ORGANIC LETTERS

2005
Vol. 7, No. 16
3585−3588

Concise Synthesis of All Stereoisomers of β-Methoxytyrosine and Determination of the Absolute Configuration of the Residue in Callipeltin A

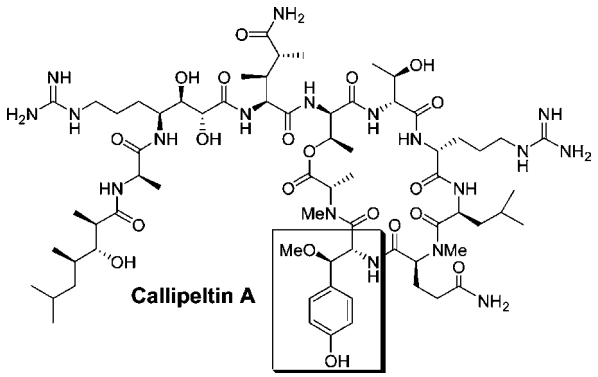
Angela Zampella,[†] Rosa D'Orsi,[†] Valentina Sepe,[†] Agostino Casapullo,[‡] Maria Chiara Monti,[‡] and Maria Valeria D'Auria*,[†]

Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli "Federico II", via D. Montesano 49, 80131, Napoli, Italy, and Dipartimento di Scienze Farmaceutiche, Università di Salerno, via Ponte don Melillo, Fisciano (SA) 84084, Italy

madauria@unina.it

Received June 10, 2005

ABSTRACT



All stereoisomers of β-methoxytyrosine (β-OMeTyr), a stereo-undefined component of callipeltin A, were synthesized from L- and D-tyrosine. The stereochemistry of β-OMeTyr in callipeltin A was determined to be 2*R*,3*R* by an oxidative procedure and Marfey's analysis.

Cyclodepsipeptide callipeltin A[1] (**1**) and its congeners[2,3] were isolated in our laboratories from the marine sponge *Callipelta* sp., collected in New Caledonia. Callipeltin A is a decapeptide containing three unusual amino acid residues: β-methoxytyrosine (β-OMeTyr), (2*R*,3*R*,4*S*)-4-amino-7-guanidino-2,3-dihydroxyheptanoic acid (AGDHE), and (3*S*,4*R*)-3,4-dimethyl-L-glutamine (diMeGln). Callipeltin A (**1**) is known to exhibit antifungal and anti-HIV activity. The stereochemistry of callipeltins, however, remains to be determined because of the uncertainty regarding the β-OMeTyr unit. Interestingly, the β-OMeTyr residue was also found as a component of papuamides[4] and neamphamide.[5] Like callipeltin A, they show anti-HIV activities. In each instance, the stereochemistry of the β-methoxytyrosine remains undetermined because of degradation of this residue under acidic hydrolysis.

[†] Università degli Studi di Napoli "Federico II".
[‡] Università di Salerno.

(1) Zampella, A.; D'Auria, M. V.; Gomez-Paloma, L.; Casapullo, A.; Minale, L.; Debitus, C.; Henin, Y. *J. Am. Chem. Soc.* **1996**, *118*, 6202−6209.
(2) D'Auria M. V., Zampella, A.; Gomez-Paloma, L.; Minale, L.; Debitus, C.; Roussakis, C.; Le Bert, V. *Tetrahedron* **1996**, *52*, 9589−9596.
(3) Zampella, A.; Randazzo, A.; Borbone, N.; Luciani, S.; Trevisi, L.; Debitus, C.; D'Auria M. V. *Tetrahedron Lett.* **2002**, *43*, 6163−6166.
(4) Ford, P. W.; Gustafson, K. R.; McKee, T. C.; Shigematsu, N.; Maurizi, L. K.; Pannell, L. K.; Williams, D. E.; de Silva, E. D.; Lassota, P.; Allen, T. M.; Van Soest, R.; Andersen, R. J.; Boyd, M. R. *J. Am. Chem. Soc.* **1999**, *121*, 5899−5909.
(5) Oku, N.; Gustafson, K. R.; Cartner; Wilson, J. A.; Shigematsu, N.; Hess, S.; Pannell, L. K.; Boyd, M. R.; McMahon, J. B. *J. Nat. Prod.* **2004**, *67*, 1407−1411.

10.1021/ol0513600 CCC: $30.25
Published on Web 07/13/2005

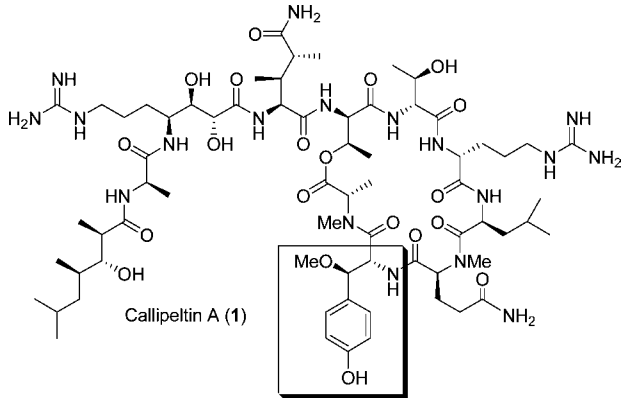


Figure 1. Callipeltin A (**1**)

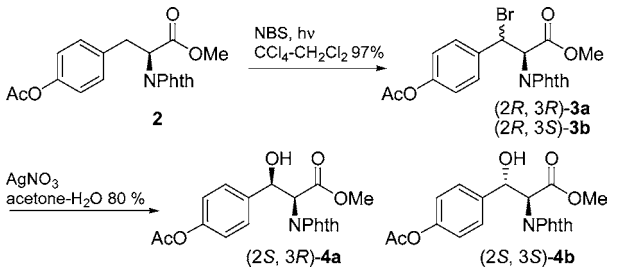
The new structure of callipeltin A, together with its stereochemical ambiguities and its intriguing biological activity, has prompted considerable interest from the synthetic community.[6] To accomplish the total synthesis as well as the structural determination of callipeltin A, all four stereoisomers of β-OMeTyr are needed in large quantities. Recently, two syntheses of all stereoisomers of β-OMeTyr have appeared in the literature.[7] They both feature a strategy based on the nucleophilic addition of arylmetal reagents to serine aldehyde equivalents followed by methylation.

In this paper we report a short, inexpensive, efficient synthesis of all four stereoisomers of β-OMeTyr from tyrosine methyl ester, using a modification of the method developed by Easton and Hutton several years ago.[8] Besides providing adequate amounts of the above amino acid unit for the planned total synthesis, the availability of all four diastereoisomers of β-OMeTyr enabled us to unambiguously assign the absolute configuration of the corresponding residue in callipeltin A, through oxidative procedure and Marfey's analysis.

Easton's method, used for the synthesis of the (2*S*,3*R*)-β-hydroxytyrosine residue in vancomycin[9] and, recently, for the synthesis of the (2*S*,3*R*)-β-methoxyphenylalanine residue in cyclomarin,[10] provides selective formation of *threo*-hydroxyphenylalanine derivatives. The corresponding *erythro* diastereomers could be obtained by oxidation of the *threo* adducts to the corresponding ketones, followed by reduction by a suitable hydride, a procedure usually used to access the not directly obtainable *erythro* β-adducts.

(2*S*)-*O*-Acetyl-*N*-phthaloyltyrosine methyl ester (**2**) was treated with *N*-bromosuccinimide to afford the corresponding 3-bromo derivatives **3a** and **3b** (Scheme 1) in 97% yield (1:1 mixture of diastereoisomers).

Scheme 1. Easton's Method[8]



Treatment of bromides with silver nitrate in aqueous acetone overnight gave the β-hydroxyl derivatives **4a**,**b** (6:1 mixture of diastereoisomers).

To obtain the corresponding β-methoxytyrosine derivative, the synthesis then required the methylation of the 3-hydroxyl group of **4a**. We tested several reported methods, but we only obtained decomposition of the starting material or unsatisfactory transformation.[11]

This failure prompted us to explore an alternative access to β-methoxy tyrosine derivatives **5a**,**b**, which involves the use of methanol in the place of water as a nucleophile for the substitution displacement of the bromide atom.

The factors determining the stereoselectivity of the hydrolysis of β-bromoarylalanine derivatives were investigated.[12] So far, no details on the stereochemical course of the methanolysis of the bromotyrosine derivatives have been reported. To gain more insight, we tested several solvolysis conditions by varying the solvent and the catalyst (Table 1).

In the presence of AgOTf, we observed the concomitant removal of the *O*-acetyl protecting group. In all tested conditions, a modest level of stereoselectivity was observed, inferring a S_N1 character with the absence of neighboring group participation. Hutton[12] reports the formation of *threo* and *erythro* adducts **4a** and **4b**, respectively, in a 6:1 ratio when the hydrolysis was performed with silver nitrate in aqueous acetone. On the same substrate, in comparable conditions (entry 1), methanolysis afforded a 6:4 ratio of *threo* and *erythro* adducts. Better stereoselectivity was observed with silver nitrate in MeOH/acetone (entries 2−4), whereas the use of silver triflate reduced the selectivity of the solvolysis reactions, giving invariably a 1:1 ratio (entries

(6) For the synthesis of the nonproteinogenic units in callipeltin A, see: (a) Liang, B.; Carroll, P. J.; Joullie, M. M. *Org. Lett.* **2000**, *2*, 4157−4160. (b) Okamoto, N.; Hara, O.; Makino, K.; Hamada, Y. *Tetrahedron: Asymmetry* **2001**, *12*, 1353−1358. (c) Acevedo, C. M.; Kogut, E. F.; Lipton, M. A. *Tetrahedron* **2001**, *57*, 6353−6359. (d) Chandrasekhar, S.; Ramachandar, T.; Rao, B. V. *Tetrahedron: Asymmetry* **2001**, *12*, 2315−2321. (e) Guerlavais, V.; Carroll, P. J.; Joullie, M. M *Tetrahedron: Asymmetry* **2002**, *13*, 675−680. (f) Thoen, J. C.; Morales-Ramos, A. I.; Lipton, M. A. *Org. Lett*. **2002**, *4*, 4455−4458. (g) Zampella, A.; Sorgente, M.; D'Auria, M. V. *Tetrahedron: Asymmetry* **2002**, *13*, 681−685. (h) Zampella, A.; D'Auria, M. V. *Tetrahedron: Asymmetry* **2002**, *13*, 1237−1239. (i) Ravi, K. A.; Venkateswara, R. B. *Tetrahedron Lett*. **2003**, *44*, 5645−5647. (j) Turk, J. A.; Visbal, G. S.; Lipton, M. A. *J. Org. Chem*. **2003**, *68*, 7841−7844.

(7) (a) Okamoto, N.; Hara, O.; Makino, K.; Hamada, Y. *J. Org. Chem.* **2002**, *67*, 9210−9215. (b) Hansen, D. B.; Wan, X.; Carrol, P. J.; Joullie, M. M. *J. Org. Chem*. **2005**, *70*, 3120−3126.

(8) Easton, C. J.; Hutton, C. A.; Roselt, P. D.; Tiekink, E. R. T. *Tetrahedron* **1994**, *50*, 7327−7340.

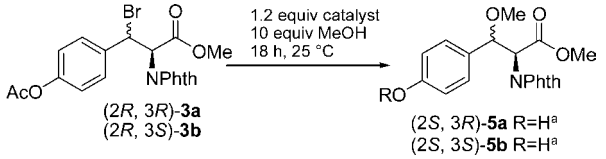
(9) Rama Rao, A. V.; Chakraborty, T. K.; Laxma Reddy, K.; Srinivasa Rao, A. *Tetrahedron Lett*. **1994**, *35*, 5043−5046.

(10) Wen, S.-J.; Yao, Z.-J. *Org. Lett.* **2004**, *6*, 2721−2724.

(11) During the preparation of this manuscript, a paper (ref 7b) appeared reporting the same difficulty in methylating a protected 3-hydroxy-tyrosine derivative.

(12) Hutton, C. A. *Tetrahedron Lett.* **1997**, *38*, 5899−5902.

Table 1. Ag^+-Promoted Methanolysis of Bromotyrosines **3a**,**b**



(2*R*, 3*R*)-**3a**
(2*R*, 3*S*)-**3b**

(2*S*, 3*R*)-**5a** R=H[a]
(2*S*, 3*S*)-**5b** R=H[a]

entry	substrate	catalyst	solvent	ratio[b] **5a**:**5b**	yield[c]
1	**3a/3b**	$AgNO_3$[d]	MeOH	60:40	75%
2	**3a/3b**	$AgNO_3$	acetone	78:22	70%
3	**3a/3b**	$AgNO_3$	acetone/MeOH 1:1	65:35	70%
4	**3a/3b**	$AgNO_3$	acetone/MeOH 2:8	59:41	77%
4	**3a/3b**	$AgNO_3$	acetone/MeOH 8:2	73:27	75%
5	**3a/3b**	AgOTf	MeOH	60:40	87%
6	**3a/3b**	AgOTf	acetone	59:41	85%
7	**3a**	$AgNO_3$	acetone	70:30	70%
8	**3a**	$AgNO_3$	MeOH	45:55	72%
9	**3b**	$AgNO_3$	acetone	68:32	70%
10	**3b**	$AgNO_3$	MeOH	50:50	73%

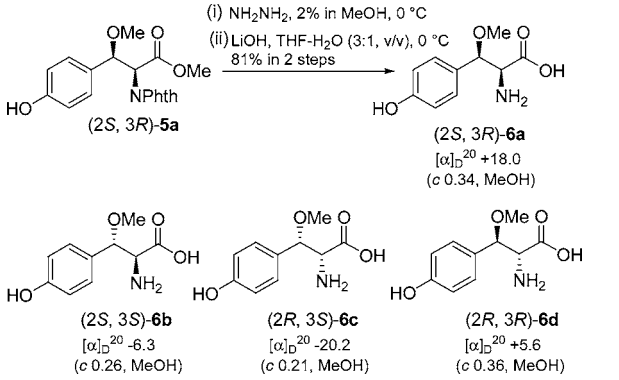
[a] R = OAc when $AgNO_3$ was used as a catalyst. [b] **5a**:**5b** ratio was determined by ^{1}H NMR analysis of the mixture. [c] Isolated yield by column chromatography. [d] Due to the poor solubility of $AgNO_3$ in MeOH/acetone, 10% water was added to the solvent when this catalyst was used.

5 and 6). Results obtained by solvolysis on isolate β-bromoarylalanine **3a** or **3b** (entries 7−10) confirm the findings of Hutton and indicate some degree of conformational restriction of the cation intermediate with the preferential formation of the *threo* adduct. Even if in all tested conditions no significant selectivity was observed, in light of our need of a rapid access to both diastereomers of β-methoxytyrosine, we selected silver triflate/methanol conditions (entry 5). In these conditions, we obtained easier synthetic elaboration in terms of yields and number of steps. The two methoxytyrosine derivatives could be easily separated by HPLC (silica gel, hexane/ethyl acetate 75:25) affording pure **5a** and **5b**. To secure the stereochemistry of the diastereoisomeric β-methoxytyrosine derivatives, a sample of (2*S*,3*R*)-β-hydroxytyrosine derivative **4a** previously obtained was methylated with CH_3I and AgOTf. The methylated derivative was obtained in a modest 10% yield; however, HPLC separation of the crude reaction mixture followed by deacetylation with *p*-TsOH afforded a product whose ^{1}H NMR and optical rotation data were superimposable with those of derivative **5a**.

To complete the synthesis of both diastereomers of β-methoxytyrosine and to access deprotected derivatives, useful as standards for the stereochemical studies on callipeltins, we explored the selective removal of both phthaloyl and methyl ester protecting groups (Scheme 2).

Quantitative removal of the phthaloyl protecting group in **5a** was achieved without observable racemization by careful

Scheme 2. Deprotection of β-Methoxytyrosine Derivatives



(2*S*, 3*R*)-**5a**

(2*S*, 3*R*)-**6a**
$[\alpha]_D^{20}$ +18.0
(*c* 0.34, MeOH)

(2*S*, 3*S*)-**6b**
$[\alpha]_D^{20}$ -6.3
(*c* 0.26, MeOH)

(2*R*, 3*S*)-**6c**
$[\alpha]_D^{20}$ -20.2
(*c* 0.21, MeOH)

(2*R*, 3*R*)-**6d**
$[\alpha]_D^{20}$ +5.6
(*c* 0.36, MeOH)

addition of a 2% hydrazine methanolic solution at 0 °C. Finally, the removal of the methyl ester was easily obtained by LiOH hydrolysis. The same deprotection procedure was applied to the *erythro* diastereomer **5b** to provide (2*S*,3*S*)-3-methoxy-tyrosine **6b** with similar results.

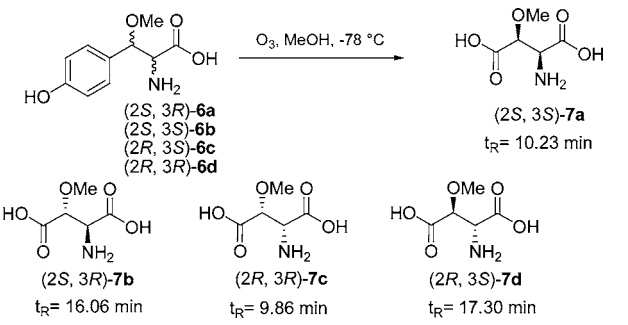
The remaining two enantiomers of β-methoxytyrosine were synthesized using the same procedures starting from (2*R*)-*O*-acetyl-*N*-phthaloyltyrosine methyl ester to afford the desired (2*R*,3*S*)-β-methoxytyrosine **6c** and (2*R*,3*R*)-β-methoxytyrosine **6d** with similar stereoselectivity and chemical yield.

Concerning the configuration of the β-methoxytyrosine residue in callipeltin A (**1**), recently we applied a new integrated NMR−quantum mechanical (QM) approach, relying on the comparison between calculated and experimental *J*-values, to the analysis of the relative configuration of four amino acid units in callipeltin A (**1**) and proposed an *erythro* arrangement (e.g., *S**,*S**) for the two stereogenic centers in the β-methoxytyrosine residue.[13] With all four stereoisomers of β-methoxytyrosine in our hands, it was then possible to confirm the above assignment and to determine the absolute configuration using a precolumn derivatization method.

We tested several acidic and basic hydrolytic conditions,[14] and unfortunately no recovery of β-methoxytyrosine was invariably observed.[15]

A literature search suggested the opportunity to transform the phenyl group, responsible of the lability of the β-meth-

Scheme 3. Ozonolysis of β-Methoxytyrosines and Their HPLC Retention Times (t_R)



(2*S*, 3*R*)-**6a**
(2*S*, 3*S*)-**6b**
(2*R*, 3*S*)-**6c**
(2*R*, 3*R*)-**6d**

(2*S*, 3*S*)-**7a**
t_R= 10.23 min

(2*S*, 3*R*)-**7b**
t_R= 16.06 min

(2*R*, 3*R*)-**7c**
t_R= 9.86 min

(2*R*, 3*S*)-**7d**
t_R= 17.30 min

(13) Bassarello, C.; Zampella, A.; Monti, C.; Gomez-Paloma, L.; D'Auria, M. V.; Riccio, R.; Bifulco, G. *Tetrahedron*, submitted.

(14) Davison, I. In *Methods in Molecular Biology*; Smith, B. J., Ed.; Totowa, NJ; Vol. 211, pp 111−122.

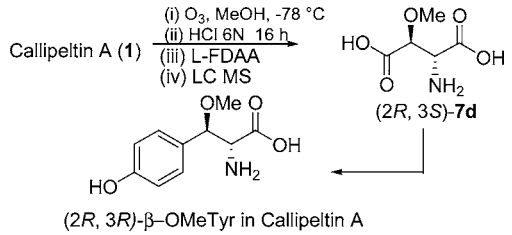
(15) As determined by LC/MS analysis (ion-selective monitoring for FDAA−MeOTyr + H^+ *m*/*z* 464) after Marfey's derivatization of the crude hydrolysate.

oxytyrosine residue, to a carboxy function, through oxidative ozonolysis.

As depicted in Scheme 3, a small sample (1 mg each) of synthetic diastereomers **6a**−**d** was transformed to the corresponding β-methoxy-aspartate derivatives **7a**−**d** by ozonolysis, followed by hydrogen peroxide workup. The compounds **7a**−**d** were derivatized with Marfey's reagent 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide; L-FDAA).

The same procedure was applied to callipeltin A (**1**). The oxidized callipeltin A (**1**) was hydrolyzed, and the amino acid residues so obtained were derivatized with Marfey's reagent (Scheme 4).

Scheme 4. Configurational Assignment of β-Methoxytyrosine in Callipeltin A



The L-FDAA derivatives of **7a**−**d** were analyzed using ESI LC/ MS in the positive ion mode. By monitoring for L-FDAA−OMeAsp at *m*/*z* 461, we detected the L-FDAA derivatives of **7a**−**d** as separate peaks at 10.23, 16.06, 9.86, and 17.30 min, respectively. The observed good separation was in contrast with that usually observed for hydrophilic hydroxy and acidic amino acids.[16] Noteworthy also is the deviation from the usual behavior for the *threo* series, with the D-isomer eluting before the L-isomer. The selective ion monitoring at *m*/*z* 461 of the hydrolysate of callipeltin A derivatized with L-FDAA showed only a peak a with retention time of 17.30 min, corresponding to the L-FDAA derivative of (2*R*,3*S*)-β-methoxy-aspartate **7d**. Thus, the (2*R*,3*R*)-configuration for the β-methoxytyrosine residue in callipeltin A was unambiguously established.

In conclusion, we have reported a concise synthesis of all four stereoisomers of β-methoxytyrosine. The absolute configuration of the above residue in callipeltin A (**1**) was assigned for the first time. In addition, our method turns out to be a useful tool for the stereochemical characterization of β-methoxy-β-aryl amino acids, often found as components of bioactive natural peptides.

Acknowledgment. This work was supported by grants from MIUR (PRIN 2003) "Sostanze Naturali ed Analoghi Sintetici ad Attività Antitumorale", Rome, Italy. Mass and NMR spectra were provided by the CRIAS, Faculty of Pharmacy, Università di Napoli Federico II. We thank Dr. Kirk R. Gustafson (Molecular Targets Development Program, NCI-Frederick) for helpful discussion.

Supporting Information Available: Experimental procedures and spectoscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0513600

(16) Fujii, K.; Ikai, Y.; Mayumi, T.; Oka, H.; Suzuki, M.; Harada, K.-I. *Anal. Chem.* **1997**, *69*, 3346−3352.