Solvent-controlled synthesis of bulky and polar-bulky galactonoamidines

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KEYWORDS. Glycosidase inhibitor, inhibitor synthesis, galactonoamidines, solvent effect

ABSTRACT: The goal of this study was the design and synthesis of bulky and polar-bulky galactonoamidines that have a potential to interact with both catalytic amino acids in the active site of human α-galactosidase. While a library of more than 25 compounds was previously synthesized following established protocols, the coupling of the selected amines with activated perbenzylated galactothionolactam yielded only small amounts for some of the perbenzylated targets. A computational approach disclosed relative energy differences of selected adducts and suggested a solvent change that then allowed a successful synthesis of the precursor compounds in 20-75%. Subsequent attempts to globally deprotect perbenzylated galactonoamidines by Pd catalyzed hydrogenation resulted in unwanted Pd coordination, incomplete debenzylation reactions, partial compound hydrolysis, and even complete decomposition. A lengthy protocol was elaborated to purify the targeted carbohydrate derivatives after modified debenzylation conditions.

Introduction.

Glycosidases are enzymes that catalyze the hydrolysis of *α*- and *β*-glycosidic bonds. To elucidate their mechanism of action, and characterize the structure of their active sites, compounds that act as inhibitors of the enzymatic activity are typically employed.[1] Very potent glycosidase inhibitors often display characteristics of the transition state of the catalyzed reaction.[2] For the hydrolysis of glycosidic bonds, these features include flattening of the chair of the glycosyl moiety,[3] a sp^2-like character at the anomeric C-atom,[3] a partial positive charge at the location of the ring O-atom of a glycosyl ring represented in an oxocarbenium-like structure,[4-6] and the lengthening of the acyclic C-O bond at the anomeric C-atom.[4]

While a myriad of inhibitors towards *β*-glycosidic bonds has been identified following these principles,[7-12] the number of available compounds to study the active sites of α-glycosidases remains limited. Notable exceptions include, among others, acarbose,[13] a tetrasaccharide which blocks α-amylase activity in intestines and is used for the treatment of type II diabetes;[14,15] and 1-deoxygalactonojirimycin and derivatives,[16] which are azasugars that ensure sufficient levels of functional *α*-galactosidases and thereby reverse a lysosomal storage disorder that otherwise enriches globotriaosylceramides in cells and tissues. The parent compound has been approved by the FDA for treatment of Fabry disease in 2018. Further classes of promising compounds under investigation as potent α-galactosidase inhibitors include pyrrolidine-triazole hybrid molecules[17] and aminohydroxycyclopentanes.[18]

Despite the significant progress, access to a large number of inhibitors with tunable activity towards specific α-galactosidases remains desirable. The availability of such compounds could provide an alternative in situations where current inhibitors are inefficient, or their prolonged use leads to side effects. Additionally, the availability of a library of structurally related α-glycosidase inhibitors enables thorough studies of the role and function of enzymes associated with the onset and progression of these and other diseases including cancer.[19]

In an unrelated study, we disclosed a library of 25 galactonoamidine **1** inhibitors with activity towards *β*-galactosidases in the nano- and picomolar concentration range.[20,21] The compounds contain aglycons composed of aromatic, linear and cyclic aliphatic hydrocarbons.[20] Supported by computational evaluations,[21,22] three of these galactonoamidines are experimentally identified as true transition state-like analogs against *β*-galactosidase from *A. oryzae* (**Chart 1**).[22-24]

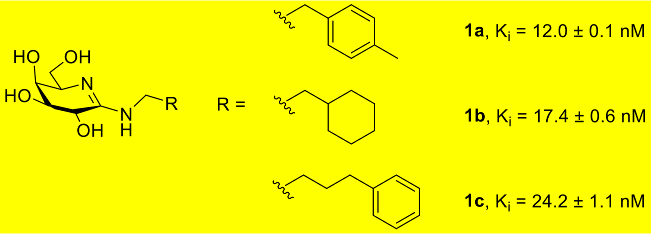


Chart 1. Galactonoamidines previously identified as transition state analogs of *β*-galactosidase (*A. oryzae*)[22-24]

In-depth kinetic studies of their interactions in the active sites of *β*-galactosidases from *A. oryzae*,[22] *E. coli*, and bovine liver[21] show competitive inhibition of the selected enzymes by all galactonoamidines. Interestingly, the most efficient inhibitors typically show a high probability to interact simultaneously with both catalytically active amino acid residues, e.g. E^{200} and E^{298},[25] in the active sites of the respective enzymes (**Figure 1**).[21]

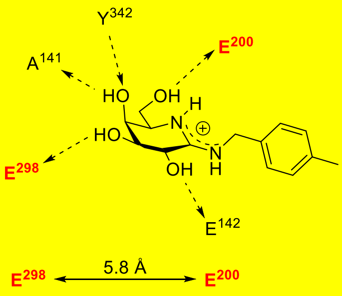


Figure 1, Key interactions of the glycon of **1a** with amino acids in the active site of *β*-galactosidase (*A. oryzae*),[22] PDB ID 4IUG.[25]

The distance between the amino acids acting as a proton donor and as a nucleophile ranges from 5.2 (PDB ID 4V45)[26] to 5.8 Å (PDB ID 4IUG)[20] in the studied *β*-galactosidases. The synthesized galactonoamidines form H-bonds with the catalytic residues using hydroxyl groups of their glycosyl moieties. Their aglycons enable stabilizing interactions in the active sites using hydrophobic, π-π or CH-π stacking interactions.[21] Additionally, induced fit deformations of the enzyme, and closed loop interactions upon interaction with the galactonoamidine inhibitors are noted.[21]

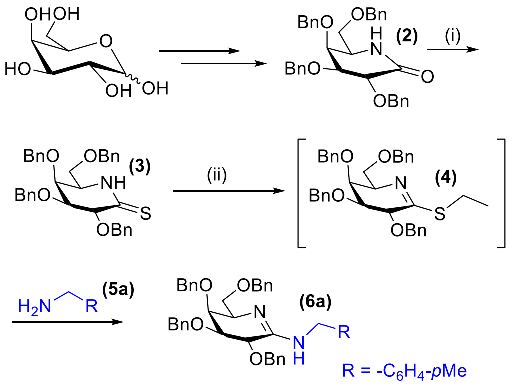
However, the distance between the catalytic amino acid residues in *α*-glycosidases is usually larger than in *β*-glycosidases, e.g. 8.2 Å in human *α*-galactosidase (PDB ID 1R47).[27] Thus, strong H-bond interactions of the available galactonoamidine inhibitors **1** with catalytically active amino acids in the active site of human α-galactosidase and possibly other α-glycosidases are unlikely. Therefore, this study aims at the development of galactonoamidines with polar groups in their aglycon to achieve competitive inhibition of α-galactosidases by allowing H-bonding with the catalytically active residues, D170 and D231, of human *α*-galactosidase.[27] The polar groups in the aglycons of the new galactonoamidines are envisioned to contain O- and N-atoms, as well as hydroxyl group functionalities to enable simultaneous H-bond formations. As a first step toward this goal, we describe here our efforts during the synthesis of the envisaged galactonoamidines with bulky and bulky-polar aglycons.

2 Results and discussion.

2.1 Synthesis of perbenzylated galactonoamidines in dichloromethane

Previously, a 10 gram-scale synthesis of perbenzylated galactonolactam (**2**) and perbenzylated galactothionolactam (**3**) from galactose was reported (**Scheme 1**) that was developed based on reports for related compounds by Overkleeft, Heck, and Vasella.[28-30] In short, after activation of **3** with Meerwein's salt perbenzylated galactoiminothioether **4** is obtained and treated with aliphatic and aromatic amines **5** to afford perbenzylated galactonoamidines **6**.[20] As a representative example, the coupling of **4** with *p*-methylbenzylamine (**5a**) in dichloromethane provides perbenzylated galactonoamidine **6a** in 51-80 % isolated yield after chromatographic purification over deactivated silica or neutral aluminum oxide.[23,28] The subsequent debenzylation of **6a** with hydrogen gas in the presence of Pd on charcoal and trifluoracetic acid in ethanol affords **1a** quantitatively without further purification.[28] ~~The amidines exist in equilibrium structures.~~[33]

Scheme 1. Synthetic approach toward perbenzylated galactonoamidines 6 from galactose



(i) Lawesson's reagent, C_6H_6, reflux,[20] 2h, 93 %[28,] (ii) Meerwein's salt, CH_2Cl_2, 0°C, 2h;[17, 20, 30] ; **5a**, CH_2Cl_2, 0°C → r.t., 8-48 h, 51-80 % over two steps;[23,28] The amines **5b-l** yield under similar reaction conditions the following isolated yields over two steps: **5b**, 24 h, 57 %; **5c**, 37 h, 52 %; **5d**, 90 h, 37 %; **5e**, 18 h, 44 %; **5f**, 136 h, 40 %; **5g**, 115 h, ≤ 8 %; **5g**, 156 h, 14 %; **5h**, 167 h, ≤ 10 %; **5i**, 144 h, ≤ 1 %; **5k**, 144 h, ≤ 1 %; **5l**, N/A.

When applying the previously developed strategy to couple bulky and bulky-polar amines **5b-l (Chart 2)** with **4**, perbenzylated amidines **6b-c** are obtained in moderate yields (52-57 %). Perbenzylated amidines **6d-f** are only obtained in 37-44 %, while compounds **6h-k** are not isolated as they are formed in less than 10 % (per TLC estimate). The synthesis of **6l** has not been attempted under these conditions. Efforts to purify perbenzylated amidines **6h-k** by column chromatography resulted in complete compound loss or in isolation of the perbenzylated galactoiminothioether **4**. The amines **5a**, **5c**, and **5g** are commercially available, all other amines **5b**, **5d-f**, and **5h-l**[31-36] were synthesized by reduction of their corresponding nitriles **7** with lithium aluminum hydride in dry diethyl ether or dimethoxyethane following literature procedures.[32 31,34,37-40]

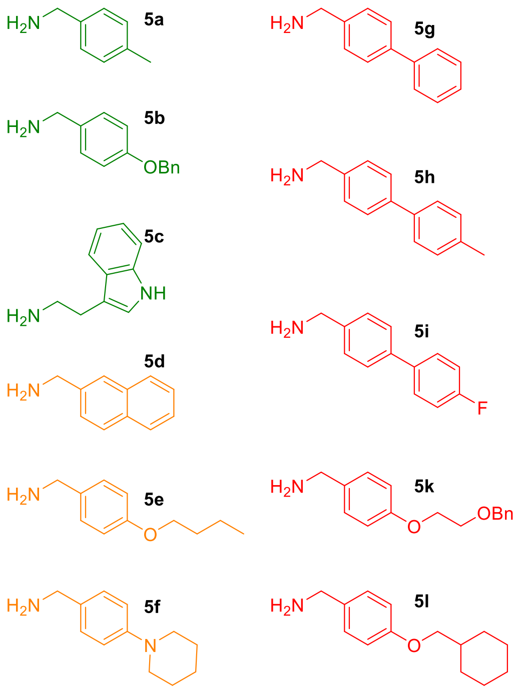


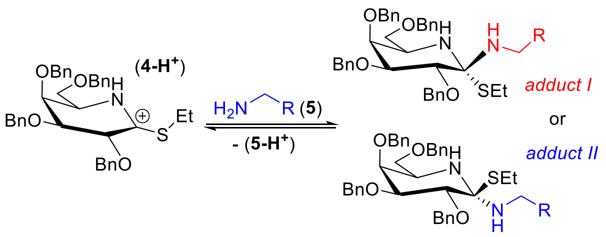
Chart 2. Structures of the bulky and bulky-polar amines 5 obtained by reduction of nitriles 7,[32] [31,34,37-40] LiALH$_4$, Et$_2$O or DME, 0 °C→ r.t, 4-16 h, 69-84% as described.[32]

To overcome the encountered pitfall, the reaction time of the transformation was extended from typical 16-48 h to over 100 h, while two equivalents of the amines relative to **4** are continued to be used for all reactions in efforts.[24,28] As a representative example for others, the isolated yield of **6g** is 8 % after a reaction time of 115 h and increases to 14 % after 156 h. While steric hindrance at the reaction side has been initially assumed as a rationale for the poor reaction yields, several amines that do not appear more bulky than others give fair or moderate amounts of products pointing at stereoelectronic reasons for the inefficient formation of perbenzylated amidines **6g-l**. Addition of **5a** to reaction mixtures with amines that do not show formation of perbenzylated amidines **6b-l** as judged by TLC analysis, yielded **6a** in less than 24 h indicating that the amine addition to **4** is the rate-determining step of the reaction. As our efforts to increase the yields of **6g-k** remained futile, the transformation of perbenzylated galactoiminothioether **4** with the selected amines **5** was evaluated in a computational approach.

2.2 Computational analysis of the addition of amines to perbenzylated galactoiminothioether

All computations are performed with the PQSmol suite based on density functional theory employing the B3LYP exchange correlation functional, the 6-31g(d) basis set, and the COSMO model under standard conditions. The structures of the perbenzylated galactoiminothioether **4**, amines **5**, their protonated forms, and addition products are calculated as energy-minimized geometries with zero imaginary frequency (**Table I**). The addition of amines **5** to protonated **4** yields two addition products, adduct I and adduct II, that are both considered throughout all calculations. The computation in the gas phase suggests that most amines approach **4** from the less hindered side of its galactosyl moiety forming adduct II, while only the reaction with amines containing a condensed aromatic ring system, **5c** and **5d**, lead preferably to adduct I.

Table 1. Changes in Gibb's free energies ΔG^a (kcal/mol) for the formation of adduct I and II in the gas phase

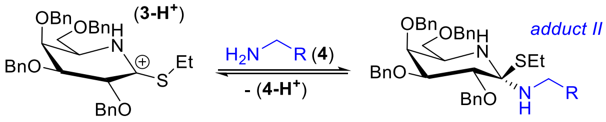


	ΔG (kcal/mol)		
Amine (**5**)	Adduct I	Adduct II	ΔΔG
5a	18.4	16.7	1.7
5b	20.3	17.3	3.0
5c	18.6	19.8	-1.2
5d	17.2	18.7	-1.5
5e	20.5	17.4	3.1
5f	18.4	17.0	1.4
5g	18.3	18.3	0.0
5h	20.4	18.6	1.8
5i	20.0	18.3	1.7
5k	20.7	19.3	1.4
5l	20.3	19.9	0.4

^{a}calculated as $\Delta G = \Delta H – T\Delta S$; $\Delta\Delta G = \Delta G_{\text{Adduct I}} – \Delta G_{\text{Adduct II}}$

To estimate the changes in Gibb's free energy of the addition reactions in solution (**Table 2**), all structures of the adducts I and II, amines **5a-l,** protonated **4**, and protonated amines **5a-l** are optimized for energy minima in dichloromethane and acetonitrile. Increased stabilization of polar intermediates in acetonitrile (ε_{CH3CN} = 36.64) than in dichloromethane (ε_{CH2Cl2} = 8.71) is expected that may facilitate the amine addition and thereby increase the reaction yield. Other polar aprotic solvents, such as DMSO, pyridine, and DMF, are excluded from this computation for experimental aspects, such as compound isolation from the reaction mixtures, and solvent preparation for water-free reactions.

Table 2. Changes in Gibb's free energies ΔΔG (kcal/mol) for the formation of the lowest energy adduct in dichloromethane and acetonitrile



	ΔΔG^a (kcal/mol)	
Amine (**5**)	CH_2Cl_2	CH_3CN

5a	-3.2	-5.7
5b	-0.84	-3.2
5c[b]	-3.0	-6.4
5d[b]	-2.3	-5.0
5e	-0.45	-2.9
5f	1.4	-0.84
5g[b]	-0.89	-3.40
5h	0.22	-2.4
5i	-1.7	-4.3
5k	0.99	-1.5
5l	2.3	-0.14

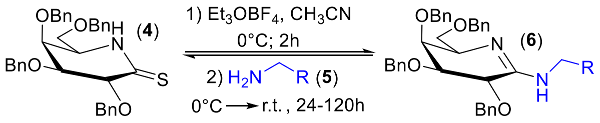
[a] $\Delta\Delta G = \Delta G_{sol}(\textbf{adduct II}) + \Delta G_{sol}(\textbf{5}\text{-H}^+) - 2\times\Delta G_{sol}(\textbf{5}) - \Delta G_{sol}(\textbf{4}\text{-H}^+)$; [b] $\Delta\Delta G$ is calculated using $\Delta G_{sol}(\textbf{adduct I})$

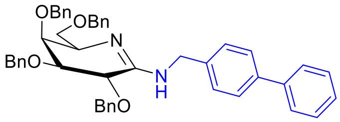
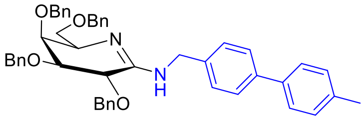
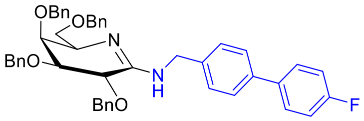
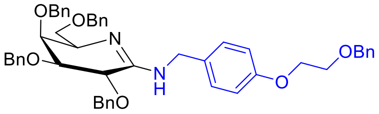
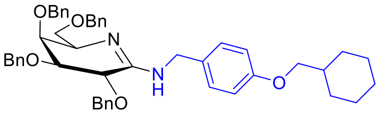
Notably, the computation suggests favored formation of all adducts in acetonitrile over dichloromethane. Only the formation of perbenzylated amidines **6a** and **6c** is computed as strongly exergonic reactions in both solvents. Moreover, reactions of **4** with amines **5f**, **5h**, **5k**, and **5l** are endergonic in dichloromethane, while the same reactions are exergonic in acetonitrile. The results point at a better stabilization of the adduct formation in acetonitrile and imply indeed stereoelectronic contributions during adduct formation. Encouraged by the computational suggestions, the syntheses of previously failed perbenzylated **6g-l** are attempted in acetonitrile.

2.3 Synthesis of galactonoamidines in acetonitrile

Using dry acetonitrile as a solvent, perbenzylated amidines **6g-l** are isolated from reactions of bulky amines **5g-l** and activated **4** in fair yields (18-35%) (**Table 3**). As a representative example, the isolated yield of **6g** increases with a change of solvent almost four-fold from 8% in dichloromethane to 30% in acetonitrile under otherwise comparable conditions. While the required reaction times of up to 120 h are still lengthy, perbenzylated amidines **6** with bulky and bulky-polar aglycons, such as substituted biphenyls and aryl ethers are obtained. The isolated compound yield increases in acetonitrile on average by about 20-30 % compared to reactions in dichloromethane. Perbenzylated amidines **6i** and **6k** are accessible under these conditions, while previous attempts in dichloromethane yielded less than 1% of the target compounds (**Table 1**). Although the overall yields of the perbenzylated amidines are still low (18-35%), the solvent change enables the synthesis of **6g-l**.

Table 3. Synthesis of selected perbenzylated galactonoamidines 6 in acetonitrile



Amine (5)	Product (6)	Time [h]	Yield[a] [%]
5g		120	30
5h		115	35
5i		120	30
5k		94	22
5l		24	75

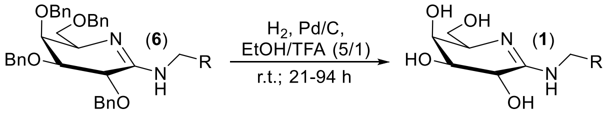
[a] isolated yield

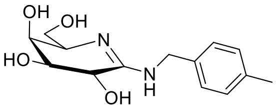
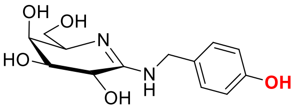
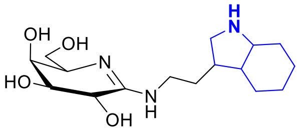
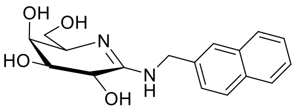
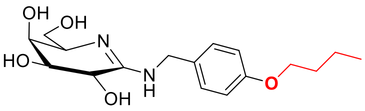
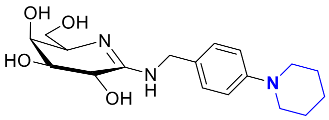
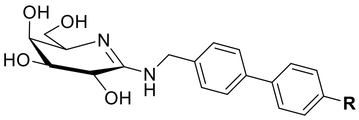
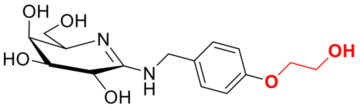
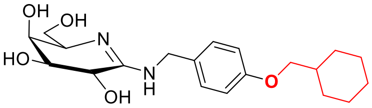
2.4 Synthesis of galactonoamidines 1

With reasonable amounts of perbenzylated amidines **6b-l** on hand, hydrogenation reactions in ethanol in presence of trifluoroacetic acid and Pd on charcoal are performed following previously established protocols.[28,30] The procedure allows the isolation of **1b** and **1e** in reasonable amount, quantity, and purity. Surprisingly, several other amidines with heteroatoms in their aglycon showed broadening of signal lines and slight shifts of the signals in their ^{1}H NMR spectrum indicating unwanted Pd coordination to the target compounds as well as incomplete debenzylation. To remedy the situation, the ratio between ethanol and TFA was altered from 5/1 to 3/1 and 2/1 to increase the TFA amount in hopes to prevent coordination of the hydrogenation catalyst. Unfortunately, the compounds completely decomposed under these conditions.

In altered attempts, the volume ratio of ethanol to TFA is kept at 5/1 or 10/1 while the reaction time is increased from the typical 12-15 h to 60-72 h. Initial ^{1}H NMR data following filtration over Celite® show overall smaller amounts of decomposition of the compounds that nevertheless require further purification. The indole aglycon of **6c** yields a fully hydrogenated aglycon in **1c** in a mixture of compounds that was characterized by LCMS. Filtration of the remaining amidine oils over 0.1 µm PFTE membranes prior to using semi-preparative HPLC on an amino phase with methanol as an eluent allowed further removal of black Pd/C residues yielding yellowish solutions. HPLC traces indicate reduction of impurities in the compounds. Successive extraction and centrifugation cycles of the oily residues with nanopure water, followed by analysis of the extract by analytical HPLC on a carbohydrate-selective Ca^{2+} phase using nanopure water as an eluent provided an estimate of the purity of the amidines. Satisfactory purity is now observed for compounds **1d**, and **1k**. The formation of unwanted galactonolactam is evident from the HPLC traces with a peak at 20 min indicating partial hydrolysis of **1f-h** and **1l**,[23] while major decomposition is observed for amidine **1i**. Overall, a massive compound loss during the extensive purification protocol is experienced that prevents recording of NMR data for amidines **1d, 1f**, and **1h-l**. Evidence for formation of the target compounds is obtained by recording of HRMS data, while an estimate of their purity is deduced from the analytical HPLC traces.

Table 4. Debenzylation of compounds 6 yielding galactonoamidines 1



Bulky non-polar amidines	Bulky amidines with O-atoms	Bulky amidines with N-atoms
 **1a, isolated**	 **1b, isolated**	 **1c, isolated**
 **1d, not isolated** **HPLC trace (100%), HRMS**	 **1e, isolated**	 **1f, not isolated** **HPLC trace (73%), HRMS**
 **1g, R = H, isolated** **1h, R = Me, not isolated** **HPLC trace (84%), HRMS** **1i, R = F, not isolated** **HPLC trace (33%), HRMS**	 **1k, not isolated** **HPLC trace (99%), HRMS**  **1l, not isolated** **HPLC trace (68%), HRMS**	

*all isolated compounds are fully characterized and purified

Conclusions

This study aims at the development of galactonoamidines as effective inhibitors with transition state-like character that allow bridging of the 8.2 Å distance between the catalytically active amino acids acting in human α-glycosidase. The envisaged inhibitors will enable stabilizing interactions with the amino acids acting as proton donor and nucleophile in the active site of the enzyme. Therefore, galactonoamidines with bulky and bulky-polar groups in their aglycons were designed to enable simultaneous H-bond formations with both amino acid residues. When employing previously developed methods,[28,30] the coupling of perbenzylated galactonolactam with amines led to incomplete reactions and low reaction yields. A computational approach provided insights into the energies of adduct formation suggesting a solvent change to remedy the situation. The altered approach allowed the synthesis of targeted perbenzylated galactonoamidine intermediates in yields between 20 and 75%. The subsequent global deprotection results in Pd coordination, incomplete reaction, decomposition, and partial hydrolysis of the target compounds. Efforts to remedy the situation triggered the development of a lengthy purification protocol and ended in near complete compound loss. Future studies will consider different protecting groups of the starting material to avoid Pd-catalyzed deprotections as required for benzyl groups. Nevertheless, our efforts resulted overall in the synthesis of three polar galactonoamidines with full characterization data (**1b**, **1c**, and **1e**), two additional galactonoamidines with evidence for high purity (**1d** and **1k**), and an account of the difficulty to obtain bulky, and bulky-polar galactonoamidines in moderate purity (**1c**, **1f-i** and **1l**). Evidence for decomposition of the target compounds at ambient temperature is not apparent, and all isolated galactonoamidines are stable towards hydrolysis when stored neat at ambient temperature over a minimum of 14 days.

3 Experimental

3.1 Instrumentation.

^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz Bruker magnet with Z gradient and 5 mm broadband head using Topspin 2.1 software. High resolution mass spectrometry data were obtained in the state-wide mass spectrometry facility at the University of Arkansas on a Bruker ultrOTOF-Q quadrupole time-of-flight (qQ-TOF) mass spectrometer equipped with an electrospray ionization source. Combustion data were obtained from Atlantic Microlab, Atlanta. IR spectra were acquired using an ATR-FTIR 8000 spectrophotometer (Shimadzu) equipped with LabSolutions IR software, version 2.15. Melting points were recorded on a Mel-Temp melting point apparatus, and the values are uncorrected. Apparent optical rotations were measured at 589 nm on an Autopol III polarimeter from Rudolph Research Analytical at ambient temperature in a 1 ml cell glass center fill stainless steel jacketed cell with an optical path length l of 100 mm. Amines were purified by distillation using a GKR-51 Kugelrohr apparatus from Büchi. Purification of final compounds was performed as needed using a HPLC system from Shimadzu equipped with SCL-10Avp system controller, 2 LC-20AD analytical pumps, DGU-20A3R three channel online degassers, SIL-20A UFLC autosampler with 96 well capability, CTO-20A/ prominence column oven and ELSD-90LT light scattering and LC solution software, version 1.25 from Shimadzu for data recording and analysis. Nanopure water at a resistance of 18.2 mΩ was obtained from a ThermoScientific Barnstead E-pure™ water purification system. Lyophilization was performed on a FreeZone 1 liter benchtop freeze dry system from Labconco.

3.2 Reagents and materials.

Chemical shifts (δ) in NMR data are expressed in parts per million (ppm) and coupling constants (J) in Hz. Signal multiplicities are denoted as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Chemical shift values are reported relative to the residual signals of these solvents ($CDCl_3$: δ_H 7.29, δ_C 77.0; CD_2Cl_2: δ_H 5.32, δ_C 54.0; acetone-d$_6$: δ_H 2.05, δ_C 29.8; DMSO-d$_6$: δ_H 2.50, δ_C 39.5; CD_3OD: δ_H 3.35, δ_C 49.0; D_2O: δ_H 4.80, δ_C 49.0 after addition of a few drops of CD_3OD.

Column chromatography was carried out using silica gel 60 from Silicycle® (40–63 µm, 230–240 mesh) and basic alumina from Sorbent Technology® (32–63 µm, 150 m2/g; pH = 9.7 ± 0.4) Thin layer chromatography (TLC) was performed using silica gel TLC plates from SORBENT Technologies, 200 µm, 4 × 8 cm, aluminum backed, with fluorescence indicator F254 and detection by UV light or by charring with an ethanolic vanillin-sulfuric acid reagent and subsequent heating of the TLC plate. Amidine pre-purification was achieved on a 250 × 10 mm Luna 5 µm NH_2 100 Å column (Phenomenex) using methanol as an eluent. Compound purity was established on a 300 × 7.5 mm Rezex RMN-Ca^{2+} column (Phenomenex), using nanopure water as an eluent with a flow rate of 0.6 mL/min at 80°C and electric light scattering detection (ELSD) at 50 °C (GAIN 5). All pH values of the buffer solutions were obtained using a Beckman Φ 250 pH meter equipped with a refillable ROSS combination pH electrode from Orion with epoxy body and an 8 mm semi-micro tip. The pH meter was calibrated before each set of readings (3-point calibration).

3.3 Chemicals.

All chemicals have reagent grade or better and are used as received from commercial supplies if not noted otherwise. Trifluoroacetic acid, ethyl acetate, and triethylamine were distilled; dichloromethane, acetonitrile, diethyl ether, and dimethoxyethane were dried dynamically over neutral aluminum oxide; the commercially available amines **5a**, **5c** and **5g** were purified by distillation on a Kugelrohr and stored at -20 °C; amidine **1a** and perbenzylated galactothionolactam **3** were prepared as described.[24,28]

3.4 Syntheses of amines 5

3.4.1 General remarks. The amines **5a**, **5c** and **5g** are commercially available; amines **5b**,[31] **5d**,[32] **5e**,[33] **5f**,[34] **5h**,[35] and **5i**[36] were synthesized by reduction of the corresponding nitriles **7**[31,34,37-40] with lithium aluminium hydride in dry diethyl ether or 1,2-dimethoxyethane using a procedure by Johansson.[32] The amines **5k** and **5l** are new compounds and synthesized likewise.

3.4.2 (4-(2-(Benzyloxy)ethoxy)phenyl)methanamine (**5k**) was prepared using a procedure by Johansson.[32] In the cold, 4-(2-(benzyloxy)ethoxy)benzonitrile (0.75 g, 2.961 mmol, 1.0 equiv) was added to a suspension of lithium aluminum hydride (0.45 g, 11.844 mmol, 4.0 equiv) in dry diethyl ether (30 mL) and allowed to warm to ambient temperature. After 40 h, the reaction mixture was cooled again in an ice bath followed by addition of water (0.45 mL), an aqueous solution of 15 % (wt/wt) of sodium hydroxide (0.45 mL) and water (1.35 mL). After 2 h of continued stirring, a formed precipitate was filtered off and rinsed with ethyl acetate. The combined organic solutions were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to afford a yellow oil. Distillation of this residue on a Kugelrohr (1.66 Torr, 150 °C) afforded the title compound as a colorless oil (0.68 g, 89%); δ_H 7.47 - 7.26 (m, 5 H), 7.25 - 7.21 (m, 2 H), 6.92 - 6.88 (m, 2 H), 4.60 (s, 2 H), 4.15 - 4.09 (m, 2 H), 3.84 - 3.79 (m, 2 H), 3.70 (s, 2 H); δ_C 159.5, 139.6, 136.2, 129.8, 129.5, 129.0, 128.9, 115.8, 74.4, 70.0, 68.8, 46.2; ν_{max} (FTIR) 2868, 1510, 1244, 1107, 816, 738 cm^{-1}; calculated for $C_{16}H_{19}NO_2$: C, 74.68; H, 7.44; N, 5.44; found C, 73.31; H, 7.43; N, 5.28.

3.4.3 (4-(Cyclohexylmethoxy)phenyl)methanamine (**5l**) was prepared using a procedure by Johansson.[32] In short, 4-(cyclohexylmethoxy)benzonitrile[39] was dissolved in the cold in a suspension of lithium aluminum hydride (0.43 g, 11.331 mmol, 4.0 equiv) in dry diethyl ether (40 mL). After 4 h, the mixture was again cooled in an ice bath followed by addition of water (0.43 mL), an aqueous hydroxide solution (0.43 mL, 15%, wt/wt), and water (1.30 mL). After 1 h, the formed slurry was filtered, and the filtrate dried over anhydrous sodium sulfate. After another filtration, the resulting solution was concentrated under reduced pressure to yield a solid residue. The residue was distilled under reduced pressure (1.66 Torr, 225 °C) on a Kugelrohr to afford the title compound as a colorless solid (0.43 g, 69%); mp 75-79 °C; δ_H (acetone-d_6) 7.23 (d, J = 8.3 Hz, 2 H), 6.85 (dd, J = 2.0, 8.6 Hz, 1 H), 4.34 (s, 1 H), 3.76 (d, J = 6.2 Hz, 2 H), 3.71 (s, 1 H), 2.75 (br. s., 1 H), 1.86 (d, J = 13.2 Hz, 2 H), 1.81 - 1.66 (m, 4 H), 1.37 - 1.18 (m, 3 H), 1.08 (dq, J = 3.0, 12.1 Hz, 2 H); δ_C (acetone-d_6) 158.3, 135.0, 128.2, 114.5, 73.6, 45.9, 37.7, 29.9, 26.5, 25.8; ν_{max} (FTIR) 2921, 1510, 1245, 1028, 829 cm^{-1}; calculated for $C_{14}H_{21}NO$: C, 76.67; H, 9.65; N, 6.39; found C, 76.51; H, 9.57; N, 6.39. Amine **5l** was recently described by others with matching spectroscopic data.[41]

3.5 Syntheses of perbenzylated galactonoamidines 6

3.5.1 General procedure. Perbenzylated galactothionolactam **3** was dissolved in dry dichloromethane or acetonitrile and treated with Meerwein's salt in the cold. After 2 h, distilled amine **5** in dichloromethane or acetonitrile was added, and the resulting solution allowed to warm to ambient temperature. After 18 h to 5 days stirring, TLC analysis of an aliquot of the reaction mixture indicated the formation of product (R_f = 0.09-0.29) and frequently the presence of trace amounts of perbenzylated galactoiminothioether (R_f = 0.93-0.96). The reaction mixtures were concentrated under atmospheric pressure and purified by flash column chromatography to afford the title compounds.

3.5.2 *N*-(4-(Benzyloxy)benzyl) 2,3,4,6-tetra-*O*-benzyl-D-galactonoamidine (6b). Galactonothionolactam **3** (0.11 g, 0.199 mmol, 1.0 equiv), dry dichloromethane (4 mL), Meerwein's salt (0.07 g, 0.369 mmol, 1.6 equiv), amine **5b**[31] (0.05 mL, 0.700 mmol, 2.2 equiv) in dry dichloromethane (1 mL) and one drop of triethylamine; 24 h stirring; TLC analysis (SiO_2, hexane/ethyl acetate, 2/1, v/v); flash column chromatography on deactivated silica, cyclohexane/ethyl acetate 10/1→4/1→2/1 (v/v); yellowish oil (0.15 g, 57%); R_f = 0.23 (SiO_2, hexane/ethyl acetate, 1/1, v/v); $\alpha_D^{21} = +14.1$, (c, 1.04, $CHCl_3$); δ_H (CD_2Cl_2) 7.19 - 7.49 (m, 25H), 7.04 - 7.17 (m, 2H), 6.82 - 6.94

(m, 2H), 5.03 - 5.08 (m, 2H), 4.78 - 4.96 (m, 3H), 4.41 - 4.70 (m, 7H), 4.28 - 4.36 (m, 1H), 4.19 (dd, *J*=24.4, 14.1 Hz, 2H), 3.97 (dd, *J*=9.7, 1.8 Hz, 1H), 3.55 - 3.78 (m, 3H); δ_C (CD_2Cl_2) 158.4, 156.3, 139.7, 139.4, 138.9, 138.7, 137.9, 132.7, 129.5, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 115.2, 82.4, 76.0, 75.2, 74.7, 74.2, 73.8, 72.0, 71.6, 70.5, 60.0, 44.8; ν_{max} (FTIR) 3062, 2865, 2174, 2022, 1813, 1668, 1509, 1237, 1092, 736 cm^{-1}; HRMS (ESI) calcd for C48H49N2O5 [M+H]+: 733.3641; found 733.3627.

3.5.3 *N*-(2-(1*H*-Indol-3-yl)ethyl) 2,3,4,6-tetra-*O*-benzyl-D-galactonoamidine (6c). Galactothionolactam **3** (0.30 g, 0.542 mmol, 1.0 equiv), dry dichloromethane (4 mL), Meerwein's salt (0.15 g, 0.813 mmol, 1.5 equiv), amine **5c**; 37 h stirring; TLC analysis (SiO_2, hexane/ethyl acetate, 2/1, v/v); starting material (R_f = 0.76), product (R_f = 0.09); flash chromatography on deactivated silica using gradient elution with cyclohexane/ethyl acetate 8/1→4/1→1/1→1/3 (v/v); beige foam (0.19 g, 52%); R_f = 0.09 (SiO_2, hexane/ethyl acetate, 2/1, v/v); $\alpha_D^{18} = +35.60$, (0.705, $CHCl_3$); δ_H (CD_2Cl_2) 8.12 (br. s., 1 H), 7.60 (d, *J* = 8.1 Hz, 1 H), 7.52 - 7.23 (m, 19 H), 7.20 - 7.11 (m, 3 H), 7.10 - 6.98 (m, 1 H), 6.85 (s, 1 H), 4.95 (d, *J* = 11.4 Hz, 1 H), 4.82 (dd, *J* = 3.0, 11.5 Hz, 2 H), 4.66 (d, *J* = 10.8 Hz, 1 H), 4.64 - 4.56 (m, 3 H), 4.53 (d, *J* = 11.2 Hz, 1 H), 4.45 (d, *J* = 9.5 Hz, 1 H), 4.32 (s, 1 H), 3.95 (dd, *J* = 1.8, 9.7 Hz, 1 H), 3.79 (dd, *J* = 3.8, 6.9 Hz, 1 H), 3.76 - 3.63 (m, 2 H), 3.44 (t, *J* = 6.3 Hz, 2 H), 2.90 (t, *J* = 6.8 Hz, 2 H); δ_C (CD_2Cl_2) 156.6, 139.8, 139.3, 138.9, 138.7, 136.9, 129.0, 128.9, 128.8, 128.6, 128.6, 128.4, 128.3, 128.2, 128.1, 128.0, 122.7, 122.3, 119.6, 119.3, 113.8, 111.6, 82.3, 76.2, 75.3, 74.6, 74.2, 73.8, 71.9, 71.6, 60.0, 41.4, 25.2; ν_{max} (FTIR) 2865, 1637, 1495, 1084, 738 cm^{-1}; HRMS (ESI) calculated for $C_{44}H_{46}N_3O_4$ [M+H]$^+$: 680.3488; found 640.3482.

3.5.4 *N*-(Naphthalen-2-ylmethyl) 2,3,4,6-tetra-*O*-benzyl-D-galactonoamidine (6d). Galactothionolactam **3** (0.16 g, 0.290 mmol, 1.0 equiv), dry dichloromethane (2 mL), Meerwein's salt (0.08 g, 0.421 mmol, 1.5 equiv), distilled napthalen-2-ylmethanamine (**5d**) (0.09 g, 0.572 mmol, 2.0 equiv) in dichloromethane (1 mL) and 1 drop of triethylamine; 90 h stirring; TLC analysis (SiO_2, hexane/ethyl acetate, 1/1, v/v); flash column chromatography on deactivated silica using cyclohexane and ethyl acetate for gradient elution from 1/0→10/1→4/1 (v/v); yellowish oil (0.0727 g, 37%); R_f = 0.13 (SiO_2, hexane/ ethyl acetate, 2/1, v/v); $\alpha_D^{24} = +13.2$ (1.030, $CHCl_3$); δ_H (CD_2Cl_2) 7.70 - 7.90 (m, 3H), 7.65 (s, 1H), 7.20 - 7.50 (m, 21H), 4.95 (d, *J*=11.0 Hz, 1H), 4.89 (d, *J* = 11.4 Hz, 1H), 4.84 (d, *J*=11.7 Hz, 1H), 4.61 - 4.73 (m, 3H), 4.52 - 4.60 (m, 3H), 4.39 - 4.51 (m, 2H), 4.33 (s, 1H), 4.01 (dd, *J* = 9.5, 1.8 Hz, 1H), 3.69 - 3.80 (m, 2H), 3.57 - 3.69 (m, 1H); δ_C (CD_2Cl_2) 156.5, 139.8, 139.4, 138.9, 138.6, 137.9, 134.0, 133.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.5, 128.4, 128.4, 128.2, 128.1, 128.1, 128.0, 126.7, 126.5, 126.4, 126.1, 82.4, 76.1, 75.3, 74.7, 74.3, 73.8, 71.9, 71.7, 60.0, 45.5; ν_{max} (FTIR) 2862, 1644, 1089, 738 cm^{-1}; HRMS (ESI) calcd. for $C_{45}H_{45}N_2O_4$ [M+H]$^+$: 677.3379; found: 677.3372.

3.5.5 *N*-(4-Butoxybenzyl) 2,3,4,6-tetra-*O*-benzyl-D-galactonoamidine (6e). Galactothionolactam **3** (0.10 g, 0.181 mmol, 1.0 equiv), dry dichloromethane (2 mL), Meerwein's salt (0.05 g, 0.263 mmol, 1.5 equiv), distilled 4-butoxybenzylamine (**5e**) (0.08 g, 0.446 mmol, 2.5 equiv) in dry dichloromethane (1 mL); 18 h stirring; TLC analysis (SiO_2, hexane/ethyl acetate, 1/1, v/v); flash column chromatography on deactivated silica using cyclohexane and ethyl acetate from 1/0→10/1→4/1 (v/v); yellowish oil (0.0556 g, 44%); R_f = 0.17 (SiO_2, hexane/ ethyl acetate, 2/1, v/v); $\alpha_D^{24} = +10.8$ (0.880, $CHCl_3$); δ_H (CD_2Cl_2) 7.18 - 7.48 (m, 20H), 7.05 - 7.15 (m, 2H), 6.75 - 6.84 (m, 2H), 4.93 (d, *J* = 11.9 Hz, 1H), 4.78 - 4.88 (m, 2H), 4.59 - 4.69 (m, 3H), 4.53 - 4.58 (m, 2H), 4.50 (d, *J* = 9.7 Hz, 1H), 4.28 - 4.33 (m, 1H), 4.19 (dd, *J* = 23.7, 14.3 Hz, 2H), 3.89 - 4.02 (m, 3H), 3.66 - 3.80 (m, 2H), 3.58 - 3.65 (m, 1H), 1.76 (quin, *J* = 7.2 Hz, 2H), 1.41 - 1.56 (m, 2H), 0.98 (t, *J* = 7.3 Hz, 3H); δ_C (CD_2Cl_2) 158.9, 156.4, 139.7, 139.4, 138.9, 138.6, 132.0, 129.5, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 114.9, 82.3, 76.0, 75.2, 74.6, 74.2, 73.8, 71.9, 71.6, 68.3, 60.0, 44.9, 31.9, 19.8, 14.2; ν_{max} (FTIR) 3429, 2865, 2362, 1966, 1640, 1502, 1088, 737 cm^{-1}; HRMS (ESI) calcd for $C_{45}H_{51}N_2O_5$ [M+H]$^+$: 699.3798; found: 699.3785.

3.5.6 *N*-(4-(Piperidin-1-yl)phenyl)methyl 2,3,4,6-tetra-*O*-benzyl-D-galactonoamidine (6f). Perbenzylated galactothionolactam **3** (0.12 g, 0.217 mmol, 1.0 equiv), dry dichloromethane (2 mL), Meerwein's salt (0.06 g, 0.316 mmol, 1.5 equiv), distilled ((4-piperidin-1-yl)phenyl)methanamine (**5f**) (0.08 g, 0.420 mmol, 1.9 equiv) in dichloromethane (1 mL); 136 h stirring; TLC analysis (SiO_2, hexane/ethyl acetate, 1/1, v/v); flash column chromatography on deactivated silica with gradient elution using hexane and ethyl acetate from 1/0→10/1→4/1→1/1 (v/v); yellowish oil (0.0623 g, 40%); $R_f = 0.19$ (SiO_2, hexane/ethyl acetate, 1/1, v/v); $\alpha_D^{21} = +5.8$ (0.625, $CHCl_3$); δ_H (CD_2Cl_2) 7.18 - 7.50 (m, 20H), 7.09 (dd, J=8.3, 1.5 Hz, 2H), 6.85 (dd, J=8.4, 1.7 Hz, 2H), 4.95 (d, J=11.6 Hz, 1H), 4.85 (t, J=9.4 Hz, 2H), 4.65 (t, J=11.4 Hz, 3H), 4.58 (s, 2H), 4.52 (d, J=9.5 Hz, 1H), 4.33 (s, 1H), 4.17 (q, J=13.4 Hz, 2H), 4.00 (d, J=8.8 Hz, 1H), 3.59 - 3.81 (m, 3H), 3.13 (t, J=5.3 Hz, 4H), 1.65 - 1.79 (m, 4H), 1.52 - 1.64 (m, 2H); δ_C (CD_2Cl_2) 156.3, 152.1, 139.8, 139.4, 138.9, 138.7, 130.4, 129.0, 129.0, 128.9, 128.7, 128.6, 128.5, 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 116.9, 116.6, 82.4, 76.1, 75.2, 74.7, 74.1, 73.8, 72.0, 71.6, 60.0, 51.3, 46.9, 45.0, 26.5, 24.9; ν_{max} (FTIR) 3745, 2929, 1645, 1509, 1234, 1089, 697 cm^{-1}; HRMS (ESI) calcd for $C_{46}H_{52}N_3O_4$ $[M+H]^+$: 710.3958; found 710.3964.

3.5.7 *N*-(4’-Phenylbenzyl) 2,3,4,6-tetra-*O*-benzyl-D-galactonoamidine (6g). Galactothionolactam **3** (0.21 g, 0.379 mmol, 1.0 equiv), dry acetonitrile (3 mL), Meerwein’s salt (0.11 g, 0.579 mmol, 1.5 equiv), 4-phenylbenzylamine (**5g**) (0.17 g, 0.928 mmol, 2.4 equiv) in acetonitrile (1 mL); 5 days stirring; TLC analysis (SiO_2, hexane/ethyl acetate, 2/1, v/v); flash chromatography on deactivated silica with gradient elution using hexane/ethyl acetate from 8/1→4/1→1/1 (v/v), and basic alumina using hexane/ethyl acetate from 10/1→4/1→1/1 (v/v); yellowish oil (0.08 g, 30%); $R_f = 0.29$ (SiO_2, hexane/ethyl acetate, 1/1, v/v); $\alpha_D^{18} = +13.4$, (c, 0.620, $CHCl_3$); δ_H (CD_2Cl_2) 7.58 - 7.64 (m, 2H), 7.51 - 7.56 (m, 2H), 7.26 - 7.50 (m, 25H), 4.95 (d, J=11.0 Hz, 1H), 4.90 (d, J=11.4 Hz, 1H), 4.85 (d, J=11.7 Hz, 1H), 4.60 - 4.71 (m, 3H), 4.51 - 4.60 (m, 3H), 4.25 - 4.39 (m, 3H), 4.00 (dd, J=9.6, 1.7 Hz, 1H), 3.69 - 3.78 (m, 2H), 3.59 - 3.69 (m, 1H); δ_C (CD_2Cl_2) 156.4, 141.4, 140.3, 139.7, 139.4, 138.9, 138.7, 129.3, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.6, 127.5, 82.4, 76.0, 75.3, 74.6, 74.4, 73.8, 71.9, 71.7, 60.0, 45.0; ν_{max} (FTIR) 3745, 2863, 2161, 1647, 1494, 1088, 696 cm^{-1}; HRMS (ESI) calcd. for $C_{47}H_{47}N_2O_4$ $[M+H]^+$: 703.3536; found: 703.3530.

3.5.8 *N*-(4’-Methylbiphenyl-4-yl)methyl 2,3,4,6-tetra-*O*-benzyl-D-galactonoamidine (6h). Perbenzylated galactothionolactam **3** (0.22 g, 0.397 mmol, 1.0 equiv), dry acetonitrile (3 mL), Meerwein’s salt (0.11 g, 0.579 mmol, 1.5 equiv) distilled ((4’-methyl-[1,1’-biphenyl])-4-yl)methanamine (**5h**) (0.17 g, 0.862 mmol, 2.2 equiv) in acetonitrile (1 mL); 115 h stirring; TLC analysis (SiO_2, hexane/ethyl acetate, 2/1, v/v) indicating side product (R_f = 0.29); flash chromatography on deactivated silica using hexane and ethyl acetate from 8/1→4/1 (v/v), and basic alumina using the same solvents from 6/1→3/1 (v/v); yellowish oil (0.10 g, 35 %)); $R_f = 0.21$ (SiO_2; hexane/ethyl acetate, 1/1, v/v); $\alpha_D^{20} = +7.05$, (0.553, $CHCl_3$); δ_H (CD_2Cl_2) 7.45 - 7.54 (m, 4H), 7.21 - 7.43 (m, 24H), 4.94 (d, J=11.0 Hz, 1H), 4.88 (d, J=11.4 Hz, 1H), 4.84 (d, J=11.7 Hz, 1H), 4.64 (dd, J=11.2, 9.2 Hz, 3H), 4.56 (s, 2H), 4.50 - 4.55 (m, 1H), 4.23 - 4.40 (m, 3H), 3.99 (dd, J=9.7, 1.8 Hz, 1H), 3.67 - 3.77 (m, 2H), 3.58 - 3.67 (m, 1H), 2.39 (s, 3H); δ_C (CD_2Cl_2) 156.3, 140.3, 140.2, 139.7, 139.7, 139.4, 139.2, 138.9, 138.7, 138.5, 137.7, 136.5, 130.0, 129.2, 129.1, 129.0, 128.9, 128.8, 128.8, 128.7, 128.7, 128.6, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.3, 127.3, 82.5, 76.1, 75.3, 74.7, 74.3, 73.8, 72.0, 71.7, 70.8, 70.2, 69.9, 60.1, 45.0, 21.4; ν_{max} (FTIR) 2863, 2164, 1649, 1496, 1209, 1090, 802, 696 cm^{-1}; HRMS (ESI) calcd for $C_{48}H_{49}N_2O_4$ $[M+H]^+$: 717.3692; found 717.3675.

3.5.9 *N*-(4’-Fluorobiphenyl-4-yl)methyl 2,3,4,6-tetra-*O*-benzyl-D-galactonoamidine (6i). Galactothionolactam **3** (0.13 g, 0.235 mmol, 1.0 equiv), dry acetonitrile (2 mL), Meerwein’s salt (0.07 g, 0.369 mmol, 1.6 equiv), distilled ((4’-fluorobiphenyl)-4-yl)methanamine (**5i**) (0.09 g, 0.447 mmol, 1.9 equiv) in acetonitrile (1 mL); 5 days stirring; TLC analysis (SiO_2, hexane/ethyl acetate, 2/1, v/v) indicating side product (R_f = 0.29); flash chromatography on deactivated silica using gradient elution with hexane and ethyl acetate from 10/1→4/1 (v/v); yellowish oil (0.05 g, 30 %); $R_f = 0.15$ (SiO_2, hexane/ethyl acetate, 1/1, v/v); $\alpha_D^{20} = +12.4$, (0.750, $CHCl_3$); δ_H (CD_2Cl_2) 7.57 - 7.65 (m, 2H), 7.51 (d, J=8.3 Hz, 2H), 7.44 - 7.48 (m, 2H), 7.27 - 7.43 (m, 20H), 7.15 - 7.22 (m, 2H), 5.13 (br. s, 1H), 4.99 (d, J=11.0 Hz, 1H), 4.94 (d, J=11.4 Hz, 1H), 4.88 (d, J=11.6 Hz, 1H), 4.72 (d, J=2.8 Hz, 1H), 4.65 - 4.70 (m, 2H), 4.55 - 4.63 (m, 3H), 4.36 (d, J=6.2 Hz, 3H), 4.04 (dd, J=9.5, 1.7 Hz, 1H), 3.72 - 3.81 (m, 2H), 3.62 - 3.71 (m, 1H); δ_C (CD_2Cl_2) 164.2, 161.8, 156.3, 139.7, 139.5, 139.4, 139.2, 138.9, 138.7, 137.7 (d, J = 3.2 Hz), 129.2, 129.1, 129.0, 128.9, 128.8, 128.7, 128.5, 128.4, 128.4, 128.3, 128.2, 128.1 (d, J = 4.4

Hz), 127.4, 116.1 (d, J = 21.5 Hz), 82.5, 76.1, 75.3, 74.7, 74.4, 73.8, 71.9, 71.7, 60.0, 45.0; ν_{max} (FTIR) 2863, 1647, 1496, 1090, 697 cm^{-1}; HRMS (ESI) calcd for $C_{47}H_{46}FN_2O_4$ $[M+H]^+$: 721.3442; found 721.3443.

3.5.10 *N*-(4-(2-(Benzyloxy)ethoxy)benzyl) 2,3,4,6-tetra-*O*-benzyl-D-galactonoamidine (6k). galactothionolactam **3** (0.14 g, 0.253 mmol, 1.0 equiv), dry acetonitrile (2 mL), Meerwein's salt (0.07 g, 0.369 mmol, 1.5 equiv), distilled amine **5k** (0.10 g, 0.389 mmol, 2.0 equiv) in dry acetonitrile (1 mL); 94 h stirring; TLC analysis (SiO_2, hexane/ethyl acetate, 2/1, v/v); flash column chromatography on deactivated silica using hexane and ethyl acetate from 10/1→4/1→1/2 (v/v); yellowish oil (0.0436 g, 22%); R_f = 0.07 (SiO_2, hexane/ethyl acetate, 2/1, v/v); $\alpha_D^{21} = +6.80$ (0.676, $CHCl_3$); δ_H (CD_2Cl_2) 7.48 - 7.19 (m, 23 H), 7.12 (d, *J* = 8.1 Hz, 2 H), 6.83 (d, *J* = 8.6 Hz, 2 H), 4.93 (d, *J* = 11.0 Hz, 1 H), 4.84 (t, *J* = 9.9 Hz, 2 H), 4.71 - 4.58 (m, 6 H), 4.58 - 4.53 (m, 2 H), 4.50 (d, *J* = 9.5 Hz, 1 H), 4.30 (t, *J* = 2.2 Hz, 1 H), 4.25 - 4.16 (m, 2 H), 4.16 - 4.09 (m, 2 H), 3.97 (dd, *J* = 1.8, 9.7 Hz, 1 H), 3.86 - 3.79 (m, 2 H), 3.78 - 3.67 (m, 2 H), 3.66 - 3.58 (m, 1 H); δ_C (CD_2Cl_2) 158.5, 156.3, 139.7, 139.4, 139.0, 138.9, 138.6, 132.6, 129.5, 129.1, 129.0, 128.9, 128.9, 128.7, 128.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 115.0, 82.4, 76.0, 75.2, 74.7, 74.2, 73.8, 73.8, 72.0, 71.6, 69.3, 68.1, 60.0, 44.8; ν_{max} (FTIR) 2864, 2158, 1645, 1506, 1091, 696 cm^{-1}; HRMS (ESI) calculated for $C_{50}H_{53}N_2O_6$ $[M+H]^+$: 777.3904; found: 777.3891.

3.5.11 *N*-(4-Cyclohexylmethoxy)benzyl 2,3,4,6-tetra-*O*-benzyl-D-galactonoamidine (6l). Galactothionolactam **3** (0.22 g, 0.397 mmol, 1.0 equiv), dry acetonitrile (3 mL), Meerwein's salt (0.11 g, 0.596 mmol, 1.5 equiv), amine **5l** (0.17 g, 0.775 mmol, 2.0 equiv) in dry acetonitrile (1 mL); 24 h stirring; TLC analysis (SiO_2, hexane/ethyl acetate, 1/1, v/v); flash column chromatography on deactivated silica using gradient elution with hexane and ethyl acetate from 1/0→10/1→4/1→1/1 (v/v) and basic alumina from 10/1→4/1→1/1 (v/v); yellowish oil (0.22 g, 75 %); R_f = 0.26 (SiO_2, hexane/ethyl acetate, 1/1, v/v); $\alpha_D^{20} = +8.50$, (0.659, $CHCl_3$); δ_H (CD_2Cl_2) 7.17 - 7.46 (m, 20H), 6.99 - 7.16 (m, 2H), 6.72 - 6.85 (m, 2H), 4.93 (d, *J*=11.0 Hz, 1H), 4.82 (dd, *J*=11.4, 7.7 Hz, 2H), 4.59 - 4.69 (m, 3H), 4.56 (s, 2H), 4.46 - 4.51 (m, 1H), 4.27 - 4.34 (m, 1H), 4.10 - 4.23 (m, 2H), 3.96 (dd, *J*=9.7, 1.8 Hz, 1H), 3.51 - 3.81 (m, 5H), 1.61 - 1.90 (m, 7H), 1.14 - 1.40 (m, 4H), 1.06 (qd, *J*=11.9, 3.1 Hz, 2H); δ_C (CD_2Cl_2) 159.0, 156.2, 139.8, 139.4, 138.9, 138.7, 132.1, 129.4, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 114.9, 114.8, 82.4, 76.1, 75.2, 74.7, 74.2, 74.1, 73.8, 72.0, 71.6, 60.1, 44.9, 38.3, 30.5, 27.2, 26.4; ν_{max} (FTIR) 2921, 2210, 1645, 1505, 1089, 697 cm^{-1}; HRMS (ESI) calculated $C_{48}H_{55}N_2O_5$ $[M+H]^+$: 739.4111; found 739.4108.

3.6 Syntheses of galactonoamidines 1

3.6.1 General procedure.[28] The perbenzylated galactonoamidines **6** were dissolved in ethanol and treated with hydrogen gas in presence of palladium (30 wt % on activated carbon) and trifluoroacetic acid at ambient temperature. After 36-72 h, the mixtures were filtered through a pad of Celite®. The pad was rinsed with 2 mL of ethanol 3-5 times. The combined filtrates were concentrated under reduced pressure to about 2 mL and centrifuged at 8500 rpm for 10-15 minutes. The supernatant was concentrated to dryness to obtain a residue that was further purified on an amino phase using high performance liquid chromatography. Acetonitrile (80%) was used as an eluent. For further purification of galactonoamidines **1**, all residues were suspended in nanopure water, separated from precipitates by centrifugation, and purified over Sephadex LH-20-100 to yield the target compounds at the specified purity. Unfortunately, compounds **1d**, **1f** and **1h-l** were isolated in insufficient amounts despite two repetitive syntheses and purification attempts preventing full compound characterization (see Supporting Information).

3.6.2 *N*-4-Hydroxybenzyl D-galactonoamidine (1b). Perbenzylated amidine **6b** (0.1017 g, 0.1388 mmol), ethanol (5 mL), 0.11 g Pd/C, trifluoroacetic acid (1 mL); 48 h stirring; colorless foam (0.0370 g, 94%); R_f = 0.29 (SiO_2, Ethyl acetate/methanol = 5/1, v/v), $\alpha_D^{24} = +17.4$, (c 1.00, H_2O); δ_H (D_2O) 7.18 (d, *J* = 8.6 Hz, 2 H), 6.84 (dd, *J* = 1.0, 7.0 Hz, 2 H), 4.55 (d, *J* = 10.1 Hz, 1 H), 4.47 (d, *J* = 2.6 Hz, 2 H), 4.22 (t, *J* = 2.2 Hz, 1 H), 3.90 (dd, *J* = 2.4, 10.1 Hz, 1 H), 3.79 - 3.60 (m, 3 H); δ_C (D_2O + DMSO-d_6) 165.6, 164.7, 157.2, 130.9, 127.2, 119.4, 117.5, 116.6, 72.2, 68.6, 68.1, 61.7, 58.9, 46.5: ν_{max} (FTIR) 3258, 1672, 1184, 1120 cm^{-1}; HRMS (ESI) *m/z* calcd for $C_{13}H_{19}N_2O_5$ [M+H]$^+$: 283.1294; found 283.1285; purity determined on Rezex RMN-Ca^{2+} (eluent 100% H_2O) 96.1 %.

3.6.3 *N*-(2-(Octahydro-1*H*-indol-3-yl)ethyl) D-galactonoamidine (1c). Perbenzylated amidine **6c** (0.0607 g, 0.0893 mmol), ethanol (6 mL), 0.06 g Pd/C, trifluoroacetic acid (2 mL); 61 h stirring; yellowish oil (4.46 mg, 0.140 mmol, 15.6%); R_f = 0.26 (SiO_2, dichloromethane/methanol = 25/1, v/v); δ_H (D_2O) 4.52 (d, *J* = 10.1 Hz, 1 H), 4.27 (s., 1 H), 3.91 (d, *J* = 10.5 Hz, 1 H), 3.86 - 3.72 (m, 3 H), 3.70 - 3.57 (m, 1 H), 3.37 (m, 3 H), 3.11 - 2.90 (m, 1 H), 2.87 - 2.72 (m, 1 H), 2.57 - 2.34 (m, 1 H), 2.34 - 2.24 (m, 1 H), 2.24 - 2.12 (m, 1 H), 2.11 - 1.88 (m, 2 H), 1.86 - 1.66 (m, 3 H), 1.60 (d, *J* = 10.8 Hz, 3 H), 1.43 - 1.03 (m, 4 H), 0.99 - 0.74 (m, 1 H); δ_C (D_2O + CD_3OD) 165.0, 164.0, 163.7, 121.7, 118.8, 115.9, 113.0, 71.5, 68.0, 67.3, 64.5, 61.0, 59.3, 58.2, 48.0, 42.8, 41.3, 40.0, 39.5, 36.9, 31.2, 30.1, 28.8, 25.8, 25.4, 24.9, 23.7, 22.6, 21.3, 21.1, 20.0; HRMS (ESI) *m/z* calcd for $C_{16}H_{30}N_3O_4$ [M+H]$^+$: 328.2236; found 328.2230; purity determined on Rezex RMN-Ca^{2+} (eluent 100% H_2O) 99.8%

3.6.4 *N*-4-Butoxybenzyl D-galactonoamidine (1e). Perbenzylated amidine **6e** (0.0905 g, 0.1295 mmol), ethanol (2 mL), 0.05 g Pd/C, trifluoroacetic acid (0.5 mL); 15 h stirring; yellowish foam (0.0437 g, quantitative); R_f = 0.38 (SiO_2; Ethyl acetate/methanol = 5/1, v/v); $\alpha_D^{24} = +26.7$ (0.823, H_2O); δ_H (D_2O) 7.26 (d, *J*=8.6 Hz, 2H), 6.96 (d, *J*=8.6 Hz, 2H), 4.59 (d, *J*=10.1 Hz, 1H), 4.52 (br. s., 2H), 4.25 (br. s., 1H), 4.01 (t, *J*=6.4 Hz, 2H), 3.94 (d, *J*=9.7 Hz, 1H), 3.62 - 3.82 (m, 3H), 1.68 (quin, *J*=6.7 Hz, 2H), 1.38 (dq, *J*=14.5, 7.0 Hz, 2H), 0.88 (t, *J*=7.2 Hz, 3H); δ_C (D_2O/DMSO-d_6) 165.6, 159.9, 130.7, 127.9, 116.9, 72.2, 70.1, 68.6, 68.1, 61.7, 58.9, 46.3, 32.0, 20.2, 14.7; ν_{max} (FTIR) 3283, 1667, 1193, 723 cm^{-1}; HRMS (ESI) calcd for $C_{17}H_{27}N_2O_5$ [M+H]$^+$: 339.1920; found 339.1909; purity determined on Rezex RMN-Ca^{2+} (eluent 100% H_2O) 100%.

3.6.5 *N*-*N*-4'-Phenylbenzyl D-galactonoamidine (1g). Perbenzylated amidine **6g** (0.08 g, 0.114 mmol), ethanol (5 mL), 0.08 g Pd/C, trifluoroacetic acid (1 mL); 41 h stirring; colorless foam (0.0390 g, 0.1139 mmol, quantitative); R_f = 0.07 (SiO_2, ethyl acetate/methanol = 5/1 v/v); $\alpha_D^{25} = +25.4$, (c 0.528, water); δ_H (CD_3OD) 7.71 - 7.58 (m, 4 H), 7.52 - 7.40 (m, 4 H), 7.38 - 7.30 (m, 1 H), 4.74 - 4.54 (m, 3 H), 4.21 (t, *J* = 2.5 Hz, 1 H), 3.87 (dd, *J* = 2.3, 9.6 Hz, 1 H), 3.84 - 3.73 (m, 2 H), 3.70 (dd, *J* = 2.7, 6.3 Hz, 1 H); δ_C (CD_3OD) 166.8, 142.7, 141.8, 134.9, 130.1, 129.1, 128.8, 128.7, 128.1, 72.9, 68.8, 68.6, 61.6, 59.7, 46.0; ν_{max} (FTIR) 3278, 1661, 1186 1131 cm^{-1}; HRMS (ESI) *m/z* calcd for $C_{19}H_{23}N_2O_4$ [M+H]$^+$: 343.1658; found 343.1657; purity determined on Rezex RMN-Ca^{2+} (eluent 100% H_2O) 77%.

4. Computational details

All electronic structure calculations are performed on a Dell Precision T7500 workstation equipped with the PQSmol suite.[42] Low energy conformers of the adducts derived from perbenzylated thionolactam **3** and amines **5** were calculated with density functional theory using the B3LYP method and the 6-31G(d) basis set.[43,44] All stationary points were examined with vibrational analyses and confirmed as minima with zero imaginary frequency. The solvation free energies are computed with the COSMO feature in PQSmol at 298.15 K and 1 atm using a dielectric constant of 36.64 for acetonitrile and 8.93 for dichloromethane.[45,46] The Gibb's free energy in the gas phase and the change in Gibb's free energy in solution are deduced from the computed energies, enthalpies and entropies as described: $G_{gas} = H_{gas} - T \times S_{gas}$; $\Delta G_{sol} = E_{sol}^{298} - E_{gas}^{298} + G_{gas} + G_{nes}$; $E_{gas}^{298} = E_{total} + E_{zero,\ vib}$; $\Delta\Delta G = \Delta G_{sol}$(**adduct II**)+$\Delta G_{sol}$(**H-5**)–2×$\Delta G_{sol}$(**5**)–$\Delta G_{sol}$(H-**4**)._ENREF_51[47]

ASSOCIATED CONTENT

SUPPORTING INFORMATION.

NMR, IR and MS spectra of all new amines, perbenzylated galactonoamidines **6** and amidines **1b-c**, **1e** and **1g**; HRMS chromatograms and HPLC traces of galactonoamidines **1**; geometries, energies and thermodynamic parameters of all computed structures. This material is available free of charge via the Internet at http://pubs.acs.org

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The authors declare no competing financial interest.

ACKNOWLEDGMENT

Support of this research to S.S. from the National Science Foundation (CHE-1854304) and the Arkansas Biosciences Institute is gratefully acknowledged. The authors thank Peter Pulay for access to PQSmol and his advice during the computational analyses.

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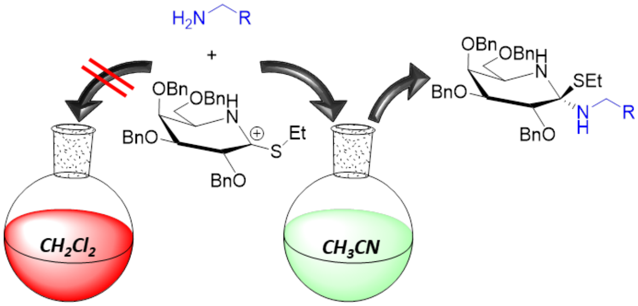
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