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Stereoselective synthesis of β-1-*C*-substituted 1,4-dideoxy-1,4-imino-D-galactitols and evaluation as UDP-galactopyranose mutase inhibitors

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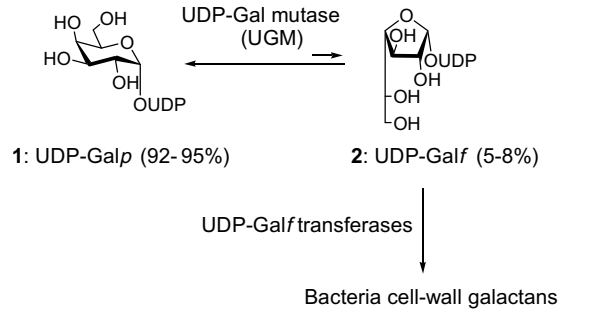
Abstract—The synthesis of 1-*C*-substituted 1,4-dideoxy-1,4-imino-D-galactitols involving nitrone *umpolung* is described. The SmI_2-induced key coupling proved highly stereoselective in favor of the β-*C*-substituted products bearing a three-carbon chain at the pseudoanomeric position. Pyrrolidines **9** and **10**, as well as the bicyclic compounds **8** and **11**, exhibit weak inhibition of the activity of the UDP-galactopyranose mutase from *Escherichia coli*.

1. Introduction

Tuberculosis, leprosy, and other diseases caused by mycobacterial infections are currently public health concerns due to multiresistances appearing against most existing treatments.[1] The necessity to investigate new therapeutic strategies against these pathologies has been widely recognized,[2] and the ones based on inhibition of the mycobacterial cell wall biosynthesis have attracted particular attention.[3] One of the main components of this rigid envelope is an arabinogalactan, that is essential to the survival of mycobacteria.[4] Since the main constituent of galactan (D-galactofuranose) is not found in mammalian metabolism, inhibition of its biosynthesis[5] constitutes an attractive approach for the development of new, selective therapeutic approaches against mycobacteria such as *Mycobacterium tuberculosis*.

In mycobacteria, galactan biopolymer arises from the action of specific enzymes having uridine-diphospho-galactofuranose (UDP-Gal*f*) as substrate, such as UDP-galactofuranose transferases[6] and UDP-galactopyranose mutase, which catalyzes the interconversion of UDP-galactopyranose (UDP-Gal*p*) and UDP-galactofuranose (UDP-Gal*f*) (Scheme 1).



Scheme 1. Biosynthesis of the galactan polymer in mycobacteria cell wall.

Keywords: Iminosugars; Pyrrolidines; UDP-galactose mutase; Nitrone; Umpolung; Samarium diiodide; Reductive coupling; Enzyme inhibition; Tuberculosis; Mycobacterium; Arabinogalactan; Pyrrolizidines; Iminogalactitols; Galactofuranose.
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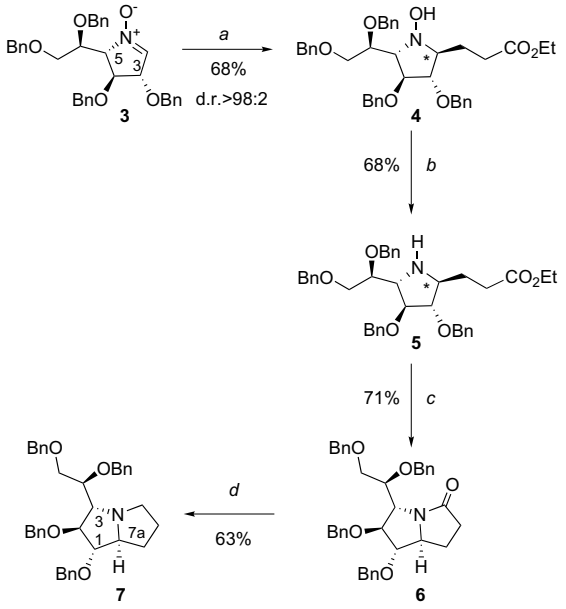
Lately, UDP-galactopyranose mutase (UGM, EC 5.4.99.9) has been the subject of extensive studies, dedicated to its characterization,[7] and to the understanding of its intriguing mechanism of action.[8] Synthetic work has also been conducted with the aim of designing suitable inhibitors[9] and/or mechanistic probes.[10] Although the details of UGM mechanism remain unclear, the presence of an oxocarbenium-type intermediate in the transition state of UDP-Gal*p* to UDP-Gal*f* isomerization is widely admitted (Scheme 2).[8f,11] Hence, strategies similar to those sought for glycosidase and glycosyltransferase inhibition,[12] i.e., the use of iminosugars as transition state analogues (oxonium mimics) or substrate analogues, have also been considered for inhibition of UGM.

2. Results and discussion

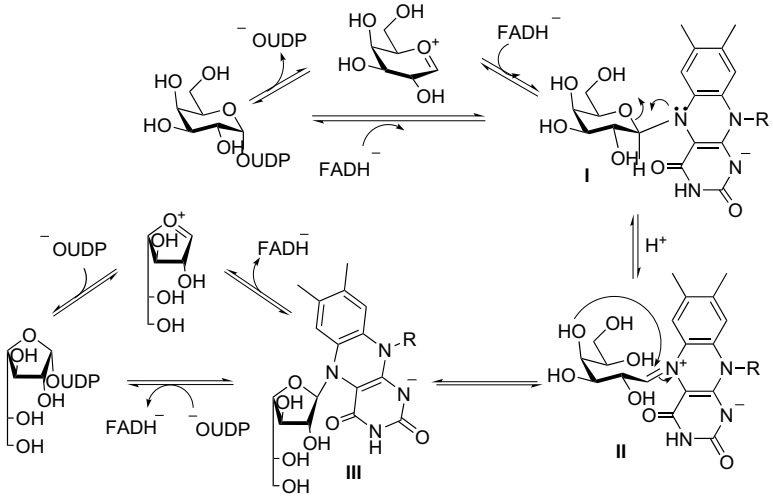
In connection with previous work aimed at the design of new UDP-Gal*f* mimics,[9d,e] we decided to investigate the utility of the SmI_2-induced nitrone *umpolung*[13] to access a new class of potential UGM inhibitors, based on the success of this synthetic methodology in the synthesis of the polyhydroxylated alkaloid (+)-hyacinthacine A_2.[14]



Scheme 3. Synthetic route to *C*-substituted β-D-1,4-dideoxy-1,4-imino-galactitol derivatives. Reagents and conditions: (a) ethyl acrylate, SmI_2/H_2O, THF, −78 °C, 3 h; (b) SmI_2/H_2O, THF, rt, 3 h; (c) K_2CO_3, $EtOH/H_2O$, 60 °C, 5 h; (d) $LiAlH_4$, THF, 66 °C, 5 h.

Nitrone **3** was prepared as previously described[9e,15] and was used as a key intermediate for the present synthesis of new 1-*C*-substituted 1,4-dideoxy-1,4-imino-D-galactitol derivatives (Scheme 3). When treated with samarium diiodide[16] (3 equiv) at low temperature, **3** was reduced to an α-amino radical species, that was trapped in situ with ethyl acrylate (1.4 equiv). As noticed before, the reaction was faster and better yielding when conducted in the presence of water (8 equiv).[13b,17] The role of this additive could stand in the protonation of intermediates, resulting in equilibrium displacement toward formation of the product. A new C–C bond was thus formed in the pseudoanomeric position, to afford *N*-hydroxypyrrolidine **4** in good yield (68%). Remarkably, the diastereoselectivity of the reaction is excellent as the minor isomer, if formed at all, could not be detected from NMR analysis of the crude material. The relative configuration at the new stereogenic center in **4** could not be assigned at this stage; however it was deduced from the structure of a cyclized derivative (vide infra).

N-Hydroxypyrrolidine **4** was next deoxygenated using samarium diiodide at room temperature to yield the corresponding pyrrolidine **5**. The latter proved quite stable and no spontaneous cyclization to the corresponding lactame was observed on standing at room temperature. Its cyclization was carried out by heating in basic medium (K_2CO_3, ethanol/water, 60 °C) to yield the pyrrolizidinone **6** in 71% yield. Reduction of **6** with lithium aluminum hydride afforded the pyrrolizidine **7**, on which



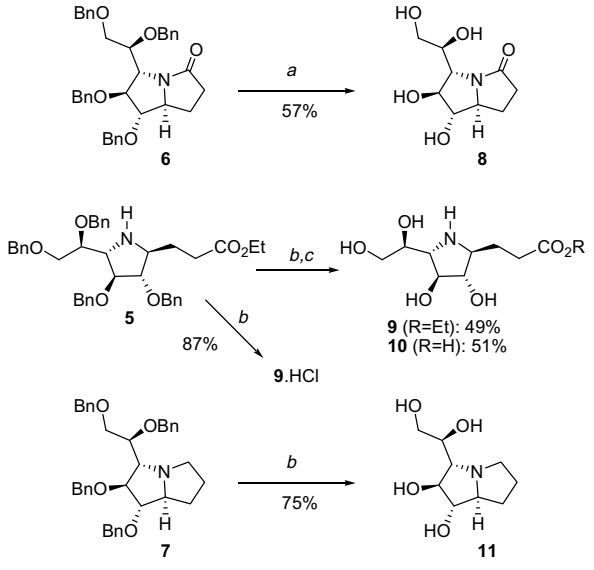
Scheme 2. Mechanistic hypotheses for UDP-Gal*p*/UDP-Gal*f* isomerization.

NOE studies allowed unambiguous assignment of the configuration at C-7a. Irradiation at the frequency of signal H-7a resulted in enhancement of the H-2 signal, in favor of a *S* configuration at C-7a. This result shows that the acrylate addition, in the C–C bond formation, occurred on the *Si* face of the nitrone, opposite to both the C-3 benzyloxy group and the C-5 substituent (pyrrolidine numbering). This 'matching' double stereoinduction may be the reason for the very high diastereoselectivity in favor of the trans adduct.

The transformation of compounds **5**–**7** into iminogalactitol derivatives was then undertaken (Scheme 4). Although classical hydrogenolytic conditions (H_2, Pd/C) allowed the preparation of **8** from lactam **6** in 57% yield, debenzylation of **5** under the same conditions only led to a complex mixture of products. The cleavage of benzylic ethers in **5** and **7** was thus performed using BCl_3. In the case of the debenzylation of **5**, initial attempt to neutralize the reaction mixture using a water suspension of Dowex 1X8 resin afforded substantial amount (51%) of the carboxylic acid **10** resulting from acid hydrolysis of the corresponding ethyl ester **9**. Finally the polyhydroxypyrrolizidine **11** could also be obtained in good yield (75%) using the same method.

In this work, because of the stereoselectivity observed in the key C–C bond forming step, 1-*C*-substituted 1,4-dideoxy-1,4-imino-D-galactitols were obtained with a β configuration at the pseudoanomeric center. Until now, a single report presents the synthesis of such D-galactofuranose mimics via a 1,3-dipolar cycloaddition of the nitrone **3** with an allylphosphonate partner.[9e]

The inhibitory activity of compounds **8**–**11** against UGM from *Escherichia coli* was then evaluated (Table 1). Inhibition assays on the purified enzyme were conducted in the reverse direction in which UDP-Gal*f* is converted to UDP-Gal*p* using two HPLC methods adapted from the work by Lee and co-workers.[18]



Scheme 4. Cleavage of benzyl ethers. Reagents and conditions: (a) H_2, Pd/C, HCl 6 M, MeOH, THF, rt, 4 days, then Dowex 1X8; (b) BCl_3, CH_2Cl_2, −78 to 0 °C, 15 h; (c) Dowex 1X8, H_2O.

Table 1. Inhibition of UGM from *Escherichia coli* by compounds **8**–**11**

Compound [I] = 25 mM	Residual activity[a]	
	Method 1	Method 2
8	65	57
9	64	57
10	78	81
11	64	57

[a] See Section 3.

The residual activities determined by method 1 are in good agreement with those of method 2. All iminogalactitol derivatives tested were found to exhibit moderate inhibition of UGM, including lactam **8**, in which the nitrogen atom cannot be protonated under the conditions of the assay. In this respect, and because of its constitution (the lactam carbonyl is not in the pseudoanomeric position), compound **8** cannot be included in the family of 'transition state analogue' inhibitors. Noticeably, compound **10** which carries a carboxylic acid function is the least active of this series. This is somewhat surprizing since the carboxylate could have been considered as a simple surrogate of the polar pyrophosphate group of the natural substrate, and a favorable interaction with a cationic residue at the UGM putative active site could have been expected.

Compounds **8**, **9**, and **11** exhibit modest activities on UGM in the same range (less than 50% inhibition at 25 mM) as other 1-*C*-substituted iminogalactitols exhibiting opposite configuration at the pseudoanomeric center.[19] This observation is in agreement with recent work suggesting that modifications of the galactose moiety in UDP-Gal*f* analogues are well tolerated while the UDP-core of the substrate seems necessary for better binding.[11c] Introduction of UDP-mimicking chains in β-1-*C*-substituted iminogalactitols is currently being studied in our laboratories.

3. Experimental

3.1. General experimental section

Reactions were performed under positive pressure of dry argon in oven-dried or flame-dried glassware equipped with a magnetic stirring bar. Standard inert atmosphere techniques were used in handling all air and moisture sensitive reagents. THF and CH_2Cl_2 were freshly distilled, respectively, from sodium and CaH_2. Reactions were monitored by thin layer chromatography (TLC) using aluminum-backed silica gel plates. Product purification by gravity column chromatography was performed using silica gel (70–230 mesh). Infrared (IR) spectra were obtained with a Fourier transform infrared spectrometer (FTIR) as neat films on sodium chloride plates. NMR chemical shifts δ are given in ppm, using tetramethylsilane as the reference. Coupling constants *J* are given with 0.5 Hz accuracy. In pseudo multiplets,

the reported J values are average values of measured constants. Mass spectra (MS) were obtained using DCI (ammonia/isobutane, 63/37).

3.2. 3-((2*S*,3*S*,4*S*,5*S*)-5-((*S*)-1,2-Bis(benzyloxy)ethyl)-3,4-bis(benzyloxy)-1-hydroxy-pyrrolidin-2-yl) propionic acid ethyl ester (4)

A stirred and carefully deoxygenated solution of nitrone **3**[9e] (102 mg, 0.19 mmol) in dry THF (4 mL) was cooled to −78 °C under argon. Freshly distilled ethyl acrylate (28 μL, 0.26 mmol), degassed water (27 μL, 1.50 mmol), and a 0.1 M solution of SmI_2 in THF (5.6 mL, 0.56 mmol) were then added. The temperature was kept at −78 °C during 3 h, then air was introduced in the reaction mixture until disappearance of its blue color. A saturated aqueous solution of $Na_2S_2O_3$ (10 mL) and ethyl acetate (30 mL) were then added; the yellow mixture was extracted with AcOEt (3× 30 mL), and the combined organic layers were washed with brine (30 mL), dried over $MgSO_4$, filtered, and concentrated under vacuum. Purification of the resulting residue by chromatography on silica gel (pentane/AcOEt, 4/1, then 1/1) afforded the expected *N*-hydroxyamino ester as a colorless oil (81 mg, 68%). LRMS (DCI) *m*/*z* (%): 640 (100) $[M+H]^+$. IR: ν_{max} (cm^{-1}) 3412, 3090, 3063, 3034, 2981, 2869, 1737. ^1H NMR (300 MHz, $CDCl_3$): δ 1.21 (t, 3H, J = 7.0 Hz); 1.76–1.87 (m, 1H); 2.11–2.23 (m, 1H); 2.40 (ps t, 2H, J = 7.0 Hz); 3.20–3.27 (m, 1H); 3.49 (ps t, 1H, J = 4.5 Hz); 3.67–3.78 (m, 3H); 3.96–4.19 (m, 4H); 4.30–4.54 (m, 7H), 4.74 (d, 1H, J = 12.0 Hz); 5.63 (br s, 1H); 7.11–7.53 (m, 20H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.2 (CH_3); 22.8 (CH_2); 32.0 (CH_2); 60.4 (CH_2); 70.0 (CH); 71.4 (CH_2); 71.7 (CH_2); 71.9 (CH_2); 72.8 (CH); 72.9 (CH_2); 73.3 (CH_2); 78.4 (CH); 85.5 (CH); 86.7 (CH); 127.5–128.3 (CH); 138.1 (C_q); 138.3 (C_q); 138.4 (C_q); 174.2 (C═O). Anal. Calcd for $C_{39}H_{45}NO_7$: C, 73.22; H, 7.09; N, 2.19. Found: C, 73.16; H, 7.01; N, 2.15.

3.3. 3-((2*S*,3*S*,4*S*,5*S*)-5-((*S*)-1,2-Bis(benzyloxy)ethyl)-3,4-bis(benzyloxy)pyrrolidin-2-yl) propionic acid ethyl ester (5)

To a stirred and carefully deoxygenated solution of *N*-hydroxypyrrolidine ester **4** (105 mg, 0.16 mmol) in dry THF (3 mL), degassed water (24 μL, 1.31 mmol) and a 0.1 M solution of SmI_2 in THF (4.9 mL, 0.49 mmol) were added under argon. The solution was stirred at room temperature during 30 min. Then air was introduced in the reaction mixture until disappearance of its blue color, whereupon a saturated aqueous solution of $Na_2S_2O_3$ (10 mL) and ethyl acetate (30 mL) were added. The yellow mixture was extracted with AcOEt (3× 30 mL) and the combined organic layers were washed with brine (30 mL), dried over $MgSO_4$, filtered, and concentrated under vacuum. Purification of the resulting residue by chromatography on silica gel (pentane/AcOEt, 4/1, then 1/1) afforded the expected pyrrolidine ester **5** as a colorless oil (70 mg, 68%). $[\alpha]_D^{20}$ − 25 (c 1.00, $CHCl_3$); LRMS (DCI) *m*/*z* (%): 624 (100) $[M+H]^+$; 578 (85) $[M+H-EtOH]^+$. IR: ν_{max} (cm^{-1}) 3061, 3028, 2920, 2862, 1728. ^1H NMR (300 MHz, $CDCl_3$): δ 1.22 (t, 3H, J = 7.5 Hz); 1.71–1.88 (m, 2H); 2.19 (br s, 1H); 2.25–2.44 (m, 2H); 3.05–3.11 (m, 1H); 3.20 (ps t, 1H, J = 5.0 Hz,); 3.65–3.74 (m, 4H); 3.88 (dd, 1H, J = 3.0, 5.5 Hz); 4.09 (q, 2H, J = 7.0 Hz); 4.39 (d, 1H, J = 12.0 Hz); 4.36–4.54 (m, 6H); 4.74 (d, 1H, J = 11.5 Hz); 7.26–7.32 (m, 20H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.2 (CH_3); 28.8 (CH_2); 31.5 (CH_2); 60.2 (CH_2); 61.3 (CH); 63.4 (CH); 71.4 (CH_2); 71.9 (CH_2); 72.0 (CH_2); 73.0 (CH_2); 73.3 (CH_2); 76.8 (CH); 86.0 (CH); 89.6 (CH); 127.5–128.3 (CH); 138.2 (Cq); 138.3 (Cq); 173.5 (C═O). Anal. Calcd for $C_{39}H_{45}NO_6$: C, 75.09; H, 7.27; N, 2.25. Found: C, 75.10; H, 7.14; N, 2.19.

3.4. (5*S*,6*S*,7*S*,7a*S*)-5-((*S*)-1,2-Bis(benzyloxy)ethyl)-6,7-bis(benzyloxy)-hexahydropyrrolizin-3-one (6)

Compound **5** (46 mg, 0.07 mmol) was dissolved in ethanol (8 mL) and treated with a solution of potassium carbonate (12 mg, 0.11 mmol) in water (1 mL) at 60 °C temperature during 5 h. The mixture was then concentrated under vacuum, and the residue was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under vacuum. Purification of the resulting residue by chromatography on silica gel (pentane/AcOEt, 4/1, then 1/1) afforded the expected pyrrolizidinone **6** as a colorless oil (30 mg, 71%). $[\alpha]_D^{20}$ + 3 (c 0.98, $CHCl_3$); LRMS (DCI) *m*/*z* (%): 578 (100) $[M+H]^+$. IR: ν_{max} (cm^{-1}), 3057, 3028, 2920, 2866, 1691. ^1H NMR (300 MHz, $CDCl_3$): δ 1.76–1.89 (m, 1H); 2.17–2.39 (m, 2H); 2.56 (td, 1H, J = 10.0, 17.0 Hz); 3.65 (dd, 1H, J = 5.5, 8.0 Hz,); 3.69–3.78 (m, 3H); 3.96 (ps q, 1H, J = 8.0 Hz); 4.01 (dd, 1H, J = 2.0, 4.0 Hz); 4.29 (dd, 1H, J = 4.0, 5.0 Hz); 4.39 (d, 1H, J = 11.5 Hz); 4.46 (d, 1H, J = 11.5 Hz); 4.48 (d, 1H, J = 11.5 Hz); 4.52 (d, 2H, J = 11.5 Hz); 4.59 (d, 1H, J = 12.0 Hz); 4.60 (d, 1H, J = 11.5 Hz); 4.79 (d, 1H, J = 11.5 Hz); 7.17–7.39 (m, 20H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.8 (CH_2); 32.5 (CH_2); 60.6 (CH); 64.2 (CH); 72.1 (CH_2); 72.2 (CH_2); 72.2 (CH_2); 73.4 (CH_2); 73.6 (CH_2); 79.1 (CH); 87.8 (CH); 89.6 (CH); 127.5–128.4 (CH); 137.7 (C_q); 137.9 (C_q); 138.2 (C_q); 138.4 (C_q); 175.3 (C═O). Anal. Calcd for $C_{37}H_{39}NO_5$: C, 76.92; H, 6.80; N, 2.42. Found: C, 77.31; H, 7.07; N, 2.61.

3.5. (1*S*,2*S*,3*S*,7a*S*)-3-((*S*)-1,2-Bis(benzyloxy)ethyl)-1,2-bis(benzyloxy)-hexahydro-1*H*-pyrrolizine (7)

A solution of pyrrolizidinone **6** (90 mg, 0.16 mmol) in THF (5 mL) was cooled to 0 °C under argon, then lithium aluminum hydride (10 mg, 0.26 mmol) was added. The reaction mixture was stirred at 66 °C during 5 h then it was quenched with water (10 μL), an aqueous 15% solution of NaOH (10 μL), and water (40 μL) and stirred during 1 h. Then sodium sulfate was added, the mixture was stirred during 1 h and filtered through Celite. The filtrate was concentrated under vacuum to give a residue, which upon column chromatography over basic alumina (pentane/AcOEt, 9/1, 4/1, 1/1, then 0/1) yielded the pyrrolizidine **7** as a colorless oil (55 mg; 63%). $[\alpha]_D^{20}$ − 11 (c 1.00, $CHCl_3$); LRMS (DCI) *m*/*z* (%): 564 (100) $[M+H]^+$. IR: ν_{max} (cm^{-1}) 3060, 3031, 2926, 2866.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.61–1.69 (m, 1H); 1.74–1.83 (m, 2H); 1.93–2.04 (m, 1H); 2.58–2.66 (m, 1H); 2.93–3.04 (m, 2H); 3.46 (ps q, 1H, J = 7.5 Hz); 3.66–3.72 (m, 3H); 3.75 (t, 1H, J = 7.0 Hz); 4.15 (ps t, 1H, J = 6.5 Hz); 4.33 (d, 1H, J = 11.5 Hz); 4.45–4.64 (m, 6H); 4.74 (d, 1H, J = 12.0 Hz); 7.21–7.50 (m, 20H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.2 (CH_2); 31.5 (CH_2); 56.7 (CH_2); 67.1 (CH); 69.8 (CH); 71.4 (CH_2); 71.8 (CH_2); 72.3 (CH_2); 73.0 (CH_2); 73.3 (CH_2); 78.2 (CH); 85.2 (CH); 88.9 (CH); 127.4–128.4 (CH); 138.3 (C_q); 138.3 (C_q); 138.5 (C_q); 138.7 (C_q). Anal. Calcd for $C_{37}H_{41}NO_4$: C, 78.83; H, 7.34; N, 2.48. Found: C, 78.49; H, 7.74; N, 2.62.

3.6. (5*S*,6*S*,7*S*,7a*S*)-Hexahydro-6,7-dihydroxy-5-((*S*)-1,2-dihydroxyethyl)pyrrolizin-3-one (8)

To a solution of pyrrolizidinone **6** (103 mg, 0.14 mmol) in a 4:1 mixture of methanol and THF (12.5 mL) was added Pd/C 10% (31 mg). After the reaction flask was purged with hydrogen, 13 drops of HCl 6 M were added and the reaction mixture was stirred for 4 days at room temperature under hydrogen (1 atm). The mixture was then filtered through Celite, and the filtrate was concentrated under vacuum. The residue was dissolved in a minimum of water and neutralized with DOWEX 1X8 resin (OH^- form). After filtration, the filtrate was concentrated under vacuum to give a residue, which upon column chromatography over silica gel (CH_2Cl_2/MeOH/NH_4OH: 98/2/0, 95/5/0, 90/10/0, 80/20/0, then 3/2/0.5) yielded the deprotected pyrrolizidinone **8** (22 mg, 57%) as a colorless oil. $[\alpha]_D^{20}$ + 18 (*c* 0.65, MeOH). LRMS (DCI) *m/z* (%): 218 (100) $[M+H]^+$. IR: ν_{max} (cm^{-1}) 3326, 2925, 2883, 1659. ^{1}H NMR (300 MHz, CD_3OD): δ 1.93–2.04 (m, 1H); 2.31–2.46 (m, 2H); 2.70 (td, 1H, J = 10.0, 17.0 Hz); 3.53–3.58 (m, 3H); 3.62 (dd, 1H, J = 3.0, 6.0 Hz); 3.77–3.84 (m, 2H); 4.30 (ps t, 1H, J = 6.5 Hz). ^{13}C NMR (75 MHz, CD_3OD): δ 25.0 (CH_2); 33.4 (CH_2); 63.6 (CH); 65.0 (CH_2); 66.3 (CH); 72.9 (CH); 81.0 (CH); 82.9 (CH); 179.9 (C=O).

3.7. 3-((2*S*,3*S*,4*S*,5*S*)-3,4-dihydroxy-5-((*S*)-1,2-dihydroxyethyl)pyrrolidin-2-yl) propanoic acid ethyl ester (9)

To a stirred solution of pyrrolidine ester **5** (61 mg, 0.10 mmol) in CH_2Cl_2 (10 mL) cooled to −78 °C, under argon, a solution of BCl_3 (1 M in hexanes, 1.20 mL, 1.2 mmol) was added at −78 °C. The temperature was slowly raised to 0 °C overnight. After 15 h, ethanol was added and the reaction mixture was concentrated under vacuum. The residue was purified by chromatography on silica gel (CH_2Cl_2/MeOH: 90/10, then 80/20) to afford the deprotected polyhydroxylated pyrrolidine ester **9** as its hydrochloride salt (23 mg, 87%): $[\alpha]_D^{20}$ − 33 (*c* 0.98, MeOH). LRMS (DCI) *m/z* (%): 264 (100) $[M+H]^+$. IR: ν_{max} (cm^{-1}) 3446, 2985, 2925, 1720. ^{1}H NMR (300 MHz, CD_3OD): δ 1.26 (t, 3H, J = 7.0 Hz); 2.01–2.2 (m, 2H); 2.58 (td, 2H, J = 1.5, 7.5 Hz); 3.33–3.38 (m, 1H); 3.46 (dd, 1H, J = 4.5, 7.5 Hz); 3.63–3.73 (m, 2H); 3.82 (dd, 1H, J = 6.5, 8.0 Hz); 3.92 (q, 1H, J = 4.5 Hz); 4.04 (ps t, 1H, J = 6.5 Hz,); 4.16 (q, 2H, J = 7.0 Hz). ^{13}C NMR (75 MHz, CD_3OD): δ 14.5 (CH_3); 27.1 (CH_2); 31.4 (CH_2); 61.9 (CH_2); 62.9 (CH); 64.9 (CH_2); 65.1 (CH); 69.3 (CH); 77.2 (CH); 80.1 (CH); 174.3 (C=O).

3.8. 3-((2*S*,3*S*,4*S*,5*S*)-3,4-Dihydroxy-5-((*S*)-1,2- dihydroxyethyl)pyrrolidin-2-yl)propanoic acid (10)

To a stirred solution of pyrrolidine ester **5** (92 mg, 0.15 mmol) in CH_2Cl_2 (15 mL) cooled to −78 °C, under argon, a solution of BCl_3 (1 M in hexanes, 1.8 mL, 1.80 mmol) was added at −78 °C. The temperature was slowly raised to 0 °C overnight. After 15 h, ethanol was added and the reaction mixture was concentrated under vacuum. The residue was dissolved in a minimum of water and neutralized with DOWEX 1X8 (OH^- form). After filtration, the filtrate was concentrated under vacuum and purification of the resulting residue by chromatography on silica gel (CH_2Cl_2/MeOH: 90/10, 80/20, then 70/30) afforded the deprotected pyrrolidine acid **10** (18 mg, 51%) and the pyrrolidine ester **9** (16 mg, 49%), both as colorless oils. Compound **10**: $[\alpha]_D^{20}$ − 20 (*c* 1.01, MeOH). LRMS (DCI) *m/z* (%): 236 (20) $[M+H]^+$; 218 (100) $[M+H-H_2O]^+$. IR: ν_{max} (cm^{-1}) 3333, 2921, 1722. ^{1}H NMR (300 MHz, CD_3OD): δ 2.06 (ps t, 2H, J = 6.0 Hz); 2.39–2.62 (m, 2H); 3.30–3.39 (m, 1H); 3.44 (dd, 1H, J = 4.0, 8.0 Hz); 3.64–3.66 (m, 2H); 3.87 (t, 1H, J = 6.5 Hz); 3.92 (dd, 1H, J = 5.0, 9.0 Hz); 4.04 (dd, 1H, J = 6.5, 8.0 Hz). ^{13}C NMR (75 MHz, CD_3OD): δ 27.0 (CH_2); 34.5 (CH_2); 63.9 (CH); 64.0 (CH); 64.8 (CH_2); 69.2 (CH); 77.2 (CH); 79.7 (CH).

3.9. (1*S*,2*S*,3*S*,7a*S*)-Hexahydro-3-((*S*)-1,2-dihydroxyethyl)-1*H*-pyrrolizine-1,2-diol (11)

To a stirred solution of pyrrolizidine **7** (38.3 mg, 0.068 mmol) in CH_2Cl_2 (7 mL) cooled to −78 °C, under argon, a solution of BCl_3 (1 M in hexanes, 820 μL, 0.820 mmol) was added at −78 °C. The temperature was slowly raised to 0 °C overnight. After 15 h, methanol was added and the reaction mixture was concentrated under vacuum. The residue was dissolved in a minimum of water and neutralized with DOWEX 1X8 (OH^- form). After filtration, the filtrate was concentrated under vacuum and purification of the resulting residue by chromatography on silica gel (CH_2Cl_2/MeOH: 90/10, then 80/20) afforded the deprotected polyhydroxypyrrolizidine **11** (10.3 mg, 75%) as a colorless oil. $[\alpha]_D^{20}$ − 11 (*c* 1.54, MeOH). LRMS (DCI) *m/z* (%): 204 (100) $[M+H]^+$. IR: ν_{max} (cm^{-1}) 3300, 2962. ^{1}H NMR (300 MHz, CD_3OD): δ 2.03–2.21 (m, 4H); 3.40–3.46 (m, 2H); 3.54–3.61 (m, 1H); 3.66 (dd, 1H, J = 5.5, 11.5 Hz); 3.72 (dd, 1H, J = 5.0, 11.5 Hz); 3.84–3.95 (m, 2H); 3.98 (ps q, 1H, J = 5.0 Hz); 4.07 (ps t, 1H, J = 7.0 Hz). ^{13}C NMR (75 MHz, CD_3OD): δ 25.7 (CH_2); 29.8 (CH_2); 59.1 (CH_2); 64.6 (CH_2); 69.9 (CH); 72.8 (CH); 74.6 (CH); 78.2 (CH); 79.7 (CH).

3.10. Inhibition assay

UDP-galactopyranose mutase (UGM) was purified according to the previously reported procedure.[8c] Iminogalactitol derivatives (25 mM) were individually pre-

incubated with UGM (8.2 nm) for 10 min at room temperature in 50 μL of 100 mM potassium phosphate buffer (pH 7.5). After the pre-incubation, 10 μL of freshly prepared sodium dithionite (100 mM) was added to the solution to reduce UGM and then 20 μL of UDP-galactofuranose (UDP-Gal*f*, 0.2 mM) was added as a substrate. The concentrations of sodium dithionite and UDP-Gal*f* in the reaction mixture were 12.5 mM and 50 μM, respectively. The reaction was carried out at 37 °C for 2 min, and the resulting mixture was immediately frozen by liquid nitrogen to terminate the reaction. The reaction mixture was analyzed by HPLC using a C_{18} column (Microsorb-MV, Varian, 4.6×250 mm) with the detector set at 262 nm. Two eluting methods were examined to determine the conversion of UDP-Gal*f* to the product UDP-galactopyranose (UDP-Gal*p*).

3.10.1. Method 1. Mobile phase was 0.5% acetonitrile in 50 mM triethylammonium acetate buffer, pH 6.8. Flow rate was at 1.0 mL/min. Under these conditions, UDP-Gal*p* and UDP-Gal*f* were eluted at 6.1 and 7.6 min, respectively.

3.10.2. Method 2. *Mobile phase A:* 50 mM potassium phosphate buffer (pH 7.0) containing 2.5 mM tetrabutylammonium hydrogen sulfate (TBAHS). *Mobile phase B:* 50% acetonitrile in 50 mM potassium phosphate buffer (pH 7.0) containing 2.5 mM TBAHS.[20] The sample was loaded on the column and eluted isocratically with mobile phase A containing 4% mobile phase B. The elution rate was 0.55 mL/min. Under these conditions, UDP-Gal*p* and UDP-Gal*f* were eluted at 10.9 and 12.6 min, respectively.

The residual activity of UGM in the presence of individual iminogalactitol derivative was evaluated by dividing the conversion in the presence of each derivative with that in the absence of the derivatives. The results are summarized in Table 1.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.06.059.

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