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Revised Stereochemistry of Ceramide-Trafficking Inhibitor HPA-12 by X-ray Crystallography Analysis

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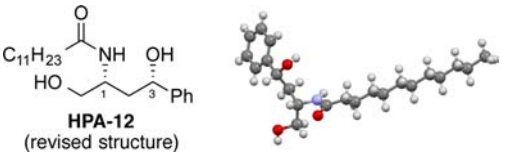
ABSTRACT



In response to Berkeš's report revising the stereochemistry of HPA-12, an important ceramide-trafficking inhibitor that was discovered and synthesized and its stereochemistry determined in 2001, the synthesis and the stereochemistry were reinvestigated. A large-scale synthetic method for HPA-12 based on a Zn-catalyzed asymmetric Mannich-type reaction in water was developed. Single crystals of HPA-12 for X-ray crystallographic analysis were obtained from ethyl propionate/*n*-hexane, and the stereochemistry was definitely determined to be 1*R*,3*S*, consistent with Berkeš's revised structure.

Sphingolipids are one of the constituents of membrane lipids in mammalian cells and play important roles in cell growth, differentiation, and apoptosis.[1] Ceramide produced in the endoplasmic reticulum (ER) is transported to the Golgi apparatus for conversion to sphingomyelin (SM). *N*-(3-Hydroxy-1-hydroxymethyl-3-phenylpropyl)dodecanamide (HPA-12) is a novel inhibitor of ceramide trafficking from the ER to the site of SM synthesis.[2] HPA-12 does not inhibit the activity of SM synthase or the ER-to-Golgi trafficking of proteins,[2a] suggesting specific inhibition of ceramide transport. Later, HPA-12 was demonstrated to be a direct antagonist of the ceramide transport protein CERT, which mediates interorganelle trafficking of ceramide from the ER to the Golgi site for the synthesis of SM.[3] Since the target was specified, the CERT inhibitor HPA-12 has been used as a convenient tool for various biological in vitro studies.[4]

HPA-12 was discovered from our amino alcohol library in 2001. We accomplished the first total synthesis and determined the absolute stereochemistry of HPA-12 (**1**, 1*R*,3*R*) in the same year.[5] Subsequently, in 2011, Berkeš et al.

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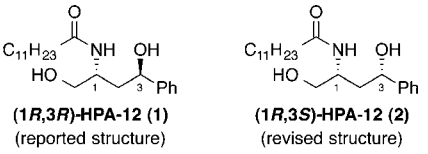
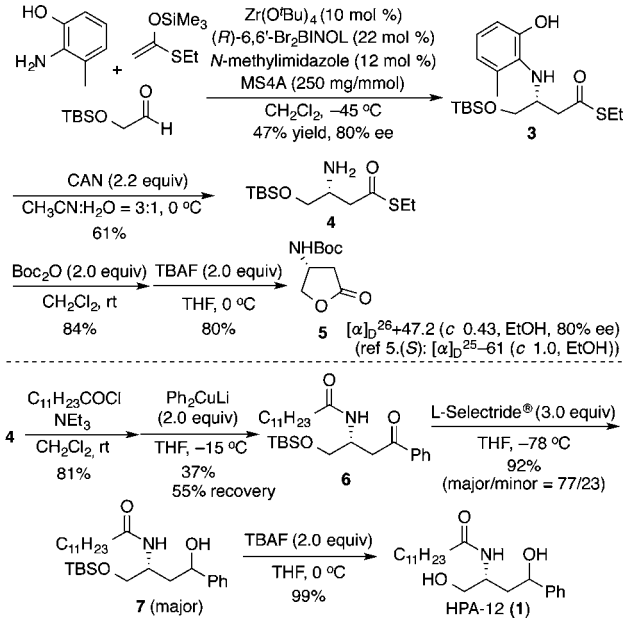
reported a synthesis of HPA-12 and also proposed a revision of the stereochemistry (**2**, 1*R*,3*S*).[6] In response to Berkeš's report, we reviewed our total synthesis and the determination of the stereochemistry.



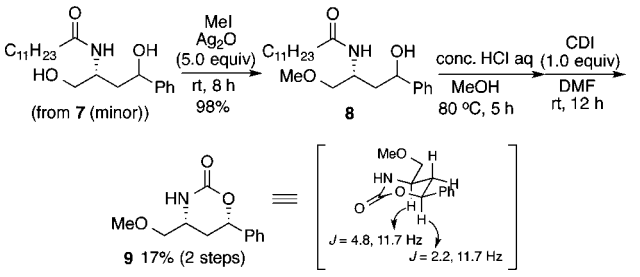
Our total synthesis started from a chiral Zr-catalyzed three-component Mannich-type reaction (Scheme 1).[5] The absolute configuration of Mannich adduct **3** was determined after conversion to amino lactone **5**[7] via **4**. Amino thioester **4** was then converted to amino ketone **6**, which was reduced with L-Selectride to form two stereoisomers of amino alcohol **7** (major/minor = 77/23). Finally, the major stereoisomer of **7** was converted to HPA-12. To determine the stereochemistry of **7**, the minor stereoisomer of **7** was further converted to **8** and then six-membered carbamate **9** via conventional transformations (Scheme 2). The ^{1}H NMR analysis of **9** indicated the syn stereochemistry, and thus, the stereochemistry of HPA-12 was determined to be 1*R*,3*R*.[5]

Scheme 1. First Total Synthesis of HPA-12 via a Three-Component Mannich-Type Reaction



During our reinvestigation of this stereochemical assignment, it was discovered that benzylic positions of compound **9** as well as even **7** and **8** were relatively easy to epimerize. We therefore thought that, if the stereochemistry was determined using one of the synthetic intermediates

Scheme 2. Determination of Relative Configuration of HPA-12



or derivatives, epimerization might occur during further conversions to HPA-12 and others, and thus, the determination of the stereochemistry might not be reliable. On the other hand, HPA-12 is a white solid, and we decided to make single crystals of HPA-12 for X-ray crystallographic analysis to determine the stereochemistry.

To make single crystals of HPA-12, a large-scale synthesis of HPA-12 was needed. Because the first total synthesis is not suitable for a large-scale preparation, we decided to develop a new method for a large-scale preparation of HPA-12.[8–10] As an intermediate in the synthesis of HPA-12, we selected hydrazone ester **13**, which was previously converted to HPA-12 via three steps.[9] Catalytic asymmetric synthesis of **13** was conducted using ZnF_2 (50 mol %), chiral ligand **11** (10 mol %), and triflic acid (1 mol %) in water/THF (1/9). To avoid the use of a relatively large amount of ZnF_2 and the organic solvent on the basis of recent regulation of green sustainable chemistry, we followed an improved method using a catalytic amount of cetyltrimethylammonium bromide (CTAB) in 100% water. Because the silyl enol ether derived from acetophenone (**12**) is labile in water, we optimized the reaction conditions for the asymmetric Mannich-type reaction (Table 1).

When 100 mol % of ZnF_2 and 10 mol % of chiral ligand **11** were used without CTAB, the desired Mannich-type adduct **13** was obtained in 25% yield with 85% enantiomeric excess (ee) (entry 1). The yield was improved markedly, to 91%, by the addition of 2 mol % of CTAB (entry 2). Finally, the amount of ZnF_2 was reduced to 10 mol %, and the desired product **13** was obtained in 81% yield with 82% ee (entry 3). Under these conditions, a gram-scale preparation of **13** was successful. Hydrazone ester **13** was successfully converted to HPA-12 following the literature.[9]

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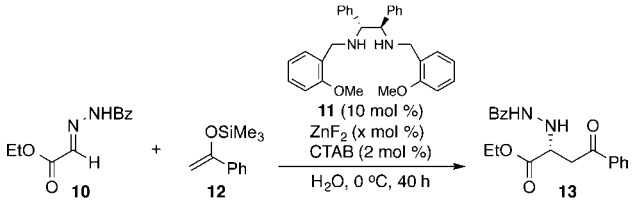
(9) For the HPA-12 synthesis via ZnF_2-catalyzed asymmetric Mannich-type reactions of hydrazone esters with silyl enol ether in water, see: (a) Kobayashi, S.; Hamada, T.; Manabe, K. *J. Am. Chem. Soc.* **2002**, *124*, 5640–5641. (b) Hamada, T.; Manabe, K.; Kobayashi, S. *J. Am. Chem. Soc.* **2004**, *126*, 7768–7769. (c) Hamada, T.; Manabe, K.; Kobayashi, S. *Chem.—Eur. J.* **2006**, *12*, 1205–1215.

(10) Raghavan and Rajender also reported a synthesis of HPA-12; see: Raghavan, S.; Rajender, A. *Tetrahedron* **2004**, *60*, 5059–5067.

Table 1. Asymmetric Mannich-Type Reaction of Hydrazone Ester **10** with Silyl Enol Ether **12** Using a Catalytic Amount of Chiral Zinc Catalyst in Aqueous Media



entry	ZnF_2 (mol %)	yield (%)	ee (%)
1[a]	100	25	85
2	100	91	85
3	10	81	82
4[b]	10	80	81

[a] Without CTAB. [b] Gram-scale (4.19 mmol) synthesis.

We then examined formation of single crystals for X-ray crystallographic analysis. Because it was found that *n*-hexane was a poor solvent for HPA-12, we decided to screen a second solvent combined with *n*-hexane. We tested dichloromethane (DCM), diethyl ether (Et_2O), chloroform ($CHCl_3$), benzene (C_6H_6), methanol (MeOH), ethanol (EtOH), ethyl acetate (AcOEt), and *t*-Bu methyl ether (TBME) at 5 °C and room temperature (rt, 15−20 °C). While powders were formed using DCM, Et_2O, and TBME at 5 °C, amorphous crystals were generated using $CHCl_3$, C_6H_6, MeOH, EtOH, and AcOEt at 5 °C and DCM, Et_2O, $CHCl_3$, and TBME at rt. Only AcOEt/*n*-hexane gave crystals; however, they were too thin for X-ray crystallographic analysis. We then examined three solvent systems including AcOEt, *n*-hexane, and a third solvent; however, all attempts to obtain suitable crystals failed. We then examined other acetic acid esters. *t*-Bu acetate/*n*-hexane and *i*-Pr acetate/*n*-hexane gave crystals; however, they were not suitable for the analysis. Finally, the combination of ethyl propionate and *n*-hexane gave beautiful single crystals, whose analysis gave the X-ray crystallographic structure (Figure 1). The stereochemistry was definitely determined to be 1*R*,3*S*.

This stereochemistry of HPA-12 is consistent with our previous structure−activity relationship studies on de novo synthesis of SM in CHO cells.[2b] We tested compounds **14a**−**d** (Figure 2), and the best activity was observed in **14a**, whose stereochemistry at the 1 and 3 positions is consistent with those of HPA-12. It is noted that the stereochemistry of **14a** is also consistent with that of phytoceramide, a natural ceramide type (**15**).

In summary, in response to Berkeš's report on the revision of the stereochemistry of HPA-12, we reexamined our previous synthesis and determination of the stereochemistry. It was found that the benzylic positions of the intermediates for the synthesis of HPA-12 were relatively easy to epimerize, and therefore, any stereochemical determination using synthetic intermediates and derivatives might not be reliable because of the possibility of such epimerization. Therefore, we decided to make single crystals of HPA-12 for the X-ray crystallographic analysis. Because a large-scale preparation of HPA-12 was needed to obtain the crystals, a novel Zn-catalyzed asymmetric Mannich-type reaction in water was developed. After many crystallization trials, beautiful single crystals of HPA-12, which has a surfactant-like structure with a long hydrophobic alkyl chain and hydrophilic alcohol functional groups, showed the stereochemistry 1*R*,3*S*, consistent with the revised structure proposed by Berkeš's group. Because of the importance of HPA-12 not only in chemistry, but also in biology and related fields, this definite stereochemical assignment will contribute to the development and further investigations in those fields.

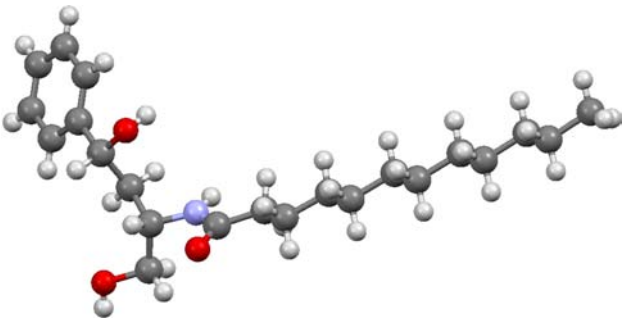


Figure 1. X-ray crystallographic analysis of HPA-12.

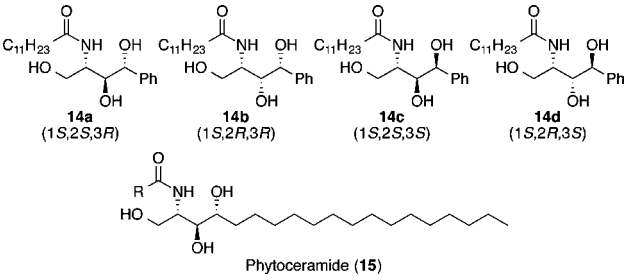


Figure 2. Structures of some HPA-12 analogues (**14a**−**d**) and phytoceramide (**15**).

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Supporting Information Available. General experimental procedures and X-ray crystal structure of HPA-12 (**2**) (CCDC 896928). This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.