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Concise syntheses of potent chaperone drug candidates, *N*-octyl-4-*epi*-β-valinenamine (NOEV) and its 6-deoxy derivative, from (+)-*proto*-quercitol

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ABSTRACT

Described are the efficient syntheses of β-galactose-type unsaturated carbasugar amine, *N*-octyl-4-*epi*-β-valienamine (**1a**, **NOEV**) and **6-deoxy NOEV** (**12**), starting from (+)-*proto*-quercitol (**2**), which is readily provided by the bioconversion of *myo*-inositol. **NOEV** is a potent chemical chaperone drug candidate for G_{M1}-gangliosidosis. An intermediate alkadiene benzoate was prepared from **2** in five steps, with the key step being a Wittig reaction with an enol ester. The 6-deoxy derivative **12** was conveniently synthesized from the versatile intermediate dibromo compound **6**, which was also an intermediate in the synthesis of **NOEV**. Enzyme inhibition assays demonstrated that **12** possessed stronger inhibitory activity than the parent **1a**, suggesting that the C-6 position of the 4-*epi*-β-valienamine-type inhibitor could have hydrophobic interactions at the β-galactosidase active site residues.

1. Introduction

In some cases the activities of enzymes resulting from genetic mutations can be raised using small amounts of inhibitors. This paradoxical behavior can be taken advantage of for the treatment of genetic lysosomal storage disease in a method referred to as chemical chaperone therapy.[1] Lowered activities of enzymes in lysosomes may result in the accumulation of substrates inside and outside of somatic cells, which can cause organ dysfunction. However, enzymes can be stabilized by the introduction of small molecular inhibitors and then transported to the lysosomes, where they express their normal enzymatic activities. This therapeutic strategy has been examined in an experimental model to treat some lysosomal diseases such as Fabry disease (α-galactosidase mutation), G_{M1} gangliosidosis and Morquio B disease (β-galactosidase mutation), Pompe disease (α-glucosidase mutation) and Gaucher disease (β-glucosidase mutation) by using the corresponding inhibitors as chemical chaperones.[1d,2]

We have previously synthesized several bioactive cyclitol derivatives, resulting in the preparation of anhydro-quercitols, carbasugar amines, deoxyinosamines, and others.[3] Among them, valienamine-type unsaturated carbaglycosylamine derivatives, *N*-octyl-4-*epi*-β-valienamine (**NOEV**, **1a**[4]) and *N*-octyl-β-valienamine (**NOV**, **1b**[5]), have been shown to possess potential curative effects for β-galactosidase and β-glucosidase deficiencies, respectively (Fig. 1). **NOEV** has been demonstrated to be a strong inhibitor of human β-galactosidase (IC_{50} = 0.2 μM[4b]) and bovine liver β-galactosidase (IC_{50} = 4.5 μM by **NOEV** hydrochloride[4d]) as well as a potent chemical chaperone for in vitro and in vivo G_{M1} gangliosidosis models.[4b]

NOEV was first synthesized from **NOV** via epimerization at C-4 through an oxidation–reduction sequence.[4a] At that time, preparation of **NOV** was established via a versatile conjugate alkadiene derived from an (−)-*endo*-adduct of furan and acrylic acid. Alternatively, starting from the (+)-*endo*-adduct, an isomeric alkadiene suitable for the second-generation synthesis of **NOEV** was effectively developed.[1e,4c] These routes recognized the wide adaptability of the *endo*-adducts for carbasugar synthesis. However, to access chiral carbasugars, optically pure *endo*-adducts have to be prepared through a cumbersome optical resolution.[6]

Bioconversion of *myo*-inositol has been shown to produce optically pure deoxyinositols, quercitols. These are versatile starting materials for the synthesis of several biologically active compounds.[7] Recently, our group has briefly reported a convenient synthetic route to optically pure **NOEV** using the commercially available deoxyinositol **2**, (+)-*proto*-quercitol, as a chiral building block and a new preparation of **NOV** from (−)-*vibo*-quercitol.[4d]

In this paper, synthesis of **NOEV** starting from (+)-*proto*-quercitol through a key protected alkadiene **5**, which was successfully

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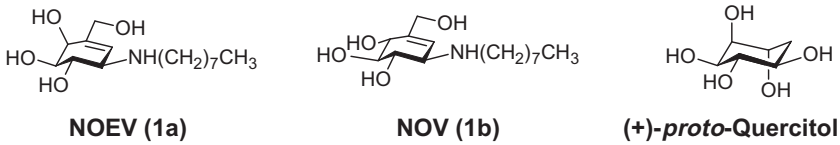
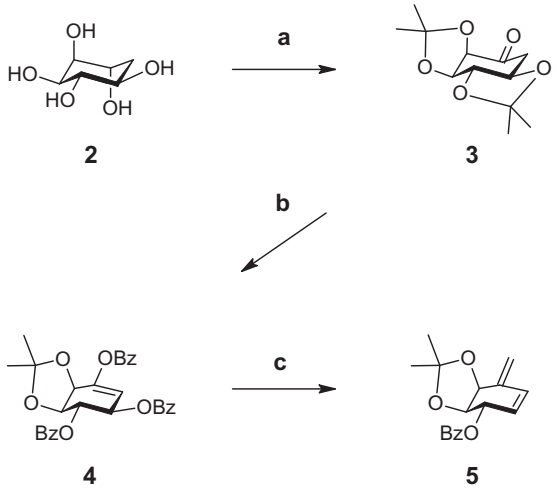


Figure 1. *N*-Octyl-4-*epi*-β-valienamine (**NOEV**, **1a**), *N*-octyl-β-valienamine (**NOV**, **1b**), and (+)-*proto*-quercitol (**2**).

converted into **NOEV** via an α-bromo intermediate, an epoxide, or a mixture of both, is described in full detail along with the isolation of the intermediates and determination of their chemical properties. Furthermore, synthesis of the 6-deoxy derivative **12** as the hydrochloride was readily attainable using the versatile intermediate. The synthesis of **12** and its β-galactosidase inhibitory activity are described herein.

2. Results and discussion

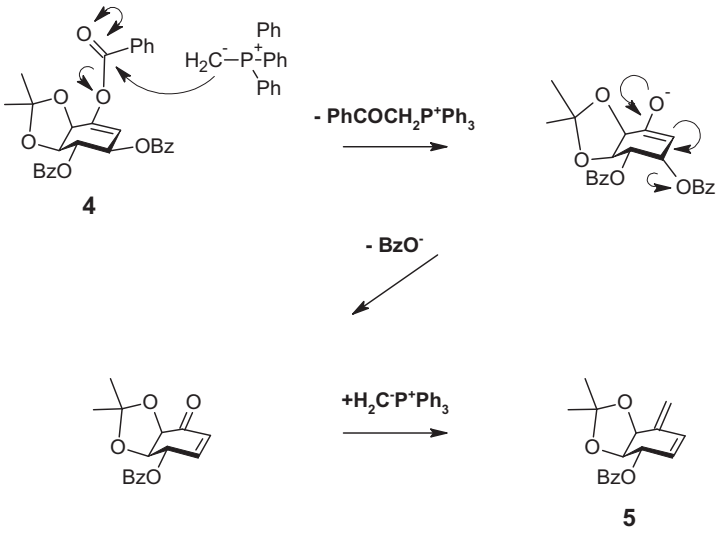
Acetonation of (+)-*proto*-quercitol (**2**) with 2,2-dimethoxypropane in the presence of (±)-10-camphorsulfonic acid in acetone, followed by oxidation with a pyridine–sulfur trioxide complex and triethylamine, afforded crystalline ketone **3**[7b] in a 53% yield (Scheme 1). Next, the *trans*-isopropylidene group of **3** was selectively removed with a catalytic amount of pyridinium-*p*-toluenesulfonate in methanol to give a dihydroxyketone that was, after treatment with benzoyl chloride in pyridine, converted into the single enol-benzoate **4** in 60% yield. The ^{1}H NMR spectrum of **4** showed a doublet (J = 2.6 Hz) at δ 5.95, attributable to the alkene proton of the assigned structure. It is noteworthy that the enol-benzoate **4** could be readily prepared from **2** on a ten gram scale without column purification. A similar acylation with acetic anhydride produced a syrupy mixture of products, most likely composed of unsaturated ketones and enol-acetates. Direct treatment of **4** under Wittig reaction conditions with an excess of methyltriphenylphosphonium bromide (6 M equiv)/*n*-BuLi (4 M equiv) in THF smoothly resulted in the desired conjugated alkadiene **5** in 66% yield after purification on a silica gel column. In the ^{1}H NMR spectrum of **5**, two new singlet protons at δ 5.42 and 5.45 were assigned as the exo-olefin protons. The two coupled signals at δ 5.78 (broad doublet, J = 10.1 Hz) and 6.32 (doublet of doublets, J = 1.8, 10.1 Hz) could be attributed to the alkene protons on the ring. Moreover, the two ring protons attached to the isopropylidene group appeared at δ 4.37 (triplet, J = 5.3 Hz) and 4.76 (doublet, J = 5.5 Hz); their coupling was confirmed in an H–H COSY measurement, clearly supporting the proposed structure.



Scheme 1. Synthesis of an alkadiene benzoate **5** from (+)-*proto*-quercitol (**2**). Reagents and conditions: (a) 2,2-dimethoxypropane (10 M equiv), (±)-10-camphorsulfonic acid (0.2 M equiv), acetone, rt, 19 h; pyridine–sulfur trioxide complex (3 M equiv), Et_3N (2 M equiv), DMSO, 0 °C to rt, 4.5 h, 53% based on **2**; (b) pyridinium-*p*-toluenesulfonate (0.2 M equiv), MeOH, 4 °C, 23 h; benzoyl chloride (8 M equiv), pyridine, 0 °C to rt, 21 h, 60% based on **3**; (c) methyltriphenylphosphonium bromide (6 M equiv), *n*-BuLi (4 M equiv), THF, −78 °C to 4 °C, 21 h, 66%.

It is known that enol-ester type lactones can be converted into the corresponding enolates with reactive phosphoranes.[8] In this case, after formation of the enolate, elimination of the benzoate may occur to give rise to the unsaturated ketone, which is successively methylenated by the phosphorous ylide to give **5** (Scheme 2). This Wittig-type one-pot elimination of the benzoate and exo-olefination reaction must be conducted under an inactive gas atmosphere with a large excess of the base and the phosphonium salt. When less than 3 M equiv of the reagents was used, a considerable amount of unreacted **4** was recovered (Table 1). Preliminarily, side reactions were shown to occur when the reaction was conducted at room temperature or the base was equimolar to the phosphonium salt. When *n*-BuLi was replaced by LiHMDS or NaHMDS, the isolated yields were 57% and 41%, respectively. The higher yields in the presence of the lithium cation might result from the coordination of the carbonyl oxygen to the lithium cation, enhancing the reactivity of the enol-ester toward the nucleophilic attack of the phosphonium ylide. These data support a plausible mechanism of the reaction as shown in Scheme 2.

The alkadiene **5** was treated with a slight excess of bromine in carbon tetrachloride to give a 68% yield of an epimeric mixture of the 1,4-addition products **6α** and **6β** (Scheme 3). The ^{1}H NMR spectra of **6α** and **6β** revealed a doublet at δ 6.20 (J = 6.0 Hz) and a doublet at δ 6.17 (J = 2.7 Hz), which could be assigned as the protons attached to the carbon atom bonded to α- and β-bromine atoms, respectively. The ratio of **6α** and **6β** was estimated to be approximately 1:1 based on the integrals of the CHBr signals. Because separation of the epimers was rather difficult, the intact mixture was used directly in the next reaction. The primary bromo group was easily replaced with a benzoate anion to give the monobrominated compounds. Thus, a selective nucleophilic substitution of **6α** and **6β** was conducted with sodium benzoate (1.2 M equiv) in DMF at room temperature affording, after separation on a silica gel column (95:5 hexane/ethyl acetate), the monobromo compounds **7α** (48%) and **7β** (27%). The ^{1}H NMR signal from **7α** at δ 5.15, which appeared as a double of doublets with J = 3.9 and 8.5 Hz, was assigned as H-2. H-2 from **7β** appeared as a triplet at δ 5.78 with J = 7.8 Hz. The configuration was further confirmed by the NOE correlation observed in the NOESY spectra of **7α** and **7β** (see Supplementary data). An NOE correlation was observed between H-1 and H-2 from **7α,** whereas its correlation was not shown from **7β**. These results suggest the stereo configuration of the bromine atom at C-1 position in **7α** and **7β**. Zemplén deacylation of **7α** and **7β** with sodium methoxide (using 0.56 and 1.1 M equiv, respectively) in methanol gave diol **8α** (65%) and epoxide **9** (44%), respectively. Under these conditions, the expected β-bromodiol could not be obtained, but instead, α-bromodiol **8α** and epoxide **9** were isolated. First, the β-bromodiol was formed, followed by a possible neighboring-group participation reaction where the *trans*-hydroxyl moiety at the adjacent carbon atom displaced the bromine to give **9**. In studies on amination of the corresponding diacetyl derivatives, the β-



Scheme 2. A plausible mechanism for the one-pot elimination of benzoate and the exo-olefination reaction.

Table 1
Optimization of the base in the preparation of **5** under Wittig reaction conditions

Base	Yield[c]
n-BuLi[a]	65%
n-BuLi[b]	22% (39% recovered)
LiHMDS[a]	57%
NaHMDS[a]	41%

[a] Ph_3PCH_3Br (6 equiv), base (4 equiv).
[b] Ph_3PCH_3Br (3 equiv), base (2 equiv).
[c] Isolated yield.

bromine was displaced by the nucleophilic amine to give the β-amino compound through the neighboring participation of the acetoxyl group.[4c,d] Therefore, in this case, the reaction course was controlled by the nucleophilicity and the stereochemical preference of the neighboring hydroxyl group under the basic conditions.

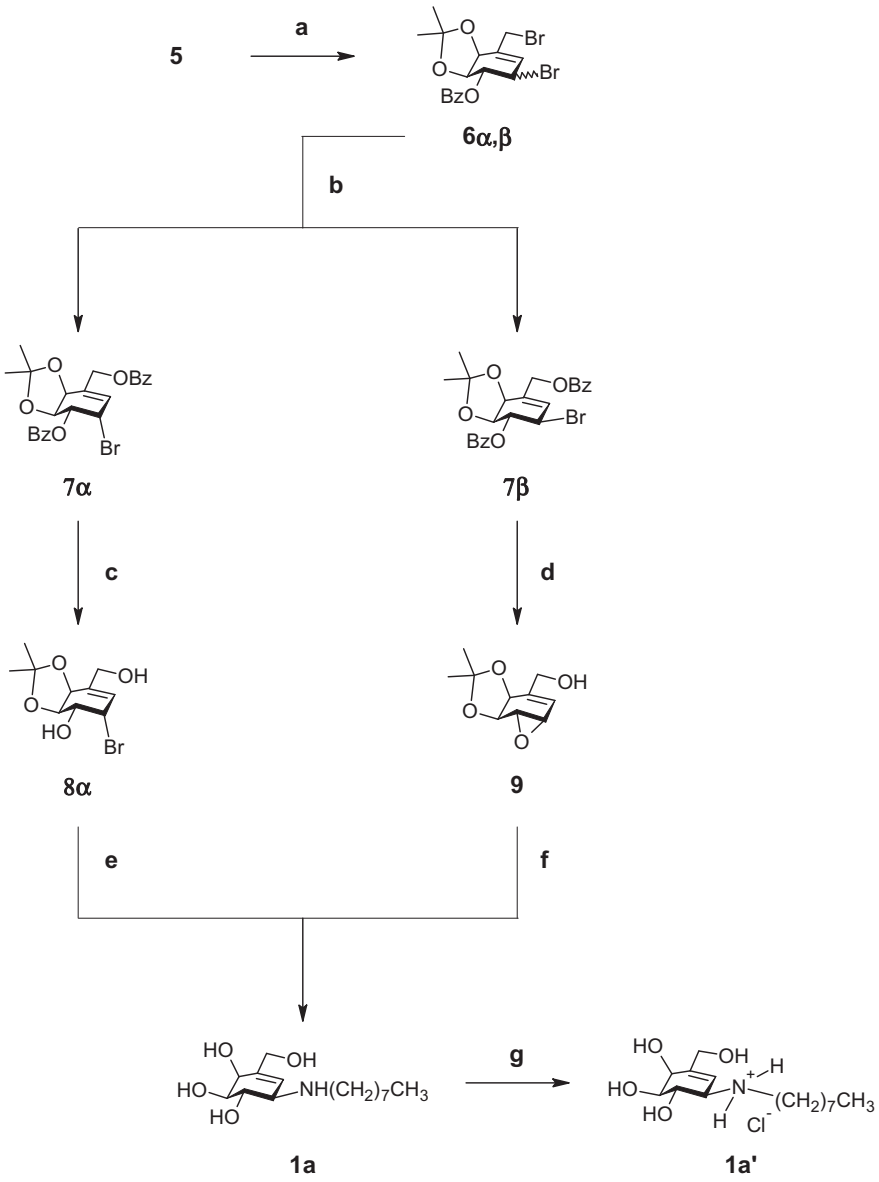
Incorporation of an alkylamino functionality at C-1 of α-bromodiol **8α** was accomplished by direct nucleophilic substitution with *n*-octylamine (2.5 M equiv) in acetonitrile in the presence of potassium carbonate (1.5 M equiv) at approximately 60 °C. The coupling products were treated with aqueous acetic acid and the resulting aminoalcohol was purified by a silica gel column with a solvent system of acetic acid/chloroform/methanol followed by a Doulite C20 (H^+) resin column with a gradient elution of 80% aqueous methanol to methanol/conc. aqueous ammonia (4:1). This afforded free **NOEV** (**1a**) at a 52% yield. This compound was unambiguously identified by comparing its spectral data, specific rotation, and biological activity, including its inhibitory activity toward β-galactosidase and chemical chaperone activity.[4b] Likewise, epoxide **9** underwent regioselective cleavage with *n*-octylamine (2.5 M equiv) to afford, after a similar purification process, the free base **1a** (48%). Thus, both isomeric intermediates formed by bromination could be successfully transformed into the target compound.

The above studies suggested that compounds **6α** and **6β** could be directly converted into the desired **NOEV** through an identical process without separation of each set of isomers (as we briefly introduced in[4d]). Optimization of the above process was attempted to improve the potential of this practical synthesis of **1a**. Specifically, the yield of the bromine addition to alkadiene **5** was increased to 85% in the presence of two equivalents of sodium hydrogen carbonate as a scavenger for the hydrobromic acid formed during the reaction. Increasing the reaction time of the selective substitution of **6α** and **6β** (1:1) with the benzoate anion improved the yields (75%→91%) of bromo compounds **7α** and **7β** (1.7:1). The change in the α:β ratio is likely attributable to epimerization at C-1, which occurs via nucleophilic attack of the bromide ion, reaching an equilibrium between **7α** and **7β**. Subsequent O-debenzoylation of the mixture afforded, after separation, **8α** (47%) and **9** (26%). The above mixture of **8α** and **9** was similarly treated with *n*-octylamine (3.5 M equiv), which afforded, after the usual processing and purification, the free amine **1a** at a 47% overall yield. Thus, the synthesis of **1a** could be readily conducted from **5** at an approximately 30% yield without separation of the isomeric products. The practical combined yield from **2** was 6% over nine steps. Moreover, the free amine **1a** could be converted into the hydrochloride salt **1a′** by treatment with a 1 M HCl aqueous solution (98%). This hydrochloride salt was used in the following biochemical assay.

The versatility of intermediate alkadiene **5** gave us an opportunity to develop some derivatives of **NOEV**. 6-Deoxy **NOEV** (**12**) is a particularly important derivative because previous research revealed that the 6-deoxy galactopyranosylamine (fucopyranosylamine)-type carbasugars tended to show stronger galactosidase inhibitory activities when compared to the intact 6-hydroxy parent.[9b] Therefore, we synthesized 6-deoxy **NOEV**, which was expected to be a potent β-galactosidase inhibitor. The mixture of **6α** and **6β** readily prepared from alkadiene **5** was reduced with sodium borohydride (2.2 M equiv) in HMPA/H_2O (4:1) at room temperature (Scheme 4). Because the primary bromo groups in **6α** and **6β** had a higher reactivity than the secondary bromo groups in selective acylation reactions, selective reduction of the primary bromo groups under appropriate conditions successfully gave **10α** (68%) and **10β** (23%) after purification over a silica gel column.[9a] The following de-acylation of **10α** with sodium methoxide (0.23 M equiv) in methanol yielded alcohol **11** (61%, with 11% recovery of **10α**). S_N2 substitution with *n*-octylamine (3.5 M equiv) in acetonitrile followed by de-acetalization under acidic conditions with hydrochloric acid and THF in a one pot reaction afforded the hydrochloride of 6-deoxy **NOEV** (**12**, 90%). Similarly, β-bromo compound **10β** is thought to be selectively transformed into **12**.

3. Biochemical assay

Hydrochlorides **1a′** and **12** were assayed for inhibitory activity against a commercially available β-galactosidase (bovine liver, EC 3.2.1.23). The IC_{50} value of **12** was 0.20 μM, which indicated that



Scheme 3. Synthesis of **NOEV** (**1a**) and its hydrochloride salt (**1a'**) from an alkadiene (**5**). Reagents and conditions: (a) bromine (1.2 M equiv), $NaHCO_3$ (1.1 M equiv), CCl_4, rt, 0.5 h, 68%; (b) anhydrous sodium benzoate (1.2 M equiv), DMF, rt, 22 h, 75% [**7α** (48%) and **7β** (27%)]; (c) sodium methoxide (0.56 M equiv), MeOH, rt, 2.5 h, 65%; (d) sodium methoxide (1.1 M equiv), MeOH, rt, 2 h, 44%; (e) *n*-octylamine (2.5 M equiv), K_2CO_3 (1.5 M equiv), MeCN, 60–65 °C, 22 h; 80% aqueous acetic acid, 80 °C, 4 h, 52%; (f) *n*-octylamine (2.5 M equiv), MeCN, 60–70 °C, 22 h; 80% aqueous acetic acid, 80 °C, 4 h, 48%; (g) 1 M HCl (aq), 98%.

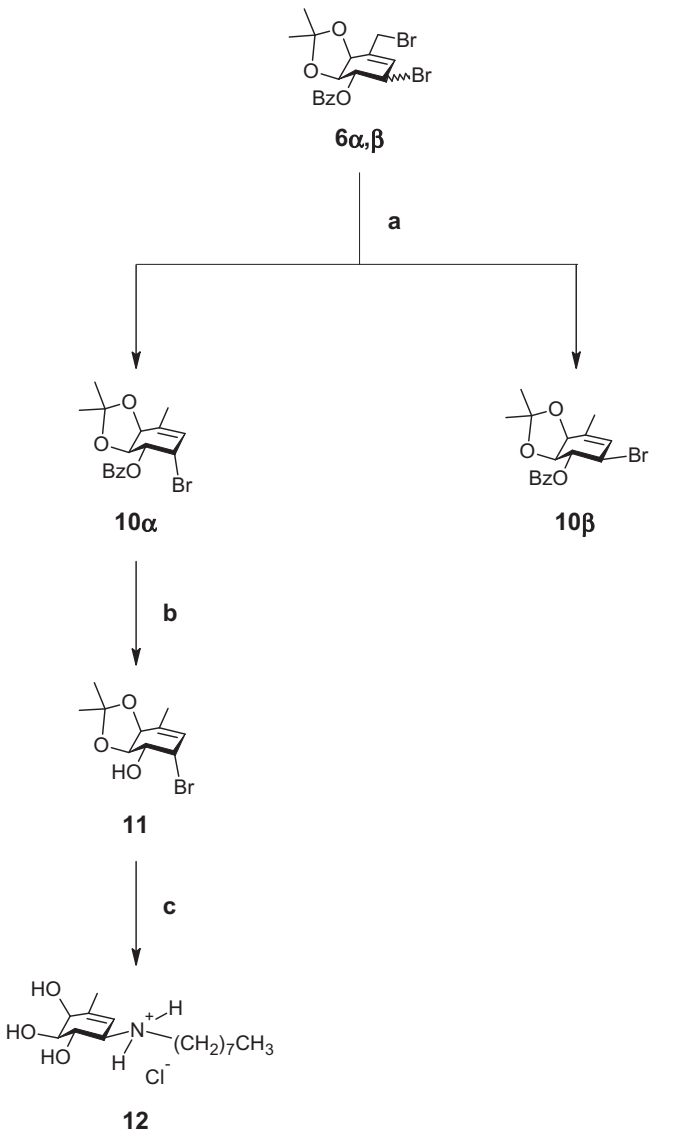
12 was approximately ten times more potent than **NOEV**, which has an IC_{50} of 2.6 μM[10] (Supplementary data Graph 1). This suggests that removal of the 6-hydroxy group in the 4-*epi*-β-valienamine-type inhibitor can increase the inhibitory activity toward β-galactosidase. This could be interpreted in the context of the interactions between the hydrophobic residues in the active site of β-galactosidase and the C-6 position of the inhibitor. Further studies examining its chaperone activity against human lysosomal β-galactosidase could be interesting. The present paper contributes to rational design concerning the development of both novel galactosidase inhibitors and chaperone therapy drugs.

Here, we have described convenient syntheses of the N-alkylated unsaturated carbagalactosylamine **1a** and its 6-deoxy derivative, **12**. The alkadiene intermediate **5** is expected to serve as a versatile intermediate for the development of interesting derivatives such as **12**. It was also revealed that removal of the hydroxyl group at the C-6 position of **1a** enhanced the inhibitory activity against bovine liver β-galactosidase. In addition, both the α-bromo compound **8α** and epoxide **9** were isolated in this study. They may be applied for the synthesis of side-chain-modified derivatives of **1a**, for example, N-arylated, azidated, and O- or S-substituted compounds. Furthermore, the other intermediates and the starting material, (+)-*proto*-quercitol, may possess great potential for the preparation of glycosidase inhibitors[11] and molecular chaperones, such as conduritols, conduritol epoxides, aminocyclitols, and conduramines. The compounds reported herein should inspire the synthesis of novel glycosidase inhibitors and pharmacological chaperones.

4. Experimental methods

4.1. General methods

Optical rotations were measured with a HORIBA SEPA-200 high sensitivity polarimeter, and $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Melting points were measured on a Yamato



Scheme 4. Synthesis of 6-deoxy **NOEV** (**12**) from the bromo compounds **6α** and **6β**. Reagents and conditions: (a) $NaBH_4$ (2.2 M equiv), HMPA/H_2O (4:1), rt, 3 h, 91% (α: 68%, β: 23%); (b) sodium methoxide (0.23 mol equiv), MeOH, 4 °C, 19 h and rt and 2 h, 61% (recover 10%); (c) *n*-octylamine (3.5 M equiv), MeCN, 60 °C, 22 h; 2 M HCl (aq)/THF (1:1), rt, 2.5 h, 90%.

Melting Point Apparatus Model MP-21 and are uncorrected. ^{1}H NMR spectra were recorded in solutions of $CDCl_3$ and CD_3OD with a JEOL JNM-ECS 400 (400 MHz) instrument; δ(H) values are reported in parts per million relative to the solvent's residual ^{1}H signal $CDCl_3$, δ(H) 7.24; CD_3OD, δ(H) 3.30. ^{13}C NMR spectra (100 MHz) were recorded on the same instrument as for ^{1}H spectra; δ(C) is reported in ppm relative to the solvent's C signal $CDCl_3$, δ(C) 77.0; CD_3OD, δ(H) 49.0, unless otherwise noted. High-resolution electron spray ionization mass spectra (HR-ESI-MS) were obtained on a LTQ Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). TLC was performed using silica gel 60 F-254 (Merck, Drmstadt, Germany). The silica gel used for chromatography was Wakogel C-300 (Wako Junyaku Kogyo Co., Osaka, Japan, 200–300 mesh). Organic solutions were dried over anhydrous Na_2SO_4 and concentrated with a rotary evaporator under reduced pressure at a temperature less than 60 °C. In Sections 4.1.4. to 4.1.11., carbasugar nomenclature following the IUPAC–IUBMB Nomenclature of Carbohydrates (Recommendation 1996: *Carbohydr. Res.*, **1997**, *297*, 1–92) was adopted for the sake of general understanding.

4.1.1. 2,3:4,5-Di-*O*-Isopropylidene derivative of (2*R*,3*R*,4*S*,5*R*)-2,3,4,5-tetrahydroxy-cyclohexanone (3)

A mixture of (+)-*proto*-quercitol (**2**) (5.00 g, 30.5 mmol), 2,2-dimethoxypropane (36.8 mL, 301 mmol), and (±)-10-camphorsulfonic acid (1.42 g, 6.09 mmol) in acetone (350 mL) was stirred for 19 h at rt. Saturated aqueous $NaHCO_3$ (30 mL) was added to the mixture, and stirring was continued for 10 min. The solution was filtered, and the filtrate was concentrated. The residue was extracted with EtOAc twice, and the extracts were concentrated. The residual product was dissolved in DMSO (30 mL) and Et_3N (12.7 mL, 91.5 mmol) and pyridine–sulfur trioxide complex (9.71 g, 61.0 mmol) was added at 0 °C. The mixture was stirred for 4.5 h at rt and then the reaction was quenched by the addition of saturated aqueous $NaHCO_3$ (30 mL). The solution was extracted with diethyl ether (150 mL × 2), and the extracts were washed with water and brine, dried, and concentrated. Crystallization of the residue from ethanol gave **3** (3.94 g, 53% for 2 steps) as white crystals: mp 153–155 °C; $[\alpha]_D^{25}$ −80° (*c* 1.0, $CHCl_3$); R_F = 0.47 (1:1 hexane/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.35, 1.43, 1.45, 1.47 (4s, each 3H, 2 CMe_2), 2.45 (dd, 1H, $J_{5,6eq}$ = 11.0, $J_{6ax,6eq}$ = 18.8 Hz, H-6 equiv), 2.96 (dd, 1H, $J_{5,6ax}$ = 7.6, $J_{6ax,6eq}$ = 18.5 Hz, H-6ax), 3.53 (dd, 1H, $J_{3,4}$ = 7.6, $J_{4,5}$ = 10.3 Hz, H-4), 4.09 (td, 1H, $J_{5,6ax}$ = 7.0, $J_{4,5}$ = $J_{5,6eq}$ = 10.6 Hz, H-5), 4.46 (d, 1H, $J_{2,3}$ = 8.7 Hz, H-2). 4.61 (dd, 1H, $J_{3,4}$ = 7.3, $J_{2,3}$ = 8.7 Hz, H-3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.69, 26.62, 26.95, 27.09, 41.06, 70.60, 75.10, 78.65, 82.18, 112.24, 113.51, 203.60; HR-ESI-MS: 265.1046 ($C_{12}H_{18}O_5Na^+$, $[M+Na]^+$; calcd 265.1046).

4.1.2. 3,4-*O*-Isopropylidene derivative of (1*R*,2*S*,3*R*,4*R*)-1,2,5-tribenzoyloxycyclohex-5-ene-3,4-diol (4)

Pyridinium-*p*-toluenesulfonate (2.07 g, 8.25 mmol) was added portionwise to an ice-cold solution of compound **3** (10.0 g, 41.3 mmol) in methanol (400 mL). The mixture was kept at 4 °C for 23 h and then neutralized with Et_3N. The solution was concentrated and co-evaporated with acetone. The residue was dissolved in pyridine (400 mL) and cooled in an ice bath, and benzoyl chloride (38.4 mL, 330 mmol) was added. The mixture was stirred at rt for 21 h. After the addition of cold saturated aqueous $NaHCO_3$, the mixture was stirred for another 0.5 h at rt. The precipitates were collected by filtration, washed with water, and reprecipitated from ethanol/water. The amorphous product was dried by co-evaporation with ethanol/toluene to give **4** (12.8 g, 60% for 2 steps) as a white powder: $[\alpha]_D^{25}$ −43° (*c* 1.0, $CHCl_3$); R_F = 0.35 (4:1 hexane/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.38 and 1.62 (2s, each 3H, CMe_2), 4.63 (dd, 1H, $J_{3,4}$ = 6.0, $J_{2,3}$ = 7.8 Hz, H-3), 5.01 (broad d, 1H, $J_{3,4}$ = 6.0 Hz, H-4), 5.83 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 7.8 Hz, H-2), 5.89 (broad dd, 1H, $J_{1,6}$ = 2.3, $J_{1,2}$ = 7.3 Hz, H-1), 5.95 (broad d, 1H, $J_{1,6}$ = 2.3 Hz, H-6), 7.36–7.42 (m, 4H, Ph), 7.45–7.52 (m, 4H, Ph), 7.59–7.63 (m, 1H, Ph), 7.97–8.04 (m, 4H, Ph), 8.10–8.12 (m, 2H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.40, 27.87, 69.43, 71.31, 72.23, 75.02, 112.21, 117.01, 128.35, 128.38, 128.57, 128.85, 129.28, 129.46, 129.84, 129.86, 130.25, 133.22, 133.29, 133.84, 146.26, 164.42, 165.53, 165.79; HR-ESI-MS: 537.1522 ($C_{30}H_{26}O_8Na^+$, $[M+Na]^+$; calcd 537.1520).

4.1.3. 2,3-*O*-Isopropylidene derivative of (1*R*,2*S*,3*S*)-1-benzoyloxy-4-methylenecyclohex-5-ene-2,3-diol (5)

n-BuLi (1.65 M solution in hexane, 14.2 mL, 23.4 mmol) was added to a solution of methyltriphenylphosphonium bromide (12.5 g, 35.0 mmol) in dry THF (12 mL) under a nitrogen atmosphere cooled with an acetone/dry ice bath (−78 °C). The mixture was then stirred for 1 h in an ice bath. The mixture was cooled again in an acetone/dry ice bath, and a solution of compound **4** (3.00 g, 5.83 mmol) in dry THF (46 mL) was slowly added via syringe. After stirring at 4 °C for 21 h, the reaction solution was loaded onto a silica gel column, and the column was eluted with 4:1 hex-

ane/EtOAc. The eluate was passed through a Celite bed with EtOAc and concentrated. The residue was purified on a silica gel column (95:5 hexane/EtOAc) to give **5** (1.11 g, 66%) as a colorless syrup: $[\alpha]_D^{25}$ +220° (*c* 1.0, $CHCl_3$); R_F = 0.45 (4:1 hexane/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.42 and 1.50 (2s, each 3H, CMe_2), 4.37 (t, 1H, $J_{1,2} = J_{2,3}$ = 5.3 Hz, H-2), 4.76 (d, 1H, $J_{2,3}$ = 5.5 Hz, H-3), 5.42, 5.45 (2s, each 1H, CH_2), 5.66–5.68 (m, 1H, H-1), 5.78 (broad d, 1H, $J_{5,6}$ = 10.1 Hz, H-5), 6.32 (dd, 1H, $J_{1,6}$ = 1.8, $J_{5,6}$ = 10.1 Hz, H-6), 7.40–7.43 (m, 2H, Ph), 7.52–7.55 (m, 1H, Ph), 8.03–8.05 (m, 2H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.22, 27.89, 71.06, 73.30, 76.43, 109.25, 120.54, 125.23, 128.29, 129.71, 129.88, 130.15, 133.08, 138.31, 165.84; HR-ESI-MS: 309.1100 ($C_{17}H_{18}O_4Na^+$, $[M+Na]^+$; calcd 309.1097).

4.1.3.1. Procedure for comparative studies of Wittig reaction conditions. The corresponding base (0.780 or 1.56 mmol) was added to a solution of methyltriphenylphosphonium bromide (1.17 or 2.34 mmol) in dry THF (1 mL) under a nitrogen atmosphere in an acetone/dry ice bath. The mixture was then stirred for 1 h in an ice bath. The mixture was cooled again in an acetone/dry ice bath, and a solution of **4** (200 mg, 0.390 mmol) in dry THF (3 mL) was slowly added via syringe. After stirring overnight at 4 °C for 22 h, the reaction solution was charged onto a short pad of silica gel and Celite, and the pad was eluted with 1:1 hexane/EtOAc. The filtrate was concentrated and the residue was purified on a silica gel column (95:5 hexane/EtOAc) to give **5**.

4.1.4. 2-*O*-Benzoyl-3,4-*O*-isopropylidene-6-bromo-6-deoxy-5a-carba-β-L-*arabino*-hex-5(5a)-enopyranosyl bromide (6α) and 2-*O*-Benzoyl-3,4-*O*-isopropylidene-6-bromo-6-deoxy-5a-carba-α-L-*arabino*-hex-5(5a)-enopyranosyl bromide (6β)

Bromine (280 μL, 5.46 mmol) was added dropwise to a stirred mixture of compound **5** (1.36 g, 4.75 mmol) and $NaHCO_3$ (748 mg, 5.23 mmol) in CCl_4 (48 mL). The resulting mixture was stirred at rt for 0.5 h. After dilution with $CHCl_3$ (100 mL), the reaction mixture was washed with saturated aqueous $NaHCO_3$ (50 mL) and water (50 mL), dried, and evaporated. The residue was chromatographed on a silica gel column (95:5→9:1 hexane/EtOAc) to give a mixture of the isomeric dibromo compounds **6α** and **6β** (1.45 g, 68%) as a colorless syrup. The ratio of **6α** to **6β** was estimated to be approximately 1:1 by ^{1}H NMR analysis.

For **6α** and **6β**: R_F = 0.63 (95:5 toluene/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.39, 1.41, 1.45 and 1.52 (4s, each 3H, $CMe_{2(6\alpha)}$ and $CMe2_{(6\beta)}$), 4.02 and 4.04 (2s, each 1H), 4.21 and 4.24 (2s, each 1H), 4.37 (dd, 1H, *J* = 6.0, 7.8 Hz), 4.61–4.64 (m, 1H), 4.71 (dd, 1H, *J* = 6.6, 8.5 Hz), 4.88 (t, 1H, *J* = 4.6 Hz), 4.93 (d, 1H, *J* = 5.5 Hz), 5.02 (d, 1H, *J* = 6.4 Hz), 5.10 (dd, 1H, *J* = 3.9, 8.5 Hz), 5.64 (t, 1H, *J* = 7.3 Hz), 6.17 (d, 1H, $J_{1,5a\ (6\beta)}$ = 2.7 Hz, $H_{(6\beta)}$-5a), 6.20 (d, 1H, $J_{1,5a\ (6\alpha)}$ = 6.0 Hz, $H_{(6\alpha)}$-5a), 7.41–7.46 (m, 4H, Ph), 7.54–7.59 (m, 2H, Ph), 8.03–8.04 (m, 2H, Ph), 8.10–8.12 (m, 2H, Ph), assigned by H-H COSY.

4.1.5. 2,6-Di-*O*-Benzoyl-3,4-*O*-isopropylidene-5a-carba-β-L-*arabino*-hex-5(5a)-enopyranosyl bromide (7α) and 2,6-di-*O*-Benzoyl-3,4-*O*-isopropylidene-5a-carba-α-L-*arabino*-hex-5(5a)-enopyranosyl bromide (7β)

A solution of a mixture of **6α** and **6β** (1.41 g, 3.15 mmol) and anhydrous sodium benzoate (522 mg, 3.62 mmol) in DMF (32 mL) was stirred at rt for 22 h. After dilution with EtOAc (300 mL), the solution was washed with water (100 mL × 2) and brine, dried, and evaporated. The residue was fractionated on a silica gel column (95:5 hexane/EtOAc) to give monobromo compounds **7α** (732 mg, 48%) as a white solid and **7β** (419 mg, 27%) as a colorless syrup.

For **7α**: $[\alpha]_D^{25}$ +190° (*c* 1.0, $CHCl_3$); R_F = 0.36 (4:1 hexane/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.39 and 1.47 (2s, each 3H, CMe_2), 4.74 (dd, 1H, $J_{3,4}$ = 6.4, $J_{2,3}$ = 8.7 Hz, H-3), 4.83 (d, 1H, $J_{3,4}$ = 6.4 Hz, H-4), 4.91–5.06 (3H, H-1, CH_2), 5.15 (dd, 1H, $J_{1,2}$ = 3.9, $J_{2,3}$ = 8.5 Hz, H-2), 6.22 (d, 1H, $J_{1,5a}$ = 6.0 Hz, H-5a), 7.42–7.48 (m, 4H, Ph), 7.55–7.60 (m, 2H, Ph), 8.07–8.13 (m, 4H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.67, 27.58, 44.61, 64.24, 71.67, 72.38, 73.82, 110.28, 125.66, 128.41, 128.47, 129.47, 129.69, 129.77, 130.01, 133.25, 133.39, 134.48, 165.85, 165.93; HR-ESI-MS: 509.0570 ($C_{24}H_{23}O_6BrNa^+$, $[M+Na]^+$; calcd 509.0570).

For **7β**: $[\alpha]_D^{25}$ +16° (*c* 1.0, $CHCl_3$); R_F = 0.24 (4:1 hexane/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37 and 1.56 (2s, each 3H, CMe_2), 4.35 (dd, 1H, $J_{3,4}$ = 6.0, $J_{2,3}$ = 7.8 Hz, H-3), 4.66–4.68 (m, 1H, H-1), 4.72 (d, 1H, $J_{3,4}$ = 5.5 Hz, H-4), 4.99 (br s, 2H, CH_2), 5.68 (t, 1H, $J_{1,2} = J_{2,3}$ = 7.8 Hz, H-2), 6.16 (br s, 1H, H-5a), 7.40–7.47 (m, 4H, Ph), 7.53–7.60 (m, 2H, Ph), 8.03–8.07 (m, 4H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.28, 27.70, 44.06, 64.37, 71.97, 74.10, 75.22, 111.50, 127.94, 128.37, 128.50, 129.55, 129.68, 129.73, 129.89, 130.02, 132.85, 133.28, 165.23, 165.98; HR-ESI-MS: 509.0559 ($C_{24}H_{23}O_6BrNa^+$, $[M+Na]^+$; calcd 509.0570).

4.1.6. 3,4-*O*-Isopropylidene-5a-carba-β-L-*arabino*-hex-5(5a)-enopyranosyl bromide (8α)

A 1 M sodium methoxide/methanol (498 μL, 0.50 mmol) solution and methanol rinse (200 μL) were added to a solution of **7α** (692 mg, 1.40 mmol) in dry methanol (14 mL). After stirring at rt for 2 h, another portion of 1 M sodium methoxide/methanol (280 μL, 0.28 mmol) was added to the mixture and it was stirred for an additional 0.5 h. The solution was neutralized with Duolite C20 (H^+) resin, filtered, and evaporated. The residual product was chromatographed on a silica gel column (4:1→2:1 hexane/EtOAc) to give the bromodiol **8α** (254 mg, 65%) as a colorless solid: $[\alpha]_D^{25}$ +350° (*c* 1.0, $CHCl_3$); R_F = 0.20 (1:1 hexane/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37 and 1.45 (2s, each 3H, CMe_2), 2.20 (broad s, 1H, OH), 2.64 (broad d, 1H, OH), 3.68–3.70 (m, 1H, H-2), 4.19–4.35 (3H), 4.69–4.74 (2H), 6.07 (d, 1H, $J_{1,5a}$ = 5.5 Hz, H-5a), assigned by H-H COSY; ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.56, 27.70, 50.41, 63.77, 70.19, 72.73, 109.87, 124.10, 138.57; HR-ESI-MS: 301.0054 ($C_{10}H_{15}BrO_4Na^+$, $[M+Na]^+$; calcd 301.0046).

4.1.7. 1,2-Epoxy-3,4-*O*-isopropylidene-5a-carba-β-L-*arabino*-hex-5(5a)-enopyranose (9)

A 1 M sodium methoxide/methanol solution (580 μL, 0.58 mmol) and methanol rinse (200 μL) were added to a solution of **7β** (270 mg, 0.554 mmol) in dry methanol (4.4 mL). After stirring at rt for 2 h, the mixture was neutralized with 0.1 M hydrochloric acid in methanol and evaporated. The residue was chromatographed on a silica gel column (4:1→2:1 hexane/EtOAc) to give **9** (48 mg, 44%) as a colorless syrup: $[\alpha]_D^{25}$ −23° (*c* 1.0, $CHCl_3$); R_F = 0.41 (1:1 hexane/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37 (s, 6H, CMe_2), 2.20 (broad s, 1H, OH), 3.34 (t, 1H, $J_{1,2} = J_{1,5a}$ = 3.9 Hz, H-1), 3.53 (dd, 1H, $J_{2,3}$ = 1.8, $J_{1,2}$ = 3.7 Hz, H-2), 4.20 (br s, 2H, CH_2), 4.46 (d, 1H, $J_{3,4}$ = 7.3 Hz, H-4), 4.77 (d, 1H, $J_{3,4}$ = 6.9 Hz, H-3), 6.01–6.04 (m, 1H, H-5a), assigned by H–H COSY; ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.73, 27.52, 47.00, 50.08, 63.60, 71.19, 71.38, 110.71, 118.94, 141.85; HR-ESI-MS: 221.0779 ($C_{10}H_{14}O_4Na^+$, $[M+Na]^+$; calcd 221.0784).

4.1.8. *N*-Octyl-5a-carba-α-L-*arabino*-hex-5(5a)-enopyranosylamine (1a)

From **8α**: A mixture of **8α** (187 mg, 0.670 mmol), K_2CO_3 (140 mg, 1.01 mmol), *n*-octylamine (279 μL, 1.68 mmol), and acetonitrile (3.4 mL) was stirred at 60–65 °C for 22 h. It was cooled to rt, filtered, and evaporated. The residue was chromatographed on a silica gel column (97:3 $CHCl_3$/MeOH) and the major fractions were concentrated. The residue was dissolved in aqueous 80% acetic acid (6.5 mL) and the solution was stirred at 80 °C for 4 h. The mixture was co-evaporated with toluene and the residue was puri-

fied on a silica gel column (10:86:4→1:6:3 AcOH/$CHCl_3$/MeOH) to give the acetate salt of **1a**. It was chromatographed on a column of Duolite C20 (H^+) resin (80% aqueous methanol→4:1 methanol/conc. aqueous ammonia) to give **1a** (100 mg, 52%) as a white solid: $[\alpha]_D^{25}$ +3.0° (*c* 1.0, MeOH); R_F = 0.41 (1:6:3 AcOH/$CHCl_3$/MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 0.89 (t, 3H, $J_{7',8'}$ = 6.9 Hz, H-8′), 1.30–1.37 (10H, H-3′, 4′, 5′, 6′, and 7′), 1.48–1.56 (m, 2H, H-2′), 2.54–2.58, 2.72–2.76 (each m, each 1H, H-1′), 3.11 (dd, 1H, $J_{1,5a}$ = 1.8, $J_{1,2}$ = 8.2 Hz, H-1), 3.44 (dd, 1H, $J_{3,4}$ = 4.1, $J_{2,3}$ = 10.1 Hz, H-3), 3.70 (dd, 1H, $J_{1,2}$ = 8.2, $J_{2,3}$ = 10.1 Hz, H-2), 4.12 (broad s, 2H, CH_2), 4.15 (d, 1H, $J_{3,4}$ = 4.1 Hz, H-4), 5.71 (d, 1H, $J_{1,5a}$ = 2.3 Hz, H-5a); ^{13}C NMR (100 MHz, CD_3OD): δ 14.43, 23.71, 28.42, 30.38, 30.60, 30.88, 32.98, 46.87, 61.78, 63.89, 68.13, 70.78, 73.85, 125.13, 140.73; HR-ESI-MS: 310.1987 ($C_{15}H_{29}O_4NNa^+$, $[M+Na]^+$; calcd 310.1989).

From **9**: A mixture of **9** (48 mg, 0.24 mmol) and *n*-octylamine (100 μL, 0.606 mmol) in acetonitrile (2.4 mL) was stirred at 60–70 °C for 22 h. The solution was cooled to rt and evaporated. The residue was chromatographed on a silica gel column (97:3 $CHCl_3$/MeOH), and the fractions containing the major product were collected and concentrated. The residue was dissolved in aqueous 80% acetic acid (2.5 mL) and the solution was stirred at 80 °C for 4 h. The mixture was processed as described above and the product was purified on a silica gel column (10:86:4→1:6:3 AcOH/$CHCl_3$/MeOH) to give the amine as the acetate salt. A similar workup as above gave **1a** (33 mg, 48%).

From **5** (without separation of the bromo compounds): Bromine (407 μL, 7.93 mmol) was slowly added dropwise to a stirred mixture of **5** (2.06 g, 7.21 mmol) and $NaHCO_3$ (1.21 g, 14.4 mmol) in CCl_4 (70 mL) at rt. The solution was stirred for 20 min, diluted with $CHCl_3$ (140 mL), washed with saturated aqueous $NaHCO_3$ (70 mL) and water (70 mL), dried, and evaporated. The residue was chromatographed on a silica gel column (95:5→9:1 hexane/EtOAc) to give an isomeric mixture of **6α** and **6β** (2.74 g, 85%, α/β = *ca.* 1:1 as determined by ^{1}H NMR analysis). A solution of the mixture of **6α** and **6β** (1.15 g, 2.57 mmol) and anhydrous sodium benzoate (425 mg, 2.95 mmol) in DMF (25 mL) was stirred at rt for 47 h. The reaction mixture was diluted with EtOAc (300 mL), washed twice with both water and brine, dried, and evaporated. The residue was fractionated over a silica gel column (95:5→9:1 hexane/EtOAc) to give **7α** (693 mg, 57%) and **7β** (419 mg, 34%). A 0.5 M sodium methoxide/methanol solution (4.3 mL, 2.2 mmol) was added to a mixture of **7α** and **7β** (1.01 g, 2.14 mmol) in dry methanol (15 mL). After the mixture was stirred at rt for 2 h, the solution was carefully neutralized with 0.1 M hydrochloric acid in methanol and evaporated. The products were chromatographed on a silica gel column (4:1→2:1→1:1 hexane/EtOAc) to give **8** (278 mg, 47%) and **9** (110 mg, 26%). Compounds **8** (278 mg, 0.996 mmol) and **9** (110 mg, 0.555 mmol) were dissolved in acetonitrile (16 mL), and to this mixture, *n*-octylamine (899 μL, 5.43 mmol) was added. After stirring at 60–70 °C for 16 h, the solution was cooled to rt and evaporated. The product was dissolved in aqueous 80% acetic acid (16 mL) and stirred at 80 °C for 4 h. The mixture was co-evaporated with toluene and the residual product was purified on a silica gel column (10:86:4→1:6:3 AcOH/$CHCl_3$/MeOH) to give the amine as the acetate salt, which was further chromatographed on a column of Duolite C20 (H^+) resin (80% aqueous methanol→4:1 methanol/conc. aqueous ammonia) to give **1a** (208 mg, 47%).

4.1.8.1. Amine hydrochloride (1a′). The free amine **1a** (78 mg, 0.27 mmol) was dissolved in 6 mL of 1 M HCl aqueous solution. The solution was azeotroped with ethanol several times and concentrated to dryness. The residue was dried under reduced pressure to yield **1a′** (86 mg, 98%) as an amorphous white powder: $[\alpha]_D^{25}$ +9.8° (*c* 1.0, MeOH); R_F = 0.41 (1:6:3 AcOH/$CHCl_3$/MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 0.90 (t, 3H, $J_{7',8'}$ = 6.9 Hz, H-8′), 1.31–1.43 (10H, H-3′, 4′, 5′, 6′, and 7′), 1.68–1.75 (m, 2H, H-2′), 3.09 (t, 2H, $J_{1',2'}$ = 7.8 Hz, H-1′), 3.53 (dd, 1H, $J_{3,4}$ = 3.9, $J_{2,3}$ = 9.8 Hz, H-3), 3.72 (dd, 1H, $J_{1,5a}$ = 2.3, $J_{1,2}$ = 8.2 Hz, H-1), 3.95 (dd, 1H, $J_{1,2}$ = 8.2, $J_{2,3}$ = 9.6 Hz, H-2), 4.11–4.25 (3H, H-4, CH_2), 5.75 (d, 1H, $J_{1,5a}$ = 1.8 Hz, H-5a); ^{13}C NMR (100 MHz, CD_3OD): δ 14.40, 23.67, 27.46, 27.66, 30.17, 32.88, 45.80, 61.43, 63.26, 67.63, 68.30, 72.96, 116.61, 146.98; HR-ESI-MS: 310.1987 ($C_{15}H_{29}O_4NNa^+$, $[M+Na]^+$; calcd 310.1989).

4.1.9. 2-*O*-Benzoyl-3,4-*O*-isopropylidene-6-methyl-6-deoxy-5a-carba-β-L-*arabino*-hex-5(5a)-enopyranosyl bromide (10α) and 2-*O*-Benzoyl-3,4-*O*-isopropylidene-6-methyl-6-deoxy-5a-carba-α-L-*arabino*-hex-5(5a)-enopyranosyl bromide (10β)

The isomeric mixture of **6α** and **6β** (375 mg, 0.841 mmol) was dissolved in 5 mL of HMPA/H_2O (4:1). Sodium borohydride (70 mg, 1.85 mmol) was added to the solution portionwise. With 3 mL of HMPA/H_2O (4:1), the rinse was added to the reaction solution. The solution was stirred at rt for 3 h. After dilution with H_2O (20 mL), the solution was extracted with diethyl ether (60 mL × 3). The combined organic phases were dried and evaporated. The residue was purified on a silica gel column (97:3→95:5 hexane/EtOAc) to give **10α** (211 mg, 68%) as a colorless syrup and **10β** (71 mg, 23%) as a white powder.

For **10α** [(purified by silica gel column chromatography (hexane/toluene = 1:2) for analysis]: $[\alpha]_D^{25}$ +340° (*c* 1.0, $CHCl_3$); R_F = 0.54 (4:1 hexane/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.39 and 1.47 (2s, each 3H, CMe_2), 1.94 (s, 3H, Me), 4.61 (d, 1H, $J_{3,4}$ = 6.4 Hz, H-4), 4.69 (dd, 1H, $J_{3,4}$ = 6.4, $J_{2,3}$ = 8.7 Hz, H-3), 4.86 (dd, 1H, $J_{1,2}$ = 3.9, $J_{1,5a}$ = 5.7 Hz, H-1), 5.03 (dd, 1H, $J_{1,2}$ = 3.9, $J_{2,3}$ = 8.9 Hz, H-2), 5.85 (br d, 1H, $J_{1,5a}$ = 6.4 Hz, H-5a), 7.42–7.45 (m, 2H, Ph), 7.54–7.58 (m, 1H, Ph), 8.11–8.13 (m, 2H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.53, 25.56, 27.58, 46.34, 71.89, 73.83, 75.57, 109.58, 124.24, 128.33, 129.60, 129.96, 133.24, 136.40, 166.00; HR-ESI-MS: 389.0352 ($C_{17}H_{19}O_4BrNa^+$, $[M+Na]^+$; calcd 389.0359).

For **10β**: $[\alpha]_D^{25}$ −35° (*c* 1.0, $CHCl_3$); R_F = 0.42 (4:1 hexane/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37 and 1.55 (2s, each 3H, CMe_2), 1.93 (s, 3H, Me), 4.27 (dd, 1H, $J_{3,4}$ = 5.5, $J_{2,3}$ = 8.2 Hz, H-3), 4.47 (d, 1H, $J_{3,4}$ = 5.5 Hz, H-4), 4.62 (br d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 5.62 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 8.0 Hz, H-2), 5.79 (br s, 1H, H-5a), 7.41–7.45 (m, 2H, Ph), 7.53–7.57 (m, 1H, Ph), 8.04–8.06 (m, 2H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.39, 26.31, 27.77, 45.81, 74.68, 75.38, 75.52, 110.96, 126.07, 128.34, 129.88, 133.15, 134.04, 165.35; HR-ESI-MS: 389.0365 ($C_{17}H_{19}O_4BrNa^+$, $[M+Na]^+$; calcd 389.0359).

4.1.10. 3,4-*O*-Isopropylidene-6-methyl-6-deoxy-5a-carba-β-L-*arabino*-hex-5(5a)-enopyranosyl bromide (11)

A 0.5 M sodium methoxide/methanol solution (68 μL, 34 μmol) was added to a solution of **10α** (55 mg, 0.15 mmol) in dry methanol (1.4 mL) at 4 °C. After stirring at 4 °C for 19 h and at rt for 2 h, the reaction was carefully quenched with a 0.1 M HCl solution in methanol. The solution was evaporated, and the residue was chromatographed on a silica gel column (9:1→4:1 hexane/EtOAc) to give **11** (24 mg, 61%) as a colorless syrup with recovery of 6 mg of **10α** (10%); $[\alpha]_D^{25}$ +100° (*c* 1.0, $CHCl_3$); R_F = 0.20 (4:1 hexane/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.38 and 1.46 (2s, each 3H, CMe_2), 1.89 (s, 3H, Me), 2.31 (broad s, 1H, OH), 3.60 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 8.2 Hz, H-2), 4.28 (dd, 1H, $J_{3,4}$ = 6.6 Hz, $J_{2,3}$ = 8.0 Hz, H-3), 4.52 (d, 1H, $J_{3,4}$ = 6.4 Hz, H-4), 4.67–4.70 (m, 1H, H-1), 5.79 (d, 1H, $J_{1,5a}$ = 5.0 Hz, H-5a); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.45, 25.51, 27.76, 51.94, 70.15, 75.59, 76.78, 109.34, 124.20, 136.64; HR-ESI-MS: 285.0097 ($C_{10}H_{15}O_4BrNa^+$, $[M+Na]^+$; calcd 285.0097).

4.1.11. *N*-Octyl-6-methyl-6-deoxy-5a-carba-α-L-*arabino*-hex-5(5a)-enopyranosylamine hydrochloric salt (12)

A mixture of **11** (62 mg, 0.236 mmol), *n*-octylamine (137 μL, 0.826 mmol), and acetonitrile (2.3 mL) was added to a sealed tube and allowed to stand at 60 °C for 22 h. The reaction solution was cooled to rt and evaporated. The residue was chromatographed on a silica gel column (99:1→97:3 $CHCl_3$/MeOH). The obtained product was dissolved in 4 mL of 1 M HCl/THF (1:1) and stirred at rt for 2.5 h. The reaction solution was concentrated under reduced pressure and the residue was re-dissolved in 1.5 mL of methanol. The solution was passed through a short column of activated carbon and washed with 40 mL of methanol. The combined solution was evaporated to yield **12** (65 mg, 90%) as a light yellow oil: $[\alpha]_D^{25}$ +10° (*c* 1.0, MeOH); R_F = 0.53 (1:6:3 AcOH/$CHCl_3$/MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 0.90 (t, 3H, $J_{7',8'}$ = 6.9 Hz, H-8′), 1.31–1.42 (10H, H-3′, 4′, 5′, 6′, and 7′), 1.67–1.75 (m, 2H, H-2′), 1.89 (s, 3H, CH_3), 3.06–3.10 (m, 2H, H-1′a and H-1′b), 3.54 (dd, 1H, $J_{3,4}$ = 4.1, $J_{2,3}$ = 9.6 Hz, H-3), 3.62–3.64 (m, 1H, H-1), 3.89 (dd, 1H, $J_{1,2}$ = 8.2, $J_{2,3}$ = 9.6 Hz, H-2), 4.04 (d, 1H, $J_{3,4}$ = 4.1 Hz, H-4), 5.49 (s, 1H, H-5a); ^{13}C NMR (100 MHz, CD_3OD): δ 14.39, 21.08, 23.66, 27.44, 27.64, 30.16, 32.87, 45.88, 61.44, 68.11, 71.04, 72.84, 117.87, 143.72; HR-ESI-MS: 272.2225 ($C_{15}H_{30}O_3N^+$, $[M+H]^+$; calcd 272.2220).

4.2. Biochemical assay

Compounds were assayed for enzyme inhibitory activity (IC_{50}) against the commercially available bovine liver β-galactosidase (Sigma–Aldrich). Various concentrations (0, 0.01, 0.1, 1, 10, 100, and 1000 μM) of compounds **1a**′ or **12** in water (20 μL) were added to the assay mixture with a 10 mM *p*-nitrophenyl-β-D-galactopyranoside (Sigma–Aldrich) aqueous solution (5 μL) and a 200 mM sodium phosphate buffer (5 μL, pH 7.0). Then 20 μL of bovine liver β-galactosidase in sodium phosphate buffer (0.1 U/mL) was added to the mixture, which was incubated at 37 °C. After 30 min, the reaction was quenched by addition of a 200 mM aqueous sodium carbonate solution (150 μL), and the absorbance of the liberated *p*-nitrophenol was measured at 410 nm by a Sunrise-Basic Tecan microplate reader (Tecan, Salzburg, Austria). The percentage of inhibition was calculated.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carres.2012.12.010.

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10. This IC_{50} value is in agreement with the previously reported in Ref. [4d].
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