JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

Synthesis of Eight Stereoisomers of Pochonicine: Nanomolar Inhibition of β-*N*-Acetylhexosaminidases

Jian-She Zhu,[†,#] Shinpei Nakagawa,[§] Wei Chen,[‡] Isao Adachi,[§] Yue-Mei Jia,[†] Xiang-Guo Hu,[†] George W. J. Fleet,[∥] Francis X. Wilson,[⊥] Teruhiko Nitoda,[▽] Graeme Horne,[⊥] Renate van Well,[⊥] Atsushi Kato,*[,§] and Chu-Yi Yu*[,†]

[†]Beijing National Laboratory of Molecular Science (BNLMS), CAS Key Laboratory of Molecular Recognition and Function, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China

[‡]College of Chemistry and Chemical Engineering, Jiang Xi Normal University, Nanchang, Jiangxi 330022, China

[§]Department of Hospital Pharmacy, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan

[∥]Chemistry Research Laboratory, Department of Chemistry, University of Oxford, Mansfield Road, Oxford OX1 3TA, U.K.

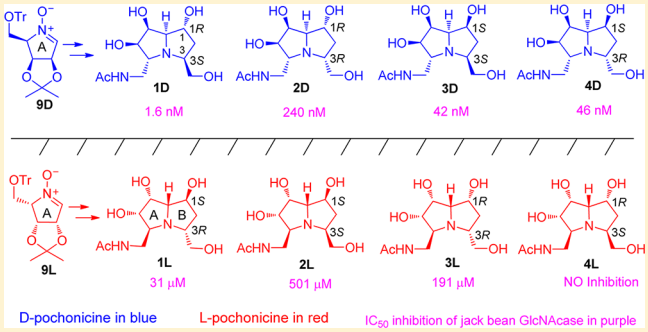
[⊥]Summit PLC, 85b Park Drive, Milton Park, Abingdon, Oxon OX14 4RY, U.K.

[#]University of the Chinese Academy of Sciences, Beijing 100049, China

[▽]Laboratory of Bioresources Chemistry, the Graduate School of Environmental and Life Science, Okayama University, 1-1-1 Tsushima-naka, Kita-ku, Okayama 700-8530, Japan

Supporting Information

ABSTRACT: Pochonicine, the first naturally occurring polyhydroxylated pyrrolizidine containing an acetamidomethyl group, which was isolated from *Pochonia suchlasporia* var. *suchlasporia* TAMA 87, together with its enantiomer and their C-1 and/or C-3 epimers, have been synthesized from the sugar-derived cyclic nitrones **9D** and **9L**, respectively. An in-depth NMR study showed that both the ^{1}H and ^{13}C NMR spectra of the synthetic pochonicines (**1D** and **1L**) matched very well with those of natural pochonicine in D_2O, which unequivocally determined the relative configuration of the natural product as **1D** or **1L**. In addition, comparison of the optical rotations of the synthetic pochonicines and that of the natural product, but more convincingly their glycosidase inhibition profiles, confirmed the absolute configuration of natural pochonicine as 1*R*,3*S*,5*R*,6*R*,7*S*,7a*R*. Thereby, the structure of natural pochonicine was unequivocally determined as (+)-(1*R*,3*S*,5*R*,6*R*,7*S*,7a*R*)-pochonicine (**1D**). Glycosidase inhibition experiments showed that natural pochonicine **1D** and its epimers **2D**, **3D**, and **4D** all are powerful inhibitors of hexosaminidases (five β-*N*-acetylglucosaminidases and two β-*N*-acetylgalactosaminidases) while their enantiomers **1L**, **2L**, **3L**, and **4L** are much weaker inhibitors of the same enzymes. (-)-3-*epi*-Pochonicine (**2L**) was found to be a potent and selective inhibitor of α-L-rhamnosidase. None of the compounds showed any inhibition of α-GalNAcase.



INTRODUCTION

Pochonicine, the first naturally occurring polyhydroxylated pyrrolizidine containing an acetamidomethyl group (Figure 1), was isolated from *Pochonia suchlasporia* var. *suchlasporia* TAMA 87 by Nitoda and co-workers in 2009.[1] Glycosidase inhibition assays showed that it is a powerful inhibitor (with IC_{50} on the nanomolar scale) against β-*N*-acetylglucosaminidases (β-GlcNAcases) from various organisms, including insects, fungi, mammals, and plants. Its inhibition potency is similar to that of the known powerful β-GlcNAcase inhibitor nagstatin. Pochonicine represents the first example of a naturally occurring pyrrolizidine alkaloid with potent inhibition of β-GlcNAcases. In fungi and insects, chitinase combined with β-GlcNAcase hydrolyzes the polysaccharide chitin into the monosaccharide β-GlcNAc; this process is known to be necessary for the normal

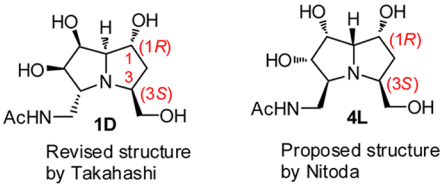


Figure 1. Structure of (+)-pochonicine.

Received: August 2, 2013
Published: September 13, 2013

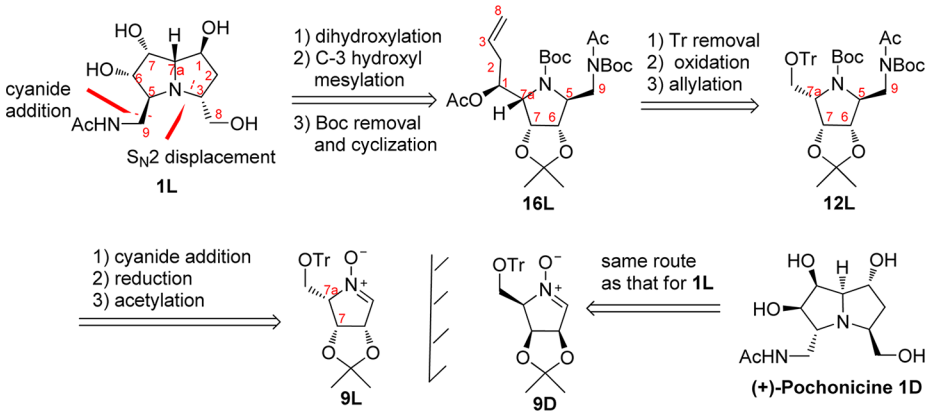


Figure 2. Retrosynthetic analysis of pochonicine.

growth of these organisms. Inhibitors of such enzymes are potentially useful in the discovery of novel pesticides and fungicides.[2] Furthermore, in mammals, especially in humans, *O*-linked *β*-*N*-acetylglucosaminidase (*O*-*β*-GlcNAcase, OGA) plays an important role in the regulation of insulin signaling and as a mediator of glucose toxicity.[3] Therefore, specific inhibitors of OGA have shown great promise in the treatment of many diseases such as insulin resistance,[4] type-II diabetes,[5] Alzheimer's disease,[6] and cancer metastasis.[7]

The structure of pochonicine was originally proposed as (1*R*,3*S*,5*S*,6*S*,7*R*,7a*S*)-**4L** (Figure 1) on the basis of NOE correlations, but its absolute configuration was not determined. Takahashi and co-workers have recently completed a synthesis of the proposed structure of pochonicine and its C-1 and/or C-3 epimers (i.e., compounds **1L**, **2L**, **3L**, and **4L**); this work revised the relative configuration and determined the absolute configuration as (1*R*,3*S*,5*R*,6*R*,7*S*,7a*R*)-**1D** (Figure 1).[8] However, a slight uncertainty in the determination of the relative and absolute configurations of pochonicine remains. First, the relative configuration of pochonicine was determined by comparison of the ^{1}H and ^{13}C NMR spectra of compound **1L** with those of the natural sample of pochonicine. However, the reported ^{1}H and ^{13}C NMR spectra of compound **1L** were similar but not identical to those of the natural sample of pochonicine. Second, the absolute configuration of pochonicine was determined by comparison of the optical rotation of compound **1L** and that of the natural sample of pochonicine. Since the values of the optical rotation of the free bases are very small and change around zero, the comparison of optical rotations may not be helpful. The optical rotations of the hydrogen chloride salts of compound **1L** and the natural sample of pochonicine were also compared. However, the determination of absolute configurations via such kinds of comparison is not definitive: while the sign of optical rotation of the hydrogen chloride salt is often the same as that of the corresponding free base for many iminosugar alkaloids, there are many with opposite specific roatations.[9] Therefore, it was necessary to make a further investigation to determine unambiguously both the relative and absolute configurations of pochonicine through the synthesis of both enantiomers of pochonicine and their C-1 and/or C-3 epimers, followed by an in-depth study of their ^{1}H and ^{13}C NMR spectra as well as an examination of the glycosidase inhibition profiles of all these isomers. In view of our continued interest in the synthesis and glycosidase inhibition of iminosugars,[10] pochonicine was an intriguing challenge to us because of its novel structure and exceptional inhibition of GlcNAcase. As has been reported in many cases, the glycosidase inhibition profiles of the enantiomers of many naturally occurring iminosugars are strikingly different from those of the natural products.[11] An in-depth evaluation of the glycosidase inhibition of pochonicine, its enantiomer, and their C-1 and/or C-3 epimers provides a preliminary structure−activity relationship of pochonicine and eight of its 64 stereoisomers and will allow the design and synthesis of even more potent inhibitors.

Herein we present our work on the synthesis of both enantiomers of pochonicine and their C-1 and/or C-3 epimers, an in-depth study of their ^{1}H and ^{13}C NMR spectra, and the glycosidase inhibition profiles of all these stereoisomers in the hope of developing an efficient method for the synthesis of pochonicine and its analogues.

RESULTS AND DISCUSSION

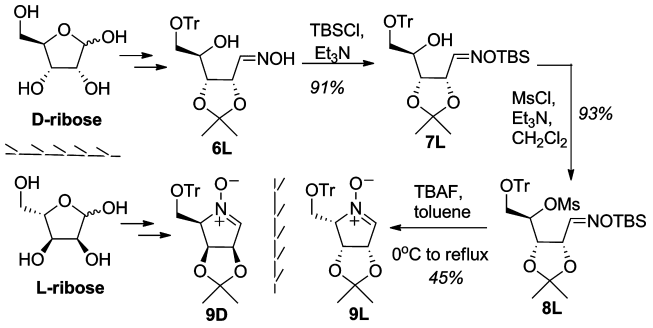
Retrosynthetic Analysis. The retrosynthetic analysis of pochonicine is depicted in Figure 2. In view of the *cis* configuration between H-7 and H-7a and the *trans* configuration between H-5 and H-6 in pochonicine, it can be envisaged that **1L** and its C-1 and/or C-3 epimers could be synthesized starting from the all-*cis* D-ribose-derived cyclic nitrone **9L**[12] having the required *cis* configuration between H-7 and H-7a. The key intermediate **12L**, with a unique acetamidomethyl group, could be synthesized by nucleophilic *anti* addition of cyanide to cyclic nitrone **9L**[9d,13] followed by reduction and acetylation. The intermediate **16L** could be derived from **12L** through selective removal of the trityl protecting group, oxidation of the hydroxymethyl group to the aldehyde, and subsequent allylic addition. Finally, the second pyrrolidine ring could be assembled through dihydroxylation of the allyl group followed by intramolecular S$_N$2 substitution of the secondary cyclic amine and the secondary hydroxyl group, affording the desired target compound. The enantiomer **1D** would be formed from cyclic nitrone **9D** via an identical synthetic route.

Preparation of Cyclic Nitrones 9L and 9D. The starting materials, cyclic nitrones **9L** and **9D**, were prepared from D-ribose and L-ribose, respectively, in 22−30% overall yield on multigram scales by the known procedure[14] (Scheme 1).

Synthesis of Both Enantiomers of Pochonicine and Their C-1 and/or C-3 Epimers. From cyclic nitrone **9L**, we investigated the synthesis of **1L** and its C-1 and/or C-3 epimers. Addition of trimethylsilyl cyanide to the all-*cis* cyclic nitrone **9L** provided hydroxylamine **10L** in 96% yield as a

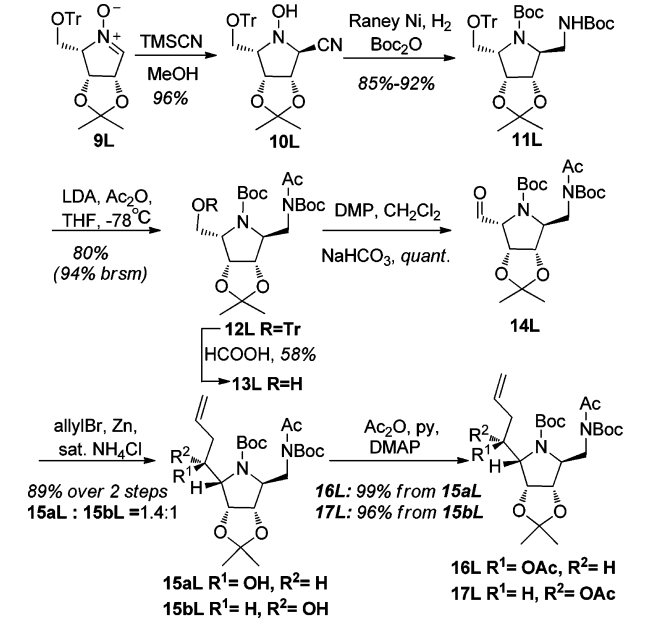
dx.doi.org/10.1021/jo401694e | J. Org. Chem. 2013, 78, 10298−10309

Scheme 1. Preparation of Cyclic Nitrones 9L and 9D



single diastereoisomer (Scheme 2). The excellent yield and selectivity were consistent with that reported by Goti and co-workers.[13a] Reduction of both the cyano and hydroxylamine groups in **10L** by Raney Ni-catalyzed hydrogenation[13b] and in situ protection of the two amino groups with Boc_2O furnished di-*tert*-butyl carbamate **11L** in excellent yield (85−92%). Deprotonation of **11L** with lithium diisopropylamide (LDA) at −78 °C followed by the addition of Ac_2O gave *N*-Boc-*N*-acyl amine **12L** in 80% yield. The conventional method for acetylation (DMAP, Ac_2O, rt or reflux)[15] was unsuccessful. It was necessary to introduce an acetyl group on this amino group as a protecting group that is stable under the conditions for removal of the Boc group at a later stage of the synthesis.[16]

Scheme 2. Synthesis of Homoallylic Alcohols 16L and 17L



With the acetamidomethyl group installed, we then turned our attention to the synthesis of the homoallylic alcohols **16L** and/or **17L** (Scheme 2). As had been expected, the selective removal of the trityl group proved to be a troublesome task because of the disturbance of the other three acid-sensitive protecting groups in **12L**. After optimization of the reaction conditions and screening of several reagent systems (e.g., Pd/C−H_2,[17] CH_3COOH,[18] HCl/CH_3OH,[19] $CuSO_4$,[20] CBr_4/CH_3OH,[21] $InBr_3/CH_3OH$,[22] etc.), it was found that the trityl group could be selectively removed via treatment of **12L** with the $HCOOH/Et_2O$[23] in 50−58% yield to give the desired alcohol **13L**. Although the yield was moderate, the reaction could be carried out on a decagram scale, as needed for the multistep synthesis. After oxidation of the unmasked primary hydroxyl group in **13L** with Dess−Martin periodinane (DMP), the resulting aldehyde **14L** was subjected to aqueous Luche-type[24] allylation to afford a separable mixture of two products, the homoallylic alcohols **15aL** (more polar) and **15bL** (less polar), in excellent total yield over the two steps (89%) with a **15aL**:**15bL** ratio of 1.4:1, slightly favoring the desired stereoisomer.

It is noteworthy that allylation of **14L** by the usual Grignard addition led to complicated results, with the major product (ca. 40% yield) being the homoallylic alcohol with loss of the acetyl group. Unlike the normal aqueous Luche allylation, which can usually be run easily at room temperature or below, the allylation of **14L** had to be carried out at elevated temperature (about 60 °C) with a large excess of zinc powder and allylic bromide, reflecting the low reactivity of aldehyde **14L**.

The structures of **15aL** and **15bL** were characterized by MS, IR, and ^1H and ^{13}C NMR analysis. The configuration of the newly formed stereocenter during the allylation was assigned unambiguously by X-ray crystallographic analysis of **15bL** (Figure 3). Acetylation of **15aL** and **15bL** with Ac_2O and DMAP in pyridine gave the key acetylated intermediates **16L** and **17L** in excellent yields (99% and 96%, respectively).

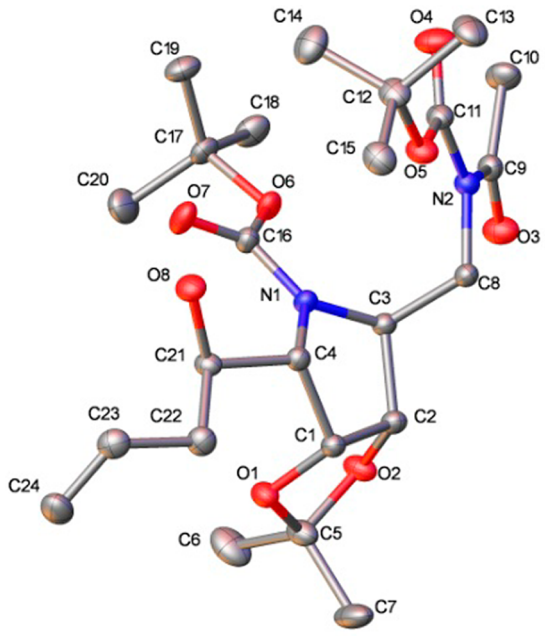
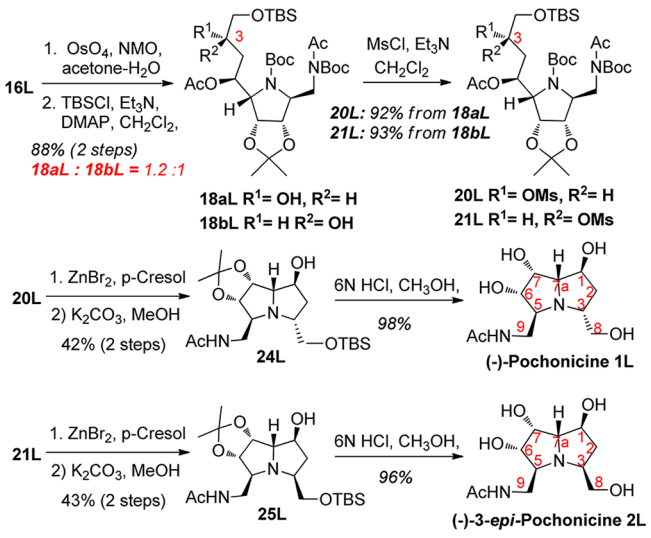


Figure 3. Crystal structure of compound **15bL**.

Dihydroxylation of **16L** by OsO_4 (cat.) and *N*-methylmorpholine *N*-oxide (NMO) in acetone resulted in an inseparable mixture of epimeric diols. However, after selective protection of the resulting primary hydroxyl group, the *O*-TBS ethers could be separated by silica gel column chromatography to yield **18aL** and **18bL** (1.2:1 favoring **18aL**, 88% overall yield in two steps starting from **16L**; Scheme 3). The relative configuration of the C-3 position in **18aL** and **18bL** could be deduced from NOE experiments on the corresponding final pyrrolizidine alkaloids. Mesylation of the remaining secondary hydroxyl group of **18aL** and **18bL** under standard conditions (i.e., MsCl and Et_3N in DCM) gave the desired products **20L** and **21L** in 92% and 93%

dx.doi.org/10.1021/jo401694e | J. Org. Chem. 2013, 78, 10298−10309

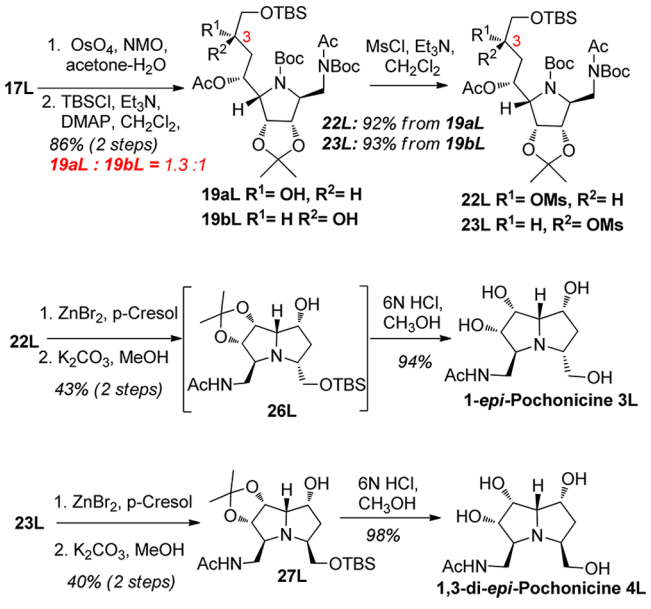
Scheme 3. Synthesis of (−)-Pochonicine (1L) and (−)-3-*epi*-Pochonicine (2L)



yield, respectively. Deprotection of the two *N*-Boc groups of mesylate **20L** by treatment with $ZnBr_2$ in CH_2Cl_2[25] followed by cyclization under basic conditions (K_2CO_3/MeOH) yielded protected pyrrolidine **24L** in moderate yield (42% over two steps). After global deprotection of **24L**, the target compound (−)-pochonicine (**1L**) was obtained in 98% yield. By a similar procedure, 3-*epi*-pochonicine (**2L**) was synthesized from **21L** (Scheme 3).

The other two epimers of (−)-pochonicine **1L**, compounds **3L** and **4L**, were synthesized staring from **17L** through the same procedure as for the synthesis of **1L** and **2L** (Scheme 4). Thus, dihydroxylation of **17L** and selective TBDMS protection of the primary hydroxyl group afforded **19aL** and **19bL** in 86% total yield over two steps. Mesylation of **19aL** and **19bL** gave mesylates **22L** and **23L** in 92% and 93% yield, respectively. Deprotection of the two *N*-Boc groups of mesylates **22L** and

Scheme 4. Synthesis of (−)-1-*epi*-Pochonicine (3L) and (−)-1,3-Di-*epi*-pochonicine (4L)



23L followed by cyclization under basic conditions yielded the protected pyrrolidines **26L** and **27L** in moderate yields (43% and 40%, respectively, over two steps). Global deprotection of **26L** and **27L** in acidic methanol provided the target compounds 1-*epi*-pochonicine (**3L**) and 1,3-di-*epi*-pochonicine (**4L**) in excellent yields (94% and 98%, respectively). The intermediates **24L**, **25L**, **26L**, and **27L** were relatively unstable at room temperature, especially compound **26**, which needed to be handled carefully and was best used directly in the next reaction step immediately after purification.

The structures of (−)-pochonicine **1L**, 3-*epi*-pochonicine **2L**, 1-*epi*-pochonicine **3L**, and 1,3-di-*epi*-pochonicine **4L** were characterized spectroscopically, including detailed 600 MHz NOESY experiments (Figure 4). The NOESY spectrum of **1L**

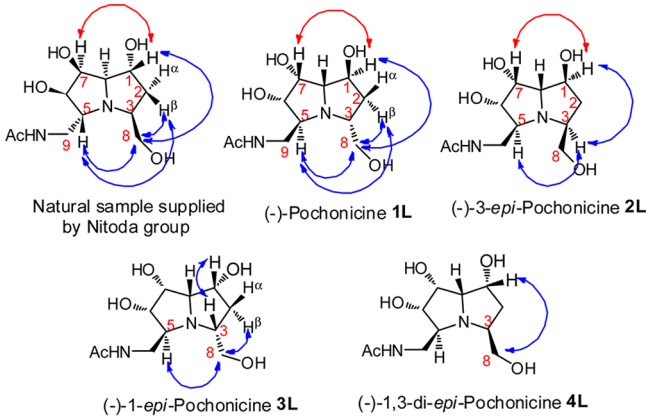


Figure 4. Main NOE interactions for natural pochonicine, **1L**, **2L**, **3L**, and **4L**.

showed strong NOE interactions between H-1 and H-8, H-5 and H-8, H-7 and H-9, and H-6 and H-7a. The characterization of compound **2L** was confirmed by NOE interactions between H-1 and H-3 and H-3 and H-5. Interestingly, an NOE interaction between H-1 and H-7 could be observed in **1L** and **2L**, which was contradictory to the *trans* configuration between H-1 and H-7 proved by the X-ray crystal structure of compound **15bL**. This unusual NOE interaction in **1L** and **2L** might be ascribed to the folding arrangement of the two pyrrolidine rings to give a twisted conformation of the bicyclic skeleton, making H-1 close to H-7. Similarly, the characterization of **3L** was also confirmed by interactions between H-1 and H-3 and H-5 and H-8; the characterization of **4L** was confirmed by the NOE interaction between H-1 and H-8.

In order to determine the absolute configuration of the natural product pochonicine and to perform a preliminary investigation of the structure−activity relationships of pochonicine and its analogues, the corresponding enantiomers of **1L**, **2L**, **3L**, and **4L**, namely, (+)-pochonicine (**1D**), 3-*epi*-pochonicine (**2D**), 1-*epi*-pochonicine (**3D**), and 1,3-di-*epi*-pochonicine (**4D**), were synthesized through the same synthetic route from the L-ribose-derived cyclic nitrone **9D**.

Unequivocal Structure Determination of Natural Pochonicine. The determination of the relative configuration of natural pochonicine was made by comparison of the ^1H and ^{13}C NMR spectra of the synthetic compounds **1L**−**4L** with those of the natural product. As has been reported by Takahashi and co-workers,[8] the ^1H and ^{13}C NMR spectra of compound **4L**, the structure of pochonicine proposed by Nitoda, are not consistent with those of the isolated natural

dx.doi.org/10.1021/jo401694e | J. Org. Chem. 2013, 78, 10298−10309

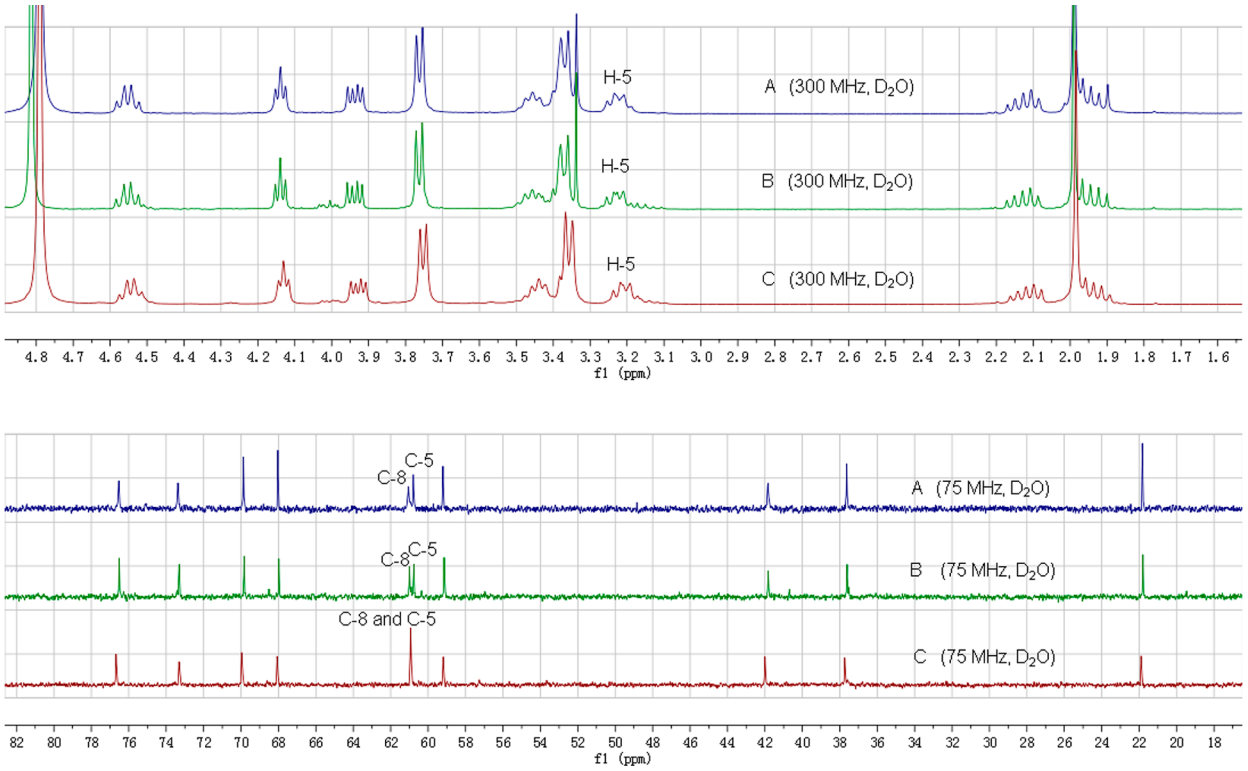


Figure 5. Comparison of the NMR spectra (300 MHz, D_2O) of **1D** and **1L** with those of the natural sample: (A) natural sample supplied by the Nitoda group; (B) synthetic (+)-pochonicine **1D**; (C) synthetic (−)-pochonicine **1L**.

Table 1. Concentrations of Synthetic (+)-Pochonicine and Its Isomers Giving 50% Inhibition of Various Glycosidases

	IC_{50} (μM)				
enzyme	(+)-pochonicine **1D**	(+)-3-*epi*-pochonicine **2D**	(+)-1-*epi*-pochonicine **3D**	(+)-1,3-di-*epi*-pochonicine **4D**	natural sample[c]
α-L-rhamnosidase from *P. decumbens*	NI[a] (0.3%)[b]	694	NI (5.1%)	674	NI (0.3%)
β-*N*-acetylglucosaminidases					
A. oryzae	**0.33**	NI (44.4%)	11	11	**0.31**
bovine kidney	**0.021**	1.2	0.51	0.49	**0.019**
HL-60	0.018	0.61	0.38	0.46	0.014
human placenta	**0.012**	0.38	0.22	0.30	**0.011**
jack bean	**0.0016**	0.24	0.042	0.046	**0.0013**
α-*N*-acetylgalactosaminidase from chicken liver	NI (9.0%)	NI (11.8%)	NI (4.6%)	NI (7.5%)	NI (12.9%)
β-*N*-acetylgalactosaminidases					
A. oryzae	0.30	NI (47.4%)	9.1	12	0.24
HL-60	0.049	2.0	1.4	1.9	0.038

[a]NI: No inhibition (less than 50% inhibition at 1000 μM). [b]( ): inhibition % at 1000 μM. [c]Natural sample: our tested results for the natural pochonicine supplied by the Nitoda group.

pochonicine. Among the four synthetic isomers of pochonicine, compounds **1L**, **2L**, **3L**, and **4L**, the ^1H and ^{13}C NMR spectra of **1L** were the most similar to those of the natural pochonicine. This suggested that the natural product pochonicine may possess the same relative configuration as **1L**. However, the reported ^1H and ^{13}C NMR spectra of compound **1L** were quite different from those of the natural sample of pochonicine, although they looked similar at first glance. After an in-depth study of the NMR spectra of the natural product pochonicine and the synthetic samples, it was found that when measured in D_2O, the ^1H and ^{13}C NMR spectra of **1L** matched very well with those of the natural pochonicine (Figure 5), which unambiguously confirmed the relative configuration of the natural pochonicine as that of **1L**. This was further confirmed by comparison of the ^1H and ^{13}C NMR spectra of **1D** with those of natural pochonicine, which showed that the two matched perfectly with each other (Figure 5)

As mentioned in the Introduction of this article, the absolute configuration of natural pochonicine was determined to be that of compound **1D** (the enantiomer of **1L**) by Takahashi and co-workers.[8] Although the optical rotation of compound **1D** {$[\alpha]_D$ +3.0 (*c* 1.74, MeOH)}[8] had the same sign as that of the natural sample of pochonicine{$[\alpha]_D$ +9.2 (*c* 0.89, MeOH)},[1] the very small values of the optical rotations of the free bases made the determination of the absolute configuration on the basis of such a comparison of optical rotation unreliable; the determination of the absolute configuration through the optical rotations of the hydrogen chloride salts of compound **1L** and the natural

dx.doi.org/10.1021/jo401694e | J. Org. Chem. 2013, 78, 10298−10309

Table 2. Concentrations of Synthetic (−)-Pochonicine and Its Isomers Giving 50% Inhibition of Various Glycosidases

enzyme	IC_{50} (μM) (−)-pochonicine **1L**	(−)-3-*epi*-pochonicine **2L**	(−)-l-*epi*-pochonicine **3L**	(−)-1,3-di-*epi*-pochonicine **4L**	Nitoda's results[d]
α-L-rhamnosidase from *P. decumbens*	291	**1.2**	323	82	ND[c]
β-*N* -acetylglucosaminidases					
A. oryzae	NI[a] (39.6%)[b]	NI (0%)	NI (0%)	NI (0%)	**0.0203**
bovine kidney	90	NI (27.6%)	NI (33.7%)	NI (29.4%)	**0.00106**
HL-60	85	NI (38.1%)	NI (32.2%)	NI (18.3%)	ND
human placenta	62	NI (36.5%)	NI (29.9%)	NI (19.9%)	**0.00239**
jack bean	31	501	191	NI (32.7%)	**0.000288**
α-*N* -acetylgalactosaminidase from chicken liver	NI (14.6%)	NI (36.8%)	NI (31.8%)	NI (44.4%)	ND
β-*N*-acetylgalactosaminidases					
A. oryzae	NI (40.2%)	NI (0%)	NI (0%)	NI (0%)	ND
HL-60	274	NI (10.5%)	NI (11.8%)	NI (7.4%)	ND

[a]NI: no inhibition (less than 50% inhibition at 1000 μM). [b]( ): % inhibition at 1000 μM. [c]ND: not detected. [d]Nitoda's result: Nitoda and co-workers[1] originally reported these results in 2009.

sample of pochonicine was also not to be trusted. With both enantiomers of pochonicine (**1D** and **1L**) in hand, the determination of the absolute configuration of the natural product pochinicine became more reliable. The optical rotations of **1L**, **1D**, and natural pochonicine {$[\alpha]_D^{35}$ −1.07 (*c* 0.90, MeOH), $[\alpha]_D^{20}$ +0.74 (*c* 0.70, MeOH), and $[\alpha]_D^{20}$ +9.2 (*c* 0.89, MeOH),[1] respectively} confirmed the absolute configuration of the natural pochonicine as (1*R*,3*S*,5*R*,6*R*,7*S*,7a*R*)-**1D**, in agreement with the results reported by Takahashi and co-workers. This was to be further proved by the comparison of the glycosidase inhibition profiles of **1L** and **1D** with that of the natural product.

Glycosidase Inhibition. To compare the glycosidase inhibition activities of the synthetic compounds with those of the natural pochonicine, all the synthetic compounds **1D**−**4D** and **1L**−**4L** were assayed together with natural pochonicine as potential glycosidase inhibitors against a range of enzymes (Tables 1 and 2). It was found that natural pochonicine is indeed a powerful inhibitor (with IC_{50} at the nanomolar level) against jack bean, human placenta, HL-60, bovine kidney, and *Aspergillus oryzae* β-GlcNAcases (IC_{50} = 1.3, 11, 14, 19, and 310 nM, respectively). Furthermore, it also exhibited potent inhibition against HL-60 and *A. oryzae* β-*N*-acetylgalactosaminidases (β-GalNAcases), with IC_{50} = 38 and 240 nM, respectively. The synthetic (+)-pochonicine **1D** showed potent inhibition against jack bean, human placenta, HL-60, bovine kidney, and *A. oryzae* β-GlcNAcases (IC_{50} = 1.6, 12, 18, 21, and 330 nM, respectively) and HL-60 and *A. oryzae* β-GalNAcases (IC_{50} = 49 and 300 nM, respectively). These nanomolar inhibition potencies were almost exactly the same. It is noteworthy that all three of the epimers **2D**, **3D**, and **4D** are about 10-fold weaker inhibitors than (+)-pochonicine **1D** and the natural product but are still potent. It is also noteworthy that none of the compounds mentioned above showed any inhibition of α-GalNAcase.

In contrast, the enantiomers (−)-pochonicine **1L**, (−)-3-*epi*-pochonicine **2L**, (−)-1-*epi*-pochonicine **3L**, and (−)-1,3-di-*epi*-pochonicine **4L** were found to be much weaker inhibitors (Table 2). Compound **1L** displayed only moderate inhibition of jack bean, human placenta, HL-60, and bovine kidney GlcNAcases (IC_{50} = 31, 62, 85, and 90 μM, respectively) and HL-60 β-GalNAcase (IC_{50} = 274 μM). Furthermore, the other three isomers **2L**, **3L**, and **4L** did not show any significant inhibition against these enzymes. However, 3-*epi*-pochonicine **2L** is a potent and selective inhibitor of *Penicillium decumbens* α-L-rhamnosidase, with IC_{50} = 1.2 μM. This inhibition potency is similar to that of the known potent α-L-rhamnosidase inhibitor L-swainsonine[26] but weaker than that of (6*R*)-C-methyl-L-swainsonine.[27] These glycosidase inhibition data strongly support the assignment of the absolute configuration of natural pochonicine as (+)-(1*R*,3*S*,5*R*,6*R*,7*S*,7a*R*)-pochonicine (**1D**).

CONCLUSIONS

(+)-Pochonicine (**1D**) and its C-1 and/or C-3 epimers **2D**, **3D**, and **4D** as well as the corresponding enantiomers **1L**, **2L**, **3L**, and **4L** have been synthesized from sugar-derived nitrones **9D** and **9L**, respectively. The key features of this synthetic strategy include (1) the use of the initial nitrone to provide the trihydroxylated pyrrolidine ring with the desired stereochemistry, (2) the installation of the required acetamidomethyl group at an early stage of the synthesis via acetylation of the primary amide by the reduction of a CN group and acylation, and (3) construction of the second pyrrolidine ring through an intramolecular S_N2 displacement. The relative configuration of the natural product pochonicine has been unequivocally determined through an in-depth NMR study, which showed that both the ^1H and ^{13}C NMR spectra of the synthetic pochonicines (**1D** and **1L**) matched with those of the natural pochonicine in D_2O, thereby confirming the relative configuration of the natural product as that of **1D** or **1L**. In addition to a comparison of the optical rotations of the synthetic pochonicines with that of the natural product, a comparison of the glycosidase inhibition profiles of both enantiomers of each pochonicine with that of the natural product further confirmed the absolute configuration of the natural pochonicine as 1*R*,3*S*,5*R*,6*R*,7*S*,7a*R*. Thus, the structure of the natural pochonicine has unequivocally bee determined as (+)-(1*R*,3*S*,5*R*,6*R*,7*S*,7a*R*)-pochonicine (**1D**) in agreement with the data reported by Takahashi and co-workers. Glycosidase inhibition experiments showed that natural pochonicine **1D** and its epimers **2D**, **3D**, and **4D** all are powerful inhibitors of hexosaminidases (five β-GlcNAcases and two β-GalNAcases), but their enantiomers **1L**, **2L**, **3L**, and **4L** are much weaker inhibitors of the same enzymes. (−)-3-*epi*-Pochonicine (**2L**) was found to be a potent and selective inhibitor of α-L-rhamnosidase. None of the compounds showed

dx.doi.org/10.1021/jo401694e | J. Org. Chem. 2013, 78, 10298−10309

any inhibition of α-GalNAcase. The results obtained from the preliminary structure−activity relationship study are valuable for future work on the design and synthesis of pochonicine-based inhibitors of β-*N*-acetylhexosaminidases.

EXPERIMENTAL SECTION

General Methods. All reagents were used as received from commercial sources or prepared as described in the literature. TLC plates were visualized by ultraviolet light or by treatment with a spray of Pancaldi reagent or a solution 0.5% ninhydrin in acetone. Acidic ion exchange chromatography was performed on Amberlite IR-120 (H^+) or Dowex 50WX8-400, H^+ form. Melting points were determined using an electrothermal melting point apparatus. NMR spectra were measured in $CDCl_3$ (with TMS as an internal standard) or D_2O on a magnetic resonance spectrometer (^1H at 300 or 600 MHz, ^{13}C at 75 or 150 MHz). Chemical shifts (δ) are reported in parts per million, and coupling constants (*J*) are in hertz. The following abbreviations are used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. High-resolution mass spectrometry (HRMS) was performed on an LTQ/FT linear ion trap mass spectrometer. Optical rotation measurements were made with an Autopol VI instrument at the sodium D-line using a cell with a path length of 0.25 dm. Concentrations (*c*) are given in grams per 100 mL.

2,3-*O*-Isopropylidene-5-*O*-trityl-D-ribose-*O*-(*tert*-butyldimethylsilyl) Oxime (7L). According to the reported procedure,[14] oxime **6L** was obtained in three steps from D-ribose (**6D** from L-ribose is an unknown compound). To an ice-cold solution of oxime **6L** (300.0 g, 0.53 mol) in CH_2Cl_2 (700 mL) was added triethylamine (112.0 mL, 0.81 mol) followed by dropwise addition of a solution of *tert*-butylchlorodimethylsilane (96.0 g, 0.64 mol) in CH_2Cl_2 (150.0 mL). After the addition, the mixture was stirred at the same temperature overnight. The reaction was quenched carefully with H_2O (200 mL). The mixture was transferred to a funnel. The organic layer was washed with H_2O (3 × 50 mL), dried ($MgSO_4$), and concentrated under vacuum to give 406 g of colorless syrup. About 200 mg of the syrup was purified by column chromatography on silica gel (petroleum ether/EtOAc 20:1) to give an inseparable *E*/*Z* mixture of **7L**.

Data for **6D** (*E*/*Z* mixture, *E*/*Z* = 3.2 as determined by ^1H NMR): $[\alpha]_D^{25}$ −8 (*c* 1.0, CH_2Cl_2). IR (neat, cm^{-1}): 3556 (OH), 3087, 3059, 3033, 2987, 2935, 1597(C═N), 1491, 1448, 1220, 1065. ^1H NMR (300 MHz,$CDCl_3$): δ 7.48−7.42 (m, 8H), 7.33−7.23 (m, 11H), 6.83 (d, *J* = 6.0 Hz, 0.23H), 5.34 (t, *J* = 6.0 Hz, 0.25H), 4.77 (t, *J* = 6.0 Hz, 1H), 4.42−4.37 (m, 0.24H), 4.22 (dd, *J* = 9.0 and 6.0 Hz, 1H), 3.79−3.74 (m, 1H), 3.42−3.23 (m, 2H), 2.81−2.79 (m, 0.2H), 2.60−2.58 (m, 1H), 1.65−1.56 (m, 1H), 1.52−1.50 (m, 0.33H), 1.40 (m, 2H), 1.35−1.31 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 150.4, 148.5, 144.1, 143.9, 128.9, 128.8, 128.0, 127.9, 127.3, 127.1, 110.0, 109.6, 87.1, 86.9, 78.3, 75.4, 71.3, 70.3, 69.0, 65.3, 27.9, 27.4, 25.6, 25.3. HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{27}H_{29}NO_5Na^+$ 470.1938; found 470.1937.

Data for **7L** (*E*/*Z* mixture, *E*/*Z* = 2.9 as determined by ^1H NMR): $[\alpha]_D^{15}$ +20 (*c* 1.0, CH_2Cl_2). IR (neat, cm^{-1}): 3483 (OH), 3059, 2930, 2857, 1598 (C═N), 1491, 1449, 1252, 1220, 1066, 934, 839, 706. ^1H NMR ($CDCl_3$, 300 MHz): δ 7.35 (d, *J* = 7.5 Hz, 1H), 7.30−7.27 (m, 8H), 7.16−7.05 (m, 17H), 6.76 (d, *J* = 5.4 Hz, 0.34H), 5.17−5.13 (m, 0.34H), 4.62 (dd, *J* = 7.5 and 6.3 Hz, 1H), 4.28−4.23 (m, 0.34H), 4.08 (dd, *J* = 9.0 and 6.3 Hz, 1H), 3.62−3.56 (m, 1H), 3.24−3.15 (m, 2H), 3.13−3.05 (m, 1H), 2.56 (d, *J* = 3.6 Hz, 0.27H), 2.35 (d, *J* = 6.1 Hz, 0.27H), 1.41−1.38 (m, 2H), 1.37−1.35 (m, 0.43H), 1.22−1.16 (m, 8H), 0.80 (s, 3H), 0.77 (s, 9H), 0.05−0.04 (m, 2H), 0 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 154.1, 152.4, 144.0, 143.8, 128.8, 128.7, 127.9, 127.2, 127.0, 109.8, 109.3, 86.9, 86.7, 78.6, 77.9, 75.4, 71.8, 70.4, 69.1, 65.3, 65.0, 27.8, 27.3, 26.1, 26.07, 25.5, 25.1, 18.2, 18.1, −5.2, −5.24. HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{33}H_{43}NO_5SiNa^+$ 584.2803; found 584.2799.

Data for **7D** (*E*/*Z* mixture, *E*/*Z* = 2.3 as determined by ^1H NMR): $[\alpha]_D^{25}$ = +2 (*c* 1.0, CH_2Cl_2). HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{33}H_{44}NO_5Si^+$ 562.2983; found 562.2982.

2,3-*O*-Isopropylidene-4-*O*-methanesulfonyl-5-*O*-trityl-D-ribose-*O*-(*tert*-butyldimethylsilyl) Oxime (8L). The syrup **7L** (406 g) was dissolved in dry CH_2Cl_2 (500 mL), and triethylamine (112.0 mL, 0.81 mol) was added. Then methanesulfonyl chloride (52.0 mL, 0.64 mol) was added dropwise to the above solution at 0 °C. When the addition was complete, the mixture was stirred for 1 h at the same temperature. H_2O (200 mL) was added to quench the reaction. The mixture was extracted. The organic layer was washed with H_2O (3 × 50 mL), dried ($MgSO_4$), and concentrated under vacuum to give 500 g of light-yellow syrup. A 200 mg sample of the syrup was purified by column chromatography on silica gel (petroleum ether/EtOAc 30:1) to give an inseparable *E*/*Z* mixture of **8L**.

Data for **8L** (*E*/*Z* mixture, *E*/*Z* = 1.6 as determined by ^1H NMR): $[\alpha]_D^{15}$ +26 (*c* 1.0, CH_2Cl_2). IR (neat, cm^{-1}): 3080, 3033, 2929, 1598 (C═N), 1492, 1449, 1362, 1178, 930. ^1H NMR ($CDCl_3$, 300 MHz): δ 7.46−7.40 (m, 11H), 7.33−7.22 (m, 22H), 6.68 (d, *J* = 3.6 Hz, 1H), 5.18 (dd, *J* = 8.1 and 3.9 Hz, 1H), 4.95−4.90 (m, 2H), 4.80−4.72 (m, 2H), 4.55−4.51 (m, 1H), 3.42−3.40 (m, 2H), 3.25 (dd, *J* = 11.7 and 8.4 Hz, 1H), 3.07 (s, 2H), 2.99 (s, 3H), 1.54 (s, 3H), 1.34−1.32 (m, 6H), 1.28−1.25 (m, 2H), 1.0 (s, 6H), 0.92 (s, 9H), 0.29 (s, 4H), 0.16 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 152.4, 151.2, 143.4, 128.8, 128.7, 128.1, 127.4, 109.8, 109.4, 87.43, 87.37, 81.7, 79.2, 78.7, 77.5, 76.7, 74.7, 72.1, 64.7, 62.0, 39.1, 39.0, 29.8, 27.1, 26.3, 26.1, 25.8, 25.0, 23.9, 18.3, 18.2, −5.1, −5.13, −5.2. HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{34}H_{45}NO_7SSiNa^+$ 662.2578; found 662.2575.

Data for **8D** (*E*/*Z* mixture, *E*/*Z* = 1.8 as determined by ^1H NMR): $[\alpha]_D^{15}$ −36 (*c* 1.0, CH_2Cl_2). HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{34}H_{45}NO_7SSiNa^+$ 662.2578; found 662.2573.

1-Amino-1,4-anhydro-1,*N*-didehydro-2,3-*O*-isopropylidene-5-*O*-trityl-D-ribitol *N*-Oxide (9L). Tetrabutylammonium fluoride (152.16 g, 0.58 mol) was added to a solution of **8L** (500 g) in toluene (500 mL) at 0 °C. The mixture was stirred for 30 min, transferred to an oil bath and heated at reflux overnight, and then cooled to room temperature. The solvent was removed under vacuum to give a black residue, which was extracted with EtOAc (3 × 50 mL), dried ($MgSO_4$), concentrated under vacuum, and purified by column chromatography on silica gel (petroleum ether/EtOAc 3:1 then 2:1) to afford **9L** (50 g) as a yellow foam. The overall yield from D-ribose to nitrone **9L** was 22%.

Data for **9L**: Mp 88−90 °C. $[\alpha]_D^{15}$ +20 (*c* 1.0, CH_2Cl_2). IR (neat, cm^{-1}): 3058, 2936, 1574 (C═N), 1491, 1448, 1211, 1075, 706. ^1H NMR ($CDCl_3$, 300 MHz): δ 7.50−7.44 (m, 7H), 7.28−7.16 (m, 8H), 6.76 (m, 1H), 5.09−5.07 (m, 1H), 4.89−4.85 (m, 1H), 4.11−4.09 (m, 1H), 3.92 (dd, *J* = 9.0 and 4.2 Hz, 1H), 3.68−3.62 (m, 1H), 1.32 (s, 3H), 1.22 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 143.5, 132.8, 128.6, 127.6, 126.9, 111.7, 87.1, 77.6, 74.8, 73.9, 58.6, 26.9, 25.9. HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{27}H_{27}NO_4Na^+$ 452.1832; found 452.1833.

Data for **9D**: 25% yield from L-ribose, mp 85−87 °C. $[\alpha]_D^{15}$ −20 (*c* 1.0, CH_2Cl_2). HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{27}H_{27}NO_4Na^+$ 452.1832; found 452.1830.

(2*S*,3*S*,4*R*,5*S*)-2,3-*O*-Isopropylidene-5-(trityloxymethyl)-1-hydroxypyrrolidine-2-carbonitrile (10L). To a solution of nitrone **9L** (40.00 g, 0.09 mol) in dry methanol (100.0 mL) was added trimethylsilyl cyanide (13.0 mL, 0.095 mol), and the resulting solution was stirred at 50 °C under an argon atmosphere overnight. The reaction solvent was removed under vacuum to give a light-yellow spumescent solid that could be crystallized from methanol to yield a white solid (30.00 g, 73%). The mother liquid was purified by column chromatography on silica gel (petroleum ether/EtOAc 6:1) to afford hydroxylamine **10L** (9.30 g), total yield 96%.

Data for **10L**: Mp 162−165 °C. $[\alpha]_D^{15}$ +60 (*c* 1.0, CH_2Cl_2). IR (neat, cm^{-1}): 3420 (br, OH), 3058, 2939, 1491 (m), 1448 (m), 1380 (m), 1214 (s), 1076 (s), 705 (s). ^1H NMR ($CDCl_3$, 300 MHz): δ 7.46−7.43 (m, 6H), 7.28−7.17 (m, 9H), 5.69 (s, 1H), 4.79 (dd, *J* = 6.6 and 5.1 Hz, 1H), 4.72−4.70 (m, 1H), 4.25−4.23 (m, 1H), 3.56−3.51 (m, 1H), 3.36 (dd, *J* = 8.7 and 5.4 Hz, 1H), 3.18−3.12 (m, 1H), 1.26−1.23 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 143.7, 128.8, 127.9, 127.2, 114.8, 112.4, 87.3, 78.7, 76.9, 67.9, 62.4, 60.9, 25.6, 24.9. HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{28}H_{28}N_2O_4Na^+$ 479.1941; found 479.1946.

dx.doi.org/10.1021/jo401694e | J. Org. Chem. 2013, 78, 10298−10309

Data for **10D**: 88% yield, mp 164−167 °C. $[\alpha]_D^{22}$ −56 (*c* 1.0, CH_2Cl_2). HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{28}H_{28}N_2O_4Na^+$ 479.1941; found 479.1937.

(2*S*,3*S*,4*R*,5*S*)-1-*N*-*tert*-Butoxycarbonyl-2-(*tert*-butoxycarbonylamidomethyl)-3,4-*O*-isopropylidene-5-(trityloxymethyl)pyrrolidine (11L). To a solution of hydroxylamine **10L** (20.00 g, 0.044 mol) in THF/MeOH (10:1) was added di-*tert*-butyl dicarbonate (24.00 g, 0.11 mol) followed by Raney nickel (10.00 g). After being stirred under a hydrogen atmosphere at room temperature for 4 days, the reaction mixture was filtered through Celite, and the filtrate was concentrated and extracted with EtOAc (3 × 100 mL) and water (50.0 mL). The combined organic extracts were dried ($MgSO_4$) and then concentrated to give a white foam, which was purified by column chromatography on silica gel (petroleum ether/EtOAc 8:1) to give **11L** (26.10 g, 92%) as a white foam.

Data for **11L**: Mp 76−78 °C. $[\alpha]_D^{15}$ +40 (*c* 1.0, CH_2Cl_2). IR (neat, cm^{-1}): 3360 (NH), 2978 (m), 1764 (m), 1710 (s, C═O), 1495 (m), 1449 (m), 1359 (m), 1249 (m), 1164 (m), 704 (m). ^1H NMR ($CDCl_3$, 300 MHz): δ 7.42−7.39 (m, 5H), 7.26−7.10 (m, 10H), 4.84−4.82 (m, 1H), 4.73−4.70 (m, 1H), 4.58−4.56 (m, 1H), 4.48−4.39 (m, 1H), 3.81−3.79 (m, 1H), 3.73−3.70 (m, 1H), 3.50−3.48 (m, 1H), 3.39−3.14 (m, 3H), 1.36−1.19 (m, 23H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 156.2, 153.1, 144.3, 144.1, 128.9, 128.88, 128.7, 128.5, 127.9, 127.7, 127.6, 127.5, 127.2, 126.9, 126.87, 112.9, 112.5, 86.9, 86.8, 83.2, 80.4, 80.05, 79.5, 68.1, 65.2, 64.5, 61.2, 60.5, 40.5. HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{38}H_{48}N_2O_7Na^+$ 667.3354; found 667.3342.

Data for **11D**: 89% yield, mp 76−77 °C. $[\alpha]_D^{15}$ −46 (*c* 1.0, CH_2Cl_2). HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{38}H_{48}N_2O_7Na^+$ 667.3354; found 667.3347.

(2*S*,3*S*,4*R*,5*S*)-1-*N*-*tert*-Butoxycarbonyl-2-(*N*-*tert*-butoxycarbonylacetylamidomethyl)-3,4-*O*-isopropylidene-5-(trityloxymethyl)pyrrolidine (12L). Commercially available lithium diisopropylamide (13.0 mL, 0.27 mol, 2 M in hexane) was added dropwise at −78 °C under an argon atmosphere to a solution of **11L** (12.00 g, 0.019 mol) in freshly distilled THF (110 mL). After the addition, the mixture was stirred for 20 min, and then acetic anhydride (6.3 mL, 0.057 mol) was added dropwise. The mixture and stirred vigorously for 20 min. **Caution: the mixture turned extremely sticky at −78 °C when Ac_2O was added, so it is important to stir vigorously for better conversion.** The mixture was allowed to warm to room temperature, and the reaction was quenched with saturated aq. NH_4Cl. The mixture was extracted with EtOAc (3 × 50 mL), dried ($MgSO_4$), concentrated under vacuum, and purified by column chromatography on silica gel (petroleum ether/EtOAc 8:1) to afford **12L** (10.40 g, 79.8%) as a white foam and recovered starting material **11L** (1.62 g).

Data for **12L**: Mp 66−68 °C. $[\alpha]_D^{15}$ +18 (*c* 1.0, CH_2Cl_2). IR (neat, cm^{-1}): 2979 (m), 1737 (s, C═O), 1608 (s, C═O), 1449 (m), 1369 (m), 1218 (m), 1150 (m), 1069 (m), 704 (s). ^1H NMR ($CDCl_3$, 300 MHz): δ 7.50−7.48 (m, 6H), 7.30−7.19 (m, 9H), 4.98−4.96 (m, 1H), 4.41−4.39 (m, 1H), 3.93−3.90 (m, 1H), 3.75−3.73 (m, 3H), 3.61−3.59 (m, 1H), 2.44 (s, 3H), 1.44−1.22 (m, 24H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.4, 172.8, 152.7, 152.65, 152.59, 144.2, 144.0, 128.8, 127.6, 127.5, 126.7, 112.2, 111.0, 86.7, 86.6, 83.3, 82.3, 80.8, 79.5, 67.1, 62.5, 59.9, 42.4, 28.1, 27.9, 27.6, 26.9, 26.5, 26.0, 25.4. HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{40}H_{50}N_2O_8Na^+$ 709.3459; found 709.3468.

Data for **12D**: 72% (77% brsm) yield, mp 65−68 °C. $[\alpha]_D^{15}$ −20 (*c* 1.0, CH_2Cl_2). HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{40}H_{50}N_2O_8Na^+$ 709.3459; found 709.3453.

(2*S*,3*S*,4*R*,5*S*)-1-*N*-*tert*-Butoxycarbonyl-2-(*N*-*tert*-butoxycarbonylacetylamidomethyl)-3,4-*O*-isopropylidene-5-(hydroxymethyl)pyrrolidine (13L). To an ice-cold solution of **12L** (15.00 g, 0.022 mol) in dry ethyl ether (200.0 mL) was added HCOOH (200.0 mL). The mixture was stirred at the same temperature for 1 h and then warmed to room temperature with stirring for another 30 min. Then the reaction mixture was diluted with EtOAc (200 mL), and the reaction was carefully quenched with solid $NaHCO_3$ until there was no effervescence. The organic layer was washed with water and brine, dried ($MgSO_4$), and concentrated under vacuum. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc 4:1) to give alcohol **13L** (5.63 g, 58%).

Data for **13L**: $[\alpha]_D^{25}$ −18 (*c* 1.0, CH_2Cl_2). IR (neat, cm^{-1}): 3432 (br, OH), 2980 (m), 1737 (s, C═O), 1679 (s, C═O), 1370 (m), 1148 (m). ^1H NMR ($CDCl_3$, 300 MHz): δ 5.12 (dd, *J* = 9.0 and 4.8 Hz, 1H), 4.67−4.64 (m, 1H), 4.41−4.31 (m, 2H), 3.87−3.85 (m, 2H), 3.79−3.60 (m, 3H), 2.44 (s, 3H), 1.51−1.24 (m, 24H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.8, 155.7, 152.9, 111.5, 84.4, 81.1, 80.5, 79.9, 64.1, 62.4, 61.3, 43.1, 28.4, 28.1, 27.3, 26.3, 24.9. HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{21}H_{37}N_2O_8^+$ 445.2544; found 445.2542.

Data for **13D**: 57% yield, $[\alpha]_D^{24}$ +16 (*c* 1.0, CH_2Cl_2). HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{21}H_{37}N_2O_8^+$ 445.2544; found 445.2542.

(2*S*,3*S*,4*R*,5*S*)-1-*N*-*tert*-Butoxycarbonyl-2-(*N*-*tert*-butoxycarbonylacetylamidomethyl)-3,4-*O*-isopropylidene-5-(((1*S*)-1-hydroxy-3-butenyl))pyrrolidine (15aL) and (2*S*,3*S*,4*R*,5*S*)-1-*N*-*tert*-Butoxycarbonyl-2-(*N*-*tert*-butoxycarbonylacetylamidomethyl)-3,4-*O*-isopropylidene-5-(((1*R*)-1-hydroxy-3-butenyl))pyrrolidine (15bL). To Dess−Martin periodinane (5.00 g, 11.8 mmol) suspended in dry CH_2Cl_2 (20.0 mL) was added $NaHCO_3$ (1.22 g, 14.8 mmol) followed by alcohol **13L** (2.62 g, 5.9 mmol) dissolved in dry CH_2Cl_2 (20.0 mL). After being stirred at room temperature for 1 h, the reaction mixture was diluted with diethyl ether (30 mL). The reaction was quenched with saturated sodium thiosulfate solution (20 mL), and the mixture was stirred vigorously until two clear layers appeared. It was then extracted with ethyl ether (3 × 50 mL). The combined organic extracts were washed with water and brine, dried over magnesium sulfate, and concentrated in vacuo to give aldehyde **14L** as a light-yellow residue that was directly used in the next step without further purification. To a solution of aldehyde **14L** in THF (5 mL) were added allylic bromide (4.1 mL, 29.5 mmol) and zinc dust (3.11 g, 59 mmol) followed by the dropwise addition of saturated NH_4Cl solution. After 5 min, more allylic bromide (16.4 mL, 118 mmol) and zinc dust (12.4 g, 236 mmol) were carefully added in four portions. Then the reaction was allowed to stir at room temperature for 4 h. The reaction mixture was filtered through Celite, and the filtrate was extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with water and brine, dried ($MgSO_4$), and then concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc 5:1) to afford a mixture of alcohol **15aL** (1.49 g) as a white solid and **15bL** (1.09 g) as a colorless oil, total yield 89%.

Data for **15aL**: $[\alpha]_D^{31}$ +7 (*c* 1.15, CH_2Cl_2). IR (neat, cm^{-1}): 3440 (OH), 2979 (m), 2935 (m), 1737 (s, C═O), 1682 (s, C═O), 1369 (s), 1147 (s). ^1H NMR ($CDCl_3$, 300 MHz): δ 5.92−5.81 (m, 1H), 5.04−4.94 (m, 2H), 4.72−4.68 (m, 1H), 4.52−4.51 (m, 1H), 4.37−4.17 (m, 3H), 3.87 (dd, *J* = 13.8 and 7.5 Hz, 1H), 3.72−3.58 (m, 1H), 2.60−2.55 (m, 2H), 2.39 (m, 4H), 2.27−2.18 (m, 1H), 1.48−1.20 (m, 24H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.9, 155.7, 152.7, 136.3, 115.9, 111.4, 84.0, 80.8, 79.4, 69.6, 65.3, 61.7, 43.1, 38.4, 28.2, 28.0, 27.0, 25.7, 24.1. HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{24}H_{41}N_2O_8^+$ 485.2857; found 485.2853.

Data for **15aD**: $[\alpha]_D^{14}$ −8 (*c* 1.0, CH_2Cl_2). 70% combined yield over two steps, **15aD**:**15bD** = 1.7:1. HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{24}H_{41}N_2O_8^+$ 485.2857; found 485.2855.

Data for **15bL**: Mp 107−108 °C. $[\alpha]_D^{31}$ −38 (*c* 1.0, CH_2Cl_2). IR (neat, cm^{-1}): 3383 (s, OH), 2979 (m), 2938 (m), 1737 (s, C═O), 1676 (s, C═O), 1422 (m), 1370 (m), 1225 (m). ^1H NMR ($CDCl_3$, 300 MHz): δ 6.02−5.97 (m, 1H), 5.91 (s, 1H), 5.11−5.00 (m, 2H), 4.52 (dd, *J* = 6.0 and 3.6 Hz, 1H), 4.44 (dd, *J* = 9.6 and 6.0 Hz, 1H), 4.24−4.20 (m, 2H), 3.71−3.57 (m, 2H), 3.39 (dd, *J* = 8.7 and 3.3 Hz, 1H), 2.60−2.55 (m, 1H), 2.41 (s, 3H), 2.36−2.29 (m, 1H), 1.47−1.20 (m, 24H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.8, 155.5, 152.8, 135.4, 116.7, 111.4, 84.2, 81.0, 80.2, 79.7, 67.7, 67.3, 63.5, 42.8, 38.0, 28.3, 28.0, 27.2, 26.6, 25.0. HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{24}H_{41}N_2O_8^+$ 485.2857; found 485.2849.

Data for **15bD**: Mp 105−106 °C. $[\alpha]_D^{28}$ +40 (*c* 1.0, CH_2Cl_2). HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{24}H_{41}N_2O_8^+$ 485.2857; found 485.2855.

General Procedure for the Synthesis of 16L and 17L. *(2S,3S,4R,5S)-1-N-tert-Butoxycarbonyl-2-(N-tert-butoxycarbonyl-*

dx.doi.org/10.1021/jo401694e | J. Org. Chem. 2013, 78, 10298−10309

*acetylamidomethyl)-3,4-O-isopropylidene-5-(((1S)-1-acetoxy-3-butenyl))pyrrolidine (**16L**).* Alcohol **15aL** (900.0 mg, 1.86 mmol) was dissolved in dry pyridine (3 mL), and 4-dimethylaminopyridine (90 mg) followed by acetic anhydride (3.72 mmol, 0.35 mL) was added. After being stirred at the same temperature for 1.5 h, the mixture was concentrated at reduced pressure to remove pyridine. Then it was extracted with EtOAc (3 × 15 mL), washed with water and brine, and concentrated under vacuum. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc 6:1) to afford compound **16L** (970.1 mg, 99%) as a white solid.

Data for **16L**: Mp 85−87 °C. $[\alpha]_D^{31}$ +10 (*c* 0.83, CH_2Cl_2). IR (neat, cm^{-1}): 2918 (s), 1739 (s, C═O), 1701 (s, C═O), 1370 (s), 1147 (s). ^1H NMR ($CDCl_3$, 300 MHz): δ 5.86−5.75 (m, 1H), 5.72 (s, 1H), 5.12−5.00 (m, 2H), 4.73−4.69 (m, 1H), 4.35−4.30 (m, 2H), 3.97 (dd, *J* = 13.5 and 7.8 Hz, 1H), 3.81−3.77 (m, 1H), 3.68 (dd, *J* = 13.5 and 8.1 Hz, 1H), 2.60−2.46 (m, 2H), 2.44 (s, 3H), 1.99 (s, 3H), 1.53−1.43 (m, 24H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.3, 170.5, 154.3, 153.0, 134.6, 117.3, 112.0, 83.9, 80.5, 80.0, 79.5, 70.9, 62.5, 62.0, 43.2, 36.3, 28.4, 28.2, 27.1, 26.2, 24.9, 21.4. HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{26}H_{42}N_2O_9Na^+$ 549.2783; found 549.2791.

Data for **16D**: 96% yield, mp 85−86 °C. $[\alpha]_D^{31}$ −8 (*c* 1.0, CH_2Cl_2). HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{26}H_{43}N_2O_9^+$ 527.2963; found 527.2962.

*(2S,3S,4R,5S)-1-N-tert-Butoxycarbonyl-2-(N-tert-butoxycarbonyl-acetylamidomethyl)-3,4-O-isopropylidene-5-(((1R)-1-acetoxy-3-butenyl))pyrrolidine (**17L**).* Following the above general method for **16L**, homoallylic alcohol **15bL** (713.0 mg, 1.5 mmol) gave **17L** (740.2 mg, 96%) as a colorless oil.

Data for **17L**: $[\alpha]_D^{31}$ −8 (*c* 1.0, CH_2Cl_2). IR (neat, cm^{-1}): 2980 (m), 1738 (s, C═O), 1701 (s, C═O), 1370 (s), 1238 (s), 1149 (s). ^1H NMR ($CDCl_3$, 300 MHz): δ 5.83−5.75 (m, 2H), 5.11−5.02 (m, 2H), 4.67−4.63 (m, 1H), 4.35 (dd, *J* = 6.6 and 1.8 Hz, 1H), 4.26−4.20 (m, 1H), 4.02−4.00 (m, 1H), 3.91 (dd, *J* = 7.5 and 5.4 Hz, 1H), 3.85−3.78 (m, 1H), 2.60−2.55 (m, 2H), 2.48 (s, 3H), 2.05 (s, 3H), 1.56−1.28 (m, 24H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.9, 170.0, 153.7, 152.6, 134.5, 116.8, 111.9, 83.4, 80.2, 79.9, 79.1, 70.9, 61.9, 60.6, 42.7, 35.8, 28.1, 27.7, 26.7, 26.0, 24.4, 20.9. HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{26}H_{42}N_2O_9Na^+$ 549.2783; found 549.2788.

Data for **17D**: 97% yield, $[\alpha]_D^{31}$ +8 (*c* 1.0, CH_2Cl_2). HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{26}H_{43}N_2O_9^+$ 527.2963; found 527.2960.

General Procedure for the Synthesis of 18aL, 18bL, 19aL, and 19bL. *Mesylation Product Precursors **18aL** and **18bL**.* To a solution of **16L** (960.0 mg, 1.83 mmol) in acetone (4 mL) were added a catalytic amount of OsO_4 (9.3 mL, 0.183 mmol, 0.5% in water) and *N*-methylmorpholine *N*-oxide (357.0 mg, 1.83 mmol, 60% in water). When the starting material had disappeared, the reaction was quenched with sat. $NaHSO_3$ solution, and the mixture was stirred vigorously for 1 h. The solvent was removed under reduced pressure. The residue was extracted with EtOAc (3 × 15 mL), washed with water and brine, and concentrated under vacuum to afford an inseparable mixture of diols (826.0 mg, 86%) as a colorless oil. The inseparable diol mixture was dissolved in dry CH_2Cl_2 (10 mL). Triethylamine (0.6 mL, 4.4 mmol) and DMAP (18.0 mg, 0.147 mmol) were added, followed by the addition of *tert*-butylchlorodimethylsilane (444.0 mg, 2.94 mmol). After the mixture was stirred for 4 h, the reaction was quenched with water (2 mL). CH_2Cl_2 (30 mL) was added to extract the product. Then the combined organic extracts were washed with water and brine, dried ($MgSO_4$), and then concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc 4:1) to afford as colorless oils compounds **18aL** (464.8 mg) and **18bL** (387.3 mg) in 86% yield from **16L**.

Data for **18aL**: $[\alpha]_D^{30}$ +20 (*c* 1.0, CH_2Cl_2). IR (neat, cm^{-1}): 3508 (OH), 2932, 1737 (s, C═O), 1698 (s, C═O), 1370 (s), 1251 (s), 1145 (s), 937 (s), 777 (s). ^1H NMR ($CDCl_3$, 300 MHz): δ 5.83 (s, 1H), 4.73−4.69 (m, 1H), 4.29−4.24 (m, 2H), 3.91−3.89 (m, 1H), 3.82−3.79 (m, 1H), 3.66−3.50 (m, 2H), 3.47−3.45 (m, 2H), 3.13−3.11 (m, 1H), 2.38 (s, 3H), 2.00−1.91 (m, 4H), 1.72−1.65 (m, 1H), 1.48−1.36 (m, 21H), 1.19 (s, 3H), 0.81 (s, 9H), −0.02 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.1, 171.4, 154.1, 152.8, 111.8, 83.9, 80.4, 79.6, 69.3, 68.7, 67.2, 63.1, 61.8, 43.1, 35.7, 28.3, 28.1, 27.0, 26.1, 25.9, 24.7, 21.3, 18.3, −5.3. HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{32}H_{59}N_2O_{11}Si^+$ 675.3883; found 675.3873.

Data for **18aD**: $[\alpha]_D^{30}$ −23 (*c* 1.0, CH_2Cl_2). HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{32}H_{58}N_2O_{11}SiNa^+$ 697.3702; found 697.3702.

Data for **18bL**: $[\alpha]_D^{30}$ 0 (*c* 1.0, CH_2Cl_2). IR (neat, cm^{-1}): 3459 (OH), 2932 (m), 1738 (s, C═O), 1700 (s, C═O), 1370 (s), 1250 (s), 1154 (s), 837 (s), 777 (s). ^1H NMR ($CDCl_3$, 300 MHz): δ 5.79 (s, 1H), 4.74−4.71 (m, 1H), 4.33−4.28 (m, 2H), 4.01−3.94 (m, 1H), 3.89−3.85 (m, 1H), 3.76−3.60 (m, 3H), 3.46 (dd, *J* = 9.9 and 3.9 Hz, 1H), 2.76−2.74 (m, 1H), 2.44 (s, 3H), 2.01 (s, 3H), 1.96−1.92 (m, 2H), 1.53−1.42 (m, 21H), 1.23 (s, 3H), 0.87−0.85 (m, 9H), 0.04 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.3, 170.9, 154.2, 153.0, 111.9, 83.9, 80.5, 79.9, 79.4, 70.1, 69.7, 67.1, 63.2, 62.0, 43.2, 35.4, 28.4, 28.2, 27.1, 26.2, 26.0, 24.8, 21.5, 18.4, −5.3. HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{32}H_{59}N_2O_{11}Si^+$ 675.3883; found 675.3889.

Data for **18bD**: $[\alpha]_D^{30}$ +2 (*c* 1.0, CH_2Cl_2). 70% combined yield over two steps, **18aD**:**18bD** = 1.5:1. HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{32}H_{58}N_2O_{11}SiNa^+$ 697.3702; found 697.3701.

*Mesylation Product Precursors **19aL** and **19bL**.* Following the above general method for **18aL**, **17L** (700.0 mg, 1.33 mmol) gave **19aL** (445.8 mg) and **19bL** (342.9 mg) as a colorless oil in 88% total yield from **17L**.

Data for **19aL**: $[\alpha]_D^{27}$ +8 (*c* 1.0, CH_2Cl_2). IR (neat, cm^{-1}): 3488 (OH), 2932 (m), 1739 (s, C═O), 1702 (s, C═O), 1370 (s), 1240 (s), 1148 (s), 837 (s), 778 (s). ^1H NMR ($CDCl_3$, 300 MHz): δ 5.85 (s, 1H), 4.66−4.62 (m, 1H), 4.30−4.27 (m, 1H), 4.17 (m, 1H), 3.92 (dd, *J* = 7.2 and 5.4 Hz, 1H), 3.75−3.71 (m, 2H), 3.57 (dd, *J* = 9.9 and 3.9 Hz, 1H), 3.34 (dd, *J* = 9.9 and 7.8 Hz, 1H), 2.63(s, 1H), 2.42 (s, 3H), 2.00 (s, 3H), 1.99−1.90 (m, 1H), 1.80 (dd, *J* = 11.7 and 3.9 Hz, 1H), 1.51−1.49 (m, 9H), 1.40−1.39 (m, 12H), 1.21 (s, 3H), 0.84 (s, 9H), 0.00 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.2, 170.7, 154.0, 152.9, 112.1, 83.8, 80.2, 79.6, 70.4, 70.1, 67.3, 62.3, 61.2, 43.0, 34.8, 28.3, 28.0, 27.0, 26.3, 25.9, 24.6, 21.4, 18.3, −5.36, −5.4. HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{32}H_{59}N_2O_{11}Si^+$ 675.3883; found 675.3887.

Data for **19aD**: $[\alpha]_D^{27}$ −8 (*c* 1.0, CH_2Cl_2). HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{32}H_{58}N_2O_{11}SiNa^+$ 697.3702; found 697.3702.

Data for **19bL**: $[\alpha]_D^{27}$ +18 (*c* 1.0, CH_2Cl_2). IR (neat, cm^{-1}): 3494 (OH), 2932 (m), 1738 (s, C═O), 1698 (s, C═O), 1370 (s), 1249 (s), 1148 (s), 837 (s), 777 (s). ^1H NMR ($CDCl_3$, 300 MHz): δ 5.96 (s, 1H), 4.60−4.57 (m, 1H), 4.30−4.28 (m, 1H), 4.22−4.20 (m, 1H), 391−3.87 (m, 2H), 3.74−3.67 (m, 1H), 3.55−3.53 (m, 1H), 3.50−3.44 (m, 2H), 2.43 (s, 3H), 2.03−1.97 (m, 4H), 1.79−1.71 (m, 1H), 1.51−1.40 (m, 21H), 1.22 (s, 3H), 0.84 (s, 9H), 0.00 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.7, 171.0, 153.9, 152.6, 111.7, 83.6, 80.3, 79.6, 69.7, 68.5, 67.3, 62.3, 61.1, 42.6, 35.7, 28.1, 27.8, 26.8, 26.0, 25.7, 24.4, 20.9, 18.1, −5.6. HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{32}H_{59}N_2O_{11}Si^+$ 675.3883; found 675.3892.

Data for **19bD**: $[\alpha]_D^{27}$ −12 (*c* 1.0, CH_2Cl_2). 80% combined yield over two steps, **19aD**:**19bD** = 1.3:1. HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{32}H_{59}N_2O_{11}Si^+$ 675.3883; found 675.3876.

General Procedure for the Synthesis of 20L, 21L, 22L, and 23L. *Cyclization Precursor **20L**.* To an ice-cold solution of alcohol **18aL** (100.0 mg, 0.15 mmol) in CH_2Cl_2 (3 mL) was added triethylamine (0.3 mmol, 40 μL), followed by the addition of methanesulfonyl chloride (0.225 mmol, 20 μL) dropwise. After the mixture was stirred for 30 min, the reaction was quenched with water (2 mL), and the mixture was extracted with CH_2Cl_2 (20 mL). The combined organic extracts were washed with water and brine, dried ($MgSO_4$), and then concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc 5:1) to afford compound **20L** (102.0 mg, 92%) as a colorless oil.

Data for **20L**: $[\alpha]_D^{30}$ +2 (*c* 1.0, CH_2Cl_2). IR (neat, cm^{-1}): 2934 (m), 1737 (s, C═O), 1698 (s, C═O), 1369 (s), 1250 (s), 1175 (s). ^1H NMR ($CDCl_3$, 300 MHz): δ 4.77−4.71 (m, 2H), 4.34−4.32 (m, 1H), 4.24−4.19 (m, 1H), 4.02−3.95 (m, 1H), 3.87−3.85 (m, 1H), 3.80−3.62 (m, 3H), 3.12−3.10 (m, 1H), 3.06 (s, 3H), 2.41 (s, 3H), 2.23−2.14 (m, 1H), 2.07−1.93 (m, 4H), 1.51−1.41 (m, 21H), 1.21 (s, 3H), 0.85 (s, 9H), 0.03 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.2, 171.1, 154.3, 152.9, 111.9, 84.0, 80.7, 80.6, 79.8, 79.1, 68.2, 65.3, 63.4, 61.5, 43.3, 38.5, 31.6, 28.4, 28.1, 27.1, 26.0, 25.9, 24.5, 21.3, 18.4, −5.4.

dx.doi.org/10.1021/jo401694e | *J. Org. Chem.* 2013, 78, 10298−10309

HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{33}H_{60}N_2O_{13}SSiNa^+$ 775.3478; found 775.3451.

Data for **20D**: 90% yield, $[\alpha]_D^{22}$ −2 (c 1.0, CH_2Cl_2). HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{33}H_{60}N_2O_{13}SSiNa^+$ 775.3478; found 775.3474.

*Cyclization Precursor **21L***. Following the above general method for **20L**, compound **18bL** (100.0 mg, 0.15 mmol) gave **21L** (103.7 mg, 93%) as a colorless oil.

Data for **21L**: $[\alpha]_D^{30}$ −4 (c 1.0, CH_2Cl_2), IR (neat, cm^{-1}): 2932 (m), 1739 (s, C═O), 1698 (s, C═O), 1370 (s), 1268 (s), 1174 (s), 1148 (s). ^1H NMR ($CDCl_3$, 300 MHz): δ 4.73−4.69 (m, 2H), 4.34−4.29 (m, 2H), 3.96−3.93 (m, 2H), 3.82−3.79 (m, 1H), 3.74 (dd, J = 11.7 and 6.9 Hz, 1H), 3.70−3.62 (m, 1H), 3.03 (s, 3H), 2.44 (s, 3H), 2.23−2.21 (m, 2H), 2.05 (s, 3H), 1.53 (m, 9H), 1.43 (m, 12H), 1.24 (s, 3H), 0.88 (s, 9H), 0.07 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.1, 171.0, 154.2, 153.0, 111.8, 84.1, 82.7, 80.7, 79.8, 79.7, 68.7, 64.8, 63.4, 62.3, 42.9, 38.5, 28.4, 28.2, 27.2, 26.0, 24.6, 21.4, 18.5, −5.3. HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{33}H_{60}N_2O_{13}SSiNa^+$ 775.3478; found 775.3477.

Data for **21D**: 89% yield, $[\alpha]_D^{22}$ +2 (c 1.0, CH_2Cl_2). HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{33}H_{60}N_2O_{13}SSiNa^+$ 775.3478; found 775.3475.

*Cyclization Precursor **22L***. Following the above general method for **20L**, compound **19aL** (200.0 mg, 0.3 mmol) gave **22L** (210.2 mg, 95%) as a colorless oil.

Data for **22L**: $[\alpha]_D^{30}$ +18 (c 1.0, CH_2Cl_2). IR (neat, cm^{-1}): 2934 (m), 1739 (s, C═O), 1699 (s, C═O), 1370 (s), 1219 (s), 1174 (s). ^1H NMR ($CDCl_3$, 300 MHz): δ 4.71−4.61 (m, 2H), 4.33−4.31 (m, 1H), 4.24−4.22 (m, 1H), 3.90−3.87 (m, 3H), 3.74−3.64 (m, 2H), 3.14−3.12 (m, 1H), 3.06 (s, 3H), 2.46 (s, 3H), 2.41−2.31 (m, 1H), 2.10 (s, 3H), 2.07−2.00 (m, 1H), 1.54 (s, 9H), 1.45 (s, 12H), 1.26 (s, 3H), 0.88 (s, 9H), 0.06−0.05 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.9, 170.6, 154.0, 152.7, 111.7, 84.0, 83.4, 80.4, 79.6, 79.5, 69.9, 65.0, 62.3, 60.2, 42.8, 38.1, 33.8, 31.6, 28.2, 27.9, 27.0, 26.1, 25.8, 24.3, 21.1, 18.3, −5.6, −5.7. HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{33}H_{60}N_2O_{13}SSiNa^+$ 775.3478; found 775.3478.

Data for **22D**: 97% yield, $[\alpha]_D^{22}$ −16 (c 1.0, CH_2Cl_2). HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{33}H_{60}N_2O_{13}SSiNa^+$ 775.3478; found 775.3474.

*Cyclization Precursor **23L***. Following the above general method for **20L**, compound **19bL** (485.0 mg, 0.72 mmol) gave **23L** (501.3 mg, 93%) as a colorless oil.

Data for **23L**: $[\alpha]_D^{30}$ +14 (c 1.0, CH_2Cl_2). IR (neat, cm^{-1}): 2932 (m), 1737 (s, C═O), 1698 (s, C═O), 1369 (s), 1249 (s), 1174 (s). ^1H NMR ($CDCl_3$, 300 MHz): δ 4.74−4.72 (m, 1H), 4.67−4.63 (m, 1H), 4.35 (dd, J = 6.4 and 1.7 Hz, 1H), 4.25−4.17 (m, 1H), 3.97−3.95 (m, 2H), 3.83−3.73 (m, 3H), 3.15−3.13 (m, 1H), 3.09 (s, 3H), 2.47 (s, 3H), 2.43−2.37 (m, 1H), 2.10 (s, 3H), 2.08−2.02 (m, 1H), 1.55 (s, 9H), 1.47 (s, 12H), 1.27 (s, 3H), 0.89 (s, 9H), 0.07−0.06 (m, 6H).^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.8, 170.4, 153.8, 152.6, 111.7, 83.7, 80.3, 79.7, 79.4, 68.6, 65.1, 61.9, 60.0, 42.7, 38.1, 32.5, 31.4, 28.1, 27.7, 26.8, 26.0, 25.6, 24.3, 20.9, 18.0, −5.7. HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{33}H_{60}N_2O_{13}SSiNa^+$ 775.3478; found 775.3477.

Data for **23D**: 95% yield, $[\alpha]_D^{22}$ −10 (c 1.0, CH_2Cl_2). HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{33}H_{60}N_2O_{13}SSiNa^+$ 775.3478; found 775.3474.

General Procedure for the Synthesis of Protected Pyrrolizidines 24L, 25L, 26L, and 27L. *(1S,3R,5S,6S,7R,7aS)-1-Hydroxyl-3-((tert-butyldimethylsilyl)oxy)methyl-5-acetamidomethyl-6,7-O-isopropylidenepyrrolizidine (**24L**).* To a solution of compound **20L** (200.0 mg, 0.27 mmol) in dry CH_2Cl_2 (8 mL) was added p-cresol (287.0 mg, 3.24 mmol) and anhydrous $ZnBr_2$ [410.0 mg, 1.83 mmol; prepared by dissolution of 25 g of $ZnBr_2 \cdot 7H_2O$ in 500 mL of THF (dried over 4 Å MS) for 2 days followed by removal of the solvent in vacuo]. After the mixture was stirred for 12 h, the reaction was quenched with sat. $NaHCO_3$ (3 mL). The reaction mixture was filtered through Celite, and the filtrate was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were washed with water and brine, dried ($MgSO_4$), and then concentrated. The residue was subjected to the next step without further purification. The residue was dissolved in MeOH (3 mL) and H_2O (0.5 mL). K_2CO_3 (75.0 mg, 0.54 mmol) was added to the above solution. After the solution was stirred at 60 °C for 4 h, the solvent was removed in vacuo. The residue was extracted with CH_2Cl_2 (3 × 10 mL), washed with water and brine, dried ($MgSO_4$), and concentrated. The resulting residue was purified by column chromatography on silica gel (petroleum ether/EtOAc 2:1 then CH_2Cl_2/MeOH + 1% NH_3OH 80:1) to afford compound **24L** (46.2 mg, 42%) as a colorless oil.

Data for **24L**: $[\alpha]_D^{30}$ +3.5 (c 1.15, CH_2Cl_2). IR (neat, cm^{-1}): 3296 (br, OH), 2930 (m), 1654 (s, C═O), 1377 (m), 1256 (m), 1097 (m), 838 (s). ^1H NMR ($CDCl_3$, 300 MHz): δ 6.21 (s, 1H), 4.56−4.53 (m, 1H), 4.39−4.37 (m, 2H), 3.70−3.61 (m, 4H), 3.50−3.49 (m, 1H), 3.28−3.08 (m, 2H), 2.45 (s, 1H), 2.19 (s, 3H), 1.76−1.74 (m, 2H), 1.39 (s, 3H), 1.21−1.83 (m, 3H), 0.83 (s, 9H), 0.00 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.4, 112.2, 85.8, 79.8, 75.4, 70.7, 64.7, 62.1, 61.5, 41.4, 38.7, 26.0, 25.9, 24.1, 23.4, 18.3, −5.4. HRMS (ESI) m/z: $[M + H]^+$ calcd for $C_{20}H_{39}N_2O_5Si^+$ 415.2623; found 415.2623.

Data for **24D**: 38% yield, $[\alpha]_D^{30}$ −6 (c 1.0, CH_2Cl_2). HRMS (ESI) m/z: $[M + H]^+$ calcd for $C_{20}H_{39}N_2O_5Si^+$ 415.2623; found 415.2623.

*(1S,3S,5S,6S,7R,7aS)-1-Hydroxy-3-((tert-butyldimethylsilyl)oxy)-methyl-5-acetamidomethyl-6,7-O-isopropylidenepyrrolizidine (**25L**).* Following the above general method for **20L**, compound **21L** (200.0 mg, 0.15 mmol) gave **25L** (46.2 mg, 42%) as a colorless oil.

Data for **25L**: $[\alpha]_D^{30}$ −1.5 (c 1.3, CH_2Cl_2). IR (neat, cm^{-1}): 3310 (br, OH), 2930 (m), 1656 (s, C═O), 1547 (m), 1374 (m), 1256 (m), 1209 (m), 1081 (m), 837 (s). ^1H NMR ($CDCl_3$, 300 MHz): δ 6.25 (s, 1H), 4.54−4.51 (m, 1H), 4.33−4.32 (m, 2H), 3.51−3.32 (m, 7H), 2.72−2.63 (m, 1H), 2.51−2.41 (m, 1H), 1.87 (s, 3H), 1.50−1.45 (m, 1H), 1.34 (s, 3H), 1.36 (s, 3H), 0.82 (s, 9H), 0.00 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.1, 112.1, 86.1, 81.5, 75.8, 72.1, 69.0, 68.4, 66.4, 40.9, 38.5, 26.3, 26.0, 25.8, 23.5, 23.3, 18.5, −5.2. HRMS (ESI) m/z: $[M + H]^+$ calcd for $C_{20}H_{39}N_2O_5Si^+$ 415.2623; found 415.2625.

Data for **25D**: 41% yield, $[\alpha]_D^{30}$ +2 (c 1.5, CH_2Cl_2). HRMS (ESI) m/z: $[M + H]^+$ calcd for $C_{20}H_{39}N_2O_5Si^+$ 415.2623; found 415.2625.

*(1R,3R,5S,6S,7R,7aS)-1-Hydroxy-3-((tert-butyldimethylsilyl)oxy)-methyl-5-acetamidomethyl-6,7-O-isopropylidenepyrrolizidine (**26L**).* Following the above general method for **20L**, compound **22L** (200.0 mg, 0.15 mmol) gave **26L** (47.3 mg, 43%) as a colorless oil. **Caution: compound 26L was poorly stable under conditions for removal of the solvent MeOH. Thus, no heat should be applied while the reaction mixture is concentrated, and it should be used directly in the next step.**

*(1R,3S,5S,6S,7R,7aS)-1-Hydroxy-3-((tert-butyldimethylsilyl)oxy)-methyl-5-acetamidomethyl-6,7-O-isopropylidenepyrrolizidine (**27L**).* Following the above general method for **20L**, compound **23L** (200.0 mg, 0.15 mmol) gave **27L** (44.2 mg, 40.2%) as a colorless oil.

Data for **27L**: $[\alpha]_D^{30}$ +20 (c 0.8, CH_2Cl_2). IR (neat, cm^{-1}): 3310 (br, OH), 2929 (m), 1656 (s, C═O), 1549 (m), 1374 (m), 1257 (m), 1210 (m), 1083 (m), 837 (s). ^1H NMR ($CDCl_3$, 300 MHz): δ 6.41 (s, 1H), 4.70−4.66 (m, 1H), 4.54−4.51 (m, 1H), 4.41 (dd, J = 6.0 and 3.0 Hz, 1H), 3.47−3.44 (m, 1H), 3.41−3.33 (m, 2H), 3.31−3.28 (m, 2H), 3.26−3.17 (m, 1H), 3.07−2.98 (m, 1H), 2.14 (s, 1H), 2.07−1.98 (m, 1H), 1.89 (s, 3H), 1.72−1.64 (m, 1H), 1.46 (s, 3H), 1.23 (s, 3H), 0.83 (s, 9H), 0.00 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.2, 113.0, 85.6, 82.3, 73.3, 69.9, 69.1, 68.8, 64.5, 40.4, 38.6, 26.6, 26.0, 23.8, 23.3, 18.5, −5.1. HRMS (ESI) m/z: $[M + H]^+$ calcd for $C_{20}H_{39}N_2O_5Si^+$ 415.2623; found 415.2622.

Data for **27D**: 43% yield, $[\alpha]_D^{30}$ −22 (c 1.2, CH_2Cl_2). HRMS (ESI) m/z: $[M + H]^+$ calcd for $C_{20}H_{39}N_2O_5Si^+$ 415.2623; found 415.2622.

General Procedure for the Synthesis of (−)-Pochonicine (1L), (−)-3-*epi*-Pochonicine (2L), (−)-1-*epi*-Pochonicine (3L), and (−)-1,3-Di-*epi*-pochonicine (4L). *(−)-Pochonicine (**1L**).* To a solution of compound **24L** (40.0 mg, 0.0967 mmol) in MeOH (0.5 mL) was added 6 N HCl (1.5 mL). After the mixture was stirred for 12 h, the solvent was removed, and the residue was dissolved in MeOH, neutralized with aqueous ammonium solution, and concentrated in vacuo. The above procedure was repeated three times to ensure complete neutralization. The residue was purified by an acid resin column (DOWEX 50W × 8, 100−200 mesh), eluting with distilled water (20 mL) and then 1 N NH_4OH (50 mL), affording **1L** (24.6 mg, 98%) as light-yellow oil.

dx.doi.org/10.1021/jo401694e | J. Org. Chem. 2013, 78, 10298−10309

Data for **1L**: $[\alpha]_D^{35}$ −1.07 (*c* 0.90, MeOH). IR (neat, cm^{-1}): 3308 (br, OH), 2930 (m), 1650 (s, C═O). ^1H NMR (300 MHz, D_2O): δ 4.56 (dd, *J* = 11.8 and 6.5 Hz, 1H), 4.14 (t, *J* = 4.0 Hz, 1H), 3.94 (dd, *J* = 8.1 and 4.0 Hz, 1H), 3.78−3.75 (m, 2H), 3.49−3.43 (m, 1H), 3.39−3.36 (m, 2H), 3.25−3.18 (m, 1H), 2.12 (dt, *J* = 12.5 and 6.0 Hz, 1H), 1.99−1.90 (m, 4H). ^{13}C NMR (75 MHz, D_2O): δ 174.4, 76.7, 73.3, 70.0, 68.1, 60.9, 59.2, 57.3, 42.0, 37.7, 21.9. HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{11}H_{21}N_2O_5^+$ 261.1445; found 261.1442.

Additional NMR data for **1L**: ^1H NMR (300 MHz, CD_3OD): δ 4.56 (dd, *J* = 10.9 and 6.1 Hz, 1H), 4.00 (t, *J* = 3.9 Hz, 1H), 3.84 (dd, *J* = 8.0 and 3.9 Hz, 1H), 3.80 (d, *J* = 3.4 Hz, 1H), 3.67 (dd, *J* = 11.9 and 5.4 Hz, 1H), 3.59−3.54 (m, 1H), 3.47 (t, *J* = 4.2 Hz, 1H), 3.42−3.41 (m, 2H), 3.34−3.32 (m, 1H), 2.14 (dt, *J* = 12.4 and 6.1 Hz, 1H), 2.02−1.98 (m, 4H).^{13}C NMR (75 MHz, CD_3OD): δ 174.1, 78.2, 75.9, 71.8, 69.2, 63.2, 62.2, 61.1, 42.6, 39.9, 22.6

Data for **1D**: 95% yield, $[\alpha]_D^{18}$ +0.74 (*c* 0.70, MeOH). IR (neat, cm^{-1}): 3313 (br, OH), 2931 (m), 1646 (s, C═O). ^1H NMR (300 MHz, D_2O): δ 4.53 (dd, *J* = 12.0 and 6.6 Hz, 1H), 4.12 (t, *J* = 3.9 Hz, 1H), 3.91 (dd, *J* = 8.1 and 3.9 Hz, 1H), 3.75−3.73 (m, 2H), 3.45−3.41 (m, 1H), 3.38−3.31 (m, 3H), 3.25−3.13 (m, 1H), 2.11 (dt, *J* = 12.8 and 6.2 Hz, 1H), 1.97 (s, 3H), 1.94−1.88 (m, 1H). ^{13}C NMR (75 MHz, D_2O): δ 174.4, 76.5, 73.3, 69.9, 68.0, 61.0, 60.8, 59.1, 41.8, 37.6, 21.8. HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{11}H_{21}N_2O_5^+$ 261.1445; found 261.1445.

Additional NMR data for **1D**: ^1H NMR (300 MHz, CD_3OD): δ 4.56 (dd, *J* = 12.0 and 6.6 Hz, 1H), 3.97 (t, *J* = 3.9 Hz, 1H), 3.83 (dd, *J* = 8.0 and 3.9 Hz, 1H), 3.78 (d, *J* = 3.3 Hz, 1H), 3.64 (dd, *J* = 12.0 and 5.1 Hz, 1H), 3.52−3.48 (m, 1H), 3.40−3.35 (m, 3H), 3.27−3.22 (m, 1H), 2.11 (dt, *J* = 12.0 and 6.0 Hz, 1H), 2.00−1.92 (m, 4H). ^{13}C NMR (75 MHz, CD_3OD): δ 173.9, 78.4, 75.6, 71.8, 69.2, 62.8, 62.4, 60.9, 42.8, 40.0, 22.6

Data for natural pochonicine (supplied by Nitoda): ^1H NMR (300 MHz, D_2O): δ 4.56 (dd, *J* = 11.7 and 6.5 Hz, 1H), 4.15 (t, *J* = 4.1 Hz, 1H), 3.95 (dd, *J* = 8.1 and 3.9 Hz, 1H), 3.78−3.76 (m, 2H), 3.47 (t, *J* = 5.6 Hz, 1H), 3.41−3.35 (m, 3H), 3.23 (dd, *J* = 13.6 and 5.8 Hz, 1H), 2.14 (dt, *J* = 12.8 and 6.1 Hz, 1H), 2.00−1.91 (m, 4H). ^{13}C NMR (75 MHz, D_2O): δ 174.5, 76.5, 73.4, 69.9, 68.0, 61.0, 60.8, 59.2, 41.8, 37.6, 21.8

Additional data for natural pochonicine (supplied by Nitoda): ^1H NMR (300 MHz, CD_3OD): δ 4.60 (dd, *J* = 11.8 and 6.7 Hz, 1H), 4.01 (t, *J* = 3.8 Hz, 1H), 3.87 (dd, *J* = 7.8 and 3.9 Hz, 1H), 3.81 (d, *J* = 3.6 Hz, 1H), 3.68 (dd, *J* = 12.0 and 5.1 Hz, 1H), 3.56−3.46 (m, 1H), 3.44−3.41 (m, 2H), 3.40−3.38 (m, 1H), 3.31−3.25 (m, 1H), 2.19−2.11 (m, 1H), 2.00−1.91 (m, 4H) ^{13}C NMR (75 MHz, CD_3OD): δ 173.7, 78.8, 75.4, 72.1, 69.2, 62.8, 61.5, 60.8, 43.1, 40.2, 22.6

*(−)-3-epi-Pochonicine (**2L**).* Following the above general method for **24L**, compound **25L** (40.0 mg, 0.097 mmol) gave **2L** (24.1 mg, 96%) as a colorless oil.

Data for **2L**: $[\alpha]_D^{30}$ +6 (*c* 1.0, MeOH). IR (neat, cm^{-1}): 3316 (br, OH), 2930 (w), 1637 (s, C═O). ^1H NMR (300 MHz, CD_3OD): δ 4.53 (ddd, *J* = 3.4, 5.1, and 6.4 Hz, 1H), 3.95 (t, *J* = 3.9 Hz, 1H), 3.77 (dd, *J* = 8.6 and 3.9 Hz, 1H), 3.57−3.44 (m, 2H), 3.37−3.34 (m, 2H), 3.40−3.37 (m, 2H), 3.31−3.29 (m, 1H), 3.09−3.00 (m, 1H), 2.85−2.79 (m, 1H), 2.24 (dt, *J* = 13.8 and 7.0 Hz, 1H), 1.96 (s, 3H), 1.62 (dt, *J* = 11.6 and 5.8 Hz, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 173.6, 77.3, 76.2, 72.6, 71.6, 70.0, 69.5, 66.7, 42.8, 39.1, 22.6. HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{11}H_{21}N_2O_5^+$ 261.1445; found 261.1445.

Data for **2D**: 93% yield, $[\alpha]_D^{32}$ −5.3 (*c* 1.5, MeOH). IR (neat, cm^{-1}): 3319 (br, OH), 2932 (m), 1638 (s, C═O). ^1H NMR (300 MHz, CD_3OD): δ 4.57 (ddd, *J* = 3.4, 5.0, and 6.5 Hz, 1H), 3.99 (t, *J* = 3.9 Hz, 1H), 3.81 (dd, *J* = 8.7 and 3.6 Hz, 1H), 3.62−3.55 (m, 2H), 3.44−3.43 (m, 2H), 3.39−3.35 (m, 1H), 3.16−3.13 (m, 1H), 2.92 (dt, *J* = 8.9 and 4.7 Hz, 1H), 2.29 (dt, *J* = 13.3 and 7.0 Hz, 1H), 1.98 (s, 3H), 1.66 (dt, *J* = 11.4 and 5.8 Hz, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 173.9, 76.9, 76.5, 72.4, 71.4, 70.7, 69.7, 65.9, 42.3, 39.0, 22.6. HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{11}H_{21}N_2O_5^+$ 261.1445; found 261.1445.

*(−)-1-epi-Pochonicine (**3L**).* Following the above general method for **24L**, compound **26L** (36.0 mg, 0.087 mmol) gave **3L** (21.3 mg, 94%) as a colorless oil.

Data for **3L**: $[\alpha]_D^{15}$ −10 (*c* 0.6, MeOH). IR (neat, cm^{-1}): 3299 (br, OH), 2928 (m), 1648 (s, C═O), 1129 (m). ^1H NMR (CD_3OD, 300 MHz): δ 4.42 (dd, *J* = 10.3 and 5.4 Hz, 1H), 4.19 (t, *J* = 4.0 Hz, 1H), 3.98 (dd, *J* = 11.7 and 7.2 Hz, 1H), 3.78 (dd, *J* = 7.5 and 3.9 Hz, 1H), 3.71 (dd, *J* = 11.4 and 3.9 Hz, 1H), 3.52−3.47 (m, 2H), 3.44−3.38 (m, 2H), 3.33−3.25 (m, 1H), 2.15−2.05 (m, 1H), 1.95 (s, 3H), 1.86 (dt, *J* = 12.0 and 6.0 Hz, 1H). ^{13}C NMR (CD_3OD, 75 MHz): δ 173.6, 78.8, 74.0, 73.8, 68.9, 63.4, 62.4, 61.5, 43.8, 40.9, 22.6. HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{11}H_{21}N_2O_5^+$ 261.1445; found 261.1445.

Data for **3D**: 96% yield, $[\alpha]_D^{15}$ +12 (*c* 0.5, MeOH). IR (neat, cm^{-1}): 3307 (br, OH), 2930 (m), 1651 (s, C═O), 1133 (m). ^1H NMR (300 MHz, CD_3OD): δ 4.46 (dd, *J* = 10.8 and 5.1 Hz, 1H), 4.21 (t, *J* = 4.0 Hz, 1H), 4.00 (dd, *J* = 12.0 and 7.5 Hz, 1H), 3.83 (dd, *J* = 7.8 and 3.9 Hz, 1H), 3.76 (dd, *J* = 12.0 and 3.9 Hz, 1H), 3.58 (m, 2H), 3.49−3.44 (m, 2H), 3.40−3.35 (m, 1H), 2.14 (dt, *J* = 12.6 and 6.3 Hz, 1H), 2.01 (s, 3H), 1.97−1.88 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 173.7, 78.7, 73.9, 73.7, 68.9, 63.3, 62.5, 61.6, 43.6, 40.8, 22.6. HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{11}H_{21}N_2O_5^+$ 261.1445; found 261.1443.

*(−)-1,3-Di-epi-pochonicine (**4L**).* Following the above general method for **24L**, compound **27L** (45.0 mg, 0.11 mmol) gave **4L** (27.8 mg, 98%) as a colorless oil.

Data for **4L**: $[\alpha]_D^{15}$ −1.6 (*c* 1.2, MeOH). IR (neat, cm^{-1}): 3306 (br, OH), 2928 (m), 1648 (s, C═O), 1134 (m). ^1H NMR (300 MHz, CD_3OD): δ 4.43 (dd, *J* = 8.2 and 4.8 Hz, 1H), 4.27 (t, *J* = 5.2 Hz, 1H), 3.77 (dd, *J* = 6.3 and 5.0 Hz, 1H), 3.55−3.51 (m, 1H), 3.48 (d, *J* = 4.2 Hz, 1H), 3.39 (dd, *J* = 10.9 and 6.1 Hz, 1H), 3.32−3.23 (m, 3H), 3.02 (dd, *J* = 11.7 and 5.4 Hz, 1H), 2.01 (ddd, *J* = 12.0, 6.0, and 3.0 Hz, 1H), 1.95 (s, 3H), 1.74 (ddd, *J* = 12.0, 6.0, and 3.0 Hz, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 173.5, 76.7, 74.3, 74.0, 71.4, 70.7, 68.4, 66.5, 43.1, 39.9, 22.6. HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{11}H_{21}N_2O_5^+$ 261.1445; found 261.1445.

Data for **4D**: 95% yield, $[\alpha]_D^{15}$ +3.4 (*c* 0.7, MeOH). IR (neat, cm^{-1}): 3316 (br, OH), 2931 (w), 1647 (s, C═O), 1126 (m). ^1H NMR (300 MHz, CD_3OD): δ 4.48−4.47 (m,1H), 4.30 (t, *J* = 6.0 Hz, 1H), 3.81 (t, *J* = 6.0 Hz, 1H), 3.62−3.56 (m, 1H), 3.54−3.52 (m, 1H), 3.47−3.41 (m, 1H), 3.37−3.34 (m, 3H), 3.10 (dd, *J* = 11.1 and 5.4 Hz, 1H), 2.06 (ddd, *J* = 12.0, 6.0, and 3.0 Hz, 1H), 1.97 (s, 3H), 1.79 (ddd, *J* = 12.0, 6.0, and 3.0 Hz, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 173.7, 76.6, 74.1, 73.9, 71.5, 70.8, 68.7, 66.2, 42.8, 39.8, 22.6. HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{11}H_{21}N_2O_5^+$ 261.1445; found 261.1445.

Assay of Glycosidase Inhibition. α-L-Rhamnosidase from *P. decumbens*; β-*N*-acetylglucosaminidases from *A. oryzae* bovine liver, human placenta, and jack bean; α-*N*-acetylgalactosaminidase from chicken liver; and β-*N*-acetylgalactosaminidases from *A. oryzae* were purchased from a commercial supplier. The human promyelocytic leukemia cell line, HL-60, was provided by the RIKEN BRC through the National Bio-Resource Project of the MEXT, Japan. The glycosidase activities were determined using an appropriate *p*-nitrophenyl glycoside as the substrate. The reaction was stopped by adding 400 mM Na_2CO_3. The released *p*-nitrophenol was measured spectrometrically at 400 nm.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

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

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: yucy@iccas.ac.cn.
*E-mail: kato@med.u-toyama.ac.jp.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This article is dedicated to Professor Zhi-Tang Huang on the occasion of his 85th birthday. This work was supported by the National Basic Research Program of China (2012CB822101 and 2011CB808603), the National Natural Science Foundation of China (21272240 and 21102149), a Grant-in-Aid for Scientific Research (C) (23590127 to A.K.) from the Japanese Society for the Promotion of Science (JSPS) and Summit PLC (UK), and the Leverhulme Trust (G.W.J.F.).

■ REFERENCES

(1) Usuki, H.; Toyo-oka, M.; Kanzaki, H.; Okuda, T.; Nitoda, T. *Bioorg. Med. Chem.* **2009**, *17*, 7248.

(2) (a) Horsch, M.; Mayer, C.; Sennhauser, U.; Rast, D. M. *Pharmacol. Ther.* **1997**, *76*, 187. (b) Merzendorfer, H.; Zimoch, L. *J. Exp. Biol.* **2003**, *206*, 4393. (c) Rast, D. M.; Baumgartner, D.; Mayer, C.; Hollenstein, G. O. *Phytochemistry* **2004**, *64*, 339.

(3) (a) Wells, L.; Vosseller, K.; Hart, G. W. *Cell. Mol. Life Sci.* **2003**, *60*, 222. (b) Buse, M. G. *Am. J. Physiol.: Endocrinol. Metab.* **2006**, *290*, E1. (c) Slámová, K.; Bojarová, P.; Petrásková, L.; Křen, V. *Biotechnol. Adv.* **2010**, *28*, 682.

(4) (a) Hart, G. W.; Housley, M. P.; Slawson, C. *Nature* **2007**, *446*, 1017. (b) Macauley, M. S.; He, Y.; Gloster, T. M.; Stubbs, K. A.; Davies, G. J.; Vocadlo, D. J. *Chem. Biol.* **2010**, *17*, 937.

(5) Vosseller, K.; Wells, L.; Lane, M. D.; Hart, G. W. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5313.

(6) (a) Yuzwa, S. A.; Macauley, M. S.; Heinonen, J. E.; Shan, X.; Dennis, R. J.; He, Y.; Whitworth, G. E.; Stubbs, K. A.; McEachern, E. J.; Davies, G. J.; Vocadlo, D. J. *Nat. Chem. Biol.* **2008**, *4*, 483. (b) Yuzwa, S. A.; Vocadlo, D. J. *Curr. Alzheimer Res.* **2009**, *6*, 451.

(7) (a) Woynarowska, B.; Wikiel, H.; Sharma, M.; Fleet, G. W. J.; Bernacki, R. J. *Anticancer Res.* **1992**, *12*, 161. (b) Woynarowska, B.; Wikiel, H.; Sharma, M.; Fleet, G. W. J.; Bernacki, R. J. *Proc. Am. Assoc. Cancer Res.* **1989**, *30*, 91. (c) Ma, Z.; Vosseller, K. *Amino Acids* **2013**, *45*, 719.

(8) Kitamura, Y.; Koshino, H.; Nakamura, T.; Tsuchida, A.; Nitoda, T.; Kanzaki, H.; Matsuoka, K.; Takahashi, S. *Tetrahedron Lett.* **2013**, *54*, 1456.

(9) For examples of opposite signs for the optical rotations of the free base and the hydrogen chloride salt of iminosugar alkaloids, see: (a) Nash, R. J.; Fellows, L. E.; Dring, J. V.; Fleet, G. W. J.; Girdhar, A.; Ramsden, N. G.; Peach, J. M.; Hegarty, M. P.; Scofield, A. M. *Phytochemistry* **1990**, *29*, 111. (b) Zou, W.; Szarek, W. A. *Carbohydr. Res.* **1993**, *242*, 311. (c) Restorp, P.; Fischer, A.; Somfai, P. *J. Am. Chem. Soc.* **2006**, *128*, 12646. (d) Merino, P.; Delso, I.; Tejero, T.; Cardona, F.; Marradi, M.; Faggi, E.; Parmeggiani, C.; Goti, A. *Eur. J. Org. Chem.* **2008**, 2929. (e) Fleet, G. W. J.; Ramsden, N. G.; Nash, R. J.; Fellows, L. E.; Jacob, G. S.; Molyneux, R. J.; Dibello, I. C.; Winchester, B. *Carbohydr. Res.* **1990**, *205*, 269.

(10) For recent reviews of cyclic nitrones, see: (a) Brandi, A.; Cardona, F.; Cicchi, S.; Cordero, F. M.; Goti, A. *Chem.—Eur. J.* **2009**, *15*, 7808. (b) Revuelta, J.; Cicchi, S.; Goti, A.; Brandi, A. *Synthesis* **2007**, 485. For recent applications of cyclic nitrones in the synthesis of pyrrolizidines, see: (c) Podolan, G.; Klescikova, L.; Fisera, L.; Kozfsek, J.; Fronc, M. *Synlett* **2011**, 1668. (d) Delso, I.; Tejero, T.; Goti, A.; Merino, P. *Tetrahedron* **2010**, *66*, 1220. (e) Stecko, S.; Solecka, J.; Chmielewski, M. *Carbohydr. Res.* **2009**, *344*, 167. (f) Stecko, S.; Jurczak, M.; Urbanczyk-Lipkowska, Z.; Solecka, J.; Chmielewski, M. *Carbohydr. Res.* **2008**, *343*, 2215. (g) Kaliappan, K. P.; Das, P. *Synlett* **2008**, 841. (h) Argyropoulos, N. G.; Panagiotidis, T.; Coutouli-Argyropoulou, E.; Raptopoulou, C. *Tetrahedron* **2007**, *63*, 321. (i) Desvergnes, S.; Py, S.; Vallee, Y. *J. Org. Chem.* **2005**, *70*, 1459. For the work of our group based on cyclic nitrones, see: (j) Zhao, W.-B.; Nakagawa, S.; Kato, A.; Adachi, I.; Jia, Y.-M.; Hu, X.-G.; Fleet, G. W. J.; Wilson, F. X.; Horne, G.; Yoshihara, A.; Izumori, K.; Yu, C.-Y. *J. Org. Chem.* **2013**, *78*, 3208 and references cited therein.

(11) (a) Jenkinson, S. F.; Best, D.; Saville, A. W.; Mui, J.; Martinez, R. F.; Nakagawa, S.; Kunimatsu, T.; Alonzi, D. S.; Butters, T. D.; Norez, C.; Becq, F.; Bleriot, Y.; Wilson, F. X.; Weymouth-Wilson, A. C.; Kato, A.; Fleet, G. W. *J. Org. Chem.* **2013**, *78*, 7380. (b) Glawar, A. F. G.; Best, D.; Ayers, B. J.; Miyauchi, S.; Nakagawa, S.; Aguilar-Moncayo, M.; García Fernández, J. M.; Ortiz Mellet, C.; Crabtree, E. V.; Butters, T. D.; Wilson, F. X.; Kato, A.; Fleet, G. W. J. *Chem.—Eur. J.* **2012**, *18*, 9341. (c) Hu, X.-G.; Bartholomew, B.; Nash, R. J.; Wilson, F. X.; Fleet, G. W. J.; Nakagawa, S.; Kato, A.; Jia, Y.-M.; van Well, R.; Yu, C.-Y. *Org. Lett.* **2010**, *12*, 2562 and references cited therein. (d) Best, D.; Wang, C.; Weymouth-Wilson, A. C.; Clarkson, R. A.; Wilson, F. X.; Nash, R. J.; Miyauchi, S.; Kato, A.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2010**, *21*, 311 and references cited therein. (e) D'Alonzo, D.; Guaragna, A.; Palumbo, G. *Curr. Med. Chem.* **2009**, *16*, 473.

(12) Yu, C.-Y.; Fu, Y. *Acta Crystallogr.* **2006**, *E62*, o3172.

(13) (a) Merino, P.; Tejero, T.; Revuelta, J.; Romero, P.; Cicchi, S.; Mannucci, V.; Brandi, A.; Goti, A. *Tetrahedron: Asymmetry* **2003**, *14*, 367. (b) Tsou, E.-L.; Yeh, Y.-T.; Liang, P.-H.; Cheng, W.-C. *Tetrahedron* **2009**, *65*, 93.

(14) Holzapfel, C. W.; Crous, R. *Heterocycles* **1998**, *48*, 1337.

(15) Burk, M. J.; Allen, J. G. *J. Org. Chem.* **1997**, *62*, 7054.

(16) (a) Stafford, J. A.; Brackeen, M. F.; Karanewsky, D. S.; Valvano, N. L. *Tetrahedron Lett.* **1993**, *34*, 7873. (b) Apelqvist, T.; Wensbo, D. *Tetrahedron Lett.* **1996**, *37*, 1471. (c) Kotsuki, H.; Ohishi, T.; Araki, T.; Arimura, K. *Tetrahedron Lett.* **1998**, *39*, 4869. (d) Siro, J. G.; Martín, J.; García-Navío, J.; Remuiñan, M. J.; Vaquero, J. J. *Synlett* **1998**, 147. (e) Yadav, J. S.; Subba Reddy, B. V.; Reddy, K. S. *Synlett* **2002**, 468. (f) Hernández, J. N.; Crisóstomo, F. R. P.; Martín, T.; Martín, V. S. *Eur. J. Org. Chem.* **2007**, 5050. (g) Singh, O. V.; Han, H. *Tetrahedron Lett.* **2007**, *48*, 7094. (h) Fujiwara, T.; Seki, T.; Miura, M.; Takeuchi, Y. *Heterocycles* **2009**, *79*, 427.

(17) Mirrington, R. N.; Schmalzl, K. J. *J. Org. Chem.* **1972**, *37*, 2877.

(18) Blickenstaff, R. T. *J. Am. Chem. Soc.* **1960**, *82*, 3673.

(19) Ikota, N.; Hanaki, A. *Chem. Pharm. Bull.* **1990**, *38*, 2712.

(20) Randazzo, G.; Capasso, R.; Rosaria Cicala, M.; Evidente, A. *Carbohydr. Res.* **1980**, *85*, 298.

(21) Yadav, J. S.; Subba Reddy, B. V. *Carbohydr. Res.* **2000**, *329*, 885.

(22) Yadav, J. S.; Reddy, B. V. S.; Srinivas, R.; Maiti, A. *J. Chem. Res.* **2001**, 528.

(23) Bessodes, M.; Komiotis, D.; Antonakis, K. *Tetrahedron Lett.* **1986**, *27*, 579.

(24) Petrier, C.; Luche, J. L. *J. Org. Chem.* **1985**, *50*, 910.

(25) (a) Nigama, S. C.; Mann, A.; Taddei, M.; Wermutha, C.-G. *Synth. Commun.* **1989**, *19*, 3139. (b) Donohoe, T. J.; Thomas, R. E.; Cheeseman, M. D.; Rigby, C. L.; Bhalay, G.; Linney, I. D. *Org. Lett.* **2008**, *10*, 3615.

(26) Davis, B.; Bell, A. A.; Nash, R. J.; Watson, A. A.; Griffiths, R. C.; Jones, M. G.; Smith, C.; Fleet, G. W. J. *Tetrahedron Lett.* **1996**, *37*, 8565.

(27) Håkansson, A. E.; van Ameijde, J.; Horne, G.; Nash, R. J.; Wormald, M. R.; Kato, A.; Besra, G. S.; Gurcha, S.; Fleet, G. W. J. *Tetrahedron Lett.* **2008**, *49*, 179.