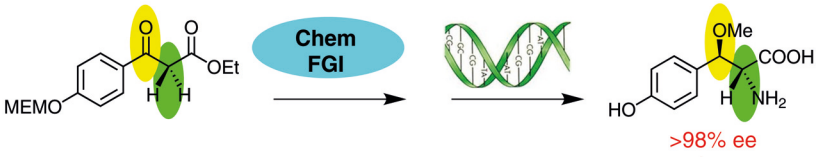
Synlett S.-M. Fan et al. Letter

An Efficient Synthesis of Enantiopure (2*R*,3*R*)-β-Methoxytyrosine

Shi-Ming Fan[a]
Xia Tian[b]
Yi-Hua Yang[b]
Long yi Jin[*a]
Shou-xin Liu[*a,b]

[a] Department of Chemistry, College of Science, Yanbian University, 977 Gongyuan Road, Yanji 133002, P. R. of China
lyjin@ybu.edu.cn
[b] State Key Laboratory Breeding Base-Hebei Province Laboratory of Molecular Chemistry for Drug, Hebei University of Science & Technology, 70 Yuhua Road, Shijiazhuang 050018, P. R. of China
chlsx@hebust.edu.cn



Received: 18.05.2015
Accepted after revision: 21.06.2015
Published online: 01.09.2015
DOI: 10.1055/s-0034-1381048; Art ID: st-2015-w0381-l

Abstract Enantiopure (2*R*,3*R*)-β-methoxytyrosine was stereoselectively synthesized from ethyl 3-(4-hydroxyphenyl)-3-oxopropanoate protected by 2-methoxyethoxymethyl (MEM) (ee >98%). L-Aminoacylase-catalyzed resolution of the corresponding *erythro*-*N*-acetyl derivatives afforded (2*S*,3*S*)-(4-MEM)-β-methoxytyrosine (ee >99%). The conversion increased to 98% by optimizing the synthesis to yield enantiopure *N*-acetyl-(2*R*,3*R*)-(4-MEM)-methoxytyrosine. *N*-Acyl cleavage was accomplished under mild conditions.

Key words amino acids, biosynthesis, asymmetric synthesis, enantioselectivity, enzymes, natural products

(2*R*,3*R*)-β-Methoxytyrosine is a common non-proteinogenic amino acid in a number of novel cyclic depsipeptides isolated from marine sponges.[1] These marine metabolites possess the similar structure, which includes callipeltins A (Figure 1) and B,[2] stellatolide A,[3] papuamides A–F,[4] mirabamides A–H,[5] and neamphamides B–D.[6] Many of these have been reported to inhibit HIV-1 viral entry in vitro as well as cytotoxicity against a number of human cancer cell lines and fungi. β-Methoxytyrosine may also play an important role in the biological activity of these depsipeptides.[5a,7] Recently, tremendous synthetic efforts have been dedicated to the preparation of β-methoxytyrosine. The strategies include the stereoselective addition reaction of arylmetal reagents with serine aldehyde equivalents followed by methylation,[8] photo-assisted bromination of D- and L-tyrosine derivatives followed by methanolysis,[9] and Sharpless asymmetric aminohydroxylation or dihydroxylation of cinnamyl ester derivatives.[10]

Although each of these approaches offer elegant advantages, they still suffer from poor stereoselectivity, the need for expensive chiral catalysts or the need for stoichiometric amounts of chiral auxiliaries. In contrast, biocatalysis is an interesting alternative that leverages the enzymatic specificity and efficiency.[11] For the first time, we report here a coupling approach for the synthesis and aminoacylase resolution to synthesize enantiopure β-methoxytyrosine as part of efforts in the total synthesis of these natural cyclic depsipeptides.

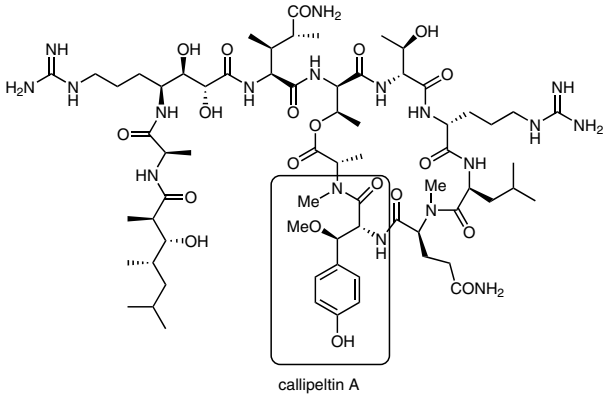
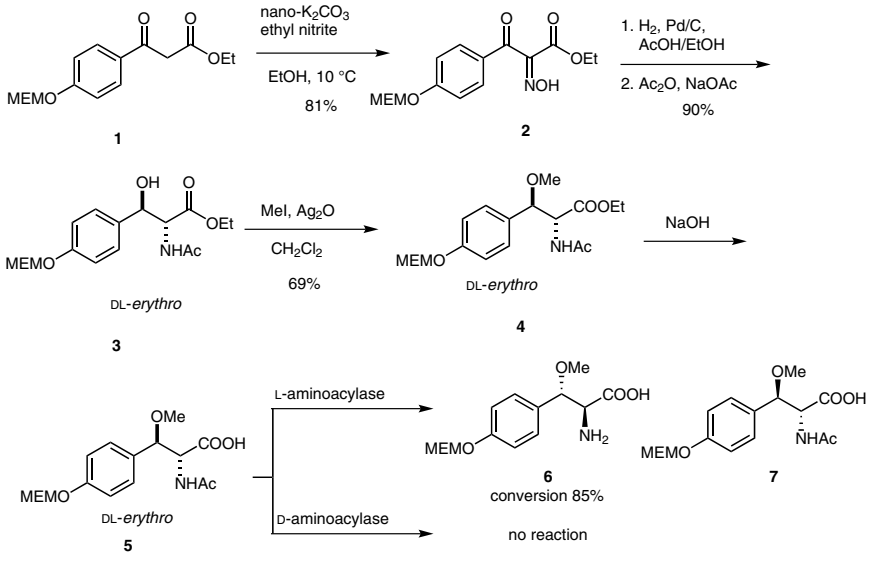


Figure 1 Structure of callipeltin A

As shown in Scheme 1, the intermediate oxime **2** was successfully achieved through the oximation of ethyl 3-(4-hydroxyphenyl)-3-oxopropanoate protected with methoxyethoxymethyl (MEM) with ethyl nitrite in the presence of nano-K_2CO_3 previously developed by our group.[12] The diastereoselective hydrogenation of oxime **2** yielded ethyl *erythro*-β-hydroxy-β-arylalanine in the presence of Pd/C and AcOH,[13] and *N*-acetylation occurred in Ac_2O/AcONa. A heterogeneous methylation of *erythro*-**3** with iodomethane and silver oxide base afforded **4**.[14] After removal of the ethyl ester, the selective hydrolysis of the *N*-acetyl from the



Scheme 1 The route to the synthesis of β-methoxytyrosine

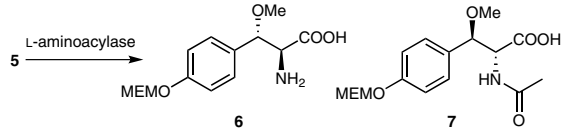
DL-*erythro*-**5** using L-aminoacylase derived from *Aspergillus oryzae* as a catalyst gave enantiopure (2*S*,3*S*)-β-methoxy-β-arylalanine.

We studied the enzyme-catalyzed hydrolysis of (DL)-*erythro*-**5** at 37 °C in aqueous solution (pH 7.5). The (2*S*,3*S*)-*N*-acetyl-β-methoxy-(4-MEM)-tyrosine could be enantioselectively hydrolyzed by L-aminoacylase to afford the corresponding (2*S*,3*S*)-**6** with excellent ee values (>99%), but low conversion (85%). Hence, the synthesis conditions were iteratively optimized by changing the temperature and pH (Table 1). Both high and low pH values decreased the conversion (Table 1, entries 2 and 3). Higher temperatures were preferable, but not higher than 40 °C (Table 1, entries 5 and 6). However, the enzyme activity likely decreases with increasing time at 40 °C due to the denaturation of L-aminoacylase at the higher temperature, and thus fresh aminoacylase was added three times at 12 hours intervals to give 98% conversion (Table 1, entry 8).

Unfortunately, no product was obtained when D-aminoacylase was used to catalyze the hydrolysis of the corresponding isomer substrate.

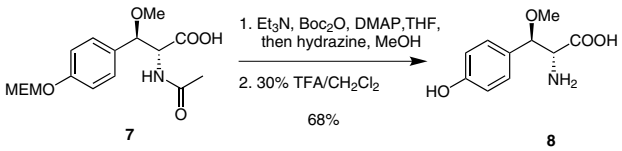
To remove the protecting acetyl group, the transformation of *N*-Ac to *N*-Boc was accomplished with a one-pot procedure.[15] The acetamide **7** was treated with equivalent amounts of triethylamine in THF, and then refluxed with Boc_2O and DMAP for four hours. The mixture was cooled to room temperature, and the same volume of MeOH and excess hydrazine (4 equiv) were added in order to both quench the unreacted Boc_2O and cleave the acetamide. Finally, (2*R*,3*R*)-β-methoxytyrosine was obtained in a one-step deprotection of the Boc and MEM groups mediated by TFA with ee >98% (Scheme 2).[16]

Table 1 Aminoacylase-Catalyzed Hydrolysis of *erythro*-*N*-Acetyl-β-methoxy-(4-MEM)-tyrosine



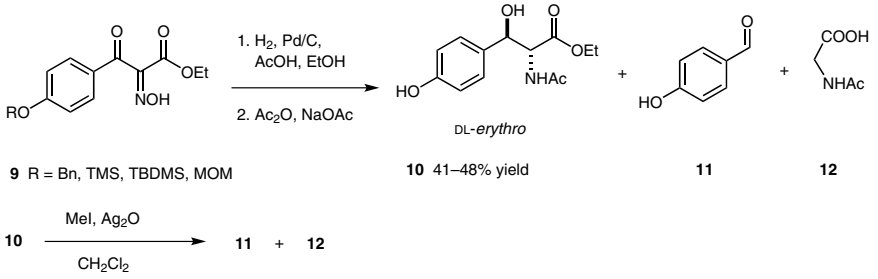
Entry	pH	Temp (°C)	Time (h)	Conv. (%)[a]
1	7.5	37	36	85
2	7.0	37	36	81
3	8.0	37	36	73
4	7.5	35	36	71
5	7.5	40	36	89
6	7.5	45	36	62
7	7.5	40	40	90
8	7.5	40	12 × 3	98

[a] The conversion was calculated at 0.5 molar quantity of *rac*-substrate and determined by HPLC on a chiral stationary phase.



Scheme 2 Deprotection of the *N*-acetyl of **7**

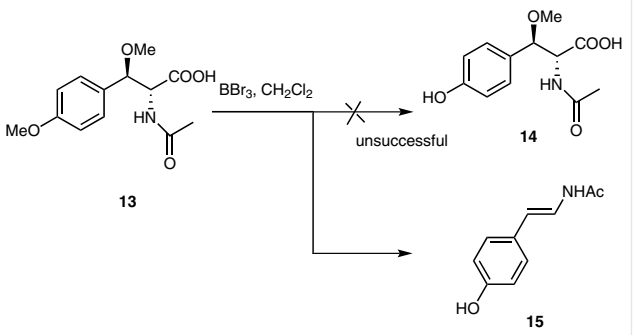
In the synthesis of enantiopure β-methoxytyrosine, considerable effort has been directed toward the selection of a suitable protecting group to protect the phenol hydroxyl.



Scheme 3 The *retro*-aldol reaction

Initially, benzyl (Bn) was selected as the protecting group, but no desired product was obtained. During the conversion from **9** to **10**, the reduced product decomposed partly with yields of 41%. The methylation of compound **10** in the presence of base converts the products via a *retro*-aldol reaction. Trimethylsilyl (TMS), *tert*-butyldimethylsilyl (TBDMS) and methoxymethyl (MOM) were also tested as protecting group for the phenol hydroxyl, but all of them were removed during the hydrogenation of the corresponding oximes (Scheme 3).

The methylation was selected to protect the phenol hydroxyl due to its stability. Following the procedure, *N*-acetyl-**13** was synthesized from ethyl 3-(4-methoxyphenyl)-3-oxopropanoate (monitored by NMR and chiral HPLC). The selective removal of the methyl from the methoxy attached on benzene ring of **13** is a critical reaction. According to the literature,[17] BBr_3 can be used to form the product **14**. However, when **13** was treated with BBr_3, the decarboxylation and elimination formed compound **15** and simultaneously removed the methyl group (Scheme 4). We then used NMR to confirm the structure of **15**, and the *trans*-configuration was unambiguously determined by the coupling pattern (J = 15.0 Hz).



Scheme 4 Demethylation of **13** with BBr_3

In summary, we have reported a novel and efficient synthetic method to prepare enantiopure (2*R*,3*R*)-β-methoxytyrosine. The key intermediate **5** was successfully synthesized using MEM as the protecting group of the phenol hydroxyl. The *erythro*-*N*-acetyl-(4-MEM)-β-methoxytyrosine was hydrolyzed selectively by L-aminoacylase catalysis to obtain the enantiopure β-methoxytyrosine.[18] This reagent will be very valuable in the future natural product synthesis efforts developing callipeltin A and its congeners.

Acknowledgment

We are grateful for the financial assistance from the National Basic Research Program of China (2011CB512007, 2012CB723501), the National Natural Science Foundation of China (Grant No. 30873139), and the Hebei Natural Science Foundation (No.12966737D, B2015208134).

Supporting Information

Supporting information for this article is available online at http://dx.doi.org/10.1055/s-0034-1381048.

References and Notes

(1) Bagavananthem Andavan, G. S.; Lemmens-Gruber, R. *Mar. Drugs* **2010**, *8*, 810.
(2) (a) Zampella, A.; D'Auria, M. V.; Paloma, L. G.; Casapullo, A.; Minale, L.; Debitus, C.; Henin, Y. *J. Am. Chem. Soc.* **1996**, *118*, 6202. (b) D'Auria, M. V.; Zampella, A.; Paloma, L. G.; Minale, L.; Debitus, C.; Roussakis, C.; Le Bert, V. *Tetrahedron* **1996**, *52*, 9589.
(3) Martín, M. J.; Rodríguez-Acebes, R.; García-Ramos, Y.; Martínez, V.; Murcia, C.; Digón, I.; Marco, I.; Pelay-Gimeno, M.; Fernández, R.; Reyes, F.; Francesch, A. M.; Munt, S.; Tulla-Puche, J.; Albericio, F.; Cuevas, C. *J. Am. Chem. Soc.* **2014**, *136*, 6754.
(4) (a) Ford, P. W.; Gustafson, K. R.; McKee, T. C.; Shigematsu, N.; Maurizi, L. K.; Pannell, L. K.; Williams, D. E.; Dilip de Silva, E.; Lassota, P.; Allen, T. M.; Van Soest, R.; Andersen, R. J.; Boyd, M. R. *J. Am. Chem. Soc.* **1999**, *121*, 5899. (b) Prasad, P.; Aalbersberg, W.; Feussner, K.-D.; Van Wagoner, R. M. *Tetrahedron* **2011**, *67*, 8529.
(5) (a) Plaza, A.; Gustchina, E.; Baker, H. L.; Kelly, M.; Bewley, C. A. *J. Nat. Prod.* **2007**, *70*, 1753. (b) Lu, Z.; Van Wagoner, R. M.; Harper, M. K.; Baker, H. L.; Hooper, J. N. A.; Bewley, C. A.; Ireland, C. M. *J. Nat. Prod.* **2011**, *74*, 185.
(6) Tran, T. D.; Pham, N. B.; Fechner, G.; Zencak, D.; Vu, H. T.; Hooper, J. N. A.; Quinn, R. J. *J. Nat. Prod.* **2012**, *75*, 2200.
(7) Ratnayake, A. S.; Bugni, T. S.; Feng, X.; Harper, M. K.; Skalicky, J. J.; Mohammed, K. A.; Andjelic, C. D.; Barrows, L. R.; Ireland, C. M. *J. Nat. Prod.* **2006**, *69*, 1582.

(8) (a) Okamoto, N.; Hara, O.; Makino, K.; Hamada, Y. *J. Org. Chem.* **2002**, *67*, 9210. (b) Hansen, D. B.; Wan, X.; Carroll, P. J.; Joullié, M. M. *J. Org. Chem.* **2005**, *70*, 3120.
(9) Zampella, A.; D'Orsi, R.; Sepe, V.; Casapullo, A.; Monti, M. C.; D'Auria, M. V. *Org. Lett.* **2005**, *7*, 3585.
(10) Konno, H.; Aoyama, S.; Nosaka, K.; Akaji, K. *Synthesis* **2007**, 3666.
(11) Fan, S.; Liu, S.; Zhang, H.; Liu, Y.; Yang, Y.; Jin, L. *Eur. J. Org. Chem.* **2014**, 5591.
(12) Li, J.; Wang, J.; Liu, S. *Fine Chemicals* **2012**, *29*, 787.
(13) Chang, Y. T.; Hartung, W. H. *J. Am. Chem. Soc.* **1953**, *75*, 89.
(14) **Characterization Data for 4**: white solid; mp 59–60 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.20 (d, *J* = 8.5 Hz, 2 H), 7.05 (d, *J* = 9.0 Hz, 2 H), 6.08 (d, *J* = 9.0 Hz, 1 H), 5.27 (s, 2 H), 4.92 (dd, *J* = 9.0, 4.5 Hz, 1 H), 4.55 (d, *J* = 4.5 Hz, 1 H), 4.12 (m, 2 H), 3.83 (m, 2 H), 3.56 (m, 2 H), 3.37 (s, 3 H), 3.21 (s, 3 H), 2.01 (s, 3 H), 1.16 (t, *J* = 7.0 Hz, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 169.9, 169.7, 157.4, 130.3, 128.0, 93.6, 83.1, 71.7, 67.8, 61.4, 59.1, 57.7, 57.1, 23.2, 14.0. HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{18}H_{28}NO_7$: 370.1866; found: 370.1857.
(15) Burk, M. J.; Allen, J. G. *J. Org. Chem.* **1997**, *62*, 7054.
(16) Akbaba, Y.; Türker Balaydın, H.; Göksu, S.; Şahin, E.; Menzek, A. *Helv. Chim. Acta* **2010**, *93*, 1127.
(17) **Characterization Data for 8**: white solid; $[\alpha]_D^{20}$ +6.1° (*c* = 0.2, MeOH). HPLC: column: Daicel Chem. Ind. Crownpak CR(+), mobile phase: MeOH–H_2O, 1:7 at pH 1.0 ($HClO_4$), 25 °C; flow rate = 0.4 mL/min, t_R = 7.9 min. ^{1}H NMR (500 MHz, CD_3OD containing 1% TFA): δ = 7.17 (d, *J* = 8.5 Hz, 2 H), 6.82 (d, *J* = 8.5 Hz, 2 H), 4.80 (d, *J* = 4.0 Hz, 1 H), 4.28 (d, *J* = 4.0 Hz, 1 H), 3.34 (s, 3 H). ^{13}C NMR (125 MHz, CD_3OD containing 1% TFA): δ = 169.1, 159.4, 129.6, 125.8, 116.7, 81.4, 59.1, 57.4. HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{10}H_{14}NO_4$: 212.0923; found: 212.0917.
(18) **Aminoacylase Resolution**: A solution of **4** (3.7 g, 10 mmol) in a mixture of EtOH (36 mL) and 1 N NaOH (10.0 mL) was stirred for 1.5 h at 0 °C. This was then concentrated under reduced pressure to 3 mL. Deionized H_2O (84 mL) and 0.05 M $CoCl_2$ (1.7 mL) were then added to the residue, and the pH of the solution was adjusted to 7.5 with 1 N HCl. The mixture was stirred at 40 °C. The L-aminoacylase (40 mg) was added three times at 12 h intervals to this magnetically stirred solution. The pH was maintained at 7.5 with 0.1 N NaOH, and the mixture was concentrated under reduced pressure to 10 mL. The residue was cooled to 0 °C and acidified with cold concentrated HCl to pH 1. It was then extracted with EtOAc (3 × 50 mL), and these extracts were washed with 2% HCl (2 × 30 mL), dried over anhyd Na_2SO_4, and concentrated to yield *N*-acetyl enantiomer **7** (1.5 g, 88%). The aqueous layer was adjusted to pH 6.5 with 4.0 N NaOH and concentrated until the volume reached 5 mL. The precipitated crystals were collected on a filter, washed with a small amount of H_2O, and dried to give a tan solid **6** (1.06 g, 71%).