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Multi gram-scale synthesis of galactothionolactam and its transformation into a galactonoamidine

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ABSTRACT

We recently proposed to conduct selective glycosylation reactions after in situ activation of a glycosyl donor promoted by a transition metal complex immobilized in a macromolecular matrix. In order to develop this catalytic entity, a feasible multi gram-scale synthesis for 2,3,4,6-tetra-*O*-benzyl-D-galactothionolactam, its transformation into galactonoamidines with aromatic aglycon, and subsequent debenzylation conditions were developed. The potential for epimerization reactions at C-2 of the glycosidic ring during the transformations from the 2,3,4,6-tetra-*O*-benzyl-D-galactonolactam into the *N*-benzyl-2,3,4,6-tetra-*O*-benzyl-D-galactonoamidines via the 2,3,4,6-tetra-*O*-benzyl-D-galactothionolactam are discussed and additionally characterized by using density functional theory calculations.

1. Introduction

A major obstacle to advances in glycobiology is the lack of pure and structurally well-defined carbohydrates and glycoconjugates.[1] Enzymatic approaches toward synthesizing these compounds are often compromised by low regioselectivity,[2] moderate thermal stability, narrow pH profiles of enzyme activity and/or low yields;[2] on the other hand, conventional multi-step syntheses involve numerous and frequently time-consuming protection/deprotection steps as well as obligatory activation of glycosyl donors. The usefulness of these synthetic methods is in addition compromised by the fact that many glycosylation reactions give mixtures of the two possible anomers.[1] If these anomers are not separated satisfactorily after each glycosylation step, complex mixtures of products are obtained in insufficient purity that cannot be used for biological studies.[1]

To overcome these shortcomings, we proposed a unique approach for selective glycosylation reactions, which relies on the in situ activation of a glycosyl donor promoted by a transition metal complex immobilized in a macromolecular matrix. This catalytic entity could be prepared by templating a suitable compound resembling features of the transition state of the glycoside hydrolysis in a polymer matrix. To show proof of concept, we are in need of such compound in gram amounts, and therefore directed initial focus on synthesizing (3*S*,4*S*,5*S*,6*R*,*Z*)-2-(benzylimino)-6-(hydroxymethyl) piperidine-3,4,5-triol, *N*-benzylgalactonoamidine **1**, as compound encompassing characteristics of the enzymatic galactoside hydrolysis (Scheme 1).[3–6]

Gluco- and mannoamidines have been reported previously as potent transition state analogs and competitive inhibitors of corresponding glycosidases.[7–12] Comparable entities with the galacto-configuration are not described yet. However, the galacto-configuration is preferable over a gluco-configuration for its intended use as template due to the structural constraints imposed by the axial hydroxyl group at C-4. While the benzyl group in **1** might not resemble the phenolate group liberated from a phenylglycoside substrate during enzymatic hydrolysis, the methylene group in the benzyl aglycon should provide additional space during the envisaged template polymerization accounting for the lengthening of the C···O bond during the catalytic reaction.

Although some procedures for the synthesis of glycosylamidine including aliphatic derivatives of glucoamidine and mannoamidine were reported,[9,12] we found several obstacles in our efforts to adapt this work for a multi gram-scale synthesis of galactothionolactam as a precursor for a galactonoamidine. Particularly, the previously suggested epimerization at C-2 during the synthesis of the glycosylthionolactam intermediate appears questionable for derivatives with the galacto-configuration.[12,13] Along with the synthesis of **1**, an optimized multi gram-scale synthesis for its key intermediate, galactothionolactam, is reported. In addition, debenzylation conditions with iron(III) chloride versus the use of hydrogen gas and palladium on charcoal are explored, and computational data on putative epimerization reactions are provided.

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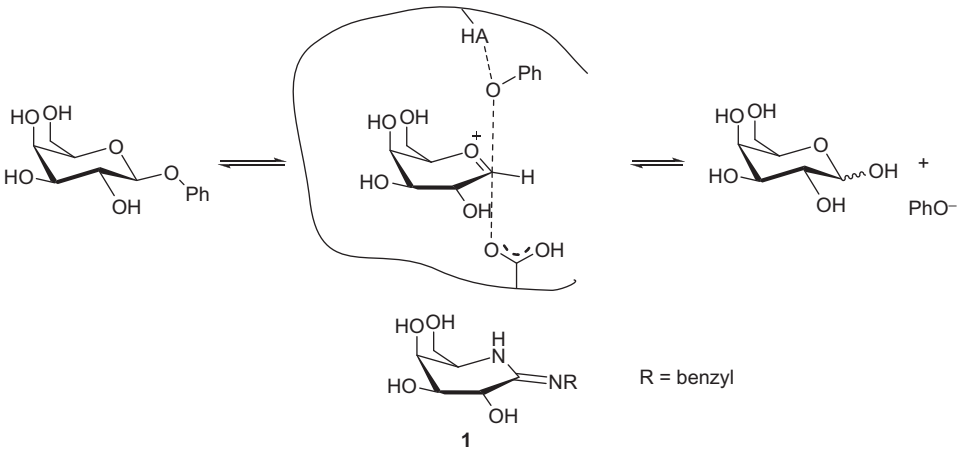


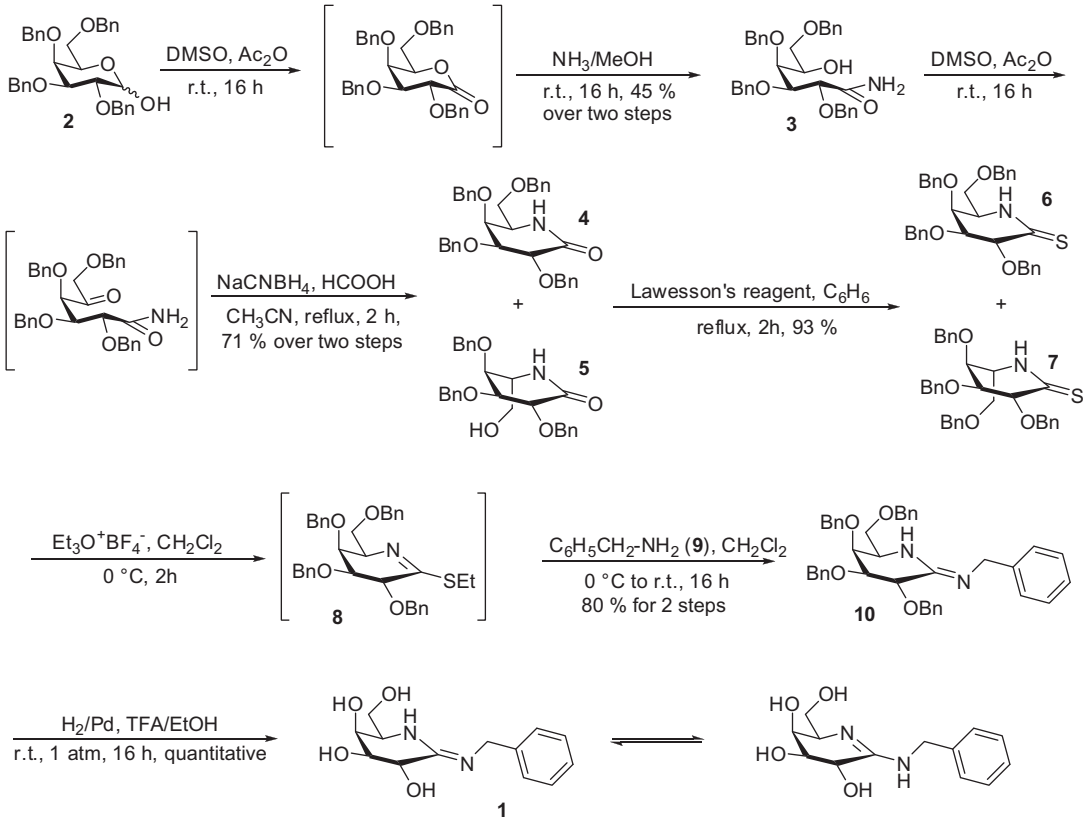
Scheme 1. Model reaction of the enzymatic galactoside hydrolysis indicating the first transition state and a putative structure for an enzyme inhibitor **1**.

2. Results and discussion

2.1. Multi-gram scale synthesis of 2,3,4,6-tetra-*O*-benzyl-D-galactothionolactam (6)

Following a procedure previously outlined by Overkleeft, 2,3,4,6-tetra-*O*-benzyl-D-galactose (**2**) was transformed via 2,3,4,6-tetra-*O*-benzyl-galactoamide (**3**) into 2,3,4,6-tetra-*O*-benzyl-D-galactonolactam (**4**);[14] lactam **4** was obtained as a colorless oil after flash chromatography (Scheme 2).[14] The overall yield for the multi gram-scale synthesis of **4** from **2** is 31%, and thus only slightly lower than the transformation described for a small-scale synthesis (43% overall yield of **4**).[14]

Analysis of the ^{1}H NMR spectrum of the residue after chromatographic purification revealed the presence of **4** as a major product. Its identity was confirmed by ^{1}H and ^{13}C NMR spectroscopy, and high resolution ESI mass spectrometry (see Supplementary data).[14] In contrast to literature reports,[14] the ESI mass spectrum of the raw material indicates the presence of another compound with the same molar mass as **4**, which is tentatively assigned to the C-5 epimer of **4**, 2,3,4,6-tetra-*O*-benzyl-L-altronolactam (**5**). The presence of **5** is furthermore evident after the integration of two doublets of doublets for the protons H-3 at 3.91 ppm (major product, **4**) and H-3 at 3.85 ppm (minor product, **5**) in the ^{1}H NMR spectrum. The splitting of the signal for H-3 of **4** indicates a *cis*-coupling to H-4 at 4.05 ppm ($J_{3,4}$ = 1.9 Hz), and a *trans*-coupling to H-2 at



Scheme 2. Synthesis of *N*-benzylgalactonoamidine **1**.

4.42 ppm ($J_{3,2}$ = 9.1 Hz); identical shifts and coupling constants between the protons H-2, H-3 and H-4 have been previously assigned to **4**.[15,14] The coupling constants for the signal of H-3 at 3.85 ppm (dd, 8.5, 2.1 Hz) for the minor product **5** reveal the same configuration of the protons at H-2, H-3 and H-4 as in **4**. The proton H-3 in 2,3,4-6-tetra-*O*-benzyl-L-altrose was previously described with a similar chemical shift and related coupling constants ($\delta_{H\text{-}3}$ 3.99 ppm; dd, 10.1, 2.7 Hz) as H-3 in **5**.[16] Lactam **5** is observed as the minor product in a 3:2 molar ratio before, and in a 10:1 molar ratio after chromatographic purification.

Hypothetical side products resulting from partial benzylation of **4** that might cause comparable chemical shifts of the proton signals in the ^{1}H NMR spectrum were not considered due to the mass identity for the compounds found in the raw material of the reaction mixture by ESI-MS. Although the formation of the L-epimer **5** would be expected during the ring closure reaction starting from **3**, it was not described for the small-scale synthesis of **4**.[14] Complete elimination of the diasteriomeric side product **5** during the purification of **4** by repetitive flash column chromatography over silica gel was not possible despite all efforts. A very small amount of an additional unidentified impurity also remained.

Subsequently, galactonolactam **4** was transformed into 2,3,4,6-tetra-*O*-benzyl-D-galactothionolactam (**6**) with Lawesson's reagent in 93% yield for a 10 g-scale synthesis following the small-scale transformation of **4** into **6** as described by Vasella and co-workers (Scheme 2).[17] Likewise, lactams with gluco- and manno-configuration were transformed into corresponding thiolactams.[12,13] However, the latter reports imply a possible epimerization at the carbon C-2 for derivatives with gluco-configuration during the reaction,[12,13] for which we did not obtain any evidence when transforming **4** into **6**. Instead, the side product **5**, obtained during the synthesis of **4**, is converted in the corresponding 2,3,4,6-tetra-*O*-benzyl-L-altrothionolactam (**7**) as concluded after analysis of ^{1}H NMR and ESI MS spectra. Attempts to purify **6** on silica gel following literature procedures resulted in compound decomposition,[13] and were consequently not further pursued. Instead, galactothionolactam **6** was efficiently purified by flash chromatography on basic aluminum oxide applying gradient elution using cyclohexane and EtOAc. Although the procedure removed some colored, unpolar impurities, the side product **7** was not eliminated. The identity of **6** with 2,3,4,6-tetra-*O*-benzyl-D-galactothionolactam described by Vasella and co-workers,[17] was confirmed by NMR and ESI mass spectrometry (see Supplementary data).

2.2. Synthesis of *N*-benzyl-2,3,4,6-tetra-*O*-benzyl-D-galactonoamidine (10)

In situ activation of **6** with Meerwein's salt afforded 2,3,4,6-tetra-*O*-benzyl-D-galactoiminothioether (**8**) as an intermediate that was not isolated from the solution,[12] but characterized by ESI mass spectrometry (see Supplementary data). The reaction of **8** with dried benzylamine (**9**) in the cold provided *N*-benzyl-2,3,4,6-tetra-*O*-benzyl-D-galactonoamidine (**10**) after purification of the residue of the reaction mixture by flash chromatography over aluminum oxide. Efforts to follow the procedures described for the purification of the corresponding aliphatic gluconoamidines over silica gel resulted in compound decomposition,[12] and were thus not further pursued.

Surprisingly, galactonoamidine **10** was isolated as the sole product of the reaction. The conclusion is based on the observation of only one signal for the proton H-3 appearing as a doublet of doublets at 4.04 ppm with a coupling constant of 9.7 Hz in the ^{1}H NMR spectrum of **10** prior to and after chromatographic purification. Evidence for the presence of C-5 or C-2 epimers was not obtained at any time indicating that **7** might have not reacted. Furthermore, the use of up to 4 mol equiv of **9** in respect to **8** did not cause any apparent epimerization of the product **10** nor was the product yield significantly increased. This finding opposes previous reports for the transformation of iminothioethers with the gluco-configuration describing epimerization of the reaction products and increasing yields in dependence of the molar amounts of aliphatic amine used.[11,12] To explore our findings further and to rationalize the lack of epimerization at C-2 during the reaction of **8** with **9**, we examined the stability of the D- and L-lactams, and of the 2-epimers of the corresponding thiolactams with a computational approach using density functional theory (see below).

2.3. Synthesis of *N*-benzyl-D-galactonoamidine

2.3.1. Debenzylation reactions with hydrogen and Pd on charcoal

Our early attempts for the debenzylation of **10** to yield **1** relied on a procedure described for the debenzylation of tetrabenzylgluco- and manno-derivatives in the presence of 30% palladium on charcoal at 1 atm;[12] however, the procedure failed to provide **1** from **10** even when using 1 or 2 wt equiv of the catalyst nor did the use of higher pressures of hydrogen gas (10 and 25 atm) afford **1**. Nevertheless, the debenzylation of galactonolactam (**4**) proceeds without difficulty under the described conditions.[12]

The failure of the debenzylation reaction of **10** is presumably due to coordination of palladium to the amidine site in **10**. The formation of a Pd-**10** complex (Chart 1) was evident from the analysis of ^{1}H and ^{13}C NMR spectra of a sample aliquot taken from the reaction mixture after 24 h. The ^{13}C signals for the imino carbon and the aliphatic carbon atom of the *N*-benzyl group are shifted downfield by 4.5 ppm and 2.9 ppm, respectively, in comparison to the corresponding ^{13}C signals of the perbenzylated amidine **10** (see Supplementary data); the proton signals of H-2, H-3, H-4 and H-5 appear as broad singlets (spectrum not shown). The integration of the proton signals in the aromatic region indicates furthermore the presence of *O*-benzyl groups and thus an unsuccessful debenzylation of **10**. The residue obtained after evaporation of the solvent was not soluble in deuterium oxide, and the NMR spectra were taken in $CDCl_3$ instead. Last, the TLC analysis of a sample aliquot on silica gel with cyclohexane–EtOAc (1:1, v/v) as eluent indicated complete transformation of the starting material **10** (R_f = 0.55) toward a slightly less polar compound (R_f = 0.6). These combined observations provide evidence for the coordination of the palladium catalyst to the amidine function in **10**, which prevents the catalytic hydrogenolysis to proceed.

As a consequence, the hydrogenation of **10** with 30% Pd on charcoal was explored in the presence of anhydrous hydrochloric acid in ethanol to preclude Pd binding to the amidine function after protonation.[18] However, the reaction failed under these conditions as well, and only the formation of a Pd-**10** complex was observed. The debenzylation of **10** was eventually achieved by using 2 wt equiv of Pd on charcoal in the presence of trifluoroacetic acid obtaining **1** quantitatively for reactions in a 30–100 mg scale. Surprisingly, **1** is stable under these strongly acidic conditions. Attempts to use stoichiometric or catalytic amounts of palladium on charcoal in the presence of TFA were futile. Consequently, the use of neat TFA for the debenzylation of **10** was not explored.[19]

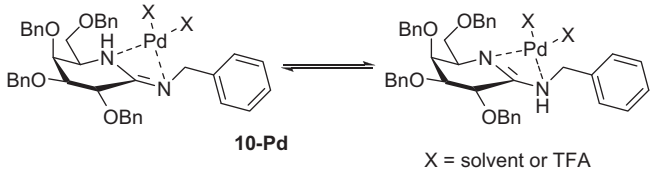


Chart 1. Putative structure of the Pd-**10** complex.

Partial O-debenzylation of **10**, N-debenzylation or a cyclization at O-4 with loss of the iminopiperidine substructure of **1** could come into mind considering the harsh reaction conditions (Chart 2). However, none of the resulting hypothetical structures **1a–c** is supported by the obtained NMR data for **1**.

The ^{13}C signals for the methylene carbon atoms of the *O*-benzyl groups in **10** are identified between 70 and 75 ppm, and are thus significantly different from the ^{13}C signal of the methylene carbon atom of the *N*-benzyl group at 45.0 ppm. This signal assignment was based on a ^{13}C DEPT experiment to distinguish the methylene carbon atoms from ring carbon atoms. Although the removal of the *N*-benzyl group of **10** might appear facile under the applied harsh conditions, the ^{13}C signal at 45.4 ppm in the NMR spectrum of **1** strongly supports the presence of the *N*-benzyl group. The integration of the proton signals in the aromatic region of the ^{1}H NMR spectrum of **1**, and the absence of the methylene carbon atoms of the *O*-benzyl groups in the ^{13}C NMR spectrum of **1** furthermore indicate the complete removal of all *O*-benzyl groups (see Supplementary data). Consequently, any compound representing partial O-debenzylation of **10**, such as **1a**, is not considered any further.

Likewise, the iminopiperidine substructure of **1** has to be considered intact due to the identified ^{13}C signal of the imino group at 155.9 ppm. If a bicyclic substructure **1b** with cyclization at O-4 was formed after the debenzylation of **10**, the hybridization of C-1 would change, and the ^{13}C signal for the imino carbon atom would disappear. Again, the obtained ^{13}C NMR data do not support this hypothesis. The ^{13}C signal of the carbon atom in the C=N group in a furanolactam derivative with a structure related to **1c** has been previously described by a chemical shift of 175.4 ppm,[20] which is about 20 ppm downfield of the chemical shift observed for the imino carbon in **1**. The hypothetical structure **1c** is therefore not considered any further.

The structure of **1** is proposed as iminopiperidine substructure based on the evidence provided above, which should encompass equilibrium structures of an exo- and an endocyclic imino group (Scheme 2). Vasella and co-workers have previously discussed such equilibria in depth using ^{15}N NMR spectroscopy and X-ray analysis concluding the presence of an exocyclic C=N group for hydroximolactams.[13] A similar arrangement for **1** is likely, but will have to be verified in future studies. An exocyclic C=N group has been previously assigned to aliphatic gluco- and mannoamidines referring to the studies by Vasella and co-workers.[12]

2.3.2. Debenzylation reactions with Fe(III) chloride

In addition to catalytic hydrogenations with Pd or Pt catalysts, Lewis acid-mediated cleavage of benzyl ethers is widely applied in multi-step organic syntheses.[21] Among others, debenzylation reactions of aromatic dibenzyloxy aldehydes and the regioselective debenzylation of monosaccharides and complex oligosaccharides have been achieved by using magnesium bromide,[22] chromium(II) chloride,[23] or iron(III) chloride.[24,25] While in need for mild reaction conditions for the encompassing deprotection of a perbenzylated carbohydrate with an amidine functionality, we explored the use of $FeC1_3$ in dichloromethane.[25] The $FeC1_3$ reagent has previously been utilized for the anomerization of glycosides,[26] and for the debenzylation of monosaccharides.[27]

While the debenzylation of **10** proceeds in the presence of 16 equiv of iron(III) chloride without difficulty,[25] the isolation of **1** was found to be tedious in the presence of such large excess of ferric ions. ESI mass spectrometry on both the aqueous and the organic layer confirmed the presence of **1** in either layer after aqueous workup of the reaction mixture. As only minor amounts of **1** were recovered from the organic layer, extraction and isolation of **1** from the aqueous layer was undertaken.

Attempts to completely remove the iron(III) ions as iron sulfide by repetitive addition of sodium sulfide to acidified or neutral aqueous solutions were futile, leading predominantly to the recovery of excess sodium sulfide, and were consequently not further pursued. The addition of aqueous sodium hydroxide solution to the reaction mixture, allowed the complete removal of the ferric ions as ferric oxide by precipitation. Further purification of **1** and removal of the sodium chloride byproduct was achieved by ion exchange yielding **1** as a yellow oil in 18% overall yield. Initial attempts to use ion exchange for a complete removal of the ferric ions from the reaction mixture failed most likely due to the large excess of metal ions relative to the amount of product formed and the strong coordination of the ferric ions to the amidine function in **1**. Thus, the tedious work-up and the low yield of the isolated product compromise the use of anhydrous iron(III) chloride for the debenzylation of **10**. Consequently, the elaborated conditions with hydrogen gas and Pd on charcoal constitute—despite the costs and the amount of the catalyst required—the only practical synthetic route toward galactonoamidine **1**. Computational efforts are summarized below to rationalize the lack of epimerization reactions along the way to synthesize **1** from **4** via **6** (vide infra).

2.4. Computational characterization of putative epimerization reactions

All computations were performed using the GAUSSIAN 09 suite.[28] The geometry of all structures was optimized using Becke's three-parameter functional coupled with the correlation function of Lee at al. (B3LYP) and the basis set 6-31+G(d).[29,30] This level of theory was previously proven to be applicable for the characterization of the $^{4}C_1$ and $^{1}C_4$ conformers of α-D-glucopyranose.[31] The use of the B3-based hybrid density functional together with a split-valence basis set has been shown to perform better than Hartree–Fock (HF) or Møeller–Plesset (MP2) procedures.[31]

The Gibbs free energies ΔG^{298} of the $^{3}H_4$ and $^{4}H_3$ half-chair conformers of galactonolactam **4**, galactothionolactam **6**, galactonoamidine **10**, and of their corresponding C-5 epimers (**5**, **7** and

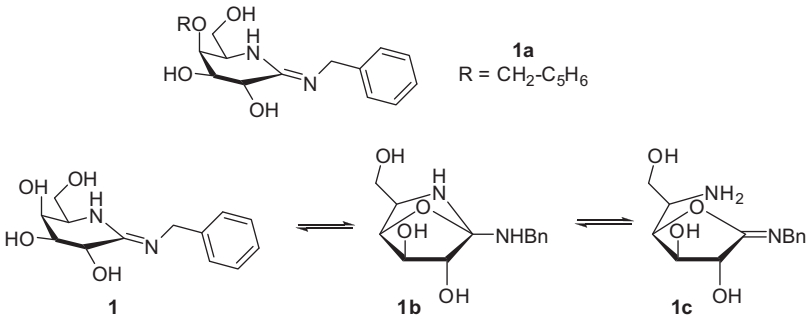


Chart 2. Hypothetical structures **1a–c**.

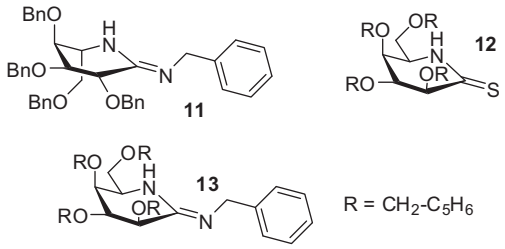


Chart 3. Structures of the compounds **11**, **12** and **13** (C-2 and C-5 epimers of **6** and **10**, respectively).

N-benzyl-2,3,4,6-tetra-*O*-benzyl-L-altronoamidine **11**) were initially calculated in the gas phase using the B3LYP/6-31G(d) level of theory. Additionally, the Gibbs free energies ΔG^{298} of the C-2 epimers of **6** and **10**, namely 2,3,4,6-tetra-*O*-benzyl-D-talothionolactam (**12**) and *N*-benzyl-2,3,4,6-tetra-*O*-benzyl-D-talonoamidine **13** were determined (Chart 3). Vibrational frequencies were computed at the same level of theory in order to define optimized geometries as minima without imaginary frequencies. Solvation free energies were subsequently computed as single point calculations of the geometry-optimized structures at the SMD/B3LYP/6-31+G(d) level of theory in acetonitrile, benzene and dichloromethane.

2.4.1. Stability of lactams

The computational data suggest that the $^4H_3$ half-chair conformer is the predominant form of lactam **4** in acetonitrile and 3.4 kcal/mol more stable than its corresponding $^3H_4$ half-chair conformer (Table 1). A similar trend is observed upon comparing the stability of the half-chair conformers for **4** in benzene, for its C-5 epimer **5** in acetonitrile and for thiolactam **6** in benzene (see Supplementary data). These results are in agreement with experimental results and computational studies for related pyranoses.[32] We consequently focus on the $^4H_3$ half-chair conformers of **4** and **6** during the following discussions.

The $^4H_3$ half-chair conformer of **5** is thermodynamically about 4.6 kcal/mol more stable in acetonitrile than the lowest energy conformer identified for **4**. This finding suggests a considerable amount of epimerization at C-5 during the ring closing reaction in acetonitrile, and the formation of the lactams **4** and **5** in this solvent confirming our experimental observations (vide infra). Nevertheless, lactam **4** is the main product of the ring closing reaction, and the following computational investigation consequently focuses on its transformation and putative epimerization at C-2.

2.4.2. Stability of thiolactams

Others previously noted unwanted epimerizations at C-2 of glycolactams with gluco- and manno-configuration during the transformation into thiolactams with Lawesson's reagent.[12,13] In contrast, our experimental results do not indicate any evidence for such a reaction. We therefore calculated the relative Gibbs free energies for the reaction of **4** into the thiolactam **6** and its C-2 epimer **12** in benzene (Table 2). The Lawesson's reagent used during the experimental setup is substituted by hydrogen sulfide during the computation for simplicity reasons.

The relative Gibbs free energies for all reactions are positive indicating non-spontaneous reactions. The Gibbs free energy for the transformation of the $^4H_3$ half-chair conformer of **4** into the $^4H_3$ half-chair conformer of **6** is about 1 kcal/mol smaller than the transformation of the same compound in a $^3H_4$ half-chair. Assuming **4** predominantly in the $^4H_3$ half-chair conformation (see above), an epimerization at C-2 leading to **12** in either half-chair conformation is thermodynamically less favored by 0.3–1.6 kcal/mol. However, lactam **4** in the $^3H_4$ half-chair conformation requires about 2–3.3 kcal/mol less than **4** in its $^4H_3$ half-chair conformation for the transformation into the C-2 epimer **12**. As we did not find any experimental evidence for such epimerization at C-2, we conclude that **4** predominantly exists and reacts in its $^4H_3$ half-chair conformation, which was characterized as the conformer with the lowest energy before.

2.4.3. Stability of amidines

Thiolactams with gluco-configuration were experimentally observed to epimerize during the reaction with amines by others previously.[12] In contrast, we isolated **10** as the sole product after the reaction of **6** with benzylamine and chromatographic purification (vide infra). We consequently computed the relative Gibbs free energies during the reactions of the thiolactam **6** with benzylamine **9** in dichloromethane resulting in galactonoamidines **10** and its C-2 epimer **13** (Table 3)

All computed reactions are non-spontaneous. Like **4**, the $^4H_3$ half-chair conformer of **6** is about 4 kcal/mol more stable than its $^3H_4$ half-chair conformer (see Supplementary data), and is therefore considered to be the predominant form of **6** during the following discussion. The Gibbs free energy for the transformation of the $^4H_3$ half-chair conformer of **6** into the same half-chair conformer of **10** requires 3.9 kcal/mol (Table 3, entry 1), while the transformation of **6** into either half-chair conformer of it's C-2 epimer **13** is additional 1.3–2.1 kcal/mol higher in energy and consequently less favored (Table 3, entries 3 and 4). The computation thus suggests that the C-2 epimerization of the major conformer of **6** is thermodynamically not favored.

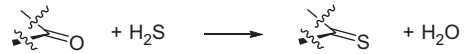
Nevertheless, after conformational change of the $^4H_3$ half-chair conformer of **6** into the $^3H_4$ half-chair conformer, epimerization reactions at C-2 and the formation of **13** would be favored over the transformation of **6** into **10** by 1.7–2.6 kcal/mol. However, as there is no experimental evidence for such epimerization products, we conclude that thiolactam **6**, like lactam **4**, exists and reacts predominantly as its $^4H_3$ half-chair conformer during the discussed reactions.

Table 1
Relative free energies $\Delta(\Delta G_f^{298})$ (in kcal/mol) of the lactams **4** and **5** in $^4H_3$ and $^3H_4$ half-chair conformation at the SMD/B3LYP/6-31+G(d) level of theory in acetonitrile ($\varepsilon = 35.69$) and benzene ($\varepsilon = 2.27$) at 298.15 K, 1 atm

Entry	Half-chair conformation	lactam	$\Delta(\Delta G_f^{298})$ (CH_3CN)	$\Delta(\Delta G_f^{298})$ (C_6H_6)
1	$^4H_3$	**4**	0	0
2	$^3H_4$	**4**	3.4	4.4
C-5 epimer:				
3	$^4H_3$	**5**	−4.6	–[a]
4	$^3H_4$	**5**	−0.7	–[a]

[a] Not determined.

Table 2
Relative free energies $\Delta(\Delta G_f^{298})$ (in kcal/mol) for the transformation of lactam **4** into the thiolactams **6** and its C-2 epimer **12** at the SMD/B3LYP/6-31+G(d) level of theory in benzene ($\varepsilon = 2.27$) at 298.15 K, 1 atm



Entry	Lactam	Thiolactam	ΔG_f^{298}	$\Delta(\Delta G_f^{298})$
1	**4** ($^4H_3$)	**6** ($^4H_3$)	16.0	0
2	**4** ($^3H_4$)	**6** ($^3H_4$)	16.8	0.8
Epimerization at C-2:				
3	**4** ($^4H_3$)	**12** ($^4H_3$)	17.6	1.6
4		**12** ($^3H_4$)	16.3	0.3
5	**4** ($^3H_4$)	**12** ($^4H_3$)	14.0	−2.0
6		**12** ($^3H_4$)	12.7	−3.3

Table 3
Relative Gibbs free energies $\Delta(\Delta G_f^{298})$ (in kcal/mol) for the transformation of the thiolactam **6** into the *N*-benzylamidines **10** and its C-2 epimer **13** at the SMD/B3LYP/6-31+G(d) level of theory in dichloromethane ($\varepsilon = 8.93$) at 298.15 K, 1 atm

Entry	Thiolactam	Amidine	ΔG_f^{298}	$\Delta(\Delta G_f^{298})$
1	**6** ($^4H_3$)	**10** ($^4H_3$)	3.9	0
2	**6** ($^3H_4$)	**10** ($^3H_4$)	4.3	0.4
Epimerization at C-2:				
3	**6** ($^4H_3$)	**13** ($^4H_3$)	6.0	2.1
4		**13** ($^3H_4$)	5.2	1.3
5	**6** ($^3H_4$)	**13** ($^4H_3$)	2.2	−1.7
6		**13** ($^3H_4$)	1.3	−2.6

3. Conclusions and summary of results

A feasible synthesis of galactonoamidines resembling features of the transition state for the cleavage of glycosidic bonds is a prerequisite for a future development of templated macromolecular catalysts that might allow selective glycosylation reactions. With this goal in mind, a practical multi gram-scale synthesis for 2,3,4,6-tetra-*O*-benzyl-D-galactothionolactam (**6**) was developed. During the course of the study, the D-galacto configuration of the carbohydrate was found to be a key element to prevent epimerization at C-2 during the formation of galactonolactam **4**, galactothionolactam **6** and galactonoamidine **10**. The rational for this observation was ascribed to the steric hindrance imposed by the hydroxyl groups at C-2 and C-4 that would be axial to each other after such epimerization. This hypothesis was further supported by computational data suggesting that the $^4H_3$ half-chair conformer is a major conformer for galactonolactam **4** and galactothionolactam (**6**). The computational data support the experimental results and indicate that the epimerization at C-2 of **4** or **6** is thermodynamically not favored for the identified major $^4H_3$ half-chair conformers.

The debenzylation of *N*-benzyl-2,3,4,6-tetra-*O*-benzyl-D-galactonoamidine (**10**) yielding the *N*-benzyl-D-galactonoamidine (**1**) was explored by using hydrogenation with palladium on charcoal, and ferric chloride in dichloromethane as reagents. Although the hydrogenolysis of **10** requires 2 wt equiv of palladium on charcoal in the presence of a strong acid, the cleavage of the benzyl ether groups affording **1** was accomplished in significantly higher isolated yields than comparable debenzylation reactions with ferric chloride. The latter reaction required a very tedious workup procedure for complete ferric ion removal. Current efforts are directed toward the characterization of the inhibitory activity of **1** during the enzymatic cleavage of phenylgalactosides, and the study of its coordination ability to metal complexes, which will be reported in due course.

4. Experimental

4.1. General methods

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV400 (400.2 MHz for ^{1}H, and 100.6 MHz for ^{13}C). Chemical shifts (δ) in ^{1}H NMR are expressed in ppm and coupling constants (J) in Hz. Signal multiplicities are denoted as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Deuterated chloroform and deuterium oxide were used as solvents, and chemical shift values are reported relative to the residual signals of these solvents ($CDCl_3$: $\delta = 7.29$ for ^{1}H and $\delta = 77.0$ for ^{13}C; D_2O: $\delta = 4.80$ for ^{1}H and $\delta = 49.0$ for ^{13}C after addition of a few drops of CH_3OD). IR spectra of the obtained oils were recorded as thin films on KBr disks with a resolution of 0.5 cm^{-1} using a Shimadzu IR Prestige-21 FT-Infrared Spectrometer; ν in cm^{-1}. MS analysis of samples was performed using an Ultra Performance LC Systems (ACQUITY, Waters Corp., Milford, MA, USA) coupled with a quadrupole time-of-flight mass spectrometer (Q-TOF Premier, Waters) with electrospray ionization (ESI) in both ESI-MS and ESI-MS/MS modes operated by the Masslynx software (V4.1). Each sample in 50% aqueous acetonitrile was directly injected into the ESI source at a flow rate of 50 μL/min. The ion source voltages were set at 3 kV for positive and negative ion mode acquisitions, respectively. The sampling cone was set at 37 V and the extraction cone was at 3 V. In both modes the source and desolvation temperature was maintained at 120 and 225 °C, respectively, with the desolvation gas flow at 200 L/h.

Thin layer chromatography (TLC) was performed using silica gel TLC plates from SORBENT Technologies, 200 μm, 4 × 8 cm, aluminum backed, with fluorescence indicator F_{254} and detection by UV light or by charring with an aqueous vanillin-sulfuric acid reagent and subsequent heating of the TLC plate. The vanillin reagent is prepared from vanillin (10 g, 0.066 mol) and concentrated sulfuric acid (20 mL) in 0.5 L of water. After stirring of this mixture at ambient temperature for 2 h, the filtrate of the suspension is used for charring. Column chromatography was carried out using Silica Gel 60 from Silicycle® (40–63 μm, 230–240 mesh) as stationary phase or basic aluminum oxide (pH range 9.7 ± 0.4, activity I), 32–63 μm, surface area 150 $m^2\,g^{-1}$ from Sorbent Technologies. Ion exchange experiments were performed on Dowex 50WX2 ion exchange resin from Sigma–Aldrich.

Benzylamine (Acros) was distilled in vacuum and stored over molecular sieves 3 Å prior to use;[33] dichloromethane (Pharmco-Aaper) and benzene (Sigma–Aldrich) were dried dynamically over neutral aluminum oxide from Acros or basic aluminum oxide from EMD; DMSO (Fisher) was dried over molecular sieves 4 Å; acetic anhydride (Fisher) was distilled prior to use. All other chemicals were reagent grade or better, and were used as received.

4.2. Calculations

All electronic structure calculations in gas phase and solution were performed with the GAUSSIAN 09 suite at the Alabama Supercomputer Center.[28] The geometry of all structures was optimized using Becke's three-parameter functional coupled with the correlation function of Lee at al. (B3LYP) and the basis set 6-31G(d).[29,30] The Gibbs free energies ΔG^{298} for all structures were calculated using the B3LYP/6-31G(d) level of theory. Vibrational frequencies were computed at the same level of theory in order to define optimized geometries as minima without imaginary frequency. The Gibbs free energies in solution ΔG_{sol}^{298} are computed as single point calculations at the B3LYP/6-31G(d) level of theory in acetonitrile, benzene or dichloromethane at 298.15 K and 1 atm using the SMD solvation model for absolute solvation energies. The SMD model is implemented in the GAUSSIAN 09 program,[34] considers non-electrostatic solvation terms, and achieves mean unsigned errors of 0.6–1.0 kcal/mol in the solvation free energies with the 6-31G(d) basis set.[34]

4.3. Syntheses

4.3.1. 2,3,4,6-Tetra-*O*-benzyl-D-galactonolactam (4)

The title compound **4** and its epimer **5** were obtained from **2** (95.5 g, 0.18 mol) following a procedure disclosed by Overkleft et al.[14] via 2,3,4,6-tetra-*O*-benzyl-galactoamide **3** (42.5 g, 76.6 mmol), and obtained as yellow oil after purification by flash chromatography on silica gel using cyclohexane–EtOAc (4:1, v/v) as eluent. ESI MS analysis revealed a molar mass of $m/z = 538.2628$ (calcd $m/z = 538.2593$) for the epimer mixture. The repeated purification of a small sample aliquot by flash chromatog-

raphy over silica gel using cyclohexane–EtOAc (1:1, v/v) allowed the isolation of **4** as major product; colorless oil; 71% yield (29.1 g, 54.2 mmol); R_f = 0.6 (SiO_2, cyclohexane–EtOAc (1:1, v/v)). The compound identity to previously described 2,3,4,6-tetra-*O*-benzyl-D-galactonolactam[14] was confirmed by ^1H and ^{13}C NMR spectroscopy and high resolution ESI mass spectrometry; δ_H ($CDCl_3$) 7.52–7.30 (m, 20H, Ph*H*), 6.04 (br s, 1H, N*H*), 5.31 (d, 11.2, 1H, PhCH_2), 4.99 (d, 11.6, 1H, PhCH_2), 4.90 (d, 11.3, 1H, PhCH_2), 4.86 (d, 11.9, 1H, PhCH_2), 4.76 (d, 12.1, 1H, PhCH_2), 4.64 (d, 11.5, 1H, PhCH_2), 4.53 (q, 11.6, 2H, PhCH_2), 4.42 (d, 9.1, 1H, *H*-2), 4.05 (br s, 1H, *H*-4), 3.91 (dd, 1.9, 9.2, 1H, *H*-3), 3.68–3.59 (m, 2H, *H*-5, *H*-6b), 3.51 (dd, 3.2, 7.8, 1H, *H*-6a); δ_C ($CDCl_3$) 170.9, 138.2, 138.0, 137.8, 137.3, 128.5–127.5, 80.6, 77.3, 75.3, 74.0, 73.50, 73.0, 72.9, 70.2, 53.5; HRMS, ESI-TOF$^+$ *m/z* calcd for $C_{34}H_{36}NO_5$ $[M+H]^+$: 538.2593; found: 538.2598.

4.3.2. 2,3,4,6-Tetra-*O*-benzyl-D-galactothionolactam (6)

A solution of Lawesson's reagent (4.5 g, 12 mmol) and **4** (9 g, 16.8 mmol) in 200 mL of dry benzene was heated to reflux under argon for 2 h. The TLC analysis (SiO_2, cyclohexane–EtOAc (1:1, v/v) of an aliquot of the reaction mixture showed complete transformation of the starting material (R_f = 0.6) after that time period, and the formation of the target compound with an R_f value of 0.85 along with the presence of a minor side product with an R_f value of 0.9. The solvent and any other remaining volatile compounds were subsequently evaporated in vacuum at 40 °C yielding an orange residue. Purification of this residue by flash chromatography on aluminum oxide using a solvent gradient from cyclohexane (100%) to cyclohexane–EtOAc (4:1, v/v) and cyclohexane–EtOAc (1:1, v/v) allowed the isolation of **6** as a yellow oil (8.6 g, 93%); δ_H ($CDCl_3$) 8.25 (br s, 1H, N*H*), 7.54–7.30 (m, 20H, Ph*H*), 5.43 (d, 10.6, 1H, PhCH_2), 4.94–4.47 (m, 8H, *H*-2, PhCH_2), 4.12–4.09 (br s, 1H, *H*-4), 3.86 (dd, 1.9, 7.7, 1H, *H*-3), 3.78–3.71 (m, 2H, *H*-5, *H*-6b), 3.57–3.54 (m, 1H, *H*-6a); δ_C ($CDCl_3$) 201.6, 137.9, 137.8, 137.5, 137.1, 128.6–127.6, 81.0, 78.8, 75.7, 73.5, 73.4, 72.9, 72.2, 69.5, 57.5; ν_{max} (KBr, thin film): 3064, 3033, 2923, 2865, 1517, 1451, 1363, 1315, 1209, 1103, 1030, 738, 697 cm^{-1}; HRMS (+TOF-MS) *m/z* calcd for $C_{34}H_{35}NO_4S$ $[M+H]^+$: 554.2365; found: 554.2396.

4.3.3. 2,3,4,6-Tetra-*O*-benzyl-D-galactoiminothioether (8)

Meerwein's salt (320 mg, 1.68 mmol, 1.5 equiv) is added to a solution of **6** (620 mg, 1.12 mmol) in 6 mL of dry CH_2Cl_2 at 0 °C. The green-yellow solution is stirred at 0 °C for 2 h, and used directly for the next step without any further purification. A (+) TOF MS-ESI analysis of the reaction solution indicates the presence of **8**; HRMS *m/z* calcd for $C_{36}H_{40}O_4NS$ $[M+H]^+$: 582.2678; found: 582.2728.

4.3.4. *N*-Benzyl-2,3,4,6-tetra-*O*-benzyl-D-galactonoamidine (10)

A solution of distilled and dried benzylamine (245 μL, 2.24 mmol) in 2 mL of dry CH_2Cl_2 is added to a solution of 2,3,4,6-tetra-*O*-benzyl-D-galactoiminothioether (**8**) under argon at 0 °C. The resulting solution is stirred over night and allowed to warm to ambient temperature. After 8 h, the TLC analysis (SiO_2, cyclohexane–EtOAc (1:1, v/v)) of a sample aliquot revealed complete transformation of the galactoiminothioether **8** (R_f = 0.8) and appearance of a more polar spot at R_f = 0.55. The reaction was subsequently stopped by evaporation of all volatile material in vacuum at ambient temperature; the remaining residue was purified by flash chromatography on basic aluminum oxide using gradient elution from cyclohexane to cyclohexane–ethyl cyclohexane (1:1, v/v) yielding **10** as a yellow oil (561 mg, 0.90 mmol) in 80%; δ_H ($CDCl_3$) 7.48–7.25 (m, 28H, Ph*H*), 5.05–4.84 (m, 4H, OCH_2), 4.79–4.61 (m, 5H, –C*H*, OCH_2), 4.41–4.27 (m, 3H, –C*H*, NCH_2), 4.04 (dd, 1.72, 9.7, 1H, –C*H*), 3.88–3.72 (m, 3H, –C*H*, –CH_2), 1.34 (br s, 1H, N*H*); δ_C ($CDCl_3$) 155.9, 139.2, 139.0, 138.4, 138.1, 137.7, 128.5–126.9, 81.9, 75.3, 74.5, 74.0, 73.7, 73,3, 71.2, 71.0, 59.6, 45.0; ν_{max} (KBr, thin film) 3439, 3029, 2922, 2860, 1654, 1495, 1453, 1091, 737, 696 cm^{-1}; ESI-TOF MS *m/z* calcd for $C_{41}H_{43}N_2O_4$ $[M+H]^+$: 627.3223; found: 627.3216.

4.3.5. *N*-Benzyl-D-galactonoamidine (1)

Procedure A. Palladium on charcoal (160 mg, 200%; w/w) and trifluoroacetic acid (1 mL, 13.5 mmol) are added to a solution of *N*-benzyl-2,3,4,6-tetra-*O*-benzyl-D-galactonoamidine (**10**) (80 mg, 0.128 mmol) in 5 mL of EtOH. The resulting suspension was stirred at room temperature in the presence of hydrogen gas at 1 atm. After 16 h, a TLC analysis (SiO_2, cyclohexane–EtOAc (1:1, v/v)) indicated complete transformation of the starting material (R_f = 0.55) into a more polar spot at the baseline. The reaction mixture was then diluted with 10 mL of EtOH, centrifuged at 6000 rpm for 6 min and filtered over Celite. The filtrate was concentrated under reduced pressure at 38 °C to afford **1** as a light yellow oil (34 mg, quantitative); R_f = 0.8 (SiO_2, MeOH); δ_H (D_2O) 7.50–7.35 (m, 5H, Ph*H*), 4.72–4.62 (m, 3H, –NCH_2, –C*H*), 4.34–4.30 (m, 1H, –C*H*), 4.01 (dd, 9.2, 2.4, 1H, –C*H*), 3.84–3.65 (m, 3H, –C*H*, –CH_2); δ_C (D_2O/CH_3OD) 164.6, 133.9, 129.3, 128.6, 127.5, 70.9, 67.2, 66.7, 60.2, 57.7, 45.3; ν_{max} (KBr, thin film) 3363, 1672, 1438, 1206, 1134, 711 cm^{-1}; ESI-TOF MS *m/z* calcd for $C_{13}H_{18}N_2O_4$ $[M+H]^+$: 267.1345; found: 267.1347.

Procedure B. Anhydrous $FeCl_3$ (332 mg, 2.04 mmol, 16 equiv) was added to a solution of **10** (80 mg, 0.127 mmol) in 5 mL of dry dichloromethane at 0 °C under argon, and the resulting reaction mixture was continued to stir in the cold. After 2 h, the reaction was quenched by addition of 1 mL of 0.1 N aqueous hydrochloric acid. After 10 min of continued stirring, the reaction mixture was treated with aqueous sodium hydroxide solution to adjust the pH of the reaction mixture to 6. The immediate formation of a brownish precipitate is observed presumably due to the precipitation of iron(III) ions as Fe_2O_3. The resulting suspension was centrifuged, and the colorless supernatant concentrated under reduced pressure to yield a colorless residue.

This residue was triturated with 10 mL of ethanol, centrifuged, and the supernatant was concentrated under reduced pressure. The remaining residue was separated in 7.40 mL fractions on a Dowex 50WX2 ion exchange resin column (2 × 5 cm). The first three fractions containing predominantly sodium chloride were eluted with a solution of 120 mL of 0.1 M aqueous hydrochloric acid. The following four fractions containing the product were then eluted with 160 mL of 0.5 M aqueous hydrochloric acid. The amidine-containing fractions were identified by ESI MS analysis, collected and evaporated under reduced pressure to yield **1** as a yellow oil (6 mg, 18%). The product is identical to one obtained with procedure A.

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Supplementary data

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