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Synthesis of tripeptide hydrolysate from papuamide A: determination of absolute stereostructure of β-methoxytyrosine

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Abstract—Four diastereomers, (2*R*,3*R*), (2*S*,3*S*), (2*S*,3*R*) and (2*R*,3*S*) at β-methoxytyrosine (β-OMeTyr), of the tripeptide hydrolysate, H-(*S*)-*N*-MeThr-β-OMeTyr-(*S*)-Hpr-OH, from papuamide A have been synthesized. Comparison of the ^{1}H NMR data of the natural hydrolysate with the four synthetic diastereomers unambiguously establishes the relative and absolute stereochemistry of the methoxytyrosine as 2*R*,3*R*.

Papuamides A (**1**) and B are a new family of novel cyclodepsipeptides isolated from the marine sponge genus *Theonella mirabilis* and *Thenella swinhoei* collected at Papua New Guinea by Boyd and co-workers.[1] Papuamides are known to strongly inhibit the infection of human T-lymphoblastoid cells by HIV-1_{RF} and also exhibit potent cytotoxicity against a number of human cancer cell lines. These cyclic heptadepsipeptides have a unique structure containing (4*Z*,6*E*)-2,3-dihydroxy-2,6,8-trimethyldecadienoic acid (Dhtda) and unusual amino acid residues, such as (2*S*,3*S*,4*R*)-3,4-dimethylglutamine (3,4-DiMeGln), (2*R*,3*R*)-3-hydroxyleucine (3-OHLeu) and β-methoxytyrosine (β-OMeTyr), as shown in Figure 1. The stereochemistry of papuamides remains to be determined because of the uncertainty regarding the stereochemistry in the β-OMeTyr and the Dhtda parts. Interestingly, two unusual amino acids, β-OMeTyr and 3,4-DiMeGln, are known as common components of the cyclodepsipeptides callipeltin A[2] and neamphamide A,[3] which show anti-HIV and antifungal activities. The intriguing structures as well as the unique biological activities led others and us to synthesize these cyclodepsipeptides.[4,5] Herein, we report synthesis of the tripeptide hydrolysate from papuamide A and structural determination of the β-methoxytyrosine in papuamides by comparison of the four synthetic tripeptides with the natural fragment.

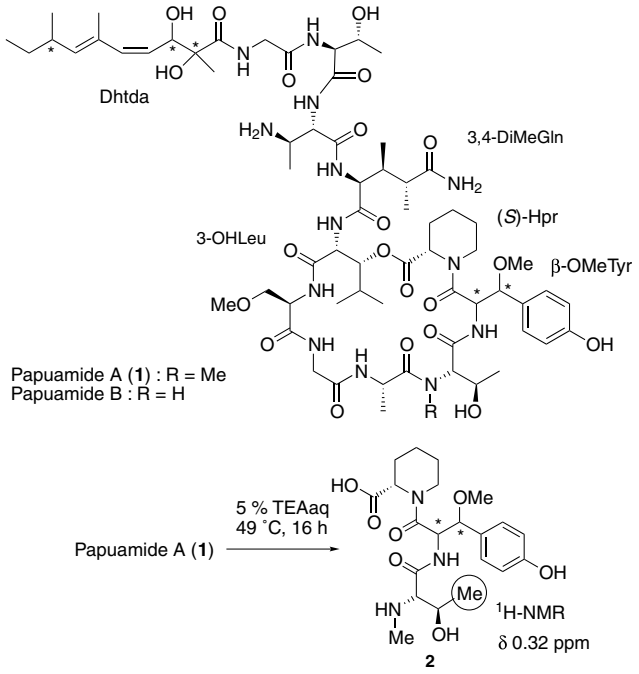

Figure 1.

In order to accomplish total synthesis as well as structural determination of papuamides, configurational assignment of two stereo-undefined components, β-OMeTyr and Dhtda, is necessary. Boyd and co-workers have reported an interesting experiment, in which the hydrolysate, H-*N*-MeThr-β-OMeTyr-Hpr-OH (**2**)

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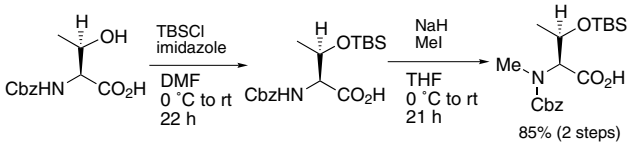
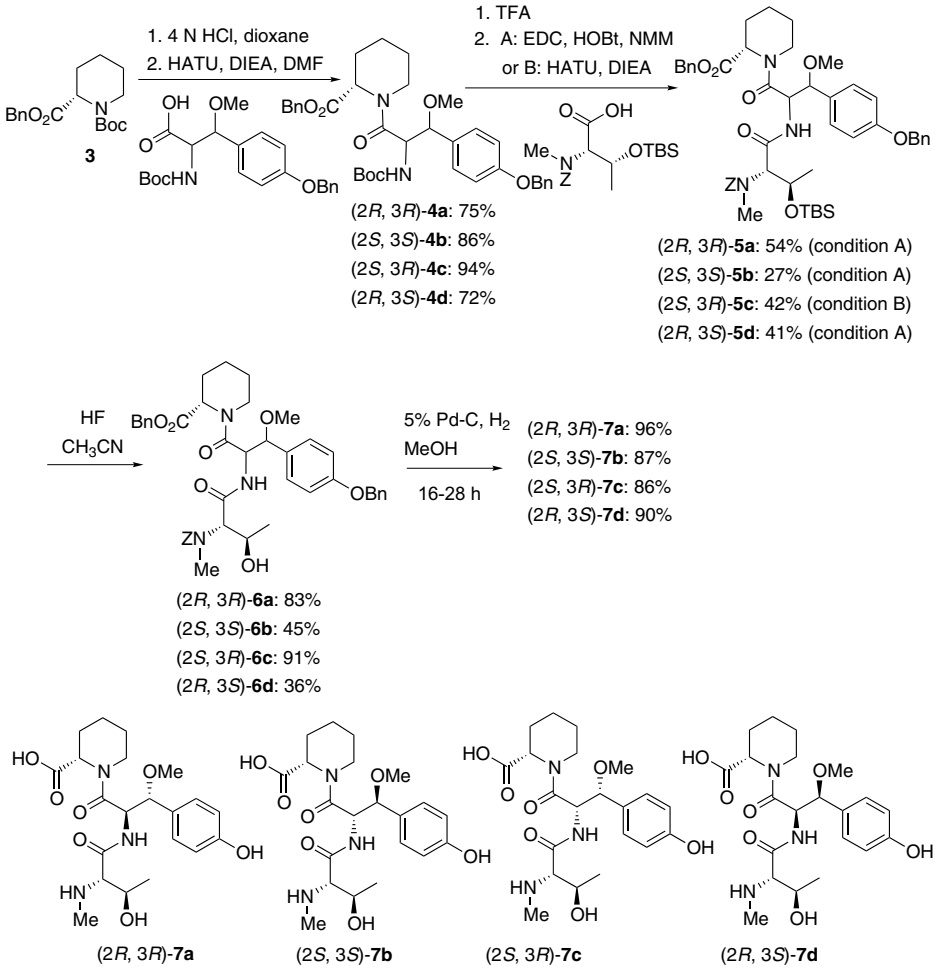
(Hpr: homoproline (pipecolic acid)), derived from hydrolysis of papuamide A with 5% aqueous triethylamine shows the anomalous high-field shift (0.32 ppm, doublet) at the methyl proton of the *N*-MeThr residue in the NMR spectrum (Fig. 1).[1] The experiment encouraged us to synthesize the four tripeptides with different stereochemistry at the β-OMeTyr residue. The required four diastereomers of the β-methoxytyrosine were prepared from (*S*)- and (*R*)-Garner aldehydes[6] by a concise route developed by us.[4e] *N*-Methylthreonine was prepared by use of Benoiton's method[7] from L-threonine because the known methods[8] were tedious or gave low overall yield. Thus, commercially available Cbz-Thr-OH was protected with *tert*-butyldimethylchlorosilane and imidazole in dimethylformamide and after workup the obtained Cbz-Thr(TBS)-OH was *N*-methylated with an excess amount of sodium hydride and iodomethane in tetrahydrofuran to give Cbz-*N*-MeThr(TBS)-OH in 85% overall yield (Scheme 1).



Scheme 1.

The four tripeptides **7a**–**d** required for structural determination of the β-OMeTyr were prepared from Boc-(*S*)-Hpr-OBn by stepwise coupling with Boc-OMeTyr(Bn)-OH and Cbz-*N*-MeThr(TBS)-OH using the HATU/DIPEA and EDCI/HOBt methods, shown in Scheme 2. Sequential deprotection of the TBS group with HF-acetonitrile and the Cbz group by hydrogenolysis furnished the free tripeptides **7a**–**d** after purification by ODS column chromatography using a mixture of 0.05% aqueous trifluoroacetic acid–acetonitrile (8:2) as an eluant. The ^{1}H NMR spectra showed that **7a** and **7d** each have an exclusive conformer containing a minor one in a ratio of >9:1, while **7b** and **7c** are a mixture of an equal amount of two conformers. It has been reported that the ^{1}H NMR spectrum of natural hydrolysate shows it to be a mixture of two conformers and the major conformer is >90% excess, which is quite similar to the conformer's ratio of **7a** and **7d**. Comparisons of the ^{1}H NMR data reported for the natural hydrolysate with those of the synthetic four tripeptides **7a**–**d** showed that the natural fragment is consistent with the (2*R*,3*R*)-isomer **7a** as shown in Table 1. The chemical shifts of the other three diastereomers at the methyl



Scheme 2.

Table 1. Comparisons of the ^{1}H NMR chemical shifts of synthetic four tripeptides with the natural hydrolysate

	δ (ppm)				
	Natural[a]	(2*R*,3*R*)-**7a**[a]	(2*S*,3*S*)-**7b**[b]	(2*S*,3*R*)-**7c**[b]	(2*R*,3*S*)-**7d**[a]
Homoproline					
2	5.14	5.15	4.99, 5.15	5.08–5.12	4.84
3	1.55	1.54–1.58	1.50–1.52	1.66–1.69	1.42–1.43
	2.15	2.16	2.13, 2.21	1.93–1.97	1.95–1.98
4	1.26	1.26			0.96–1.16
	1.67	1.68–1.70	1.21–1.41	0.84–1.27	1.16–1.43
5	1.42	1.40–1.45	1.39–1.41	1.42–1.47	0.96–1.16
	1.67	1.68–1.70		1.63–1.67	1.16–1.43
6	3.12	3.13	3.22–3.24	N.A.	3.00
	4.21	4.21	4.17–4.40	3.90, 4.10	3.78
β-*Methoxytyrosine*					
2	5.19	5.20	5.10, 5.15	5.05	5.08
3	4.31	4.32	4.17, 4.31	4.10	4.30
OMe	3.00	3.01	3.02, 3.03	3.08, 3.12	3.09
Ar-H 2, 6	7.14	7.15	7.16, 7.17	7.09, 7.15	7.10
Ar-H 3, 5	6.70	6.71	6.68, 6.69	6.69, 6.74	6.74
N-Me threonine					
2	3.27	N.A.[c]	N.A.[c]	N.A.[c]	3.16
3	3.42	N.A.[c]	N.A.[c]	N.A.[c]	3.57
4	0.32	0.35	0.98, 1.00	1.03, 1.09	1.13
N-Me	2.28	2.27	1.59, 1.65	2.07, 2.15	2.35

[a] For the major conformer.
[b] For the two conformers.
[c] Not assigned due to overlapping with water signal in DMSO-d_6.

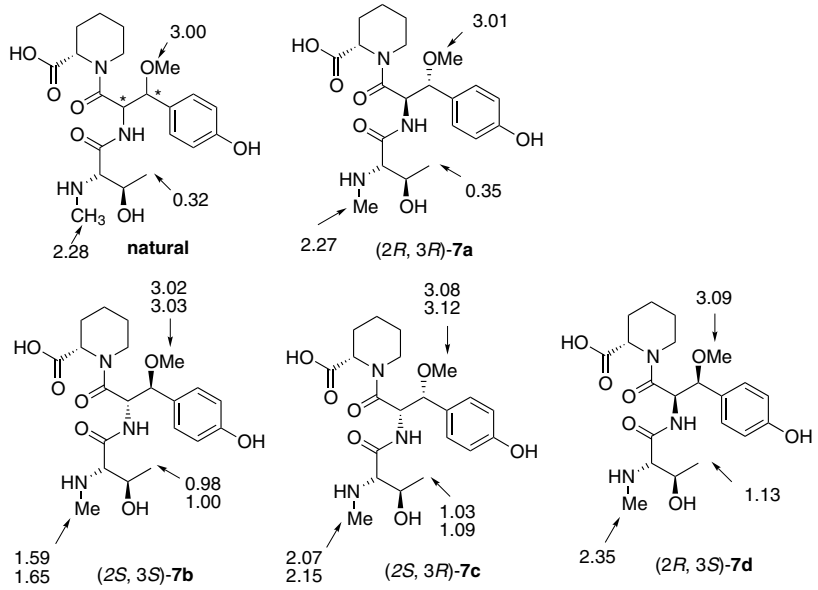


Figure 2.

proton of the *N*-MeThr residue differed largely from the 0.32 ppm of the naturally derived one (Fig. 2). In addition, chemical shifts of the other parts except that described above are in good accordance with those of the natural hydrolysate. Accordingly, the relative and absolute stereochemistry of the β-OMeTyr residue in papuamide A was unambiguously established as 2*R*,3*R*. Incidentally, efficient synthesis of Boc-(2*R*,3*R*)-β-OMeTyr(Bn)-OH, a building block for natural papuamides, has been reported by use of asymmetric hydrogenation using the chiral iridium catalyst developed by us.[4h]

In conclusion, four diastereomers of the tripeptides relating to the hydrolysate from papuamide A have been synthesized from four diastereomers of the β-methoxytyrosines. Comparison of the ^{1}H NMR data of the natural hydrolysate with those of the four synthetic

tripeptides unambiguously confirmed the relative and absolute stereostructure of the β-OMeTyr residue in papuamides as 2*R*,3*R*. Further investigation directed towards total synthesis of papuamides is underway in this laboratory.

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