The Total Synthesis of Proteasome Inhibitors TMC-95A and TMC-95B: Discovery of a New Method To Generate *cis*-Propenyl Amides**

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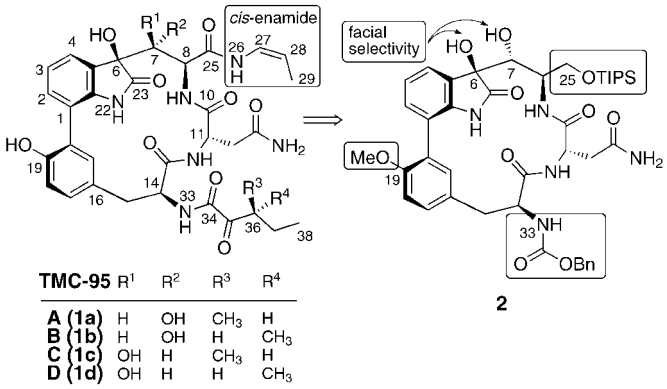
The ubiquitin proteasome pathway is critical for accomplishing proteolysis in both the cytosol and nucleus of all eukaryotic cells.[1] Understanding of the physiological roles of a particular proteasome has been aided by studying the effects of cell-permeable inhibitors. Moreover, specific proteasome inhibitors are emerging as possible drug candidates.[1c] Accordingly, the discovery of the cyclic peptides TMC-95A–D (**1a–d**; see Scheme 1), first isolated as fermentation products of *Apoispora montagnei* Sacc TC 1093 derived from soil samples,[2] was of great interest.[3, 4] The A and B isomers of **1**, which differ in stereochemistry at the remote, configuratively labile C36 center, inhibit proteasomal functions of the 20S proteasome with IC_{50} values down to low nanomolar levels.[2a]

Accordingly, a total synthesis program, which targets the more active TMC-95A and B isomers, was launched in our laboratory. We hoped to gain access to both compounds since after a successful total synthesis the groundwork would be more promising for establishing systematic SAR profiles of these TMC-95 inhibitors. Ideally, simpler structures could be designed, synthesized, and screened for similar activity in anticipation of their use as new drug leads.

Recently, we described an approach to accomplish stage I in our program, that is, the total synthesis of the TMC-95 inhibitors.[4a] The most advanced structure reported in our previous disclosure was compound **2** (Scheme 1). The attainment of this subgoal, promising as it was for our proposed program, still left unaddressed several key issues for total syntheses of **1a** and **1b**. Thus, provision would be necessary to allow exposure of a free phenolic hydroxy group at C19 and a homopyruvoyl group at N33 (see structure **2**). Moreover, the oxidation level at C25 must be upgraded from an alcohol, as found in **2**, to the carboxy state required to reach **1** (see below). This was no trivial matter, given the free hydroxy groups at C6 and C7 in our model target, **2**. We also note that in the synthesis of **2** the facial sense of *cis* dihydroxylation at

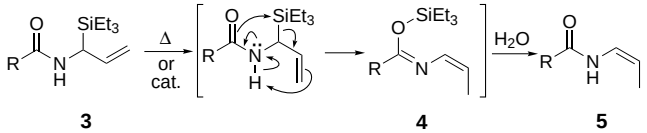


TMC-95	R^1	R^2	R^3	R^4
A (1a)	H	OH	CH_3	H
B (1b)	H	OH	H	CH_3
C (1c)	OH	H	CH_3	H
D (1d)	OH	H	H	CH_3

Scheme 1. Structures of TMC-95A–D (**1a–d**) and **2**. TIPS = triisopropylsilyl, Bn = benzyl.

C6 and C7 was virtually stereorandom.[4a] Perhaps most problematic was the *cis*-propenyl amide linkage that encompasses atoms N26–C29 of the multifaceted molecular ensemble of **1**. It is with this latter issue that we commence our report.

Examination of the literature reveals several methods that might, in principle, be relevant to the enamide problem.[5] However, the applicability of these methods to the synthesis of the active TMC compounds is not certain. Compounds **1** have a rich diversity of functionality, particularly in the extended "pyruvoyl"-like (C34–C38) and dihydroxyindolinone (positions 22, 23, and 6–8) regions. Anticipation of potential vulnerability in these sectors, not to speak of the *cis*-enamide itself (N26–C29), prompted us to explore a new modality for reaching such a substructure appropriate for molecules with multiple sites of potential instability. We wondered whether a substance of the type **3** might, upon heating or catalysis, undergo concurrent ene- and silatropic-like bond reorganizations that would lead to **4** (Scheme 2). 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 possible that the intermediate silyl imidate linkage in **4** could be cleaved, with retrieval of general substructure **5**.



Scheme 2. Formation of *cis*-propenyl amides **5** by rearrangement–hydrolysis of α-silylallyl amides **3** (R = aryl, alkenyl, and alkyl groups).

In the event, a range of probe substrates **3** was synthesized by acylation of the known of amine **6** (Table 1).[6] As seen in entries 1–3, heating of these compounds at about 110 °C for the time periods indicated gave rise to silyl imidates **4a–c** (observed by ^{1}H NMR analysis).[7] Aqueous hydrolysis of these compounds afforded enamides **5a–c**. The reaction was also applicable to substrate **3d** (entry 4), though a longer heating time was required for its conversion to **4d**. Most significantly, the rearrangement–hydrolysis sequence proved extendable to the aminoacyl substrate **3e** (entry 5) to afford

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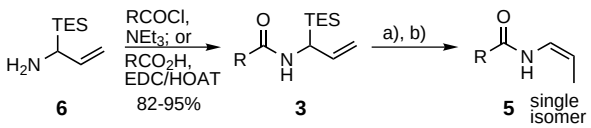
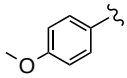
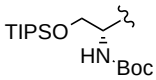
Tabelle 1. Rearrangement – hydrolysis of α-silylallyl amides **3**.[a]



6 **3** **5**

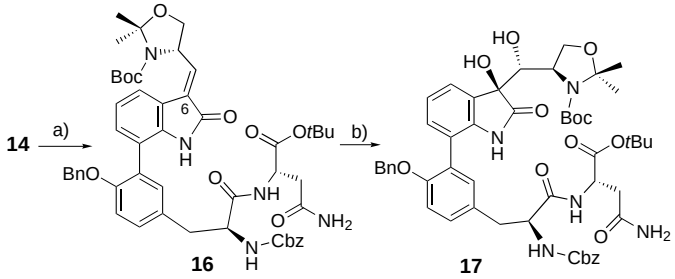
Entry	R =	Conditions	Yield [%]
1	**a** 	a) toluene, 110 °C, 10 h; b) H_2O	81
2	**b** 	a) toluene, 110 °C, 20 h; b) H_2O	73
3	**c** 	a) toluene, 110 °C, 27 h; b) H_2O	67
4	**d** 	a) toluene, 110 °C, 3 d; b) H_2O	72
5	**e** 	a) *o*-xylene, 135 °C, 4 d; b) H_2O	52

[a] TES = triethylsilyl, Boc = *tert*-butoxycarbonyl, EDC = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, HOAT = 1-hydroxy-7-azabenzotriazole.

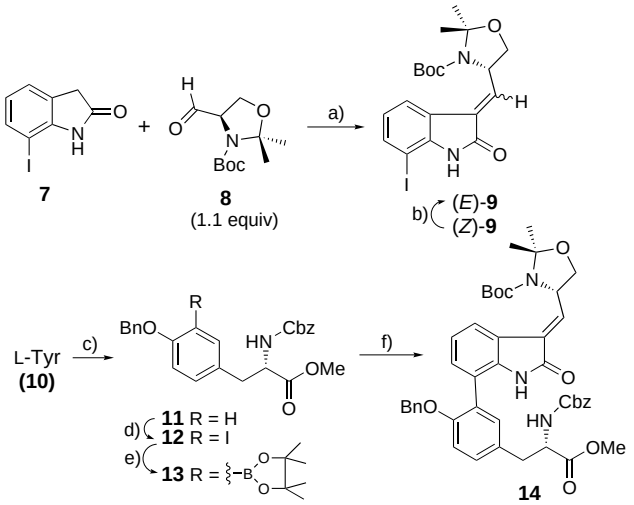
4e and thence **5e**. It was of great interest to determine whether this new method would find application at a very late stage of our projected total synthesis.

The starting material for our refashioned synthesis was the previously described iodooxindole **7** (Scheme 3).[4a] Crossed-aldol condensation of **7** with the Garner aldehyde **8**,[8] followed by β-elimination of the derived mesylate, afforded a 1:1.3 mixture of α,β-unsaturated lactams (*Z*)-**9** and (*E*)-**9**.[4a,e] Fortunately, the former isomer could be converted to the latter one through iodine-mediated isomerization.[4a,e] In a parallel sequence, L-tyrosine (**10**) was converted to **11** in three steps (Scheme 3).[9] A high-yielding *ortho* iodination[4a] of **11** led to **12** and thence, after palladium-mediated borylation,[10] to **13**. Suzuki-type coupling[11] of **13** with (*E*)-**9** afforded compound **14** (75% yield). We hoped that the biaryl domain thus presented would be serviceable in the context of our projected total synthesis (see below).

Hydrolysis of the methyl ester function of **14** led to the corresponding carboxylic acid, which, after acylation of the basic nitrogen atom of the asparagine derivative H-Asn-O*t*Bu (**15**), afforded **16**. The hydroxy groups were introduced at C6 and C7, as shown in Scheme 4. Indeed, the presence of the Garner *N,O*-acetonide served to direct, preferentially, the oxidizing agent to the *Re* face (C6) of **16** to afford **17** in a 5:1 ratio relative to its 6*R*,7*S* stereoisomer (not shown).

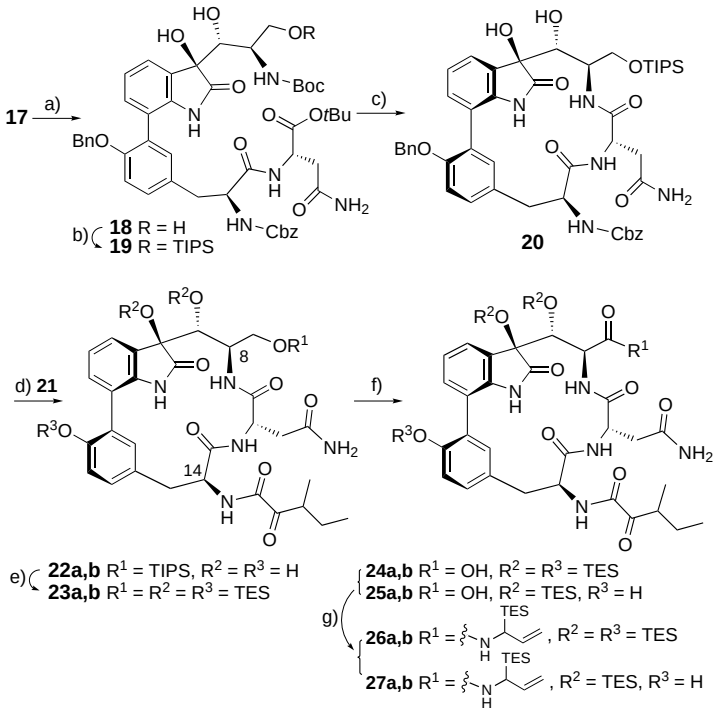


Scheme 4. Synthesis of diol **17**. a) 1) LiOH, THF/H_2O, 0 °C, 1.5 h; 2) H-Asn-O*t*Bu (**15**), EDC/HOAT, THF, RT, 2 h; 85% (two steps); b) OsO_4/NMO, $(DHQD)_2$-PHAL, *t*BuOH/H_2O, RT, 12 h; 88% (dr = 5:1). Asn = asparagine, NMO = 4-methylmorpholine *N*-oxide, $(DHQD)_2$-PHAL = 1,4-bis(9-*O*-dihydroquinidine)phthalazine.



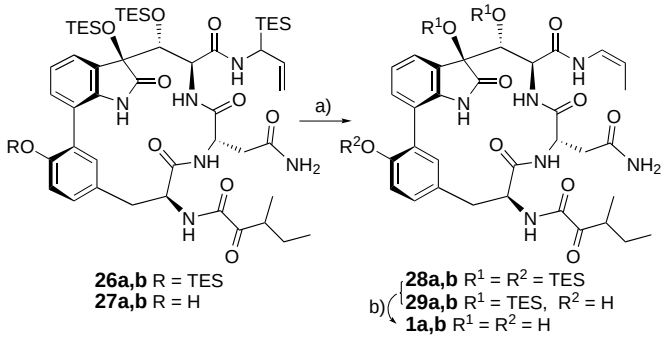
Scheme 3. Synthesis of biaryl compound **14**. a) LDA (2.0 equiv), THF, −78 °C, 1.5 h; NEt_3, MsCl, CH_2Cl_2, −70 → −50 °C, 1.5 h; 81% (*E*/*Z* = 1.3:1); b) I_2 (cat.), benzene, 80 °C, 26 h; DMP/PPTS, toluene, 65 °C, 5 h; 85% (60% conversion); c) 1) MeOH/$SOCl_2$; 2) CbzCl/K_2CO_3; 3) BnBr, Cs_2CO_3, acetone, reflux; 88% (three steps); d) Ag_2SO_4/I_2, MeOH, RT, 1 h; 99%; e) pinacolatodiborane, $[PdCl_2(dppf)]\cdot CH_2Cl_2$, KOAc, DMSO, 80 °C, 10 h, 91%; f) (*E*)-**9**, $[PdCl_2(dppf)]\cdot CH_2Cl_2$, K_2CO_3, DME, 80 °C, 2 h; 75%. Cbz = benzyloxycarbonyl, LDA = lithium diisopropylamide, DMP = 2,2-dimethoxypropane, PPTS = pyridinium *p*-toluenesulfonate, dppf = bis(diphenylphosphanyl)ferrocene.

The timing and manner in which the various heteroatom-centered functional groups were exposed and protected proved to be critical to the success of the project. We proceeded as follows (see Scheme 5): Deprotection of the *N,O*-acetonide linkage of **17** afforded *N*-Boc triol **18**. The primary alcohol at position 25 was protected as its TIPS derivative (**19**). Cleavage of the *tert*-butyl group generated a carboxylic acid at C10 which set the stage for macrolactamization (see compound **20**).[12] In the next step, the benzyl groups protecting the C19 phenol and N33 were concurrently cleaved by hydrogenolysis. Acylation of the latter basic nitrogen atom with racemic **21**[13] afforded **22a** and **22b** as a 1:1 mixture. Both compounds were advanced concurrently. The TIPS protecting group was cleaved from the primary alcohol. The four hydroxy groups (positions 6, 7, 19, and 25) were protected as the TES derivatives (see **23a** and **23b**). In a key step of the synthesis, reaction of these compounds with Jones reagent[14] led to specific oxidation at the primary center (position 25) to afford acids **24a** and **24b**. In addition, partial deprotection occurred at the C19 ether linkage which resulted in the formation of **25a** and **25b**. This four-component mixture led, after condensation with amine **6** as indicated, to the amide silyl ethers **26a** and **26b** as well as amide phenols **27a** and **27b** (**26a,b**:**27a,b** ≈ 1:1.5).



Scheme 5. Synthesis of α-silylallyl amides **26a,b** and **27a,b**. a) PPTS/MeOH, reflux, 2 h; b) TIPSCl, imidazole/DMAP, CH_2Cl_2, RT, 5 h; 88% (two steps); c) 1) TFA/CH_2Cl_2 (4:1), RT, 2 h; 2) EDC/HOAT/DIEA, CH_2Cl_2/DMF(2 mM), RT, 24 h; 52% (two steps); d) 1) Pd/C, H_2, EtOH, RT, 19 h; 2) (±)-3-methyl-2-oxopentanoic acid (**21**), EDC/HOAT, CH_2Cl_2/DMF, RT, 2 h; 85% (2 steps); e) 1) HF/Py; 2) TESOTf, 2,6-lutidine, CH_2Cl_2, 0 °C → RT, 15 h; 3) $NaHCO_3$; 4) citric acid, EtOAc/H_2O; 73% (from **22**); f) Jones reagent, acetone, 0 °C, 2 h; g) **6**, EDC/HOAT, CH_2Cl_2/DMF, RT, 13 h; 45% (two steps). DMAP = 4-dimethylaminopyridine, TFA = trifluoroacetic acid, DIEA = *N*,*N*-diisopropylethyl amine.

Construction of the (*Z*)-1-propenylamide was now achieved by thermally driven rearrangement of the α-silylallyl amides corresponding to **3** (Scheme 6). The rearrangement of



Scheme 6. Synthesis of TMC-95A (**1a**) and TMC-95B (**1b**). a) 1) *o*-xylene, 140 °C, 3 d; 2) H_2O; b) HF/Py, THF/Py; then Me_3SiOMe; 49% (two steps).

the complex mixture[15] in anhydrous *o*-xylene at 140 °C provided (*Z*)-1-propenylamides **28a,b** and **29a,b**. 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 TMC-95B (**1a** and **1b**; 1:1). This mixture was separated by RP-HPLC[16] to provide the individual compounds **1a** and **1b**. These were characterized by their high-field NMR spectra in comparison with those of authentic samples.[16]

In summary, the total syntheses of TMC-95A and TMC-95B have been achieved. The program featured a sequential assembly of oxindole **7**, Garner's aldehyde (**8**), aryl boronate **13**, asparagine derivative **15**, 3-methyl-2-oxopentanoic acid (**21**), and α-silylallyl amine **6**. Highlights of the synthesis include a Suzuki biaryl construction ((*E*)-**9** + **13** → **14**), a diastereofacial dihydroxylation reaction that took advantage of the Garner method (**16** → **17**), and a macrolactamization (formation of **20**). We also note that new chemistry to accomplish stereospecific *cis*-propenyl amide formation (**26**/**27** → **28**/**29**) was inspired by goal system **1**. The application to the delicate case at hand serves to build confidence in its generality.

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[6] Amine **6** (S.-F. Chen, E. Ho, P. S. Mariano, *Tetrahedron* **1988**, *44*, 7013 – 7026) was synthesized by using an improved procedure from allyl alcohol through a one-pot TES ether formation, retro-Brook rearrangement, and mesylation, followed by a displacement of mesylate with ammonia. Acylation in entries 1 – 4 (Table 1) was accomplished by coupling of **6** with the acid chlorides, while in entry 5 an EDC-mediated coupling with protected amino acid with amine **6** was involved. Details will be forthcoming in a full disclosure.

[7] The formation of silyl imidates **4** was clearly observed by ^1H NMR spectroscopy when these reactions were carried out in deuterated solvents.

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[9] To address the issues raised by problematic deprotection of the phenolic methyl group at a later stage (that is, compound **2**, Scheme 1), a benzyl group, instead of a methyl group, was used for the protection of the phenol (see ref. [4a]).

[10] a) T. Ishiyama, M. Murata, N. Miyaura, *J. Org. Chem.* **1995**, *60*, 7508 – 7510; b) A. M. Elder, D. H. Rich, *Org. Lett.* **1999**, *1*, 1443 – 1446.

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[12] The atropisomer shown for **20** follows from the C6 stereochemistry (see ref. [2b]).

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[13] 3-Methyl-2-oxopentanoic acid (**21**) was obtained from its commercially available sodium salt. There would be no purpose in coupling with enantiomerically defined acid **21** because the C36 stereocenter epimerizes rapidly throughout the series.
[14] a) K. Bowden, I. M. Heibron, E. R. H. Jones, B. C. L. Weedon, *J. Chem. Soc.* **1946**, 39; b) R. A. Pilli, M. M. Victor, *Tetrahedron Lett.* **1998**, *39*, 4421 – 4424.
[15] It was not feasible or necessary to separate at this stage. Rather the eight-component mixture was advanced as shown, and the separation was achieved at the stage of the two-component mixture **1a**/**1b**.
[16] HPLC conditions are as follows. Column: YMC-pack ODS-AM, 150×10 mm; eluant: 25 % MeCN in water; flow rate: 2.5 mL min^{-1}; t_R(**1a**) = 37.3 min, t_R(**1b**) = 34.3 min. The synthetic materials were identical to natural TMC-95A and TMC-95B (TLC, NMR, EIMS, and HPLC). We thank Dr. Jun Kohno, Tanabe Seiyaku Co., Japan, for generously providing us with the HPLC conditions and the samples of TMC-95A and B for comparison.

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