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Total Synthesis of TMC-95A and -B via a New Reaction Leading to *Z*-Enamides. Some Preliminary Findings as to SAR

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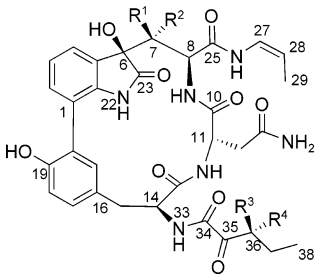
Abstract: A full account of the total syntheses of proteasome inhibitors TMC-95A and -B is provided. A key feature of the syntheses involved installation of a *cis*-propenylamide moiety by a thermal rearrangement of an α-silylallyl amide. The scope and mechanism of the enamide-forming reaction are discussed. Also provided are some preliminary results from SAR studies. It was found that simplified analogues can retain the full potency of proteasome inhibition.

Introduction

In eukaryotic cells, degradation of key regulatory proteins by the ubiquitin-proteasome pathway is crucial for many important cellular processes, including cell cycle progression, apoptosis, antigen presentation, and NF-κB activation.[1,2] Selective proteasome inhibitors are of therapeutic potential for a number of disorders, such as cancer, inflammation, and immune diseases.[3]

TMC-95A (**1a**) and its diastereoisomers TMC-95B−D (**1b**−**d**, Figure 1), recently isolated as fermentation products of *Apoispora montagnei*,[4] represent a new class of selective proteasome inhibitors. Among their defining structural characteristics are (i) the cyclic polypeptide array containing L-tyrosine, L-asparagine, and highly oxidized L-tryptophan moieties, (ii) an acylated (*Z*)-1-propenylamine substructure, and (iii) a 3-methyl-2-oxopentanoic acid substructure in the form of an amidic linkage to the tyrosine-like sector of the cyclic peptide.[5] Biological studies showed that TMC-95A inhibited the chymotrypsin-like (CT-L), trypsin-like (TL), and post-glutamyl peptide hydrolytic (PGPH) activities of the proteasome with IC_{50} values of 5.4, 200, and 60 nM, respectively.[4] TMC-95B inhibited these activities to the same extent as TMC-95A, while TMC-95C and -D were 20−150 times weaker. The binding mode of these inhibitors to the proteasome has been recently elucidated by X-ray crystallography.[6] Unlike other synthetic or natural proteasome inhibitors,[3b] TMC-95A does not modify the *N*-terminal catalytic threonine residue. It binds to the active sites of the proteasome via characteristic hydrogen bonds. TMC-95A also showed cytotoxic activities against human cancer cells HCT-116 and HL-60 with IC_{50} values of 4.4 and 9.8 μM, respectively.[4]



TMC-95	R^1	R^2	R^3	R^4
A (1a)	H	OH	Me	H
B (1b)	H	OH	H	Me
C (1c)	OH	H	Me	H
D (1d)	OH	H	H	Me

Figure 1. Structures of TMC-95A-D.

The combination of structural novelty and potency, as well as the unique inhibition mechanism of TMC-95A and -B has served to generate considerable interest among synthetic organic chemists. Not surprisingly, a variety of approaches have been pursued by several research groups.[7] Our group reported the

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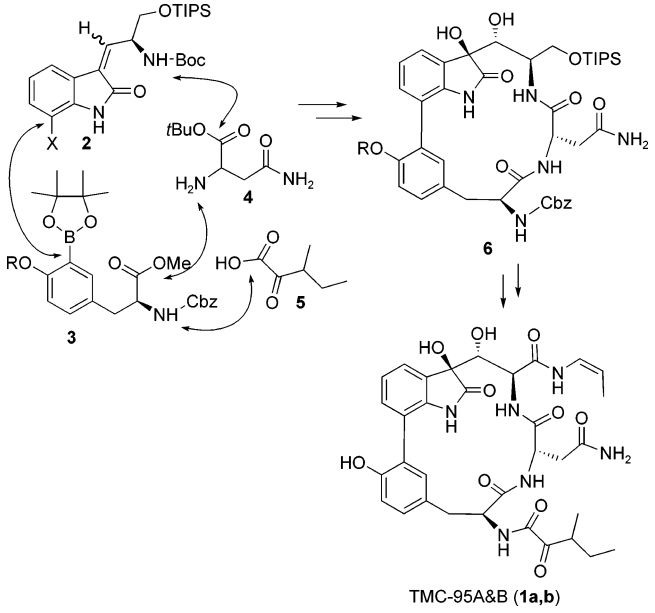
Scheme 1. Synthetic Plan[a]

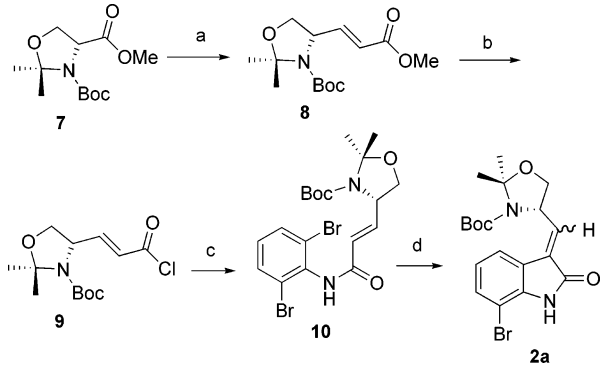


[a] TIPS = triisopropylsilyl, Cbz = benzoxycarbonyl, Boc = *tert*-butoxycarbonyl, X = Br or I.

total synthesis of TMC-95A and -B. A key phase of that synthesis involved installation of the cis-propenylamide moiety by a thermal rearrangement of an α-silylallyl amide.[8] Later, the Williams group reported a total synthesis of TMC-95A/B by linkage to compounds **41a**,**b** shown below.[9] Very recently, Hirama and co-workers reported a total synthesis of TMC-95A. In this synthesis, the *cis*-propenyl amide was produced through a decarboxylative *anti* elimination.[10] Below, we disclose experiments that resulted in the total synthesis of TMC-95A and -B. Notwithstanding some significant improvements in the synthesis, reaching the final natural targets was still prohibitively laborious in terms of a potential drug development program. We demonstrate below some positive SAR results, which serve to significantly simplify the synthetic endpoints required to realize equivalent bioactivity. The status of the project from the standpoint of a potential drug discovery program is evaluated in the light of these new findings. We also report on the scope of the enamide forming reaction, and provide some mechanistic insights as to its nature.

Overall Synthetic Strategy. Our synthetic plan, from the outset, stressed convergence in assembling a protected core system on to which the remaining required pendant functionality at C25 and N33 could be mounted. We envisioned that a Suzuki−Miyaura coupling reaction[11] could join aryl halide **2** and aryl boronate **3**. Installation of Asn residue **4** followed by macrolactamization could reach key structure **6** (Scheme 1). Obviously, the quality of the most speculative steps—(i) introduction of the 6,7-diol functions, (ii) fashioning of the C1−C20 bond by a Suzuki−Miyaura reaction, and (iii) macrolactamization—would be determined only through experimentation.

Scheme 2. Attempted Synthesis of 7-Bromooxindole **2a**[a]



[a] Conditions: (a) (1) DIBAL/toluene, −78 °C, 1 h; (2) methyl (triphenylphosphoranylidine)acetate, CH_2Cl_2, rt, 88% (two steps); (b) (1) LiOH, THF/MeOH/H_2O, (2) TBSCl, Et_3N/DMAP, (3) $(COCl)_2$, DMF (cat.); (c) 2,6-dibromoaniline, NaH, DMF/THF, 75 °C, 1.5 h, 44%; (d) $[Pd(PPh_3)_4]$ or $Pd(OAc)_2$, 5−15%. DIBAL = diisobutylaluminum hydride, TBS = *tert*-butyldimethylsilyl, DMAP = 4-(dimethylamino)pyridine.

Discussion of Results

Synthesis of the Oxindole Fragment. A synthesis of 7-bromooxindole **2a** was first attempted by recourse to a palladium-mediated intramolecular Heck reaction of substituted *N*-acyl-2,6-dibromoaniline **10** (Scheme 2). The synthesis commenced from methyl ester **7**, derived from D-serine.[12] Reduction of **7** with DIBAL gave a crude aldehyde, which was treated with methyl (triphenylphosphoranylidene)acetate to provide the α,β-unsaturated ester **8**. The latter was converted to the corresponding acid chloride **9** under neutral conditions via a three-step procedure of the type developed by Wissner.[13] Acylation of 2,6-dibromoaniline with **9** was sluggish and afforded α,β-conjugated amide **10** in only moderate yield. In light of the subsequent breakdown of this plan, this anilide formation step was not optimized.

Intramolecular Heck reactions of **10** were then investigated. Unfortunately, the desired cyclic product, 7-bromooxindole **2a**, was obtained in very low yields (5−15%), even after significant efforts at optimization. Since reactions of this general type are known,[14] we tend to ascribe the breakdown here to the presence of the second bromine function in **10** or the lack of protection of the amidic NH group.[7b] Clearly, the synthesis as proposed would be hard-pressed to progress, given the near breakdown of such a critical step at an early stage.

Accordingly, an alternative synthesis of the oxindole fragment was investigated. The key proposal envisioned a crossed-aldol reaction of serine-derived aldehyde **12** and 7-iodooxindole **16** (Scheme 3). Aldehyde **12** was synthesized in high yield from *N*-Boc-D-serine **11** following TIPS ether protection of the hydroxyl group and DIBAL reduction. The required precursor to oxindole **16**, 7-iodoisatin **15**, was obtained in good yield in two steps from 2-iodoaniline **13** via intermediate isonitrosoacetanilide **14**.[15]

We note, parenthetically, that the mechanism for this transformation is not known in detail. It might involve initial conversion of the aldoxime to a nitrile, with the latter serving

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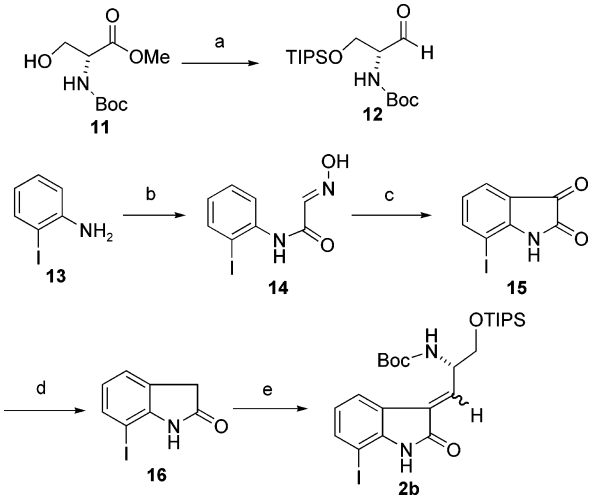
Scheme 3. Synthesis of 7-Iodooxindole **2b**[a]



[a] Conditions: (a) (1) TIPSCl, imidazole, 94%; DIBAL, −78 °C, 96%; (b) $CCl_3CH(OH)_2$, $NH_2OH{\cdot}HCl$, Na_2SO_4, H_2O, 45 °C, 12 h, 66%; (c) H_2SO_4, 70 C, 15 min, 88−98%; (d) (1) $NH_2NH_2{\cdot}H_2O$, 125 °C, 1 h, (2) HCl (6 N), 60 °C, 2 h, 81% (two steps); (e) **12**, see Table 1.

as the active electrophile. Alternatively, the oxime may be the active electrophile (with cyclization occurring at a faster rate than Beckman rearrangement (or dehydration to nitrile). The resultant hydroxylaminolactam, could in principle, suffer, ready conversion to the isatin by β-elimination of water, and hydrolysis of the monoimine.

The next phase in the progression involved conversion of isatin **15**, to the required oxindole. This was accomplished in two stages. The first apparently involves formation of the α-hydrazone lactam. This is followed by "Wolf−Kishner like" reduction, leading to the desired **16**.[16] Aldol condensation of **16** with aldehyde **12**, mediated through the use of 2 equiv of LDA, proceeded smoothly to provide alkene **2b** in good yields with 96% ee. Subsequent β-elimination of the derived mesylate afforded the desired α,β-unsaturated lactam target as an *E*/*Z* mixture of stereoisomers. The compounds were separated, thus facilitating NMR analysis at the stage of the mixtures. In addition, it was possible to evaluate the extent of racemization of the *N*-Boc-bearing asymmetric center (corresponding to C8 in **1a**−**d**). In principle, racemization might have occurred at the level of aldehyde **12** or by γ-deprotonation of product **2b**.

In Table 1, we summarize the results of varying the reaction conditions on yields, *E*−*Z* ratios, and maintenance of enantiomeric integrity of product **2b**.

Table 1. Condensation of Oxindole **16** with Aldehyde **12**

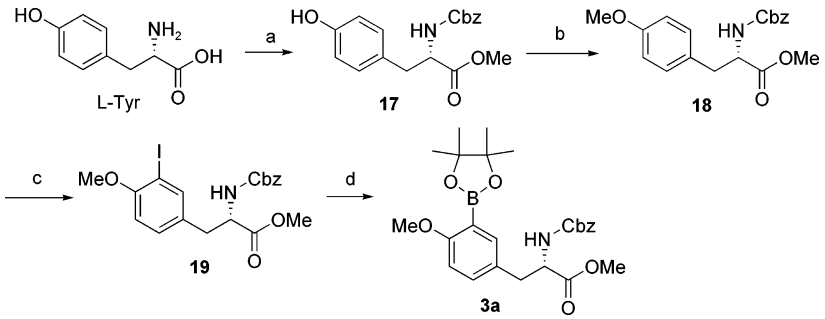
entry	conditions	yield[a] (%)	*E*/*Z*[b]	ee[c] (%)
1	piperidine (cat.), MeOH or EtOH, 65 °C, 2−3 h	44−50	2.0/1	0
2	piperidine (cat.), THF, rt, 17−44 h	55	1.7/1	∼10
3	(i) LDA (2.1 equiv), THF, −78 °C, **16**, then **12**, 1 h; (ii) TEA (2.5 equiv), MsCl (1.2 equiv), CH_2Cl_2, −60 to −30 °C, 2 h	76	1.3/1	92
4	(i) LDA (2.0 equiv), THF, −78 °C, **16**, then **12**, 1.5 h; (ii) TEA (3 equiv), MsCl (1.5 equiv), CH_2Cl_2, −70 to −50 °C, 1.5 h	74	1.2/1	96

[a] Isolated yield. [b] Determined by ^{1}H NMR; two isomers are separable. [c] Determined by chiral HPLC.

Suzuki−Miyaura Cross-Coupling. Aryl borate **3a**, the second component required for Suzuki−Miyaura cross-coupling, was prepared starting from L-tyrosine (Scheme 4). Methyl ester formation, as shown, followed by protection of the amino group as its Cbz derivative under standard conditions afforded phenol **17** in quantitative yield. Subsequent *O*-methylation of **17** with Me_2SO_4 in the presence of LiOH in THF provided **18** in 86% yield.[17] Selective iodination of **18** at the 3-position, ortho to the methoxy group, was accomplished in high yield with I_2/$AgSO_4$ in methanol (see compound **19**).[18] The required aryl borate **3a** was obtained from **19** in 95% yield following Miyaura's protocol.[19]

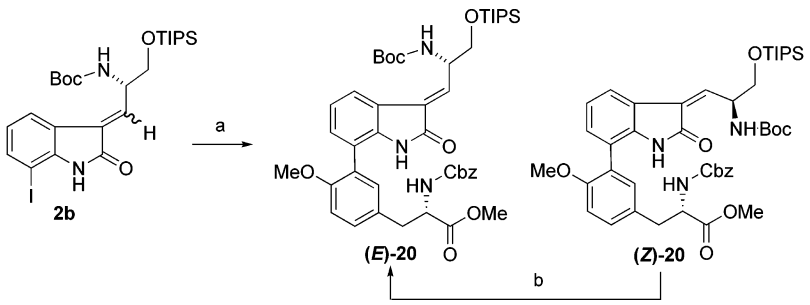
With 7-iodoxindole **2b** and aryl borate **3a** in hand, the proposed Suzuki−Miyaura cross-coupling reaction could be investigated.[19b] After an extensive survey of conditions (catalyst loading, temperature, and reaction time, equivalents of borate and base), we identified a regimen that afforded coupling product **20** in >70% yield as a 2:1 mixture of *E* and *Z* isomers, respectively (Scheme 5). The two isomers were separable by silica gel chromatography, and the stererochemistry of each was determined by NOE experiments (similar to those conducted at the stage of **2b**). Seemingly, by NMR analysis, no epimerization of the methinyl allylic urethane center at the future C-8 position had taken place under these conditions. However, *E*/*Z* isomerization apparently did occur during the Suzuki process. *The ratio of geometric isomers seen in the product (ca. 2:1) was independent of the nature of the E/Z ratio of starting material **2b***. Fortunately, recycling of *Z*-**20** to the usual 2:1 *E*/*Z* distribution could be accomplished following its thermolysis in DME in the presence of catalytic I_2.[7e]

Scheme 4. Synthesis of Aryl Borate **3a**[a]



[a] Conditions: (a) (1) MeOH/$SOCl_2$, (2) CbzCl, K_2CO_3, H_2O/acetone, 96% (two steps); (b) LiOH, Me_2SO_4, 86%; (c) I_2, Ag_2SO_4, MeOH, rt, 1.5 h, 93%; (d) bis(pinacolato)diboron, $[PdCl_2(dppf)]{\cdot}CH_2Cl_2$, KOAc, DMSO, 80 °C, 13 h, 95%. dppf = bis(diphenylphosphanyl)ferrocene.

Scheme 5. Biaryl Linkage Formation by Suzuki Coupling[a]



[a] Conditions: (a) **3a**, $[PdCl_2(dppf)]\cdot CH_2Cl_2$, KOAc, DME, 80 °C, 2 h, 72% (*E*/*Z* ~ 2/1); (b) I_2 (cat.), DME, 80 °C, 1 d, 87% (63% conv). DME = 1,2-dimethoxyethane.

Table 2. Conditions Investigated for Dihydroxylation of **23a**,**b**

			yields (%)		
entry	**23**	conditions	**24** (*S*/*R*)	**25**	**23a**
1	**a**	OsO_4 (0.4 equiv), NMO (1.2 equiv), acetone/H_2O (9:1), rt, 4 h	59 (1/2)	17	0
2	**a**	OsO_4 (0.1 equiv), NMO (1.2 equiv), $(DHQD)_2$−PHAL (0.15 equiv), *t*-BuOH/H_2O (2:1), rt, 1 h	84 (1/1.8)	<5	<5
3	**a**	OsO_4 (0.1 equiv), NMO (1.2 equiv), $(DHQ)_2$−PHAL (0.25 equiv), *t*-BuOH/H_2O (2:1), rt, 1 h	89 (1/3.7)	<3	<3
4	**a**	AD-mix-β, *t*-BuOH/H_2O, 0 °C to rt, 1 d	no reaction		
5	**a**	"Super"AD-mix-β (OsO_4: 5%), *t*-BuOH/H_2O, 0 °C to rt, 3 h	trace	29	64
6	**b**	OsO_4 (0.1 equiv), NMO (1.2 equiv), $(DHQD)_2$−PHAL (0.15 equiv), *t*-BuOH/H_2O (2:1), rt, 4 h; TIPS-Cl, imidazole/DMAP, 5 h	84 (1/1.7)	<5	<5
7	**b**	OsO_4 (0.1 equiv), NMO (1.2 equiv), $(DHQ)_2$−PHAL (0.25 equiv), *t*-BuOH/H_2O (2:1), rt, 4 h; TIPS-Cl, imidazole/DMAP, 5 h	81 (1/1.4)	<5	<5

Macrolactamization. With compound **20** in hand, the phase of the program ultimately directed to macrolactamization could begin. From the outset, we had a preference to investigate the dihydroxylation of the C6−C7 alkylidene linkage after macrolactam formation. It seemed likely that the macrocyclic structure would be more rigid than seco counterparts and that this rigidity could be exploited to provide diastereoface bias. Furthermore, it seemed that protecting group issues would be more manageable if the dihydroxylation would be postponed as late as possible.

The L-asparagine residue was installed onto the seco framework. Thus, hydrolysis of the α-methyl ester of **20** and conversion of the derived acid to the corresponding *N*-hydroxysuccinimide ester paved the way for amide formation with L-Asn O-*t*-Bu to afford **21**, as shown. Exposure of **21** to the action of 4 N HCl resulted in cleavage of the Boc protecting group. However, formation of the macrolactam amide bond between the aspargine and the ultimate C8-amino group could not be accomplished under various common peptide-coupling conditions (FDPP/DIEA in DMF or EDC/HOAT in DMF, CH_2Cl_2, or MeCN). We reasoned that perhaps the rigidity of the exo double bond at the 3-position of oxindole ring probably tilts the future C8 amino group away from the asparagine moiety, thereby disfavoring cyclization.

Accordingly, dihydroxylation was conducted prior to the proposed cyclization step. Saponification of the methyl ester (*E*)-**20** followed by coupling with L-asparagine *tert*-butyl ester (**4**) as above provided **23a** in 70% yield over two steps. Treatment of **23a** with HF/pyridine did indeed afford free alcohol **23b**. Next, the conditions for dihydroxylation of **23a**,**b** were investigated (Table 2). It was found that using either ligand $(DHQD)_2$PHAL or $(DHQ)_2$PHAL accelerated the reactions, thereby minimizing the formation of isatin **25**.[20] However, little effect on facial selectivity was observed with or without ligand. Under the optimized conditions, dihydroxylation of **23a** (entry 2) provided the diols **24** in 84% yield (*S*/*R* ≈ 1/1.8), along with a small amount of isatin **25** (<5%). Dihydroxylation of homoallyl alcohol **23b** (entry 7) in the presence of $(DHQ)_2$PHAL, followed by selective reprotection of the primary hydroxyl, gave similar results (81%, *S*/*R* ≈ 1/1.4, Scheme 6). The *R* configuration of **24** was assigned by conversion to its primary−secondary diol acetonide (see (*R*)-**24a**), whereupon the coupling constant between H7 and H8 is 0 Hz, indicating their *syn* relationship.

Treatment of **24*S*** with TFA in CH_2Cl_2 resulted in concurrent removal of the Boc protecting group and hydrolysis of the *tert*-butyl ester. The crude amino acid was then submitted to macrolactamization using EDC/HOAT under highly dilute conditions (4 mM) in CH_2Cl_2/DMF (4/1). Cyclization progressed smoothly, providing the desired product **6a** in 55% yield over two steps (Scheme 7). The large coupling constant (10.4 Hz) observed for H7−H8 in **5** is similar to those observed in TMC-95A and -B (**1a** and **1b**),[2] further confirming the configurational assignments at C6 and C7. *It was most interesting to discover that treatment of the R-isomer (24R) under the same reaction conditions did not afford any of the cyclization product.* This observation strongly suggested that the stereoconfiguration of

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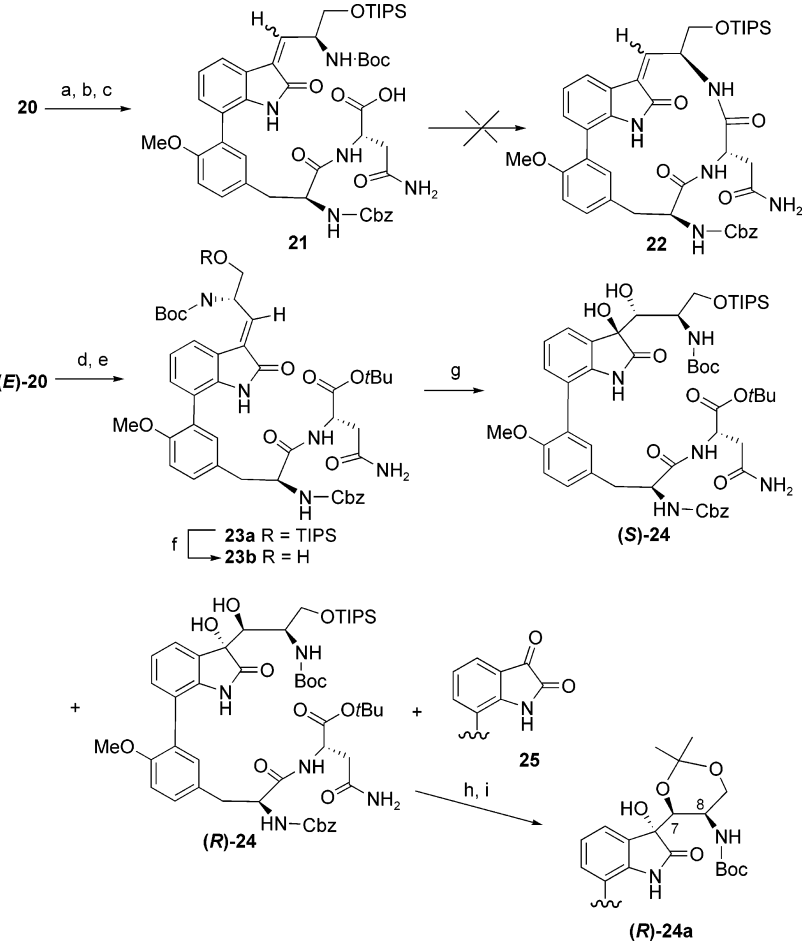
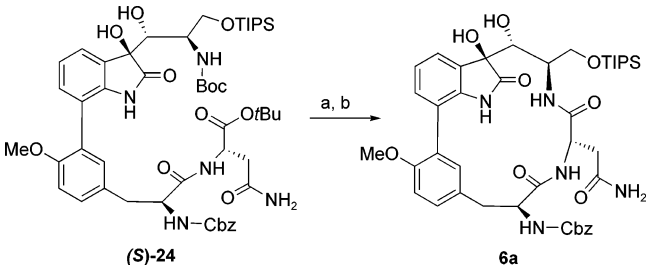
Scheme 6. Synthesis of Diols[a]



[a] Conditions: (a) LiOH, THF/MeOH/H_2O; (b) hydroxysuccinimide, DDC, THF, 55% (two steps); (c) L-Asn·H_2O, Et_3N, THF/H_2O, rt, 4 h, 70%; (d) LiOH, THF/H_2O, 0 °C, 1.5 h; (e) H-Asn-O-*t*-Bu, EDC/HOAt, THF, rt, 2 h, 70% (two steps); (f) HF/Py, 84%; (g) for **23a**: OsO_4/NMO, $(DHQD)_2PHAL$, *t*-BuOH/H_2O, rt, 1 h, 84% (*S*/*R* ∼ 1/1.8); for **23b**: (1) OsO_4/NMO, $(DHQ)_2PHAL$, *t*-BuOH/H_2O, rt, 4 h, (2) TIPSCl, imidazole/DMAP, 5 h, 81% (*S*/*R* ∼ 1/1.8); (h) HF/Py; (i) DMP/PPTS, CH_2Cl_2. DCC = 1,3-dicyclohexylcarbodiimide, EDC = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, HOAT = 1-hydroxy-7-azabenzotriazole, Asn = asparagine, NMO = 4-methylmorpholine *N*-oxide, $(DHQD)_2PHAL$ = 1,4-bis(9-*O*-dihydroquinidine)phthalazine, $(DHQ)_2PHAL$ = 1,4-bis(9-*O*-dihydroquinine)phthalazine, DMP = 2,2-dimethoxypropane, PPTS = pyridinium *p*-toluenesulfonate.

Scheme 7. Macrolactamization of (*S*)-**24**[a]



[a] Conditions: (a) TFA/CH_2Cl_2 (4:1), rt, 2 h; (b) EDC, HOAT, DIEA, CH_2Cl_2/DMF (4/1, 4 mM), 20 h, 55% (two steps). DIEA = *N*,*N*-diisopropylethylamine.

the diol was critical for macrocyclizations, at least with this particular arrangement of protecting groups.

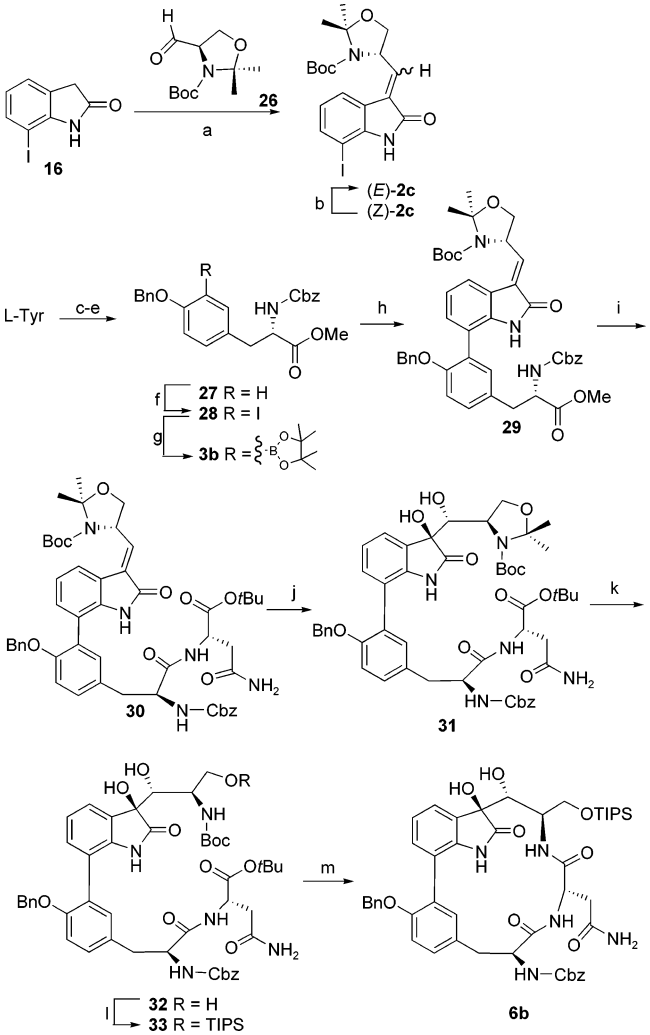
Modification of the Synthetic Strategy. Considering the low facial selectivity of dihydroxylation, we decided to modify our sequence. Thus, Garner aldehyde **26**[21] was to be employed in place of aldehyde **12**, since it had been shown earlier that such an oxazolidine could serve to direct stereoselective reactions at proximal sites of unsaturation (specifically, the dihydroxylation of (*E*)-**2a**).[7e]

To this end, a crossed-aldol condensation of 7-iodooxindole **16** with **26** was conducted. Following β-elimination of the mesylate derived from the resulting β-aldol, a 1:1.3 mixture of α,β-unsaturated lactams **2c(*Z*)**/**2c(*E*)** was obtained. As described above, the former isomer could be converted to the latter via iodine-mediated isomerization as shown (Scheme 8).

In anticipation of the need to achieve ultimate deprotection under the mildest conditions, the protection of the phenol hydroxyl of the tyrosine residue was changed from a methyl to a benzyl group. Thus, L-tyrosine was transformed to a suitably protected derivative, **27**, in three steps as shown. A high-yielding ortho iodination of **27** led to **28** and, thence, following palladium mediated borylation, to **3b**. Suzuki-type coupling of **3b** with **2c(*E*)** afforded compound **29** (75% yield). The biaryl domain of TMC-95A and -B was thus assembled. It was noted that no *E*/*Z* isomerization was observed during this Suzuki process, in contrast to that described in Scheme 5. This was likely due to the presence of the constraining *N*,*O*-acetonide moiety. Hydrolysis of the methyl ester function of **29** led to the corre-

(21) (a) Garner, P.; Park, J. M. *J. Org. Chem.* **1987**, *52*, 2361−2364. (b) McKillop, A.; Taylor, R. K.; Watson, R. J.; Lewis, N. *Synthesis* **1994**, 31−33.

Scheme 8. Synthesis of Macrolactam **6b**[a]



[a] Conditions: (a) LDA (2.0 equiv), THF, −78 °C, 1.5 h; then Et_3N, MsCl, CH_2Cl_2, −70 to −50 °C, 1.5 h, 81% (*E*/*Z* = 1.3/1); (b) I_2 (cat.), benzene, 80 °C, 26 h; DMP/PPTS, toluene, 65 °C, 5 h; 85% (60% conv); (c) MeOH/SO_2Cl_2; (d) CbzCl/K_2CO_3; (e) BnBr, Cs_2CO_3, acetone, reflux, 88% (three steps); (f) Ag_2SO_4/I_2, MeOH, rt, 1 h, 99%; (g) pinacolatodiborane, [$PdCl_2$(dppf)]CH_2Cl_2, K_2CO_3, DME, 80 °C, 10 h, 91%; (h) (*E*)-**2c**, [$PdCl_2$(dppf)]CH_2Cl_2, K_2CO_3, DME, 80 °C, 2 h, 75%; (i) (1) LiOH, THF/H_2O, 0 °C, 1.5 h, (2) H-Asn-O-*t*-Bu, EDC/HOAT, THF, rt, 2 h, 85% (two steps); (j) OsO_4/NMO, $(DHQD)_2$PHAL, *t*-BuOH/H_2O, rt, 12 h, 88% (dr = 5:1); (k) (1) PPTS/MeOH, reflux, 2 h; (l) TIPSCl, imidazole/DMAP, CH_2Cl_2, rt, 5 h, 88% (two steps); (m) (1) TFA/CH_2Cl_2 (4:1), rt, 2 h, (2) EDC/HOAT/DIEA, CH_2Cl_2/DMF (2 mM), rt, 24 h, 36%. DMP = 2,2-dimethoxypropane, PPTS = pyridinium *p*-toluenesulfonate, dppf = bis-(diphenylphosphanyl)ferrocene.

sponding carboxylic acid which served to acylate the basic nitrogen of asparagine O-*t*-Bu to afford **30**.

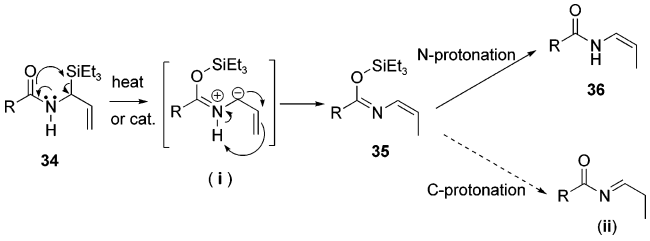
The facial selectivity of the dihydroxylation reaction was investigated next. The hydroxyl groups were introduced at carbons 6 and 7 as shown (Scheme 4). Indeed, the presence of the Garner *N*,*O*-acetonide did preferentially direct the dihydroxylating agent to the *Re* face (C6) of **30**, thus affording **31** in a 5:1 ratio relative to its 6*R*,7*S* stereoisomer (not shown).

Though the stereochemistry at carbons 6 and 7 was not rigorously known at this stage, our assignment followed well-established precedents with the Garner directing system.[7e] With the successful conclusion of the synthesis of TMC-95A (vide infra) the assignment was vindicated. Deprotection of the *N*,*O*-isopropylidene acetal linkage of **31** afforded *N*-Boc triol **32**.

Table 3. Conditions Investigated for Macrolactamization

entry	reagents	solvents, concentration	yield (%)
1	EDC/HOBT/DIEA	CH_2Cl_2/DMF (6:1), 2 mM	23
2	EDC/HOAT/DIEA	CH_2Cl_2/DMF (6:1), 4 mM	30
3	EDC/HOAT/DIEA	CH_2Cl_2/DMF (6:1), 2 mM	36
4	EDC/HOAT/DIEA	CH_2Cl_2/DMF (6:1), 1 mM	27
5	EDC/HOAT/DIEA	CH_2Cl_2, 2 mM	29
6	HATU/DIEA	CH_2Cl_2/DMF (6:1), 2 mM	32
7	HATU/DIEA	DMF, 2 mM	25
8	HATU/DIEA	THF, 5 mM	24
8	FDPP/DIEA	DMF, 5 mM	0
9	FDPP/DIEA	CH_2Cl_2/DMF (6:1), 5 mM	0
10	PyBOP/HOBT/DIEA	CH_2Cl_2/DMF (4:1), 4 mM	<10

Scheme 9. Proposed Strategy for *cis*-Enamide Formation



The primary alcohol at position 25 was selectively protected as its TIPS ether (see **33**). Cleavage of the *tert*-butyl and Boc groups generated a free amino acid, thereby setting the stage for macrolactamization studies to reach **6b**.

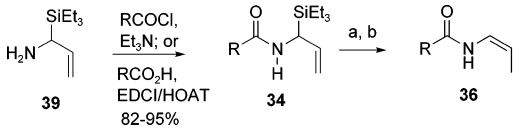
We investigated a variety of conditions to macrocyclization, and the results are listed in Table 3. Unfortunately, the best yield obtained is only 36%, when the combination of reagents of EDC/HOAT/DIEA was used and the reaction was carried out in a mixture of CH_2Cl_2/DMF (6:1, 2 mM)) (entry 3). A slightly lower yield (32%, entry 6) was obtained by using HATU/DIEA in a mixture of CH_2Cl_2/DMF (6:1, 2 mM).

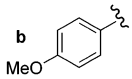
Installation of the *cis*-Propenylamide and Completion of the Synthesis. Having assembled the macrocyclic core, the next key issue to be addressed to reach the target was that of installing the *cis*-enamide side chain. Examination of the literature revealed several methods which might, in principle, be relevant to the enamide problem.[22] However, the applicability of these methods to the active TMC compounds was not certain. Considering the rich diversity of functionality presented by TMC95A and -B (**1a**,**b**), we set out to explore a new and mild modality for reaching such a substructure. We proposed that a substance of the type **34** might, upon heating or catalysis, undergo concurrent ene- and silatropic-like bond reorganizations that would lead to **35** (Scheme 9). If this hypothesis were to be fruitful, the *cis* character of the enamide would be virtually assured at the kinetic level. Moreover, it seemed at least conceivable that the intermediate silyl imidate linkage in **35** could be cleaved, with retrieval of general substructure **36**.

The idea is advanced in Scheme 9. Of course, this portrayal is intended as an overall accounting of the ultimate disposition of functional groups in going from starting material (**34**) to proposed product **36**, rather than as a mechanistic statement of concertedness. We envisioned the key step to be the transfer of the triethylsilyl group from C → O. As the TES group migrates

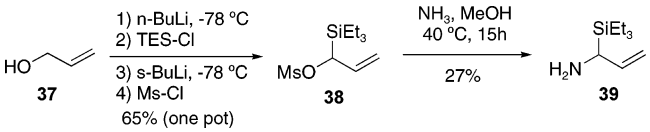
(22) (a) J. K. Stille, Y. Becker, *J. Org. Chem.* **1980**, *45*, 2139−2145. (b) Ribereau, P.; Delamare, M.; Celanire, S.; Queguiner, G. *Tetrahedron Lett.* **2001**, *42*, 3571−73. (c) Kuramochi, K.; Watanabe, H.; Kitahara, T. *Synlett* **2000**, *3*, 397−399. (d) Chen, R.; Porco, J. A., Jr. *Org. Lett.* **2000**, *2*, 1333−1336.

Table 4. Rearrangement−Hydrolysis of α-Silylallyl Amides **34**



Entry	R =	Conditions	Yield
1	**a** 	a. toluene, 110 °C, 10 h; b. H_2O	81%
2	**b** 	a. toluene, 110 °C, 20 h; b. H_2O	73%
3	**c** 	a. toluene, 110 °C, 27 h; b. H_2O	67%
4	**d** 	a. toluene, 110 °C, 3 d; b. H_2O	72%
5	**e** 	a.*o*-xylene, 110 °C, 4 d; b. H_2O	52%

Scheme 10. Synthesis of α-Silyl Allylamine



there is generated an allyl carbanionoid species linked to a positively charged imino ether like nitrogen (see species (i)). Whether species (i) is an actual intermediate or represents a phase of an overall reorganization cascade leading to the first uncharged intermediate **35** would be an interesting computational issue. We could foresee a significant synthesis level risk in getting from **35** → **36**. Thus, success would require *N*-protonation of the silyl intermediate without interrupting the *cis*-enamide functionality. Clearly, *C*-protonation of the conjugated imidate linkage of **35** would lead to (ii) with likely unraveling of the whole scheme.

To evaluate these conjectures, a range of probe substrates **34** (Table 4) was synthesized. The method involved appropriate acylations of the known amine **39**.[23] This compound was prepared by using an improved procedure, starting with allyl alcohol **37**. The scheme involves one-pot TES ether formation, retro-Brook rearrangement, and mesylation, followed by displacement of mesylate **38** with ammonia (Scheme 10). As seen in entries 1−3 (Table 4), thermolysis of these compounds at ca. 110 °C, for the time periods indicated, gave rise to silyl imidates **35a**−**c** (observed via ^{1}H NMR analysis). Aqueous hydrolysis of these compounds did indeed afford enamides **36a**−**c**. The reaction was applicable to substrate **34d** (entry 4), though a longer thermolysis time was required for its conversion to **35d**. Most significantly, the thermolysis−hydrolysis sequence proved to be extendable to the aminoacyl substrate **34e**, leading to **35e** and thence to **36e**. It was of great interest to determine whether this new method would find application at the very late stage of our projected total synthesis.

With the core hypothesis vindicated at the model level, we proceeded to convert **6b** to the corresponding α-silylallyl amide derivative. In this process, we found the choice of the protecting groups was crucial to the success of the project. Hydrogenolysis of **6b** in EtOH in the presence of Pd−C removed the benzyl and Cbz groups concurrently. Interestingly, EtOH was found to be a superior solvent than MeOH in that use of the latter resulted in significant methylation at N33 position. Subsequent acylation at N33 was achieved using racemic 3-methyl-2-oxopentanoic acid (**5**). This acid was obtained from its commercially available sodium salt. We saw no purpose to attempt acylation of the ultimate N33 with enantiomerically pure acid **5** since the C36 stereocenter epimerizes rapidly in this series. The TIPS protecting group was cleaved from the primary alcohol. Next, the four hydroxyl groups (positions 6, 7, 19, and 25) were protected as the tetra-TES derivatives (see **41a** and **41b)**. In a key step of the synthesis, reaction of these compounds with Jones reagent[24] led to specific oxidation at the primary center (position 25) to afford the corresponding acids. These closely related acids gave rise to the desired α-silylallyl amide derivatives **42a**,**b** and **43a**,**b**, following condensation with amine **39** (Scheme 11).

Happily, construction of the (*Z*)-1-propenylamide was accomplished by thermally driven rearrangement of the α-silylallyl amides **42a**,**b** and **43a**,**b**. It was not feasible or necessary to separate this complex mixture at this stage. Rather the eight component mixture was advanced, as shown, to provide the corresponding (*Z*)-1-propenylamides. The crude mixture of these compounds was globally deprotected with pyridine-buffered HF/pyridine to afford a mixture of our total synthesis goals: TMC-95A and -B (**1a** and **1b**; 1/1). This mixture was separated by reversed-phase HPLC to provide the individual compounds **1a** and **1b**. Their ^{1}H and ^{13}C NMR spectra were found to be identical to those of authentic samples. The completion of the synthesis of TMC-95A and B had been accomplished.

On the Mechanism of *cis*-Enamide Formation. Shortly following our report of the synthesis of TMC-95A and -B, Houk and co-workers investigated the mechanism of *cis*-enamide formation from *N*-(α-silyl)allylamides using density functional calculations.[25] Their studies strongly suggested a stepwise dyotropic rearrangement mechanism involving sequential 1,4-silyl and 1,4-hydrogen shifts. This mechanism is favored energetically relative to a fully concerted variation of Scheme 9. The two-stage mechanism explained well the experimental results in Table 2, wherein reactions are considerably faster when R = aromatic groups than those when R = alkyl groups. We note that in going from (i) to species type **35**, the transfer is almost certainly intramolecular, as hypothesized earlier, in keeping with the strict formation of *Z*-olefin.

Synthesis of Simplified Analogues. The current synthesis allowed us to produce small amounts of material, adequate for conducting the initial proteasome inhibition studies. The initial studies confirmed the activity in the fully synthetically derived compounds. However, our ultimate goal would be to develop potential drug candidates that are more accessible. Thus, we set out to design and synthesize several simplified TMC-95 analogues.[26] Even the currently improved route to TMC-95A

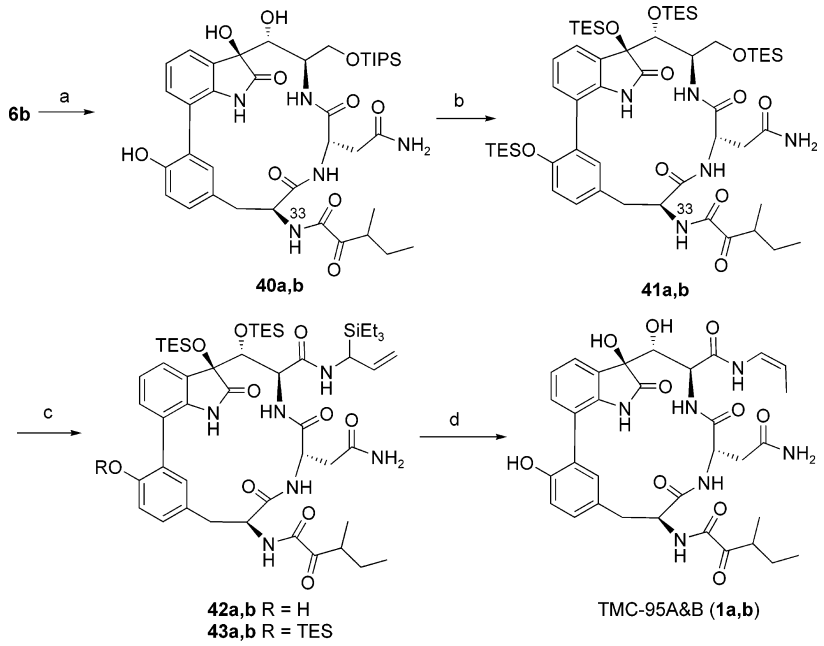
(23) Chen, S.-F.; Ho, E.; Mariano, P. S. *Tetrahedron* **1988**, *44*, 7013−7026.

(24) (a) Bowden, K.; Heibron, I. M.; Jones, E. R. H.; Weedon, B. C. L. *J. Chem. Soc.* 1946, 39. (b) Pilli, R. A.; Victor, M. M. *Tetrahedron Lett.* **1998**, *39*, 4421−4424.

(25) Zhang, X. Y.; Houk, K. N.; Lin, S.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 5111−5114.

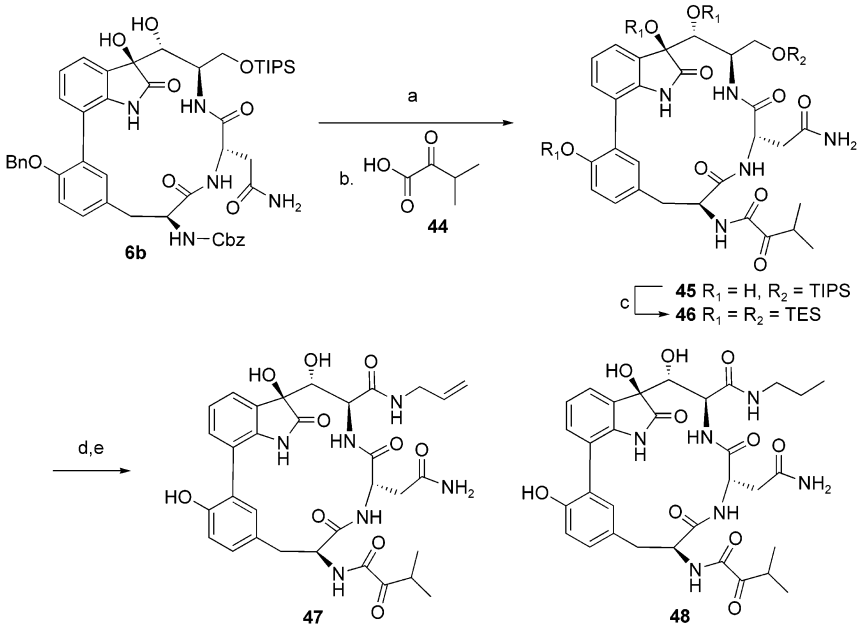
(26) Yang, Z.-Q.; Kowk, B. H. B.; Lin, S.; Koldobskiy, M. A.; Crews, C. M.; Danishefsky, S. J. *ChemBioChem* **2003**, *4*, 508−513.

Scheme 11. Completion of the Synthesis of TMC-95A/B[a]



[a] Conditions: (a) (1) Pd/C, H_2, EtOH, rt, 19 h, (2) (±)-3-methyl-2-oxopentanoic acid (**5**), EDC/HOAT, CH_2Cl_2/DMF, rt, 2 h, 85% (two steps); (b) (1) HF/Py, (2) TESOTf, 2,6-lutidine, CH_2Cl_2, 0 °C to rt, 15 h, (3) $NaHCO_3$, (4) citric acid, EtOAc/H_2O, 73% (four steps); (c) (1) Jones reagent, acetone, 0 °C, 2 h, (2) **39**, EDC/HOAT, CH_2Cl_2/DMF, rt, 13 h, 45% (two steps); (d) (1) *o*-xylene, 140 °C, 3 d, (2) HF/py, THF/py, then Me_3SiOMe, 49% (two steps).

Scheme 12. Synthesis of Analogues[a]



[a] Conditions: (a) Pd/C, H_2, EtOH, rt, 19 h; (b) **44**, EDC/HOAT, CH_2Cl_2/DMF, rt, 2 h, 57% (two steps); (c) (1) HF/Py, (2) TESOTf, 2,6-lutidine, CH_2Cl_2, 0 °C to rt, 15 h, (3) $NaHCO_3$, (4) citric acid, EtOAc/H_2O, 56% (four steps); (d) (1) Jones reagent, acetone, 0 °C, 2 h, (2) allylamine or *n*-propylamine, EDC/HOAT, CH_2Cl_2/DMF, rt, 13 h; (e) HF/py, THF/py; then Me_3SiOMe, 39% for **47**, 32% for **48** (three steps).

and -B has problematic phases which severely limit the accessibility of the natural product drugs. Two of these are (i) the *cis*-enamide formation steps and (ii) the final separation of TMC-95A and -B.

Given these problematic steps, we set out to explore the biological impact arising from replacement of the enamide at the C-8 position with a simple amide (such as allylamide or *n*-propylamide) and from circumventing of the C-36 stereogenic center. To this end, compounds **46** and **47**, were synthesized in a straightforward fashion using chemistry with which we were by now quite confident (Scheme 12). Specifically, installation of a symmetric α-ketoamide group was achieved employing **44**. Similar transformations provided the corresponding tetra-TES derivatives **46**. Jones' oxidation, followed by direct coupling reaction with allylamine or *n*-propylamine and cleavage of silyl ether groups, afforded target analogues **47** and **48**.

Proteasome Inhibition Studies. The biological activities of the synthetic analogues were evaluated on purified bovine erythrocyte proteasome.[26] The inhibitory concentrations (K_{iapp}) of the synthetic analogues against all three catalytic activities

Table 5. Inhibition Constants (K_{iapp}) of Catalytic Activities of the Proteasome by Synthetic Inhibitors[a]

	$K_{iapp} = [I]/(v_o/v_s) - 1]$[b]		
	CT-L activity (nM)	PGPH activity (nM)	TL activity (μM)
TMC-95A (**1a**)	1.1	29	0.8
TMC-95B (**1b**)	1.7	23	1.1
47	1.9	23	1.2
48	24	110	13

[a] The concentrations required for inhibition of the three proteasome catalytic activities were determined for TMC-95A and -B and their synthetic analogues. [b] The value v_o is the rate of enzyme activity in the absence of inhibitors, and v_s is the steady rate of inhibited enzyme activities.

of the proteasome are reported in Table 5 and compared with those of TMC-95A and B. Remarkably, the allylamide **47**, bearing a shorter side chain at the C14 position retains full inhibition potency of all three proteasome activities. The *N*-propylamide analogue, **48**, was however about 10 times less active. These results suggest that the side chain at C8 position still requires a certain degree of rigidity. The stereogenic center or nonstereogenic character at C36 was found to have no effect on potency. Thus a simple, much more accessible proteasome inhibitor (i.e. **47**), equipotent with TMC-95A and -B had been identified.

Conclusions

In summary, our totally synthetic route to TMC-95A and -B features aldol condensation of oxindole **16** and Garner's aldehyde **26**, Suzuki biaryl construction ((*E*)-**2c** + **3b** → **29**), facial-selective dihydroxylation (**30** → **31**), macrolactamization (**33** → **6b**), and a novel stereospecific *cis*-propenyl amide formation (**42**/**43** → **1**). We also discovered that a simplified analogue (**47**), featuring replacement of the enamide function with an allylamide and elimination of C36 stereogenic center, retains potent inhibition against proteasome activities. These findings certainly serve to provide more accessible drug candidates relative to the natural products. Future work will focus on such improvements.

However, for this work to progress to a viable platform for a feasible drug discovery program, we would have to improve the accessibility of equipotent compounds by another 1−2 orders of magnitude. This could, in principle, be accomplished by dramatic improvements in the synthesis or dramatic simplification in the structures required for activity. Thus, the field of TMC-95 inhibitors still merits continuing attention and creative advances from synthetic and pharmaceutical chemists.

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Supporting Information Available: Experimental procedure and/or physical data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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