

A New Approach to (2*S*, 3*S*, 4*S*)-3-Hydroxy-4-Methylproline, A Subunit in Echinocandin B and Related Oligopeptide Antibiotics

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Abstract: A new approach to two protected forms of (2*S*, 3*S*, 4*S*)-3-hydroxy-4-methylproline, a constituent of echinocandins and related oligopeptide antibiotics is reported. The method features a highly regioselective and diastereoselective reductive furylation of protected (2*S*, 3*S*)-3-methylmalimide and a chemoselective oxidative transformation of furyl to carboxyl as the key steps.

Key words: Proline, regioselectivity, addition, asymmetric synthesis, malic acid, echinocandins, furan, organometallics.

Since the isolation, from a strain of *Aspergillus rugulosus* and *Aspergillus nidulans*, of echinocandins (**1**) (Fig. 1) as a novel class of oligopeptide antibiotic [1], intensive studies have been undertaken in order to find related compounds with improved bioactivities by chemical modification or from natural origins, which resulted in the discovery of mulundocandin [2a], deoxymulundocandin [2a], pneumocandin A₀ [2b], cilofungin [2c], aculeacine A_γ [2d], FK901379 [2e], FR131535 [2f], LY 303366 [2g] and FK-463 [2h]. Among them LY303366 and FK-463 are currently being investigated in phase II/III clinical studies against *Candida* and *Aspergillus* species. In all these cyclohexapeptides, (2*S*, 3*S*, 4*S*)-3-hydroxy-4-methylproline (Hmp), a nonproteinogenic amino acid, is present as a common structural moiety. In addition, Hmp is also a constituent found in nostopeptins A and B [3], which are elastase inhibitors isolated from the cultured freshwater cyanobacterium *Nostoc minutum* (CNIES-26).

The syntheses of (2*S*, 3*S*, 4*S*)-3-hydroxy-4-methylproline and its derivatives have been reported by Ohfuné [4], Evans [5], Mulzer [6], Langlois [7] and Kazmaier [8]. In continuation of our efforts to develop chiral imide-based reductive alkylation methodology [9], we wish to report herein a new approach to (2*S*, 3*S*, 4*S*)-3-hydroxy-4-methylproline.

Based on the consideration that 2-lithiofuran is a useful synthetic equivalent to unpoled hydroxycarbonyl anion [10], a simple retrosynthetic analysis (Scheme 1) showed that **2** could be derived from 5-(fur-2-yl)-pyrrolidin-2-one **3**, which could in turn be prepared from protected 4-methyl malimide **4** via a reductive furylation. It is to be noted that although, the 2-furyl group is a well known synthetic equivalent to the carboxyl [10], for the transformation of **3** to **2**, we have to face with the problem of chemoselectivity, namely oxidizing the 2-furyl group to carboxyl with strong oxidating agent RuO₄, while keeping the easily oxidizable *p*-methoxybenzyl group (PMB) [11] intact. Moreover, for the conversion of **4**

to **3** via reductive 2-furylation, we have to overcome the problem of regioselectivity. This is because, although the reductive alkylation of protected (*S*)-*N*, *O*-dibenzyl malimide **5a** [9] by Grignard reagents has been shown to be a highly regioselective procedure [9], we have observed that the addition of *n*-butyllithium to (*S*)-*N*, *O*-dibenzyl malimide proceeded with poor C-2 regioselectivity [9f]. Recent results from Cha [12a] and Pilli [12b] also showed that the addition of organometallics other than organomagnesium to (*S*)-*N*, *O*-dibenzyl malimide **5a** [12a,b] or (*S*, *S*)-*N*, *O*-dibenzyl-4-methylmalimide **5b** [12a] occurred predominately at C-5 carbonyl instead of C-2 carbonyl.

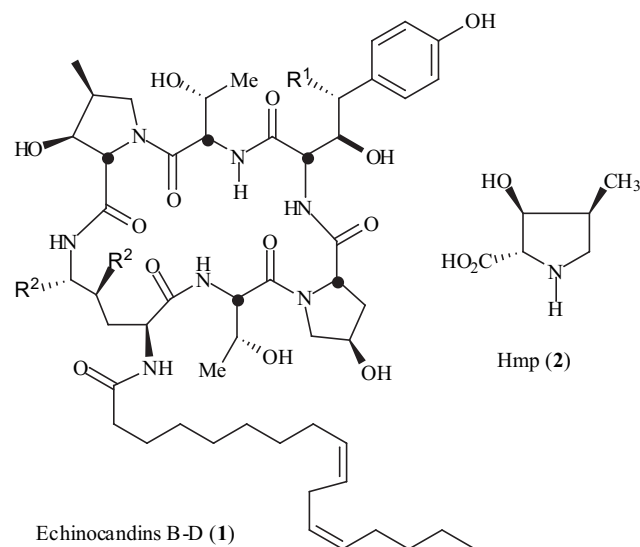
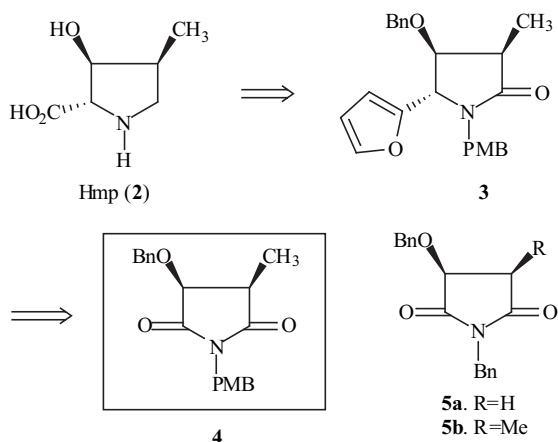


Fig. (1).

Our synthesis is depicted in Scheme 2. The requisite malimide **4** was prepared as follows. Started with (2*S*, 3*S*)-2-methylmalic acid (**7**), easily available from known (2*S*, 3*S*)-3-methylmalate **6** (as a higher than 10:1 diastereomeric mixture) [13] by saponification, and following the procedure described for its de-methyl derivative [9], **7** was converted, in one-pot and in an overall yield of 61%, to imide **8**. Higher reaction temperature and prolonged reaction time should be avoided in order to prevent epimerization at C-4

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stereogenic center. In this way **8** was obtained as an inseparable 9:1 diastereomeric mixture as indicated by its $^1\text{H-NMR}$ spectrum. Cleavage of the acetyl group under mild transesterification conditions (AcCl , EtOH , 0-rt, 30 h) allowed the isolation of crystalline **9** {mp 56°C . $[\alpha]_{\text{D}}^{28}$ -45.5 (c 1.0, CHCl_3)} as a single diastereomer in 90% yield. *O*-Benzylation (BnBr , Ag_2O , Et_2O , rt) then afforded the desired imide **4** $\{[\alpha]_{\text{D}}^{28} +59.6$ (c 1.0, CHCl_3)} in 82% yield.



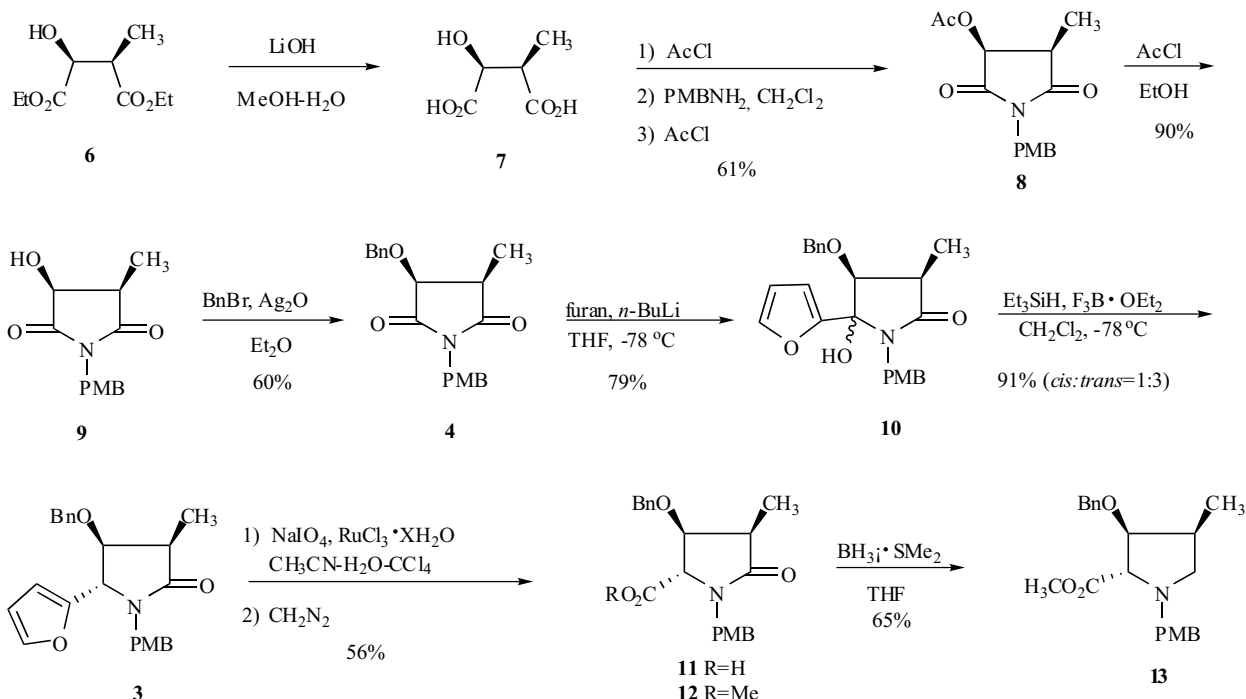
Scheme 1.

We then turned our attention to study the regio and diastereoselective reductive furylation of imide **4**. Treatment of imide **4** with 2-lithiofuran, *in situ* generated from furan and *n*-BuLi, at -78°C led smoothly to the formation of the desired C-2 adduct in 79% yield, which was then treated with boron trifluoride etherate and triethylsilane (-78°C , 8 h, then rt, 8 h). In this way, a 3:1 diastereomeric ratio was formed in a combined yield of 91%, from which the desired major *trans*-diastereomer (**3**) {oil. $[\alpha]_{\text{D}}^{28} +31.3$ (c 1.2,

CHCl_3)} was separated by column chromatography. No other regioisomer was observed. The attribution of C-2 addition instead of C-5 addition during the transformation of **4** to **10** was made based on the observed H-3 resonance appeared at 2.92 ppm of the $^1\text{H-NMR}$ spectrum of **3**, which was later confirmed by conversion of **3** to known compound **15**. The observed high C-2 regioselective 2-lithiofuran addition seems to be in contrast to above-mentioned observations [9c,12]. Although the exact reason for the preferred C-2 addition of 2-lithiofuran compared with *n*-butyllithium is not yet clear, lower reactivity 2-lithiofuran compared with *n*-BuLi would allow a pre-coordination of the former with oxygen at C-3 position of **4**.

Compared with the unexpected high regioselectivity observed in the 2-lithiofuran addition, the followed reductive deoxygenation of **10** was less stereoselective (*trans*:*cis* = 3:1), comparing with our previous results where *trans* diastereoselectivities are at least 94:6 [9]. Conversely, the presence of the *cis*-methyl should favor the formation of more *trans*-product [9c].

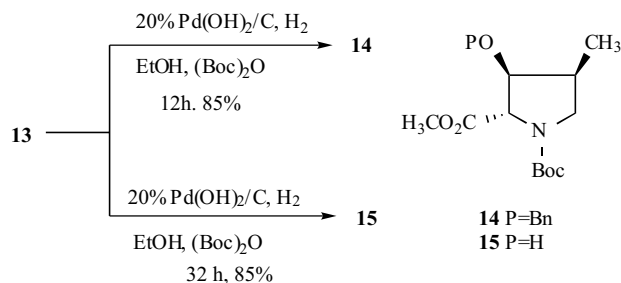
With compound **3** in hands, the un-masking of the 2-furyl to a carboxyl was undertaken. As we have mentioned earlier, chemoselective oxidation of the 2-furyl with powerful oxidant in the presence of *N*-(*p*-methoxybenzyl) group is a challenging task. Fortunately, treatment of **3** with RuO_4 , *in situ* generated from $\text{RuCl}_3 \cdot x\text{H}_2\text{O}$ - NaIO_4 system in a mixed solvent system (H_2O - MeCN - CCl_4 , 3:3:2) led to, after esterification (CH_2N_2 , Et_2O , 0°C) of the crude acid to the desired methyl ester **12** in 56% yield over two steps. This result showed that the reaction proceeded with good chemoselectivity: majority of *N*-(*p*-methoxybenzyl) group can survival from the RuO_4 oxidation conditions. Since RuO_4 is a powerful oxidant, which could even oxidize terminal olefin and phenyl group into a carboxyl, while in this case, most of *N*-*p*-methoxybenzyl group, a protective



Scheme 2

group removable under mild oxidative conditions (CAN, MeCN-H₂O, rt, 30 min or DDQ) has survived from the reaction. Next, chemoselective reduction of the amide carbonyl to methylene in the presence of an ester group was achieved by treatment of **12** with BH₃•SME₂, which provided the desired fully protected **13** in 65% yield.

Finally, chemoselective *N*-de-(*p*-methoxybenzylation) in the presence of both Pearlman's catalyst and (Boc)₂O [20%Pd(OH)₂, H₂, 1 atm, (Boc)₂O, EtOH, rt, 12 h] afforded protected Hmp **14** [**14**] in 85% yield (Scheme 3). If the catalytic hydrogenolysis was performed with prolonged time, known methyl (2*S*, 3*S*, 4*S*)-*N*-(*tert*-butyloxycarbonyl)-Hmp {**15**: [α]²⁵_D -23.4 (*c* 0.8, CHCl₃); lit.⁴ [α]²⁵_D -24.2 (*c* 1.1, CHCl₃)}, a compound which has been used in the total synthesis of echinocandin D, was obtained in 85% yield.



Scheme 3.

In summary, a new approach to protected (2*S*, 3*S*, 4*S*)-3-hydroxy-4-methylprolines was developed via a regio and diastereoselective reductive furylation of protected (2*S*, 3*S*)-3-methyl malimide. The choice of the furyl group as the masked carboxyl allowed the successful chemoselective unmask of the former in the presence of the *N*-(*p*-methoxybenzyl) group, which constituted the second key step of the synthesis.

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ABBREVIATION

Hmp = 3-hydroxy-4-methylproline

REFERENCES

- [1] (a) Benz, v. F.; Knusel, F.; Nuesch, J.; Treichler, H.; Voser, W.; Nyfeler, R.; Keller-Schierlein, W. *Helv. Chim. Acta* **1974**, *57*, 2459. (b) Traber, v. R.; Keller-Juslen, C.; Loosli, H.-R.; Kuhn, M.; v. Wartburg, A. *Helv. Chim. Acta* **1979**, *62*, 1252.
- [2] (a) Mukhopadhyay, T.; Ganguli, B. N.; Fehlhaber, H. W.; Kogler, H.; Vertesy, L. *J. Antibiot.* **1987**, *40*, 281; (b) Schwartz, R.; Sesin,

- D. F.; Joshua, H.; Wilson, K. E.; Kempf, A. J.; Goklen, K. E.; Kuehner, D.; Gailliot, P.; Gleason, C.; White, R.; Inamine, E.; Bills, G.; Salmon, P.; Zitano, L. *J. Antibiot.* **1992**, *45*, 1886. (c) Gprdee, R. S.; Debono, M.; Parr, T. R. Jr. The Fungal Cell Wall-A Target for Lipopeptide Antifungal Agents. In *New Approaches for antifungal Drugs*, Fernandes, P. B.; Ed.; Birkhauser: Boston, **1992**; pp 46-63. (d) Mizuno, K.; Yagi, A.; Sato, S.; Takada, M.; Hayashi, M.; Asano, K.; Matsuda, T. *J. Antibiot.* **1977**, *30*, 297. (e) Iwamoto, T.; Fujie, A.; Sakamoto, K.; Tsurumi, Y.; Shigematsu, N.; Yamashita, M.; Hashimoto, S.; Okuhara, M.; Kohsaka, M. *J. Antibiot.* **1994**, *47*, 1084. (f) Fujie, A.; Iwamoto, T.; Sato, B.; Muramatsu, H.; Kasahara, C.; Furuta, T.; Hori, Y.; Hino, M.; Hashimoto, S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 399. (g) Debono, M.; Turner, W. W.; LaGrandeur, L.; Burkhardt, F. J.; Nissen, J. S.; Nichols, K. K.; Rodriguez, M. J.; Zweifel, M. J.; Zeckner, D. J.; Gordee, R. S.; Tang, J.; Parr, T. R. *J. Med. Chem.* **1995**, *38*, 3171. (h) Mikamo, H.; Sato, Y.; Tamaya, T. *J. Antimicrob. Chemother.* **2000**, *46*, 485.
- [3] Okino, T.; Qi, S.; Matsuda, H.; Murakami, M.; Yamaguchi, K. *J. Nat. Prod.* **1997**, *60*, 158.
- [4] (a) Kurokawa, N.; Ohfune, Y. *J. Am. Chem. Soc.* **1986**, *108*, 6041. (b) Kurokawa, N.; Ohfune, Y. *Tetrahedron* **1993**, *49*, 6195.
- [5] Evans, D. A.; Weber, A. E. *J. Am. Chem. Soc.* **1987**, *109*, 7151.
- [6] Mulzer, J.; Becker, R.; Brunner, E. *J. Am. Chem. Soc.* **1989**, *111*, 7500.
- [7] (a) Langlois, N. *Tetrahedron Lett.* **1998**, *39*, 1333. (b) Langlois, N.; Rakotondradany, F. *Tetrahedron* **2000**, *56*, 2437.
- [8] (a) Mues, H.; Kazmaier, U. *Synlett* **2000**, 1004. (b) Mues, H.; Kazmaier, U. *Synthesis* **2001**, 487.
- [9] (a) Huang, P.-Q.; Wang, S. L.; Zheng, H.; Fei, X. S. *Tetrahedron Lett.* **1997**, *38*, 271. (b) Huang, P.-Q.; Wang, S. L.; Ruan, Y. P.; Gao, J. X. *Nat. Prod. Lett.* **1998**, *11*, 101. (c) Huang, P.-Q.; Chen, Q. F.; Chen, C.-L.; Zhang, H.-K. *Tetrahedron: Asymmetry* **1999**, *10*, 3827. (d) Huang, P.-Q.; Zheng, X. *Arkivoc* **2003**, Part ii, 7 (<http://www.arkatusa.org>). (e) Wu, T.-J.; Ye, J.-L.; Huang, P.-Q. *Chin. J. Chem.* **2003**, *21*, 723. (f) He, B.-Y.; Wu, T.-J.; Yu, X.-Y.; Huang, P.-Q. *Tetrahedron: Asymmetry*, **2003**, *14*, 2101.
- [10] (a) Mukaiyama, T.; Tsuzuki, R.; Kato, J. *Chem. Lett.* **1985**, 837. (b) Danishefsky, S. J.; DeNinno, M. P.; Chen, S. *J. Am. Chem. Soc.* **1988**, *110*, 3929. (c) Poss, H. A.; Reid, J. A. *Tetrahedron Lett.* **1992**, *33*, 1411.
- [11] (a) Yamaura, M.; Suzuki, T.; Hashimoto, H.; Yoshimura, J.; Okamoto, T.; Shin, C. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 1413. (b) Yoshimura, J.; Yamaura, M.; Suzuki, T.; Hashimoto, H. *Chem. Lett.* **1983**, 1001.
- [12] (a) Kim, S.-H.; Park, Y.; Choo, H.; Cha, J. K. *Tetrahedron Lett.* **2002**, *43*, 6657; (b) Schuch, C. M.; Pilli, R. A. *Tetrahedron: Asymmetry* **2002**, *13*, 1973.
- [13] Chamberlin, A. R.; Chung, J. Y. L. *J. Am. Chem. Soc.* **1983**, *105*, 3653. (b) Klaver, W. J.; Hiemstra, H.; Speckamp, W. N. *J. Am. Chem. Soc.* **1989**, *111*, 2588.
- [14] Selected data for **14**: colorless oil. [α]¹⁷_D -26.7 (*c* 0.9, CHCl₃), IR (film) ν_{max}: 2967, 1746, 1711, 1612 cm⁻¹. ¹H-NMR (500 MHz, CDCl₃): 1.11, 1.12 (two rotamers, each d, 3H, *J* = 6.8, 6.9 Hz, CH₃-CH), 1.44, 1.49 (each s, 9H, *t*-Boc), 2.40 (m, 1H, H-4), 3.18, 3.22 (each dd, 1H, *J* = 10.1, 10.3 Hz, H-5), 3.70 (dd, 0.5H, *J* = 8.2, 9.8 Hz, H-5, rotamer 1), 3.74 (dd, 0.5H, overlapped with CO₂CH₃, H-5, rotamer 2), 3.75, 3.76 (each s, 3H, overlapped with H-5, CO₂CH₃), 3.88, 3.91 (each dd, 1H, *J* = 1.1, 4.5 Hz, H-3), 4.38 (d, 0.5H, *J* = 1.1 Hz, H-2, rotamer 1), 4.51 (s, 0.5H, H-2, rotamer 2), 4.54, 4.56 (each d, 1H, *J* = 11.4 Hz, OCH₂Ph), 4.76, 4.78 (each d, 1H, *J* = 11.4 Hz, OCH₂Ph). ¹³C-NMR (125 MHz, CDCl₃): 171.6, 171.3, 154.6, 153.7, 137.6, 128.4, 127.8, 127.7, 127.5, 83.6, 82.6, 79.9, 71.3, 71.2, 64.7, 64.3, 52.3, 52.1, 51.4, 50.9, 36.6, 35.8, 29.7, 28.4, 28.2, 11.1, 11.0. HRMS calcd for [C₁₂H₂₁NO₅+H]⁺: 350.1967. Found: 350.1964.