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Expedient and Practical Synthesis of CERT-Dependent Ceramide Trafficking Inhibitor HPA-12 and Its Analogues

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ABSTRACT

The practical stereodivergent route to both *syn*- and *anti*-diastereomers of 1-substituted 3-aminobutane-1,4-diols based on the crystallization-induced asymmetric transformation (CIAT) approach was completed. This led to the revision of the reported stereochemistry of the first inhibitor of CERT-dependent ceramide trafficking HPA-12 from (*R*,*R*)-*anti*- to the (*R*,*S*)-*syn*-enantiomer. Due to the expeditiousness of production and inexpensive conditions developed, a series of alkyl- and aryl-substituted analogues of HPA-12 is also reported.

Sphingolipids are ubiquitous components of eukaryotic cell membranes where they play very pivotal roles in intracellular signaling and in membrane structure. Even though the biochemical pathway of sphingolipid synthesis and its compartmentalization between the endoplasmic reticulum (ER) and Golgi apparatus have been known for many years, the transport mechanistic paradigms in this pathway have only recently been elucidated. One of the most exciting developments in this field over the past few years was the identification of a protein, CERT,[1] a cytosolic 68 kDa protein, which mediates the transport of ceramide from the ER to the Golgi apparatus in a nonvesicular manner. Sphingolipid misregulation contributes to the development of variety of disease states. CERT works as one of the mediators of sphingolipids homeostasis[2] and was found likely to be relevant to Goodpasture autoimmune disease.[1b]

(1*R*,3*R*)-*N*-(3-Hydroxy-1-hydroxymethyl-3-phenylpropyl)dodecamide (commonly known as HPA-12) has been used to determine the role of CERT in sphingolipids biosynthesis. It was identified as the first specific inhibitor for sphingomyelin (SM) synthesis in mammalian cells and a potential drug that inhibits the CERT-dependent pathway of ceramide trafficking in intact cells. As was further demonstrated, the (1*R*,3*R*)-HPA-12 isomer, but not other stereoisomers, inhibits the CERT-mediated intermembrane transfer of ceramide in a cell-free assay system,

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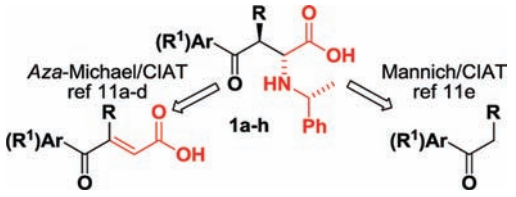
indicating this diastereomer to be a direct antagonist of CERT.[1d,3] The crystal structures of the CERT START domain, in complex with (1*R*,3*R*)-HPAs of varying acyl chain lengths outlining the more significant role of stereochemistry of the inhibitors, have been published recently.[4]

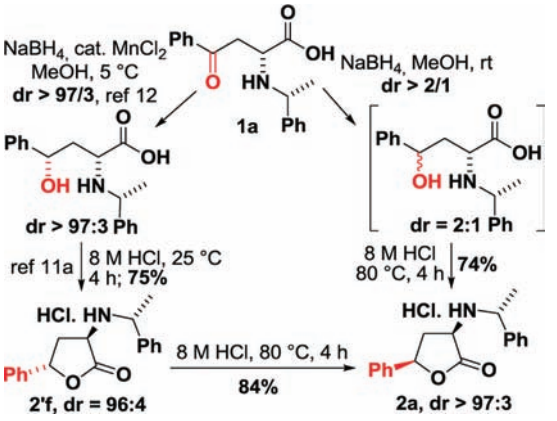
Scheme 1. Crystallization-Induced Asymmetric Transformations (CIAT) Approaches to the Starting Oxoamino Acid Derivatives



The oxoamino acids and their derivatives are now recognized as the crucial intermediates for the HPA synthesis, although other approaches have been presented.[5] The first synthesis of HPA-12 was published in 2001 by the Kobayashi group.[6] The asymmetric Mannich protocol was improved by the same authors in the following years.[7] While the C−N bond was introduced with high selectivity, the diastereoselectivity of C−OH bond formation was modest. For the last step of the synthesis, *anti*-stereoselective reduction of γ-oxoamino acid derivatives, both K-Selectride and $LiBEt_3H$ have been used to give the expected aminodiol in 9/91 (*syn*/*anti*) selectivity. Interestingly, the selectivity dropped to 40/60 with traces of moisture in the reaction media.[8] Other strategies for the *anti*-1,3-amino alcohol moiety in the same or related aminodiols provide low stereoselectivity as well.[9] Elsewhere, the stereoselective synthesis of the *anti*-1,3-amino alcohol moiety via the improved oxazolidine route in the racemic aminobutanediols was published only recently.[10] In this paper, we introduce viable scalable synthesis of (1*R*,3*R*)-HPA-12, its aryl-substituted analogues, and also the (1*R*,3*S*)-HPA-12 together with some 1-alkyl-substituted analogues of the same stereochemistry.

Our strategy is based on the use of an expedient straightforward transformation of enantiomerically and diastereomerically pure oxoamino acids easily accessible via the CIAT process from available substrates (Scheme 1).[11] The application of 1-phenylethylamine (PEA) as a chiral mediator in such a transformation is especially favorable since both (*R*)- and (*S*)-enantiomers of PEA are commercially available, with both antipodes of the final compounds attainable. The required (1*R*,3*R*)-*anti*-relationship of 3-amino-1-phenylbutane-1,4-diols was accomplished using (*R*)-PEA and another CIAT process in tandem with reversible acid-catalyzed lactonization.[11a]

Scheme 2. Expedient Approach to the *cis*-Butanolide **2a**



Since in our previous paper we described the preparation of the *trans*-lactone **2′f**,[12] we are now investigating modified conditions to reach the required (2*R*,5*R*)-derivative (**2a**, Scheme 2). We are pleased to notice that a simple raising of the reaction temperature from 25 up to 80 °C under otherwise unchanged conditions (8 M HCl, 4 h) is sufficient to achieve the transformation of *trans*-lactone **2′f** to the thermodynamically more stable *cis*-butanolide **2a** in 84% yield. Finally, optimal conditions for the required *cis*-lactone **2a** were realized in two steps—a one-pot procedure starting from oxoamino acid **1a** using a nonstereoselective reduction with sodium borohydride (dr 2/1) followed by acid-catalyzed lactonization under the formerly described conditions.[11a] Under these conditions, the lactonization occurred smoothly and the desired product **2a** started to seed within 30 min of the reaction.

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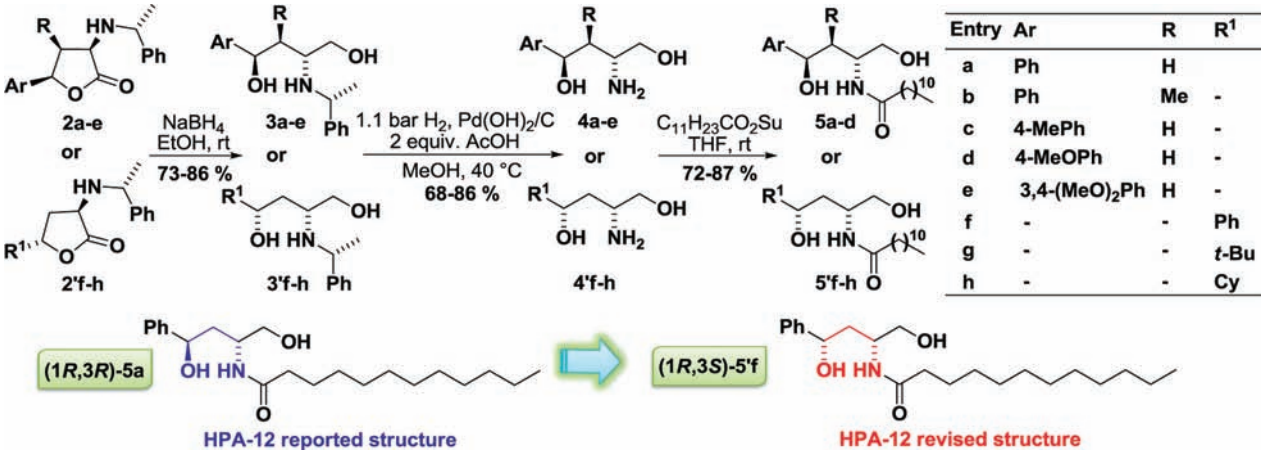
Scheme 3. Synthesis of Inhibitor of CERT-Dependent Ceramide Trafficking HPA-12 and its Alkyl and Aryl Analogues



Entry	Ar	R	R^1
a	Ph	H	
b	Ph	Me	-
c	4-MePh	H	-
d	4-MeOPh	H	-
e	3,4-$(MeO)_2$Ph	H	-
f	-	-	Ph
g	-	-	*t*-Bu
h	-	-	Cy

Having in hand a series of the required *cis*-lactones expanded by the selected *trans*-butanolides (**2′** → **2**)[11b] with tethered alkyl substituents, the simple and straightforward three-step synthesis of HPA-12 and their analogues was then achieved very easily (Scheme 3).

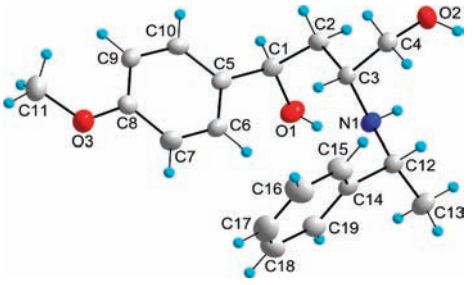


Figure 1. Molecular structure of product **3d** showing the atom-labeling scheme and displacement ellipsoids at the 30% probability level. For clarity, only one of the two symmetry-independent molecules is shown.

Along this line, the reduction of lactones **2** and **2′** proceeds with high yield and fortunately without erosion of stereochemistry (Scheme 3). The relative configuration of the *N*-substituted aminobutanediols **3** as well as **3′** was secured without ambiguity on the basis of the results of X-ray crystallographic analysis of product **3d** as a crystalline hemihydrate (Figure 1).[13] The further chemoselective catalytic *N*-debenzylation was accomplished under standard conditions using $Pd(OH)_2$ in methanol. However, an unusually long reaction time was required for full conversion of starting material (20−48 h), and furthermore, a high extent of epimerization to another diastereomer in the arylsubstituted series was observed (*anti*/*syn* ≈ 90/10 for products **4a**−**e**; *syn*/*anti* ≈ 80/20 for product **4′f**). Interestingly, the addition of 2 equiv of acetic acid shortened the reaction time (only 3−4 h are necessary for **4a**−**e** instead of 24 h for **4′f**), and it also improved the diastereomeric purity of the product (dr > 95/5).

As shown in Scheme 3, the ultimate chemoselective *N*-acylation of the diamino diols **4** and their epimers **4′** was accomplished under neutral conditions with succinimide dodecanoate at room temperature. After 1−2 days of the reaction, the resulting acylated products **5a**−**d** and **5′f**−**h** were purified by silica gel column chromatography.

To distinguish which stereogenic center is unstable under debenzylation conditions, a mixture of *syn*/*anti* diastereomers obtained from *N*-substituted amino diol **3a** and **3′f** was chromatographically separated to their pure stereoisomers. After their *N*-acylation, the optical rotation of final derivatives was compared with the literature data. The major diastereomer (from the *syn*-amino diol product **3′f**) provided the product **5′f** with $[\alpha]_D = -34$, while the minor epimer resulted in the formation of the derivative **5a** with $[\alpha]_D = +10$. In an analogous manner, the *N*-acylated product with $[\alpha]_D = +10$ was isolated from the *anti*-diastereoisomer **3a** and the isolated minor diastereomer (dr = 95/5) showed $[\alpha]_D = -33$.

Moreover, the dextrorotatory nature of the major diastereomer was also confirmed in the series of aromatic *anti*-(1*R*,3*R*)-analogues **4b**−**e** and in the aliphatic *syn*-aminodiols **4′g**,**h**; the levorotatory nature of the major diastereomer was in agreement with the derivative **4′f**.

However, the published $[\alpha]_D$ value for HPA-12 in the literature was −36.3 (*c* 0.505, $CHCl_3$)[8] and −35.1 (*c* 0.8, $CHCl_3$),[6] respectively, and was in stark contrast to those measured for the synthesized (1*R*,3*R*)-**5a** which

(13) The crystallographic coordinates have been deposited with the Cambridge Crystallographic Data Centre; deposition nos. CCDC 806264 for **3d** (Figure 1) and 805528 for **4c** (Figure 2). These data can be obtained via https://www.ccdc.cam.ac.uk/services/structure_deposit/.

Table 1. Key ^{1}H and ^{13}C NMR Data for Comparisons (δ in ppm)[a]

	(1*R*,3*R*) HPA-12[8]	(1*R*,3*S*) HPA-12[14]	(1*R*,3*R*) **5a**	(1*R*,3*S*) **5′f**
N-H	6.41 (d,*J* = 6.5 Hz)	6.27 (d,*J* = 8.3 Hz)	6.31 (d,*J* = 8.3 Hz)	6.40 (d,*J* = 6.3 Hz)
H-1	4.81 (dd, *J* = 3.4,8.3 Hz)	4.67 (brd, *J* = 10.5 Hz)	4.66 (dd, *J* = 2.7,10.3 Hz)	4.82 (dd, *J* = 3.4,8.8 Hz)
C=O	174.3	174.9	175.0	174.3
C-1	71.8	70.4	70.4	72.2
C-3	50.4	48.7	48.8	50.7

[a] All spectra were recorded in $CDCl_3$.

is $[\alpha]_D = +10.5$ (*c* 0.44, $CHCl_3$). This value, however, is in agreement with that obtained for (1*S*,3*R*)-**5′f**, which is $[\alpha]_D = -34.4$ (*c* 0.36, $CHCl_3$), and therefore, a revision of the previously proposed structure seems to be necessary.

In order to clarify this issue, we were looking for independent facts to resolve the observed discrepancy. We were delighted to observe that also the spectroscopic data for product **5a** closely matched those of (1*S*,3*R*)-HPA-12 diastereomer[14] and vice versa, while the measured product **5′f** data nearly matched the literature data of (1*R*,3*R*)-HPA-12[8] (see Table 1).

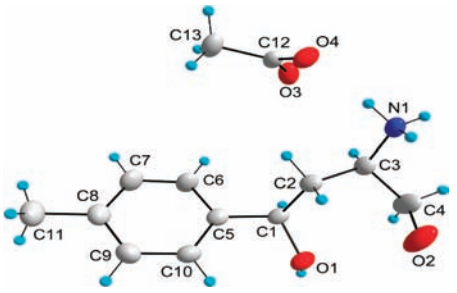


Figure 2. Molecular structure of **4c**·AcOH showing atom labeling scheme and displacement ellipsoids at the 30% probability level. The hydroxy group (O2-H) is disordered over two positions. Only a dominant position with an occupation factor of 0.71 is shown. The corresponding hydroxy group hydrogen atom could not be located because of the disorder.

Finally we succeeded in the preparation of suitable crystals for crystallographic analysis from the derivate **4c**. Thus, the X-ray analysis of **4c**·AcOH[15] confirmed the *anti*-relationship between both stereogenic centers.

Furthermore, from an ^{1}H NMR standpoint, the large coupling constants between H_2 atoms in antiperiplanar relationships were evidenced in spectra of *N*-substituted *syn*-amino diols **3′f** and **3′g** recorded in $CDCl_3$. In addition, in nonprotic solvent, a strong intramolecular hydrogen bond could be anticipated due to stabilizing effects of the R^1 group as large substituents in equatorial positions. In the *anti* derivatives, such stabilizing effects do not exist, and the favorable conformations afforded similar coupling constants for both $H_{2\alpha}$ and $H_{2\beta}$ protons in the *N*-substituted and unsubstituted amino diols enantiopure **3a**−**e** and **4a**−**e**, respectively. Finally, since the similarities in the ^{1}H NMR spectral data are evident in the both series of *anti*-amino diols (products **3a**−**e** and **4a**−**e**; see the Supporting Information for more details), the *anti*-relationship established with X-ray analysis could be predicated also for the phenyl derivatives **3a** and **4a**.

Thus, we suspect that the stereochemical assignment of the HPA-12 structure based on the original published data of Ueno et al.[6] might be incorrect, and we thus recommend a revision of the HPA-12 stereochemistry from the (1*R*,3*R*)-*N*-(3-hydroxy-1-hydroxymethyl-3-phenylpropyl)-dodecamide (**5a**) to the (1*R*,3*S*)-**5′f** diastereomer.

The absolute configuration of HPAs is crucial for their biological activities with respect to the CERT protein. In their recent crystallographic study, Kudo et al.[4] invoked interactions of the CERT START domain with three out of four possible stereoisomers of HPAs, and on the basis of their previous study[16] they favored (1*R*,3*R*)-HPA over (1*R*,3*S*)-HPA. Our findings herein demonstrate that they have been indeed using (1*R*,3*S*)-HPA. As a consequence, revision of some other stereochemical transformations can be expected.[5]

In summary, the demonstrated expeditious practical and inexpensive approach represents a short stereodivergent elegant route to both diastereomers of 1-aryl-3-aminobutane-1,4-diols (**4a**−**e** and **4′f**), which opens the door to the multigram synthesis of the first inhibitor of CERT-dependent ceramide trafficking and its analogues.

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Supporting Information Available. Experimental procedures and characteristics of all new compounds. ^{1}H and ^{13}C NMR spectra for all novel compounds. This material is available free of charge via the Internet at http://pubs.acs.org

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