Title	Absolute stereochemistry of the β-hydroxy acid unit in hantupeptins and trungapeptins
Author(s)	Deepak Kumar Gupta, Gary Chi Ying Ding, Yong Chua Teo and Lik Tong Tan
Source	*Natural Product Communications, 11,* 1-4
Published by	Natural Product Communications

NPC Natural Product Communications

2016
Vol. 11
No. 0
1 - 4

Absolute Stereochemistry of the β-Hydroxy Acid Unit in Hantupeptins and Trungapeptins

Deepak Kumar Gupta, Gary Chi Ying Ding, Yong Chua Teo and Lik Tong Tan*

Natural Sciences and Science Education, National Institute of Education, 1 Nanyang Walk, Singapore 637616

liktong.tan@nie.edu.sg

Received: July 26th, 2015; Accepted: October 2nd, 2015

The β-hydroxy/amino acid unit is a common structural feature of many bioactive marine cyanobacterial depsipeptides. In this study, the absolute stereochemistry of the β-hydroxy acid moieties in hantupeptins and trungapeptins were determined through their synthesis and HPLC analysis of the Mosher ester derivatives. Synthesis of two3-hydroxy-2-methyloctanoic acid (Hmoa) stereoisomers, (2*S*,3*R*)-Hmoa and (2*S*,3*S*)-Hmoa, were achieved using diastereoselective asymmetric method and the retention times of all four Hmoa isomers were established indirectly by RPLC-MS analysis of their Mosher ester derivative standards. Based on the retention times of the standards, the absolute configuration of the Hmoa unit in hantupeptin C (**3**) and trungapeptin C (**6**) was assigned as (2*R*,3*S*)- and (2*S*,3*R*)-Hmoa, respectively. The use of the Mosher's reagents, coupled with HPLC analysis, provided a viable alternative to the absolute stereochemical determination of β-hydroxy acid units in depsipeptides.

Keywords: β-Hydroxy acid, Hantupeptins, Trungapeptins, Depsipeptides, Marine cyanobacterium, Mosher's reagent.

Marine cyanobacteria are a rich source of novel bioactive natural products for drug discovery and development [1]. Hantupeptins (**1**-**3**) and trungapeptins (**4**-**6**) are cyclic depsipeptides recently isolated from the marine cyanobacterium, *Lyngbya majuscula* (Figure 1) [2-4]. Hantupeptins, in particular, were reported to exhibit significant *in vitro* anticancer activity [2,3]. The structures of these cyclic depsipeptides are characterized by the presence of β-hydroxy acid units, occurring as a 3-hydroxy-2-methyl-octynoic acid (Hmoya), 3-hydroxy-2-methyl-octenoic acid (Hmoea), or 3-hydroxy-2-methyl-octanoic acid (Hmoa) moiety [2-4]. Other marine-derived metabolites, such as viequeamide A [5], kulomo'opunalides [6], veraguamides [7,8], and onchidin B [9], have been reported to contain structurally related β-hydroxy acid units. In our ongoing efforts toward the total synthesis of the hantupeptins, confirmation of the absolute stereochemistry of the β-hydroxy acid units is important. Herein, we report the use of the (*R*)- and (*S*)-Mosher's reagents as chiral derivatizing agents for the absolute stereochemical determination of the Hmoa unit present in hantupeptin C (**3**) and trungapeptin C (**6**) [10].

To determine the stereochemistry of the Hmoa unit in **3**, the synthesis of two stereoisomers of Hmoa, (2*S*,3*R*)-Hmoa (**9**) and (2*S*,3*S*)-Hmoa (**14**), using a diastereoselective method was initiated [11-13]. Briefly, Evans reaction involving Evans auxiliary, (*S*)-4-benzyl-3-propionyloxazolidin-2-one (**7**), afforded (*S*)-4-benzyl-3-[(2*S*,3*R*)-3-hydroxy-2-methyloctanoyl]oxazolidin-2-one (**8**) having temporary installation of chiral auxiliary fragments of known absolute configuration. Compound **8** was then cleaved to obtain enantiomerically pure (2*S*,3*R*)-Hmoa (**9**) [14,15]. Mitsunobu reaction on **8**, which allows the inversion of configuration at the hydroxylated carbon C-3, yielded the other isomer, (2*S*,3*S*)-Hmoa (**14**) [16]. Each of the two synthesized stereoisomers of Hmoa underwent Fisher esterification to obtain methyl esterified derivatives, (2*S*,3*R*)-methyl-3-hydroxy-2-methyloctanoate (**10**) and (2*S*,3*S*)-methyl-3-hydroxy-2-methyloctanoate (**15**). Subsequently, the two synthesized methyl esters of Hmoa, **10** and **15**, were derivatized separately with either (*R*)- or (*S*)-Mosher's acid chloride to provide four synthetic diastereomers of **11**, **12**, **16**, and **17** having

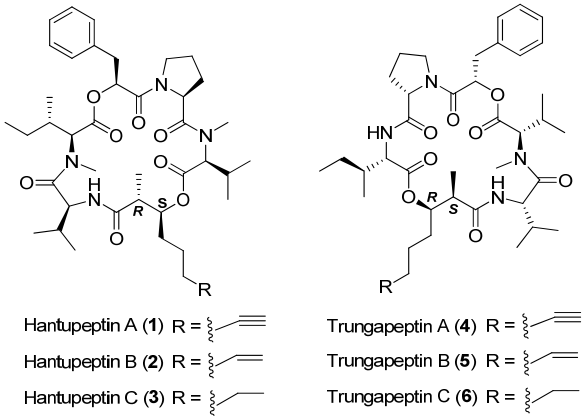
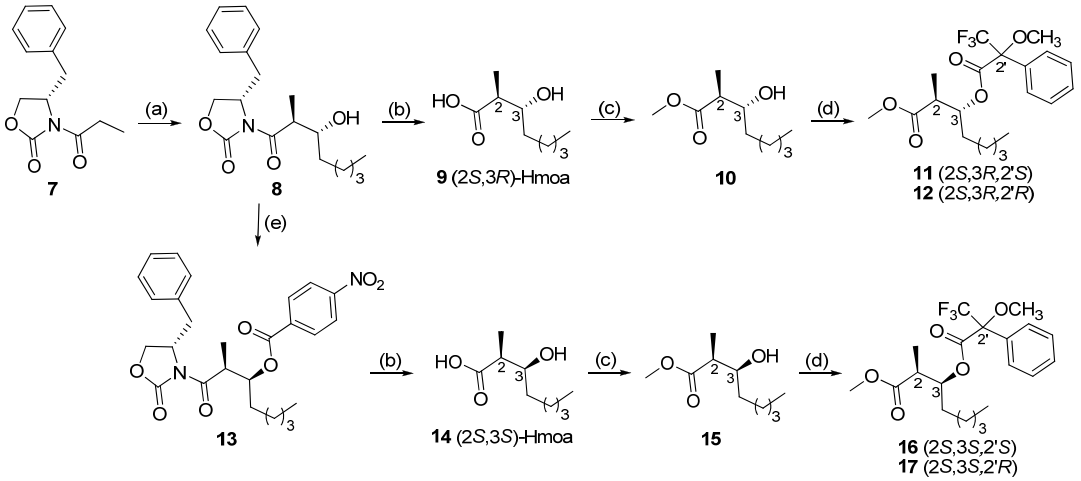


Figure 1: Structures of hantupeptins (**1**-**3**) and trungapeptins (**4**-**6**).

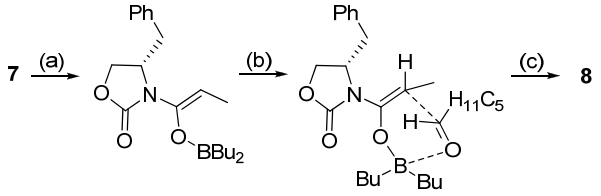
stereochemistry of (2*S*,3*R*,2'*S*), (2*S*,3*R*,2'*R*), (2*S*,3*S*,2'*S*), and (2*S*,3*S*,2'*R*), respectively (Scheme 1).

Evans oxazolidinone-containing molecule, **8** (2.5 g, 87.6% yield), was formed from a *syn* addition of *n*-hexanal with **7** via a transition state involving a BBu_2 adduct, using $(nBu)_2BOTf$ (dibutylborontriflate) as the chelating agent that favored the *Z* enolate configuration (Scheme 2). That the *R* configuration of the hydroxyl group at C-3 in **8** was obtained as the desired configuration indicated that there was no significant dipolar repulsion between the enolate C-O and oxazolidinone C=O in the transition state. This enhanced the stability of the transition state, which favored the formation of the desired product **8**.

Compound **9** (0.17 g, 66.8% yield) was obtained by removing the Evans auxiliary using H_2O_2 and then reducing the peracid intermediate with Na_2SO_3 (Scheme 1). The addition of LiOH with H_2O_2 produced LiOOH, which ensured cleavage of the auxiliary and yielded the free (2*S*,3*R*)-Hmoa (**9**) [11]. No purification of **9** was required as the removal of the auxiliary was complete based on ^{1}H NMR spectral data.



Scheme 1: Synthesis of the two isomers of Hmoa and Mosher's reagent derivatization. (a) $(nBu)_2BOTf$ in CH_2Cl_2, Et_3N, 0°C to –40°C, and hexanal in CH_2Cl_2; (b) LiOH, H_2O_2 THF-H_2O, 0°C; (c) CH_3OH, boiling chips, H_2SO_4, reflux for 1 h; (d) dry CH_2Cl_2, DMAP, rt, 12 h, *R*- or *S*-MTPCl; and (e) diethylazodiacarboxylate, triphenylphosphine, *p*-nitrobenzoic acid.



Scheme2: Mechanism of Evans *syn* addition reaction. (a) $(nBu)_2BOTf$ in CH_2Cl_2, Et_3N, 0°C; (b) *n*-hexanal in CH_2Cl_2, –40°C, 20 min; and (c) 0°C, 1 h, phosphate buffered, CH_3OH:30% H_2O_2.

In order to prepare compound **14**, (2*S*,3*S*)-Hmoa, the stereochemistry at C-3 in **8** was inverted from *R* to *S* through the use of Mitsunobu reaction (Scheme 1). During the reaction, we faced the challenge of obtaining the purified product, **13**, due to the presence of the triphenylphosphine reagent. The yield of compound **13** (0.43 g, 74.3% yield) was eventually improved by the use of resin-bound triphenylphosphine. Simultaneous hydrolysis of the benzoate ester moiety and Evans auxiliary in **13** was achieved through a combination of H_2O_2, followed by Na_2SO_3, to yield (2*S*,3*S*)-Hmoa, **14** (0.13 g, 85.9% yield). The two synthetic isomers of Hmoa with configurations (2*S*,3*R*) and (2*S*,3*S*) in **9** and **14**, respectively were synthesized.

In order to obtain distinct retention times of compounds **9** and **14** on RP-HPLC, their methyl-ester derivatives were analysized. Fisher esterification of compounds **9** and **14** in MeOH yielded (2*S*,3*R*)-methyl-3-hydroxy-2-methyloctanoate (**10**, 0.11 g, 65.6%) and (2*S*,3*S*)-methyl-3-hydroxy-2-methyloctanoate (**15**, 0.11 g, 60.1%), respectively, in substantial yields (> 80%) (Scheme 1).

The methyl esters of Hmoa were then derivatized with either (*R*)- or (*S*)-MTPA-Cl [α-methoxy-α-(trifluroromethyl)phenylacetyl chloride] to provide (2*S*,3*R*,2'*S*) **11** or (2*S*,3*R*,2'*R*) **12**, respectively from **10**, while (2*S*,3*S*,2'*S*) **16** or (2*S*,3*S*,2'*R*) **17**, respectively were obtained from **15**. The Mosher ester derivatives, **11**, **12**, **16**, and **17**, were subsequently analyzed using RPLC-MS [linear gradient of H_2O:ACN (80:20)], which gave four distinct peaks equating to the four diastereomers of Mosher derivatized methyl esters of Hmoa. These peaks were (2*S*,3*R*,2'*S*) **11**, (2*S*,3*R*,2'*R*) **12**, (2*S*,3*S*,2'*S*) **16**, and (2*S*,3*S*,2'*R*) **17**, with retention times (t_R) of 17.57 min, 10.15 min, 10.20 min, and 12.70 min, respectively (Figure 2).

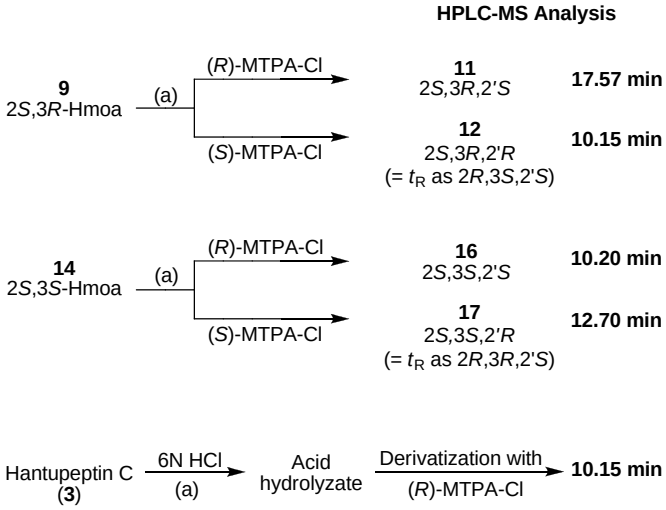


Figure 2: Absolute stereochemical analysis of the Hmoa unit inhantupeptin C (**3**). (a) CH_3OH, H_2SO_4, reflux for 1 h.

The absolute stereochemistry of the Hmoa unit in hantupeptin C (**3**) was determined by acid hydrolysis of the natural product and derivatization of the hydrolyzate with (*R*)-MTPA-Cl. Subsequent analysis by RPLC-MS and comparison with the retention times obtained for compounds **11**, **12**, **16**, and **17**, established the absolute stereochemistry of the Hmoa unit in hantupeptin C (**3**) as 2*R*,3*S* (Figure 2) [17].

Encouraged by the successful use of Mosher's reagent and HPLC analysis for the determination of the β-hydroxy unit in hantupeptin C, we proceeded to apply the method on the trungapeptins. The trungapeptins were recently isolated, along with the hantupeptins, from a later collection of *Lyngbya majuscula* from P. Hantu Besar, Singapore. Due to miniscule amount of isolated trungapeptin C (**6**), the absolute configuration of the Hmoa residue in **6** was determined indirectly via trungapeptin A (**4**) through the reduction of the terminal alkyne functionality in **4**. Subsequent analysis based on derivatization of the acid hydrolysis of the reduced **4** with (*R*)-Mosher's reagent and retention time comparison with compounds **11**, **12**, **16**, and **17** on RP-HPLC-MS, established the stereochemistry of the Hmoa unit as 2*S*,3*R*.

In conclusion, the absolute stereochemistry of the Hmoa units in hantupeptin C (**3**) and trungapeptin C (**6**) were determined as (2*R*,3*S*)- and (2*S*,3*R*)-Hmoa, respectively. This is consistent with the stereochemistry previously reported in the literature [2-4]. In addition, this study demonstrated that chiral Mosher's reagents coupled with RP-HPLC analysis can be adapted for the absolute stereochemical determination of β-hydroxy acid units in natural products.

Experimental

General: ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz Bruker NMR spectrometer in $CDCl_3$ (Cambridge Isotope Laboratories, Inc.), 99.8% D containing 0.03%, v/v, tetramethylsilane (TMS) and residual solvent signal (δ_H at 7.26 ppm and δ_C at 77.36 ppm) as internal standard. A linear gradient method, used for LCMS data to confirm the absolute stereochemical analysis of the hydrolysate, was carried out with a C_{18} RP-HPLC column [Phenomenex Sphereclone 5 µm C_{18} (2) 100 Å, 150 x 2.00 mm] using an Agilent 1100 series LC system coupled to a mass selective detector (MSD) ion trap XCT mass spectrometer equipped with an ESI interface system. TLC was performed using silica gel 60, F_{254} (green fluorescence) pre-coated glass plates with a thickness of 250 µm and a mean particle size of 10-12 µm (Merck). All solvents were HPLC grade (Sigma-Aldrich), and water was purified by a Millipore Milli-Q system before use.

Preparation of (S)-4-benzyl-3-[(2S,3R)-3-hydroxy-2-methyloctanoyl]oxazolidin-2-one (8): A stirred solution of (*S*)-4-benzyl-3-propionyloxazolidin-2-one (**7**) (2.0 g, 8.57 mmol) in dry CH_2Cl_2 (20.0 mL) was cooled to 0°C and sequentially treated dropwise via a syringe with 1.0 M dibutyl boron triflate (12 mL, 12 mmol in CH_2Cl_2) and triethylamine (2.0 mL, 14.35 mmol). The resulting solution was cooled to –40°C, and a solution of hexanal (1.5 mL) was added dropwise via a syringe. The resulting mixture was stirred at –40°C for 20 min and then an additional hour at 0°C. The reaction was quenched by addition of aqueous phosphate buffered solution (pH 7.0, 10.0 mL) and CH_3OH (10.0 mL). Then a mixture of CH_3OH:30% H_2O_2 (2:1, 13.3 mL) was added, and the resulting solution was stirred for 45 min at rt. The solvent was evaporated under reduced pressure, the residue redissolved in water (26.6 ml), and the resulting solution extracted with EtOAc (3 × 13.3 mL). The combined organic phase was washed with 5% $NaHCO_3$ (26.6 mL) and brine (26.6 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure, yielding a viscous colorless liquid. The crude was purified by flash chromatography using EtOAc:hexane (1:3) to provide **8**.

(*S*)-4-Benzyl-3-[(2S,3R)-3-hydroxy-2-methyloctanoyl]oxazolidin-2-one (8)

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.39 – 7.29 (m, 3H), 7.23 (d, *J* = 7.1 Hz, 2H), 4.70 (m, *J* = 6.9, 3.2 Hz, 1H), 4.26 – 4.16 (m, 2H), 3.33 (dd, *J* = 13.4, 3.2 Hz, 1H), 3.08 – 2.90 (m, 2H), 2.79 (dd, *J* = 13.4, 9.7 Hz, 1H), 1.59 (s, 6H), 1.23 (t, *J* = 7.3 Hz, 3H).

Preparation of (2S,3R)-3-hydroxy-2-methyloctanoic acid (9): A solution of **8** (0.5 g, 1.5 mmol) in THF:H_2O (4:1, 7.5 mL) was cooled to 0°C. To this solution was added 30% H_2O_2 (0.6 mL, 6.0 mmol) and a solution of LiOH (58.5 mg, 6.0 mmol) in H_2O (3.0 mL). The solution was stirred continuously for 1 h. After that, a solution of Na_2SO_3 (0.75 g, 5.95 mmol) in water (4.5 mL) was added, and the resulting solution was stirred for another 15 min. The organic solvent was concentrated under reduced pressure, and the resulting aqueous phase (~pH 12.0) was extracted with CH_2Cl_2 (3 × 9.0 mL). Then, the aqueous phase was cooled to 0°C, acidified to pH 1.0 with 6 N HCl, and extracted with EtOAc (5 × 6.0 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to yield the white solid compound **9**.

Preparation of (2S,3S)-1-[(S)-4-benzyl-2-oxooxazolidin-3-yl]-2-methyl-1-oxooctan-3-yl-4-nitrobenzoate (13): A solution of diethylazodicarboxylate (1.46 g, 8.4 mmol) in benzene (5.0mL) was added to **8** (0.40 g, 1.2 mmol), triphenylphosphine (2.2 g, 8.4 mmol), and *p*-nitrobenzoicacid (1.40 g, 8.4 mmol) in benzene (40.0mL) at rt. The resulting solution was stirred for 17 h at rt. The solvent was removed under reduced pressure, and the reaction crude was purified by flash chromatography using EtOAc:hexane (1:7), yielding the nitrobenzoate ester **13** as a yellow viscous liquid.

Preparation of (2S,3S)-3-hydroxy-2-methyloctanoic acid (14): A solution of **13** (0.4 g, 0.89 mmol), cooled to 0°C, in THF:H_2O (4:1, 41.4 mL) was sequentially treated with 30% H_2O_2 (0.4 mL, 3.92 mmol), followed by a solution of LiOH (71.0 mg, 2.97 mmol) in H_2O (1.7mL). The mixture was stirred for 1 h at 0°C and then for a period of 18 h at rt. Aqueous Na_2SO_3 (0.5 g, 3.97 mmol) in H_2O (2.6 mL) was added, and the organic solvent was removed under reduced pressure. The resulting aqueous phase was extracted with CH_2Cl_2 (4 × 5.3 mL), acidified to pH 1.0 with 6 N HCl and re-extracted with EtOAc (4 × 3.5 mL). The combined EtOAc extract was washed with brine (51.7 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to yield a colorless viscous liquid **14**.

Preparation of(2S,3R)-methyl-3-hydroxy-2-methyloctanoate (10) and (2S,3S)-methyl-3-hydroxy-2-methyloctanoate (15): Samples of **9** (0.15 g, 0.85 mmol) and **14** (0.18 g, 1.0 mmol) were dissolved separately in CH_3OH (20.8 g, 0.2 mol; 24.7 g, 0.37 mol) with a few boiling chips. Each mixture was heated under reflux with a few drops of concentrated H_2SO_4 for 1 h. The solution was then cooled in an ice bath, decanted into 25.0 mL of H_2O, and extracted with EtOAc (3 × 5.0 mL). The combined organic phase was washed with 2.0 M Na_2CO_3 (10.0 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to yield the methyl esters **10** and **15** as a colorless viscous liquid and yellow viscous liquid, respectively.

Preparation of 2-methyl-3-[(3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy]octanoate (11, 12, 16, and 17): Each portion of **10** and **15** in dry CH_2Cl_2 (4.0 mL) was divided into two equal portions, and small amounts of DMAP (4-dimethylaminopyridine) were added to each divided portion. Each portion was then treated with small amounts of (*R*)-MTPA-Cl and (*S*)-MTPA-Cl [α-methoxy-α-(trifluoromethyl) phenylacetyl chloride] separately and stirred for 12 h at rt, to yield the (2*S*,3*R*) ester with *S* and *R* configuration at C-2' in (2*S*,3*R*,2'*S*) **11** and (2*S*,3*R*,2'*R*) **12**, respectively. Similarly in the (2*S*,3*S*) ester with *S* and *R* configuration at C-2' in (2*S*,3*S*,2'*S*) **16** and (2*S*,3*S*,2'*R*) **17**, respectively.

Preparation of Mosher methyl ester derivative of Hmoa in hantupeptin C: Hantupeptin C (**3**) (1.0 mg) was hydrolyzed in 6N HCl at 110°C for 12 h. An aliquot of the hydrolysate underwent Fisher esterification in CH_3OH, under reflux with a few drops of concentrated H_2SO_4 for 1 h. The reaction mixture was subsequently derivatised with (*R*)-MTPA-Cl in the presence of DMAP and dry CH_2Cl_2, to obtain the Mosher methyl ester derivative of Hmoa.

Reduction of trungapeptin A and preparation of Mosher methyl ester derivative of Hmoa in trungapeptin C: The absolute configuration of the Hmoa residue in trungapeptin C was

determined through trungapeptin A (**4**). Compound **4** was hydrogenated with 10% Pd/C (palladium on carbon) to fully reduce the terminal alkyne functionality as in **6**. A stirred solution containing 1.0 mg of trungapeptin A (**4**) in 2.0 mL of EtOH was treated with 1.3 mg of 10% Pd/C, and the atmosphere was replaced with H_2 (g) by balloons pressure. The reaction mixture was stirred at rt for 3 h and filtered through glass wool into a reaction vial with 10.0 mL of CH_2Cl_2. The sample was then concentrated under reduced pressure *in vacuo* and finally dried under a stream of N_2 (g). The hydrogenated solid residues were hydrolyzed in 6N HCl at 110°C for 12 h to yield the β-hydroxy acid equivalent to the unit of trungapeptin C (**6**), which then underwent Fisher esterification and subsequent derivatization with (*R*)-MTPA-Cl to obtain the Mosher derivatized natural Hmoa unit for direct comparision of t_R with the established stereochemistry of hantupeptin C (**3**).

Absolute stereochemical analysis of Hmoa in hantupeptin C and trungapeptin C: The Mosher ester derivatives, (2*S*,3*R*,2'*S*) **11**, (2*S*,3*R*,2'*R*) **12**, (2*S*,3*S*,2'*S*) **16**, (2*S*,3*S*,2'*R*) **17**, and the Mosher derivatized natural Hmoa from the acid hydrolysis of hantupeptin C (**3**), were analyzed using RPLC-MS (linear gradient of H_2O:ACN; 80:20 to 0:100 in 45 min at 0.4 mL/min) with the mass detector in negative mode fixed at *m/z* 403. Four distinct peaks with t_R of 17.57 min, 10.15 min, 10.20 min, and 12.70 min were obtained for **11**, **12**, **16**, and **17**, respectively. Co-injections of the standards with the Mosher derivatized Hmoa from **3** (t_R at 10.15 min) confirmed the stereochemistry of (2*R*,3*S*)-Hmoa in hantupeptin C. Similar analysis were conducted on the Mosher derivatized Hmoa from trungapeptin C (t_R at 17.57 min) and the stereochemistry of (2*S*,3*R*)-Hmoa in **6** was confirmed [17].

Acknowledgments - The authors would like to acknowledge the NIE AcRF (RI 11/10 TLT and RI 02/14 TLT) for funding support. In addition, the authors would like to acknowledge Dr A. Tripathi, A. A. B. Lim-Ang, and A. S. G. Yee-Lee (NSSE, NIE) for providing valuable technical assistance.

References

[1] Tan LT, Gupta DK. (**2015**) Molecular targets of anticancer agents from filamentous marine cyanobacteria. *Handbook of Anticancer Drugs from Marine Origin*. Springer International Publishing, Switzerland, 571-592.
[2] Tripathi A, Puddick J, Prinsep MR, Lee PP, Tan LT. (**2009**) Hantupeptin A, a cytotoxic cyclic depsipeptide from a Singapore collection of *Lyngbya majuscula*. *Journal of Natural Products*, ***72***, 29-32.
[3] Tripathi A, Puddick J, Prinsep MR, Lee PP, Tan LT. (**2010**) Hantupeptins B and C, cytotoxic cyclodepsipeptides from the marine cyanobacterium *Lyngbya majuscula*. *Phytochemistry*, ***71***, 307-311.
[4] Bunyajetpong S, Yoshida WY, Sitachitta N, Kaya K. (**2006**) Trungapeptins A-C, cyclodepsipeptides from the marine cyanobacterium *Lyngbya majuscula*. *Journal of Natural Products*, ***69***, 1539-1542.
[5] Boudreau PD, Byrum T, Liu WT, Dorrestein PC, Gerwick WH. (**2012**) Viequeamide A, a cytotoxic member of the kulolide superfamily of cyclic depsipeptides from a marine button cyanobacterium. *Journal of Natural Products*, ***75***, 1560-1570.
[6] Nakao Y, Yoshida YW, Szabo MC, Baker BJ, Scheuer PJ. (**1998**) More peptides and other diverse constituents of the marine mollusk *Philinopsis speciosa*. *Journal of Organic Chemistry*, ***63***, 3272-3280.
[7] Salvador LA, Biggs JS, Paul VJ, Luesch H. (**2011**) Veraguamides A-G, cyclic hexadepsipeptides from a dolastatin 16-producing cyanobacterium *Symploca* cf. *hydnoides* from Guam. *Journal of Natural Products*, ***74***, 917-927.
[8] Mevers E, Liu WT, Engene N, Mohimani H, Byrum T, Pevzner PA, Dorrestein PC, Spadafora C, Gerwick WH. (**2011**) Cytotoxic veraguamides, alkynyl bromide-containing cyclic depsipeptides from the marine cyanobacterium cf. *Oscillatoria margaritifera*. *Journal of Natural Products*, ***74***, 928-936.
[9] Fernandez R, Rodriguez J, Quinoa E, Riguera R, Munoz L, Fernandez-Suarez M, Debitus C. (**1996**) Onchidin B: a new cyclodepsipeptide from the mollusc *Onchidium* sp. *Journal of the American Chemical Society*, ***118***, 11635-11643.
[10] Hoye TR, Jeffrey CS, Shao F. (**2007**) Mosher ester analysis for the determination of absolute configuration of stereogenic (chiral) carbinol carbons. *Nature Protocols*, ***2***, 2451-2458.
[11] Nunnery JK, Suyama TL, Linington RG, Gerwick WH. (**2011**) Expedient synthesis of α,α-dimethyl-β-hydroxycarbonyl scaffolds via Evans' aldol reaction with a tertiary enolate. *Tetrahedron Letters*, ***52***, 2929-2932.
[12] Evans DA, Bartroli J, Shih TL. (**1981**) Enantioselective aldol condensations. 2. Erythro-selective chiral aldol condensations via boron enolates. *Journal of the American Chemical Society*, ***103***, 2127-2129.
[13] Evans DA, Britton TC, Ellman JA. (**1987**) Contrasteric carboximide hydrolysis with lithium hydroperoxide. *Tetrahedron Letters*, ***28***, 6141-6144.
[14] Guzman A, Alvarado C, Diaz E. (**2007**) Total synthesis of ulongamide A, acyclic depsipeptide isolated from marine cyanobacteria *Lyngbya* sp. *Tetrahedron Lett*ers, ***48***, 603-607.
[15] Smith TE, Richardson DP, Truran GA, Belecki K, Onishi M. (**2008**) Acylation, diastereoselective alkylation, and cleavage of an oxazolidinone chiral auxiliary: a multistep asymmetric synthesis experiment for advanced undergraduates. *Journal of Chemical Education*, ***85***, 695-697.
[16] Mitsunobu O. (**1981**) The use of diethyl azodicarboxylate and triphenylphosphine in synthesis and transformations of natural products. *Synthesis*, 1-28.
[17] Tan LT, Sitachitta N, Gerwick WH. (**2003**) The guineamides, novel cyclic depsipeptides from a Papua New Guinea collection of the marine cyanobacterium *Lyngbya majuscula*. *Journal of Natural Products*, ***66***, 764-771.