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Total Synthesis of Cyclic Tetrapeptide FR235222, a Potent Immunosuppressant that Inhibits Mammalian Histone Deacetylases

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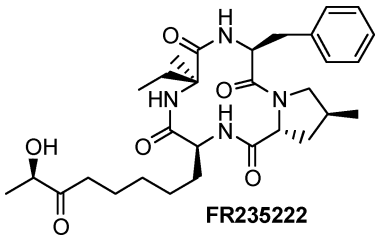
ABSTRACT



FR235222

The total synthesis of FR235222, a potent immunosuppressant with in vivo activities, has been achieved. The key steps include assembling its (2*S*,9*R*)-2-amino-9-hydroxy-8-oxodecanoic acid residue via an olefin cross-metathesis of a methyl (*R*)-lactate-derived homoallyl ketone with protected allyl amino acid and constructing its *trans*-(2*R*,4*S*)-4-methylproline unit from protected (*R*)-pyroglutamic acid in seven steps.

FR235222 (**1**, Figure 1) is a cyclic tetrapeptide that was isolated from the fermentation broth of a fungus, *Acremonium* sp. No. 27082.[1] This compound displayed a potent in vitro inhibitory effect on both lymphocyte proliferation and lymphokine production (IC_{50} = 0.35∼4.7 ng/mL).[2] In animal models, it showed marked immunosuppressive effects on mouse ex vivo splenic T-lymphocyte proliferation, mouse delayed type hypersensitivity (DTH) response, rat adjuvant-induced arthritis (AA), and rat heterotopic cardiac transplantation.[3] These facts, together with the low cytotoxicity of FR235222, make this cyclopeptide promising as a novel immunosuppressive drug. Like other cyclic tetrapeptides such as trapoxin B (**2**),[4] chlamydocin (**3**),[5] and diheteropeptin (**4**),[6] FR235222 is also a potent inhibitor for mammalian histone deacetylases (HDAC, IC_{50} = 9.7 ng/mL).[2] However, FR235222 possesses unique structural features that are distinct from the other cyclic tetrapeptides, which include isovaline, hydroxyketone, and methylproline units. The former two are rare, and the latter one is unprecedented in the other cyclic tetrapeptides. It was recognized that some of these units might play an essential role for its in vivo immunosuppressive effects because those related cyclic tetrapeptides bearing an epoxyketone element did not perform similar activities.[3] The presence of these elements in FR235222 is correlated to the agent's oral bioavailability

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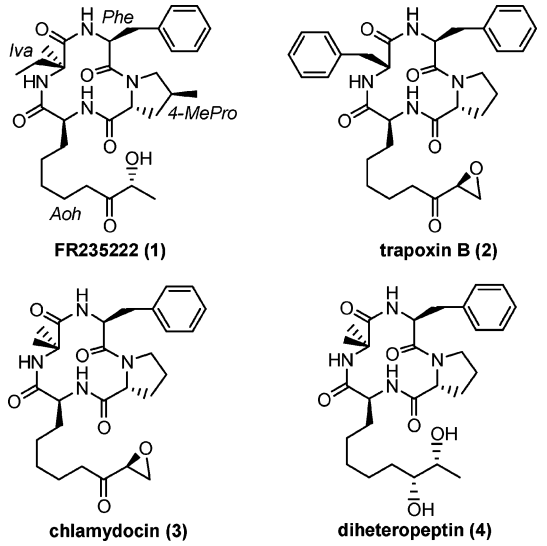
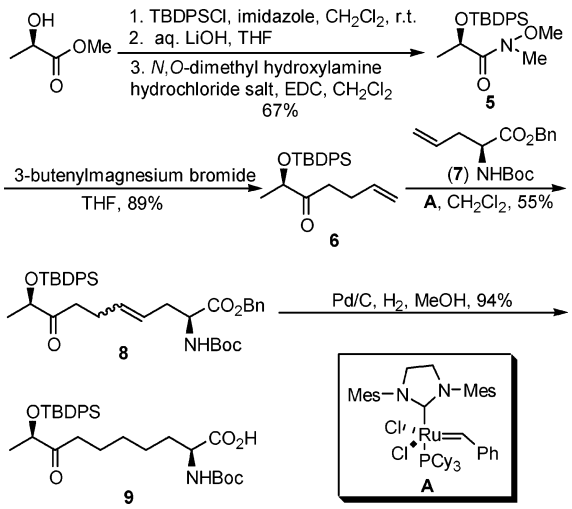


Figure 1. Structures of FR235222 and other related cyclic tetrapeptides.

and/or selectivity for immunorelated HDAC isozymes. Further SAR studies are required to clarify these issues. Toward this goal, we disclose here the first total synthesis of FR235222.[7]

On the basis of the structure of FR235222 and previous synthetic investigations toward cyclic tetrapeptides,[8] we realized that its (2*R*,4*S*)-4-methylproline site should be an ideal juncture for macrocyclization. Thus, our initial synthetic efforts were devoted to the assembly of FR235222's two unusual amino acid units, (2*S*,9*R*)-2-amino-9-hydroxy-8-oxodecanoic acid (Aoh) and (2*R*,4*S*)-4-methylproline. The production of the protected Aoh **9** is outlined in Scheme 1.

Scheme 1

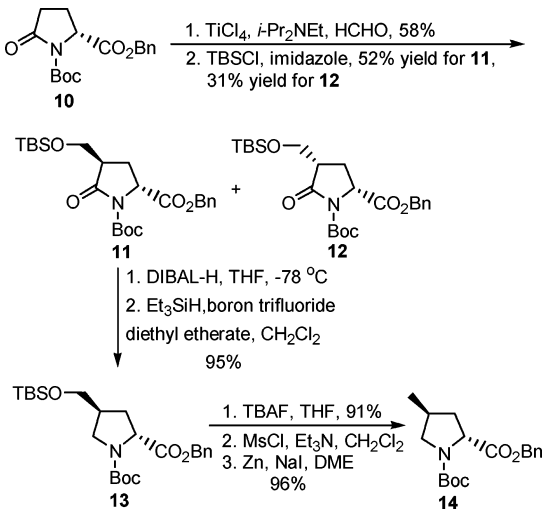


Treatment of methyl (*R*)-lactate with TBDPSCl, followed by hydrolysis of the ester group, and coupling of the resultant acid with *N*,*O*-dimethyl hydroxylamine afforded Weinreb amide **5**. Grignard reaction of **5** with 3-butenylmagnesium bromide delivered homoallyl ketone **6** in 89% yield. Next, we planned to use an intermolecular olefin cross-metathesis of **6** with protected allyl amino acid **7** to elaborate the desired olefins **8**.[9a] To our delight, reaction of **6** and **7** under the action of second-generation Grubbs RCM catalyst **A**[10] afforded **8** as a mixture of (*E*)- and (*Z*)-isomers in 55% yield. Finally, reduction of the olefin moiety of **8**, accompanied by removal of the benzyl group, upon Pd/C-catalyzed hydrogenation, provided acid **9** in 94% yield.

We next moved our attention to the elaboration of (2*R*,4*S*)-4-methylproline. Literature surveys[11−13] indicated that although considerable efforts have been directed toward the synthesis of 4-substituted prolines and their derivatives, none of them is satisfactory for assembling this *trans*-4-methylproline. For example, a recently reported protocol by Koskinen and co-workers suffers from long operation periods (10 steps from Garner aldehyde) and production of a diastereomeric mixture at a late stage.[11] Goodman's method[12] would require unnatural 4-hydroxyproline as a starting material and difficult to prepare Ir(COD)PyPCy$_3$PF$_6$ as a hydrogenation catalyst. Drawbacks such as these prompted us to develop a more practical route.

As depicted in Scheme 2, protected (*R*)-pyroglutamic acid

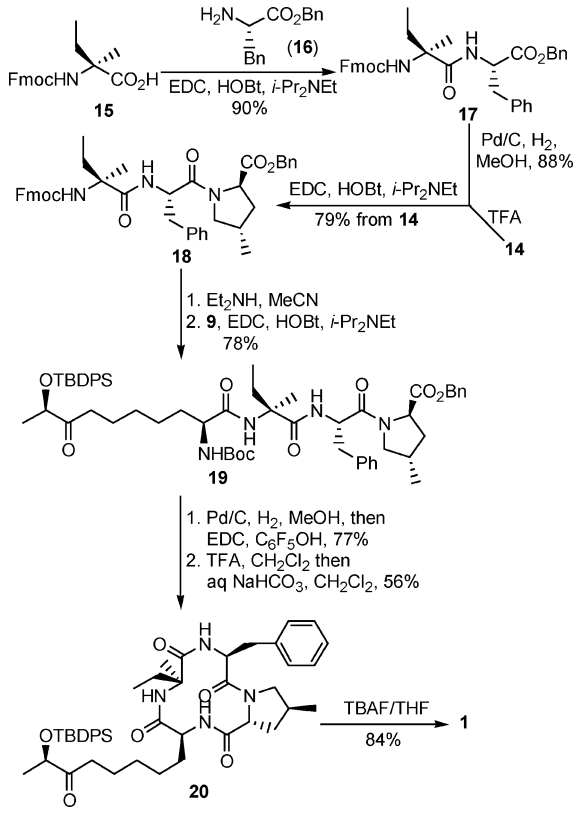
Scheme 2



10 was condensed with formaldehyde under the action of TiCl$_4$/*i*-Pr$_2$NEt-delivered aldol adducts in 58% yield,[14] which, upon treatment with TBSCl, produced a separable mixture of silyl ethers **11** and **12**. Stepwise reduction[15] of the 2-oxo unit in pyrrolidinone **11** with DIBAL-H and then Et$_3$SiH/BF$_3$·OEt$_2$ gave pyrrolidine **13** in 95% yield. Cleavage of the

(7) For other synthetic efforts toward the cyclopeptides from our group, see: (a) Yu, S.; Pan, X.; Lin, X.; Ma, D. *Angew. Chem., Int. Ed.* **2005**, *44*, 135. (b) Zhu, J.; Ma, D. *Angew. Chem., Int. Ed.* **2003**, *42*, 5348. (c) Zou, B.; Wei, J.; Cai, G.; Ma, D. *Org. Lett.* **2003**, *5*, 3503. (d) Ma, D.; Wu, Q. *Tetrahedron Lett.* **2001**, *42*, 5279. (e) Ma, D.; Wu, Q. *Tetrahedron Lett.* **2000**, *41*, 9089.

Scheme 3



silyl ether of **13** with TBAF followed by mesylation of resultant hydroxy group and reductive deoxygenation with Zn/NaI[16] afforded *N*-Boc-*O*-Bzl-*trans*-4-methylproline **14**. The overall yield for seven steps from **10** was about 25%. Substitution of formaldehyde with other aldehydes at the first step is envisioned to allow rapid assembly of other *trans*-4-alkyl-prolines. Thus, the simplicity and generality of the presented methodology should be comparable to existing methods.[11−13]

With synthons **9** and **14** in hand, we set out to construct FR235222 as shown in Scheme 3. Coupling of Fmoc-isovaline **15**, prepared by Mutter's procedure,[17] with (*S*)-phenylalanine benzyl ester **16**, afforded dipeptide **17** in 90% yield. Hydrogenolysis of the benzyl group in **17** and subsequent connection of the liberated amine from **14** mediated by EDC resulted in tripeptide **18**. Fmoc cleavage with diethylamine of **18** in acetonitrile produced the free amine of **18**, which was then coupled with the acid **9** to afford linear tetrapeptide **19** in 78% yield. Liberation of the ester moiety in **19** with Pd/C-catalyzed hydrogenolysis followed by coupling with pentafluorophenol delivered an activated ester. This ester was treated with TFA to effect cleavage of the Boc group and then subjected to macrocyclization in methylene chloride mediated by aqueous $NaHCO_3$ to provide cyclic tetrapeptide **20** in 56% yield. It is noteworthy that attempts at DPPA-mediated macrolactamization gave **20** in less than 20% yield. Finally, removal of the silyl protecting group in **20** with TBAF furnished FR235222 **1**.[18] Importantly, analytical data obtained for synthetic **1** were found to be indistinguishable from literature precedence.[1]

In conclusion, we have described here the first total synthesis of FR235222 featuring a novel and convenient protocol for elaboration of protected *trans*-4-methylproline and a facile synthesis of amino acid **9** via olefin metathesis with Grubbs RCM catalyst. Further investigations on the structure−activity relationship of this immunosuppressant made possible by the advances described here are in progress.

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Supporting Information Available: Experimental procedures and characterizations for compounds **5**−**9**, **11**−**14**, **17**−**20**, and **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Selected data for **1**: $[\alpha]^{23}_D = -129.1$ (*c* 0.5, $CHCl_3$), [lit[1]: $[\alpha]^{23}_D = -129.1$ (*c* 0.5, $CHCl_3$)]; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52 (d, J = 10.2 Hz, 1H), 7.29−7.20 (m, 5H), 7.17 (d, J = 10.6 Hz, 1H), 5.83 (s, 1H), 5.16 (ddd, J = 6.3, 9.7, 9.9 Hz, 1H), 4.24−4.17 (m, 2H), 4.05 (dd, J = 7.8, 9.7 Hz, 1H), 3.54 (d, J = 4.5 Hz, 1H), 3.24 (dd, J = 9.9, 13.6 Hz, 1H), 2.95 (dd, J = 6.1, 13.5 Hz, 1H), 2.73 (t, J = 7.8 Hz, 1H), 2.63 (m, 1H), 2.54−2.28 (m, 4H), 2.16 (m, 1H), 1.80 (m, 1H), 1.63 (m, 3H), 1.38 (d, J = 7.1 Hz, 3H), 1.33 (m, 5H), 1.28 (s, 3H), 0.88 (d, J = 6.7 Hz, 3H), 0.84 (t, J = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 214.4, 175.6, 171.1, 171.8, 137.1, 129.1, 128.6, 126.7, 72.6, 63.1, 58.0, 54.5, 53.9, 53.4, 37.3, 35.8, 33.1, 32.9, 28.8, 27.9, 25.3, 23.3, 22.4, 19.9, 18.2, 16.5, 8.4; ESIMS *m*/*z* 557 $(M + H)^+$, 579 $(M + Na)^+$; HRMS for $C_{30}H_{44}N_4O_6Na$ $(M + Na)^+$ calcd 579.3158, found 579.3156.