95B are potent proteasome inhibitors that bind to enzymes noncovalently with IC$_{50}$ values at low nanomolar levels.[3] Thus, TMC-95 may provide unique insight into the detailed biological functions of proteasomes. Recently, the X-ray crystallographic structure of a complex of **1** with a proteasome was reported,[4] which has allowed the design of novel TMC-95 analogues with tailored biological activities.[5]



Scheme 1. Synthetic approach to TMC-95A. Boc = *tert*-butoxycarbonyl

The intriguing biological profile and the structural complexity of TMC-95 immediately generated considerable interest within the synthetic community, and chemical syntheses have been pursued by several research groups.[6,7] These efforts have culminated in a total synthesis of TMC-95A/B by Lin and Danishefsky,[8] and a formal synthesis by Albrecht and Williams.[9] This report details a new solution to the stereoselective construction of TMC-95A.

The most notable features of TMC-95A from the perspective of the synthetic chemist are the highly oxidized tryptophan moiety, the (*Z*)-1-propenylamide, the 3-methyl-2-oxopentanoic acid side chain with the configurationally unstable methyl-substituted C36 center, and the aryl–oxindole ring junction (C1−C20) (Scheme 1). It was envisaged that the macrolactam structure could be assembled by a Suzuki–Miyaura coupling reaction (C1−C20) and peptide bond formation (N9−C10).[8–11] To avoid epimerization at the C36 stereocenter, we planned to introduce 3-methyl-2-oxopentanoic acid as the reduced form **3**, and to generate the C35 ketone in the final stage of the synthesis. We previously reported a stereoselective synthesis of the northern part of the molecule[6] (**7**, Scheme 2) with the following key features: 1) a *Z*-selective Mizoroki–Heck reaction to construct the oxindole structure (**4**→**5**), 2) diastereoselective epoxidation (**5**→**6**), 3) a 6-endo epoxide ring opening reaction to establish the stereochemistry of the C6 and C7 stereocenters (**6**→**2**), and 4) decarboxylative *anti* elimination under Mitsunobu conditions[12] to create the (*Z*)-1-propenylamide **7**. However, removal of the carbamate in **7** to liberate the N9 amine was unsuccessful despite extensive experimentation, which revealed the chemical instability of the propenylamide both in strongly acidic and in strongly basic media. Accordingly, we decided to construct the propenylamide after macrolactam formation, although the applicability of our method in a more complex structure remained uncertain.[13]



Scheme 2. Synthesis of the northern part of TMC-95A. DMDO = 3,3-dimethyldioxirane.[6]

Our modified route started with the previously reported compound **2** (Scheme 3). The Boc group of **2** was selectively removed with Mg(ClO$_4$)$_2$ in acetonitrile,[14] and the C7 secondary alcohol was protected as its ethoxyethyl (EE) ether to afford **8** in 94 % yield for the two steps. For effective biaryl coupling, the bromide **8** was converted into the corresponding iodide **9** in 86 % yield through metalation with *n*BuLi and subsequent addition of diiodoethane without affecting the potentially reactive carbamate. The carbamate group of **9** was in fact extremely resistant to removal under a variety of conditions in the next step. Finally, it was found that the "anhydrous hydroxide" method of Gassman et al.[15] with *t*BuOK (15 equiv) and H$_2$O (10 equiv) transformed **9** into the aniline **10** (74 % yield), in which both the carbamate and the acetonide had been hydrolyzed.

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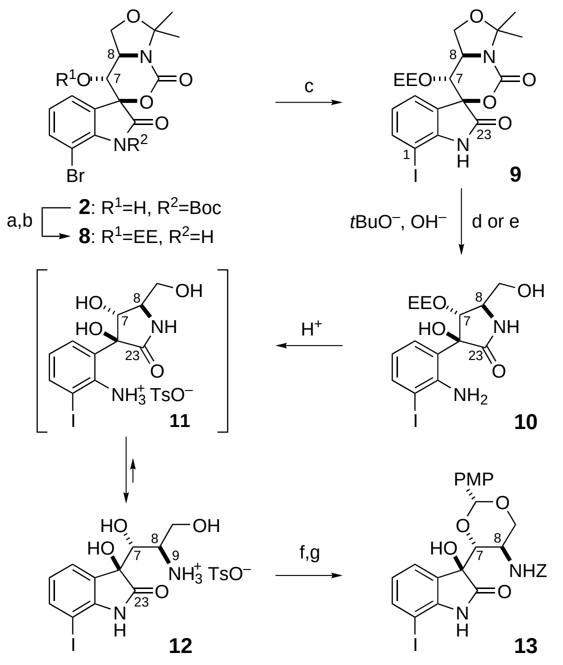
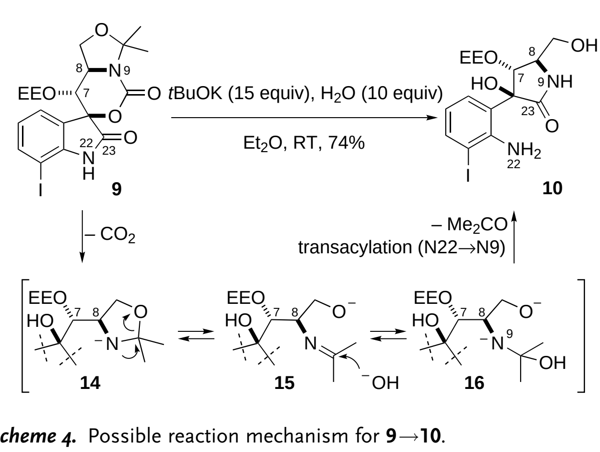


Scheme 3. Reagents and conditions: a) $Mg(ClO_4)_2$, MeCN, 50 °C; b) ethyl vinyl ether, PPTS, THF, 35 °C, 94 % from **2**; c) *n*BuLi, ICH_2CH_2I, THF, −60 °C→room temperature, 86 %; d) *t*BuOK (15 equiv), H_2O (10 equiv), Et_2O, room temperature, 74 %; e) *t*BuOK (15 equiv), H_2O (10 equiv), Et_2O, room temperature; then H_2O, TsOH, MeOH, room temperature; f) ZCl, Et_3N, DMF, 0 °C→room temperature, 55 % from **9**; g) *p*-$MeOC_6H_4CH(OMe)_2$, TsOH, THF, 84 %. DMF = dimethylformamide, PMP = *p*-methoxyphenyl, PPTS = pyridinium *p*-toluenesulfonate, TsOH = *p*-toluenesulfonic acid, Z = benzyloxycarbonyl.

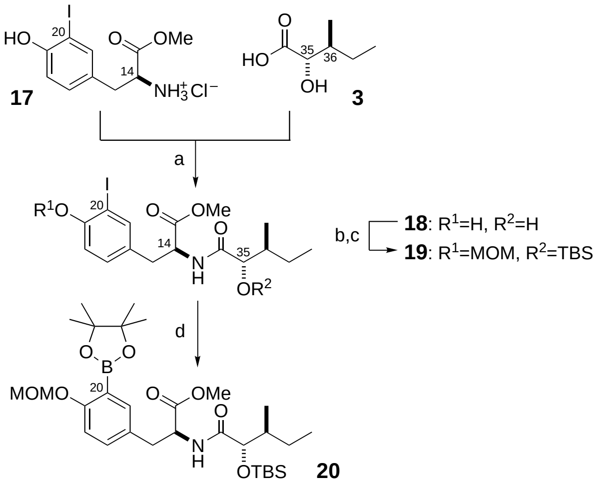
The most plausible mechanism for this interesting outcome is depicted in Scheme 4: Compound **14**, generated upon loss of the carbamate group in **9** by decarboxylation, is in equilibrium with the imine **15**, which can undergo attack by a hydroxy anion to give **16**. Expulsion of acetone from **16** and transacylation from N22 to the more nucleophilic N9 then results in the formation of **10**.



Scheme 4. Possible reaction mechanism for **9**→**10**.

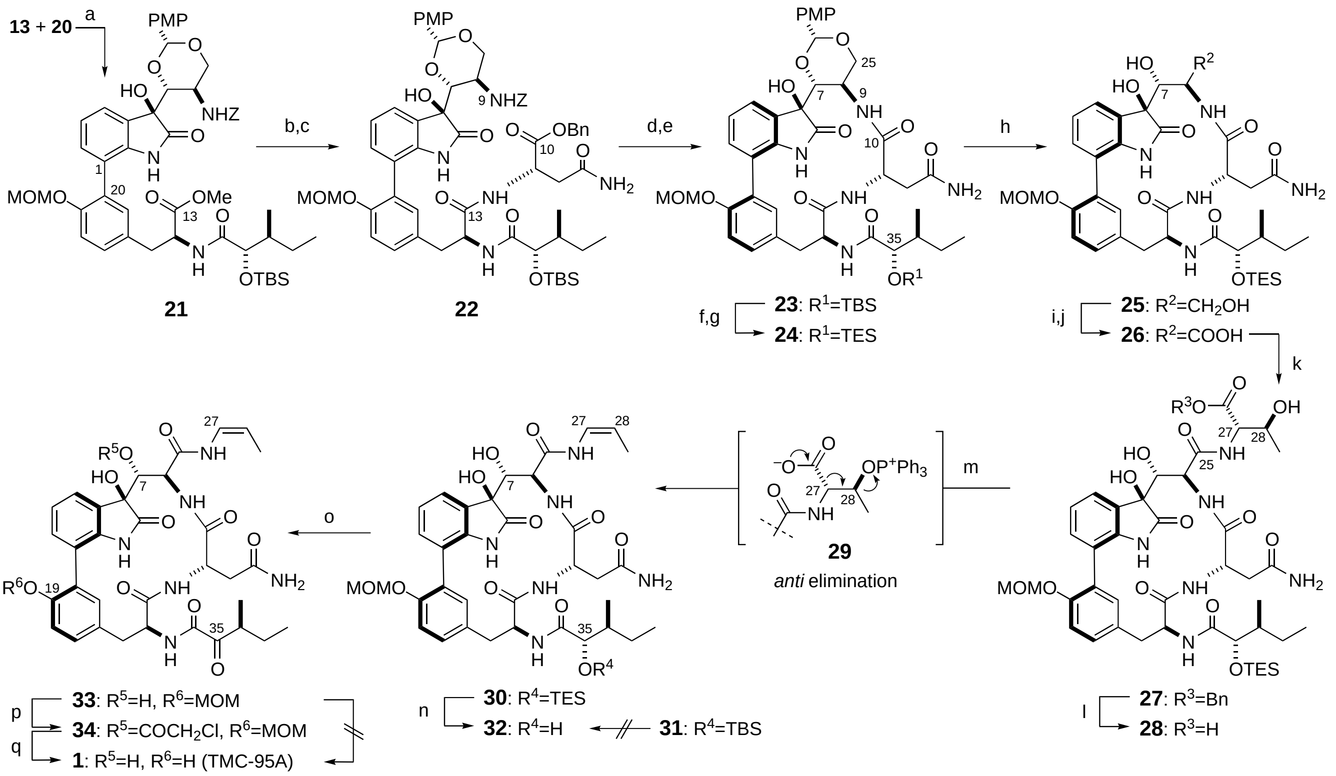
As shown in Scheme 3, regeneration of the oxindole structure from the aniline **10**, accompanied by loss of the ethoxyethyl group, was realized by lowering the pH value with *p*-toluenesulfonic acid to give **12** as a salt. The formation of **12** over **11** in acidic media is attributable to the preferential protonation of the more basic N9 amine of **12**.[16] The transformation from **9** to **12** was performed more practically in one pot by treating **9** under the Gassman conditions before sequentially introducing water, methanol, and TsOH. The liberated amine of **12** was then protected as its benzyl carbamate (55 % yield from **9**), and the 1,3-diol was masked as its *p*-methoxybenzylidene acetal to produce the tryptophan fragment **13** (84 % yield), suitably protected for the next reaction sequence.

We then turned our attention to the synthesis of the coupling partner of **13** (**20**, Scheme 5). The iodotyrosine derivative **17**[17] and the α-hydroxycarboxylic acid **3**[18] were prepared from L-tyrosine and L-isoleucine, respectively. After condensation of these two structural units in the presence of EDC and HOBt (56 % yield), selective protection of the phenolic hydroxy group of **18** as its methoxymethyl ether, followed by protection of the remaining C35 alcohol with TBS, produced **19** in 75 % yield over the two steps. The protected iodide **19** was converted into the aryl boronate **20** in 71 % yield by palladium-mediated borylation.[19]



Scheme 5. Reagents and conditions: a) **3** (2 equiv), EDC·HCl, HOBt, 4-methylmorpholine, DMF, 0 °C, 56 %; b) MOMCl, K_2CO_3, acetone, 85 %; c) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 88 %; d) bis(pinacolato)diboron, $[Pd(dppf)Cl_2]$, KOAc, DMSO, 80 °C, 71 %. DMSO = dimethylsulfoxide, dppf = 1,1′-bis(diphenylphosphany)ferrocene, EDC = ethylcarbodiimide chloride, HOBt = 1-hydroxy-1*H*-benzotriazole, MOM = methoxymethyl, TBSOTf = *tert*-butyldimethylsilyl trifluoromethanesulfonate.

The C1–C20 biaryl bond was formed by treating the two advanced intermediates **13** and **20** with a catalytic amount of $[Pd(PPh_3)_4]$ and Na_2CO_3 in aqueous DME at 95 °C to give the adduct **21** in 84 % yield (Scheme 6).[11,20] Basic hydrolysis of the methyl ester **21**, followed by amidation with L-asparagine benzyl ester, afforded **22** in 75 % yield. Hydrogenolysis of both the benzyloxycarbonyl (Z) and benzyl groups gave a seco acid, which was subjected to cyclization in the presence of EDC and HOAt to afford the macrolactam **23** in 78 % yield. In this way, the macrocyclic structure was constructed effectively through the assembly of three key fragments.



Scheme 6. Reagents and conditions: a) **20** (1.5 equiv), $[Pd(PPh_3)_4]$, Na_2CO_3, DME/H_2O (4:1), 95 °C, 84 %; b) LiOH, THF/H_2O (1:1), 0 °C; c) H-Asn-OBn·TFA, EDC·HCl, HOBt, DMF, 0 °C, 75 % from **21**; d) H_2, $Pd(OH)_2$/C, THF/H_2O (1:1); e) EDC·HCl, HOAt, DMF, 0 °C, 78 % from **22**; f) TBAF, 4-Å MS, THF, 86 %; g) TESCl, imidazole, DMF, 85 %; h) $Zn(OTf)_2$, EtSH, $NaHCO_3$, CH_2Cl_2, 100 %; i) SO_3·pyr, Et_3N, CH_2Cl_2/DMSO (3:1), room temperature; j) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*BuOH/H_2O (5:1), room temperature; k) L-allo-Thr-OBn·TFA, EDC·HCl, HOBt, DMF, 0 °C, 67 % from **25**; l) H_2, $Pd(OH)_2$/C, THF/H_2O (1:2); m) DEAD, PPh_3, 4-Å MS, 0 °C→room temperature, 59 % from **27**; n) HF·pyridine, THF, 79 %; o) Dess–Martin periodinane, CH_2Cl_2, 80 %; p) $(ClCH_2CO)O$, pyridine, CH_2Cl_2, 0 °C; q) aqueous HCl (1 N)/THF (3:1), room temperature; then saturated aqueous $NaHCO_3$, 64 % from **33**. Bn = benzyl; DEAD = diethyl azodicarboxylate, DME = 1,2-dimethoxyethane, HOAt = 1-hydroxy-7-azabenzotriazole, MS = molecular sieves, pyr = pyridine, TBAF = tetrabutylammonium fluoride, TES = triethylsilyl, TFA = trifluoroacetic acid.

We had thus reached a critical stage of the total synthesis: introduction of the (*Z*)-propenylamide, construction of the dicarbonyl moiety, and the final deprotection. First, the TBS group of the C35 alcohol was exchanged for TES (**23**→**24**) for facile deprotection at a later stage. The PMP acetal was then removed with zinc triflate and ethanethiol in the presence of $NaHCO_3$ as a buffer[21] to afford the triol **25** in 73 % yield over three steps. Selective oxidation of the C25 primary alcohol of **25** allowed differentiation of the oxidation levels of C7 and C25 and led to the carboxylic acid **26** in two steps. Attachment of L-allothreonine benzyl ester to **26** produced **27** (67 % yield from **25**), which was then subjected to hydrogenolysis to provide the β-hydroxycarboxylic acid **28**. Subsequent treatment of **28** with PPh_3 and DEAD[12] in the presence of molecular sieves induced dehydrative decarboxylation at room temperature and led to the isomerically pure (*Z*)-propenylamide **30** in 59 % yield over the two steps.[6,22] The geometry of the resultant olefin is thought to be defined through the Grob-type *anti* elimination of **29**.[23] Thus, the applicability of this method in a complex setting was clearly demonstrated. The neutral nature and high chemoselectivity of the reaction should make it suitable for use in the synthesis of a wide variety of TMC-95 analogues and other compounds containing enamides.[8,24]

To adjust the oxidation level to that of the natural product, the C35 alcohol in **30** had to be deprotected. A TBS-protected compound **31** had been prepared by a similar route to **30**, but extensive investigations had failed to identify conditions that would allow the selective removal of the TBS group while leaving the rest of the molecule intact. However, the TES group in **30** was readily removed and the alcohol **32** was obtained in 79 % yield upon treatment of **30** with HF·pyridine. Selective oxidation of the C35 alcohol of **32** in the presence of the C7 alcohol with Dess–Martin periodinane[25] then afforded MOM-protected TMC-95A **33** in 80 % yield.

The final deprotection step proved to be nontrivial because of the intrinsic instability of the enamide. Various attempts to remove the MOM group of **33** under acidic conditions led to a complex mixture of products, and TMC-95A (**1**) was obtained in only minute amounts. The free C7 alcohol may be responsible for these difficulties as it may react in an intramolecular fashion with the propenylamide to form a six-membered ring.[26] Therefore, the alcohol at C7 was temporarily protected as its chloroacetyl ester (**33**→**34**), which indeed suppressed the side reactions. Exposure of **34** to HCl (1 N)/THF and then saturated aqueous $NaHCO_3$ in one pot effectively afforded fully synthetic TMC-95A (**1**) in 64 % yield from **33** without destruction of the enamide or the stereochemistry of epimerizable C36 center. The physical data (^{1}H NMR, IR, MS) for the synthetic material were identical to those for the natural product.[3,8]

In summary, an efficient, highly convergent total synthesis of TMC-95A has been achieved. The key transformations in this synthesis are 1) a new deprotection protocol for the removal of the hindered carbamate and the acetonide of **9**, 2) conversion of the aniline **10** into the oxindole **12** by lowering the pH value, 3) a Suzuki–Miyaura coupling reaction and macrolactamization to efficiently create the macrocyclic framework **23**, and 4) a mild decarboxylative *anti* elimination to selectively produce the (*Z*)-1-propenylamide as part of a complex structure. The chemistry and biology of TMC-95A are being investigated further in our laboratory.

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Keywords: biaryls · macrocycles · natural products · olefination · total synthesis

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