Potent GH20 N-Acetyl-β-D-hexosaminidase Inhibitors: N-Substituted 3-acetamido-4-amino-5-hydroxymethyl-cyclopentanediols

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Abstract: From 1,2;3,4-di-O-isopropylidene-D-galactopyranose, a preliminary series of highly functionalized amino(hydroxymethyl)cyclopentanes was easily available. These amine-containing basic carbasugars featuring the D-galacto configuration are potent inhibitors of the GH20 β-D-hexosaminidases probed and may bear potential as regulators of N-acetyl-D-hexosaminidase activities in vivo.

Keywords: aminocyclopentane; N-acetyl-D-hexosaminidase inhibitor; Tay-Sachs

1. Introduction

In humans, N-acetyl-D-hexosaminidases (HexA, HexB, and HexS) play essential roles in the lysosomal processing of degradation-bound glycolipids as well as glycans [1]. O-GlcNAcase [2] removes O-linked GlcNAc residues from serine or threonine in nucleocytoplasmic proteins. Another N-acetylhexosaminidase, HexD, was recently reported and has been mentioned in context with rheumatoid arthritis [3].

Whereas the retaining lysosomal N-acetyl-α-D-hexosaminidases of GH89 follow the standard double-displacement mechanism, N-acetyl-β-D-hexosaminidases of GH20, as well as the GH84 O-GlcNAcase, GH18 chitinases, and GH85 endo-N-acetyl-β-D-glucosaminidases exploit anchimeric assistance by the sugar’s N-acetyl group, which upon intramolecular attack of the intermediary oxocarbenium ion forms an α-configured oxazoline intermediate. This, in turn, is attacked from the β-face by an activated water molecule [4]. Interesting reviews on hexosaminidases are available [5,6].

Potent and highly selective inhibitors are required for studying these enzymes and their physiological significance. In particular, potential therapeutic applications in context with lysosomal disorders [7], cancer [8], and Alzheimer’s disease [9] require high degrees of selectivity for any of the enzymes mentioned above over the respective other N-acetylhexosaminidases.

Amongst N-acetylhexosaminidase inhibitors (Figure 1), 2-acetamido-1,2-dideoxynojirimycin, 1 [10] and diastereomers such as the D-galacto (2) [11] and the D-erro (3) [12] analogs, PUGNAc (4) [13], and NAG-thiazoline (NGT, 5) [14] have attracted considerable attention. Furthermore, Thiame G (6) [15], nagstatin (7) [16], and 6-acetamido-6-deoxycastanospermine (8) [17], various pyrrolidine derivatives...
(for example, compound 9 [18]), as well as 2-N-acetyl glycals including 10 [19] have been reported. Amongst carbacyclic hexosaminidase inhibitors, pyranoid carbasugar acetamidodeoxy-β-valienamine (11) has to be mentioned [20]. These inhibitors are either substrate/product analogs or, in the case of bicyclic systems such as NGT, are chemically stable structural analogues of the above-mentioned intermediate generated by anchimeric assistance of the N-acetyl group at C-1 at the first transition state of enzymatic N-acetylhexosaminide hydrolysis.

![Chemical structures](image)

**Figure 1.** Examples of established as well as recently reported N-acetyl-D-hexosaminidase inhibitors.

Jäger and co-workers [21] have directed our attention toward cyclopentanoid basic sugar analogs as potentially useful inhibitors of lysosomal glycosidases. Relying on the pioneering synthetic work by Vasella and co-workers [22] based on guiding contributions by Padwa [23] as well as Oppolzer [24], we had thus investigated cyclopentane-based β-galactosidase inhibitors [25] and have recently extended our range of new compounds by addition of N-acetyl-β-D-galactosaminide and -β-D-glucosaminide analogs.

2. Results and Discussion

2.1. Synthesis

Starting from known [26] N-benzylisoxazolidine 12 (Scheme 1), by a simple oxidation/reduction sequence, ketone 13 provided epimer 14 in high yield.

Its structural identity can unambiguously be verified by X-ray structure determination (Figure 2). The corresponding triflate 15 provided azidodeoxy derivative 16 by clean inversion of configuration. Intermediate 16, upon reduction with Zn under slightly acidic conditions and subsequent conventional N-acetylation, highly selectively furnished, via free amine 17, desired acetamido compound 18, which yielded crystals of sufficient quality for XRD (Figure 2).
Scheme 1. Introduction of the acetamido group by double inversion approach. a: Swern or Dess-Martin; b: NaBH₄, MeOH; c: (1) Tf₂O, pyr., (2) NaN₃, DMF; d: (1) Zn/NH₄Cl, (2) Ac₂O, pyr.; e: Pd(OH)₂/C, H₂.

Scheme 2. Conversion of crucial intermediate 19 into free inhibitor 20 and N-alkyl derivatives thereof. f: (1) resp. halogenoalkane, NaHCO₃, DMF; (2) HCl/MeOH; g: Raney-Ni, H₂, MeOH; h: dansyl chloride, NaHCO₃, MeOH; i: HCl/MeOH. (Structures show the numbering system applied for NMR-analysis for easier comparison with other D-galactosaminide related compounds).

Chemoselective N-alkylation of intermediate 19 provided the corresponding N-hexyl (21), N-methoxycarbonylpentyl (22), as well as N-cyanopentyl (23) derivatives. By reduction of the nitrile function in 23, primary amine 24 became available, which was converted into fluorescent dansylaminohexyl derivative 25 with the aid of dansyl chloride.
2.2. Biological Evaluation

New compounds turned out particularly potent inhibitors of *Streptomyces plicatus* N-acetyl-β-hexosaminidase (SpHex) with $K_i$-values in the sub-nanomolar range (Table 1). By introduction of a dansylamido moiety into the alkyl chain, this activity was further improved as shown by the 60 pM value determined for inhibitor 25 when compared to analogue 21. With the same enzyme, compound 1 exhibited $K_i = 80 \mu M$ [26].

| Enzyme       | SpHex 0.0007 | HexA (h.lys.) n.d. | 0.88 | 0.0007 | n.d. | 0.0007 | 0.0006 | 0.30 |
|--------------|--------------|--------------------|------|--------|------|--------|--------|------|

Table 1. Inhibitory activities of new compounds with N-acetylhexosaminidases.

$^{1}$IC$_{50}$-values [µM] of compounds with: SpHex = *Streptomyces plicatus* N-acetyl-β-hexosaminidase; HexA h. lys. = IC$_{50}$ [µM] with human lysosomal N-acetyl-β-D-hexosaminidase A.; n.d., not determined.

The latter two compounds were also screened with Tay-Sachs disease-related human lysosomal N-acetyl-β-hexosaminidase A. For comparison, pyrimethamine [27], which under the same screening conditions exhibited IC$_{50} = 62 \mu M$, as well as 2-acetamido-1,2-dideoxyxojirimycin,1 (IC$_{50} 31 \mu M$ [28]) were included in these studies.

Inhibitors 21 and 25 exhibited excellent properties with this vital human enzyme, considerably exceeding the activities of the reference compounds probed in this study.

In conclusion, compounds of this new aminocyclopentanone-derived family of N-acetylglactosaminide mimetics represent a potentially interesting class of N-acetyl-D-hexosaminidase inhibitors and pharmacological chaperones for treatment of Tay Sachs disease, in particular, when also considering their simplicity of synthesis.

3. Materials and Methods

3.1. General Methods

Optical rotations were measured at 20 °C on a Perkin Elmer (Waltham, MA, USA) 341 polarimeter at a wave length of 589 nm and a path length of 10 cm.

NMR spectra were recorded on a Varian (Palo Alto, CA, USA) INOVA 500 operating at 499.82 MHz ($^1$H), and at 125.894 MHz ($^{13}$C), or on a Bruker (Billerica, MA, USA) Ultrashield spectrometer at 300.36 and 75.53 MHz, respectively. CDCl$_3$ was employed for protected compounds and CD$_3$OD as well as D$_2$O for unprotected inhibitors. Chemical shifts are listed in delta employing residual, non-deuterated solvent as the internal standard. Signals were assigned unambiguously by COSY and HMQC analysis. The signals of the N-dansyl group are located in the expected regions and are not listed explicitly. For easier comparison with other N-acetylglactosaminide analogues, interpretation of NMR-spectra was performed according to the carbohydrate-related numbering system depicted in Scheme 2. MALDI-TOF and EI-TOF mass spectrometry were performed on a Micromass (Waters Corporation, Milford, MA, USA) TofSpec 2E Time-of-Flight mass spectrometer. Analytical TLC was performed on pre-coated aluminum plates silica gel 60 F254 (E. Merck, Darmstadt, Germany 5554) and detected with UV light (254 nm). For staining, a solution of vanillin (9 g) in a mixture of H$_2$O (950 mL)/EtOH (750 mL)/H$_2$SO$_4$ (120 mL) or ceric ammonium molybdate (100 g ammonium molybdate/8 g ceric sulfate in 1 l 10% H$_2$SO$_4$) were employed, followed by heating on a hotplate. For column chromatography, silica gel 60 (230–400 mesh, E. Merck 9385) or silica gel 60 (Acros Organics (Thermo Fisher Scientific Inc., Waltham, MA, USA), AC 24036) were used. CCDC contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via
3.2. Biochemical Methods

*Streptomyces plicatus* N-acetyl-β-hexosaminidase, SpHex, was expressed and purified in *E. coli* as described previously [27]. Kinetic studies were performed at 25 °C in the assay buffer (sodium phosphate (50 mM), sodium citrate (50 mM), NaCl (100 mM), BSA (2 mg/mL), pH = 6.0). The enzyme was incubated with different concentrations of the inhibitors for 2–5 min prior to the start of the reaction by addition of the substrate (4-nitrophenyl N-acetyl-β-D-glucosaminide) and the initial rates were measured by monitoring the increase in absorbance at 405 nm for three to five minutes using a microplate reader (Synergy H1, BioTek, VT, USA). *K*<sub>i</sub> determinations were performed using two or three different substrate concentrations. For each one of these substrate concentrations a range of five to eight different inhibitor concentrations bracketing the ultimately determined *K*<sub>i</sub> value were used. Dixon plots (1/rate vs [I]) were constructed to validate the use of a competitive inhibition model. The data were then fit to a competitive inhibition model using non-linear regression analysis with GraFit 7.0 (Erithacus Software, UK). Assays were done twice using enzyme concentrations of 0.3 nM and 0.03 nM respectively in order to check compliance with the assumptions of Michaelis Menten kinetics ([E] << [I]). In addition, for compound 25, the assays were done a third time with the concentration of enzyme lowered to 0.003 nM. In all cases, the *K*<sub>i</sub> values were in good agreement.

Human skin fibroblasts (wild type) were grown in minimal essential medium (MEM) with Earle’s Salts (Sigma Aldrich, St. Louis, MO, USA) containing 10% fetal bovine serum, 400 µM L-glutamine, and 50 µg/mL gentamycin at 37 °C and 5% CO<sub>2</sub>. All cells used in this study were between the third and nineteenth passages.

All inhibitors were dissolved in DMSO in a concentration of 10 mM and diluted in 10 mM phosphate buffer (pH 7.0) containing 100 mM NaCl, 0.01% NaN<sub>3</sub>, and 0.01% Triton for the IC<sub>50</sub>-measurements.

Human N-acetyl-β-hexosaminidase A activity measurements were performed in duplicate assays unless otherwise stated. Fibroblast cells of three single 80 cm<sup>2</sup> flasks were harvested by trypsinization in 500 µL (each of them) 0.9% NaCl containing 0.01% Triton, homogenized by sonication (4 times 15 s, Bandelin (Berlin, Germany) Sonopuls ultrasound homogenator mini 20) and centrifuged at 13,000 rpm for 1 min in a table top centrifuge (Biofuge Pico, Heraeus, Hanau, Germany). Protein amounts were determined according to the method of Lowry. For assessment of N-acetyl-β-hexosaminidase A activity, 10 µL (diluted 1:10) of cell homogenate were mixed with 90 µL 0.9% NaCl and 200 µL of 1 mM (4-methyl)umbelliferyl N-acetyl-β-glucosaminide-6-sulfate. Na (Glycosynth, Warrington, UK) in McIlvains phosphate/citrate puffer, pH 4.4. After incubation at 37 °C for 60 min, the reaction was stopped by adding 2.5 mL 400 mM glycine/NaOH (pH 10.4). The amount of hydrolyzed 4-methylumbelliferone was determined with a fluorescence spectrometer (F7000 Hitachi, Chiyoda, Japan).

Modified β-hexosaminidase A assays were used to estimate the half maximal inhibitory concentration (IC<sub>50</sub>) of the particular inhibitor. For IC<sub>50</sub> determination, 0.001 to 100 µM of inhibitor was added to the assay mixture.

Activity was measured in normal fibroblasts. Data analysis was performed with Microcal™ Origin® v6.0 (Origin Lab, Northampton, MA, USA) using a non-linear curve fitting module based on sigmoid curve fitting.

3.3. (3aR,3bS,6aR,7R,7aR)-Hexahydro-5,5-dimethyl-1-phenyl-1H-[1,3]dioxolo[3,4]cyclopent[1,2-c]isoxazol-7-ol or 1-L-(1,2,3,4,5)-1,2,1-Anhydro-1-hydroxymethyl-2-(N-hydroxy)benzylamino-4,5-O-isopropylidene-3,4,5-cyclopentanetriol 14

(a) Via Swern oxidation: To a solution of oxalyl chloride (1.12 mL, 13.0 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (20 mL), DMSO (1.11 mL, 15.7 mmol) was added dropwise at −60 °C. After 15 min, a 50% solution (w/v) of
alcohol 12 (1.52 g, 5.22 mmol) in CH₂Cl₂ was added and the reaction was stirred for 15 min when Et₃N (2.89 mL, 20.9 mmol) was added. The reaction mixture was allowed to reach ambient temperature and methanol (50 mL) and NaBH₄ (0.39 g, 10.4 mmol) were added. When completed conversion of cyclopentanone 13 was observed (tlc, 30 min), solvents were removed under reduced pressure and crude alcohol 14 was dissolved in CH₂Cl₂. The organic layer was extracted with saturated aqueous NaHCO₃, dried (Na₂SO₄), filtered, and concentrated under reduced pressure. Purification on silica gel (cyclohexane/ethyl acetate 3:1 v/v) provided compound 14 as a pale yellow syrup (848 mg, 2.91 mmol, 55.8% from epimer 12).

(b) Via Dess-Martin oxidation: A 10% solution (v/v) of alcohol 12 (1.05 g, 3.60 mmol) in CH₂Cl₂ was stirred with Dess-Martin periodinane (1.68 g, 3.96 mmol) at ambient temperature for 10 min. After completed conversion, the reaction mixture was washed with saturated aqueous NaHCO₃, dried (Na₂SO₄), and filtered. Removal of solvents under reduced pressure gave the crude product 13.

To a solution of crude ketone 13 in methanol (20 mL), NaBH₄ (0.273 g, 7.21 mmol) was added. When completed conversion was detected (tlc, 30 min), solvents were removed under reduced pressure, and the crude product was dissolved in CH₂Cl₂. The organic layer was extracted with saturated aqueous NaHCO₃, dried (Na₂SO₄), filtered, and concentrated under reduced pressure. Purification on silica gel (cyclohexane/ethyl acetate 3:1 v/v) provided compound 14 as a pale yellow syrup (0.769 g, 2.64 mmol, 73.2% over two steps).

After extended storage, a compound sample provided a few minute crystals, one of which could be exploited for X-ray structure determination (CCDC 1826202).

| MS (MALDI): | Calc'd for [C₁₆H₂₁NO₄Na]: m/z 314.1368 [M + Na]+; Found [M + Na]+ 314.1368.

4.3. (3aR,3bS,6aR,7S,7aR)-Hexahydro-7-azido-5,5-dimethyl-1-phenyl-1H-[1,3]dioxololo[3,4]cyclopent[1,2-clisoaxazol or 1-1-(1,2,4,5,3)-1'-2'-Anhydro-3-azido-1-hydroxymethyl-2-(N-hydroxy)benzylamino-4,5-O-isopropylidene-4,5-cyclopentamed 16

A solution of alcohol 14 (848 mg, 2.91 mmol) in CH₂Cl₂ (20 mL) was cooled to 0 °C. Pyridine (0.940 mL, 11.6 mmol) and trifluoromethanesulfonyl anhydride (0.637 mL, 3.78 mmol) were added. When completed conversion of the starting material was observed (10 min), the reaction mixture was washed consecutively with HCl (6%) and saturated aqueous NaHCO₃. After drying with Na₂SO₄, the suspension was filtered, and the solvent was removed at room temperature under reduced pressure. Resulting crude triflate 15 was dissolved in DMF (20 mL), NaNO₂ (1.14 g, 17.5 mmol) was added and the mixture was stirred at ambient temperature for 60 min. The reaction mixture was then concentrated under reduced pressure, the residue was dissolved with CH₂Cl₂ and the solution was washed with brine. The organic layer was dried (Na₂SO₄), filtered, and concentrated under reduced pressure. Purification of the remaining residue on silica gel (cyclohexane/ethyl acetate 10:1 v/v) provided azidodeoxy compound 16 (568 mg, 1.80 mmol, 61.7% from 14)| [α]D 20: +74.8 (c = 1.09, CHCl₃); 1H-NMR (300 MHz, CDCl₃) δ = 7.44–7.23 (m, 5H, aromatic Nb), 4.58 (dd, 1H, J₂,₃ = 5.5 Hz, H-3), 4.50 (dd, 1H, J₄,₅ = 7.5 Hz, H-4), 4.33 (dd, 1H, J₅,₆a < 1 Hz, J₆a,₆b = 8.7 Hz, H-6a), 4.12 (dd, 1H, J = 13.3 Hz, N-CH₂-Ph), 4.04 (m, 1H, H-2), 3.97 (dd, 1H, J₅,₆b = 6.4 Hz, H-6b), 3.91 (dd, 1H, N-CH₂-Ph), 3.68 (dd, 1H, J₁,₂ = 7.6 Hz, H-1), 3.48 (bs, 1H, 6-CH₂-OH), 3.07 (dd, 1H, H-5), 1.57, 1.25 (2s, 3H each, C( CH₃)₂).

C-NMR (75.5 MHz, CDCl₃): δ = 137.2 (ipso NBn), 129.2, 128.6, 127.6 (aromatic Nb), 112.8 (C(CH₃)₂), 81.7 (C-3), 78.3 (C-4), 73.2 (C-1), 71.2 (C-2), 65.6 (C-6), 62.1 (N-CH₂-Ph), 49.6 (C-5), 25.4, 25.0 (C(CH₃)₂).

MS (MALDI): Calc'd for [C₁₆H₂₁NO₄Na]: m/z 314.1368 [M + Na]+; Found [M + Na]+ 314.1368.

4.3. (3aR,3bS,6aR,7S,7aR)-Hexahydro-7-azido-5,5-dimethyl-1-phenyl-1H-[1,3]dioxololo[3,4]cyclopent[1,2-clisoaxazol or 1-1-(1,2,4,5,3)-1'-2'-Anhydro-3-azido-1-hydroxymethyl-2-(N-hydroxy)benzylamino-4,5-O-isopropylidene-4,5-cyclopentamed 16

A solution of alcohol 14 (848 mg, 2.91 mmol) in CH₂Cl₂ (20 mL) was cooled to 0 °C. Pyridine (0.940 mL, 11.6 mmol) and trifluoromethanesulfonyl anhydride (0.637 mL, 3.78 mmol) were added. When completed conversion of the starting material was observed (10 min), the reaction mixture was washed consecutively with HCl (6%) and saturated aqueous NaHCO₃. After drying with Na₂SO₄, the suspension was filtered, and the solvent was removed at room temperature under reduced pressure. Resulting crude triflate 15 was dissolved in DMF (20 mL), NaNO₂ (1.14 g, 17.5 mmol) was added and the mixture was stirred at ambient temperature for 60 min. The reaction mixture was then concentrated under reduced pressure, the residue was dissolved with CH₂Cl₂ and the solution was washed with brine. The organic layer was dried (Na₂SO₄), filtered, and concentrated under reduced pressure. Purification of the remaining residue on silica gel (cyclohexane/ethyl acetate 10:1 v/v) provided azidodeoxy compound 16 (568 mg, 1.80 mmol, 61.7% from 14). | [α]D 20: +74.8 (c = 1.09, CHCl₃); 1H-NMR (300 MHz, CDCl₃) δ = 7.44–7.23 (m, 5H, aromatic Nb), 4.59 (dd, 1H, J₃,₄ = J₄,₅ = 6.9 Hz, H-4), 4.31 (dd, 1H, J₅,₆a = 3.5 Hz, J₆a,₆b = 8.9 Hz, H-6a), 4.28 (m, 1H, H-3), 4.07 (dd, 1H, H-6b), 4.01 (d, 1H, J = 12.6 Hz, N-CH₂-Ph), 3.78 (dd, 1H, J₁,₂ = 7.3 Hz, H-1), 3.70 (d, 1H, N-CH₂-Ph), 3.61 (dd, 1H, H-5, 1.54, 1.29 (2s, 3H each, C(CH₃)₂).

C-NMR (75.5 MHz, CDCl₃): δ = 136.5 (ipso NBn), 129.1, 128.6, 127.8 (aromatic Nb), 113.4 (C(CH₃)₂), 84.5 (C-3), 77.4 (C-4), 75.6 (C-1), 69.9 (C-2), 64.9 (C-6), 59.5 (N-CH₂-Ph), 45.6 (C-5).
3.5. (3aR,3bS,6aR,7S,7aR)-Hexahydro-7-acetamido-5,5-dimethyl-1-phenyl-1H-[1,3]dioxolo[3,4-c]cyclopent[1,2-c]isoxazol or 1-L-(1,2,4,5/3)-1,2′-Anhydro-3-acetamido-1-hydroxymethyl-2-(N-hydroxy)benzylamino-4,5-O-isopropylidene-4,5-cyclopentanediol 18

To a stirred suspension of zinc (1.17 g, 18.0 mmol) and NH4Cl (0.961 g, 18.0 mmol) in methanol (20 mL) a 50% solution (w/v) of azidocyclopentane 16 (848 mg, 2.91 mmol) in methanol was added. After completed conversion of the starting material (2 h), the mixture was filtered and concentrated under reduced pressure. The resulting crude amine 17 was dissolved in pyridine (20 mmol) and treated with acetic anhydride (0.255 mL, 2.69 mmol) and 4-DMAP (5 mg) at 0 °C. After completed consumption of amine 17, the reaction was quenched with methanol, and the solvents were removed under reduced pressure. The residue was dissolved in CH2Cl2 and consecutively washed with HCl (6%) and saturated aqueous NaHCO3, dried (Na2SO4) and filtered. Purification over silica gel chromatography (cyclohexane/ethyl acetate 2:1 v/v) provided aceticamide 18 (422 mg, 1.27 mmol, 70.7% from 16) as a pale yellow syrup. [α]D20 +11.1 (c = 0.82, CHCl3); 1H-NMR (300 MHz, CDCl3) δ = 7.38–7.23 (m, 5H, aromatic NBn), 6.11 (d, 1H, NHCOCH3), 4.95 (dd, 1H, J1,3 = 6.3 Hz, H-3), 4.75 (dd, 1H, J4,5 = 7.1 Hz, H-4), 4.29 (dd, 1H, J5,6a = 4.1 Hz, J6a,6b = 8.7 Hz, H-6a), 4.22 (dd, 1H, J1,2 = J1,5 = 6.2 Hz, H-1), 4.06 (dd, 1H, J3,4 = 8.9 Hz, H-6b), 3.98 (d, 1H, J = 12.9 Hz, N-CH2-Ph), 3.67 (d, 1H, N-CH2-Ph), 3.42 (m, 1H, H-5), 3.34 (dd, 1H, H-2), 1.83 (s, 3H, NHCOCH3). 13C-NMR (75.5 MHz, CDCl3): δ = 170.7 (NHCOCH3), 137.0 (ipso NBn), 129.2, 128.5, 127.6 (aromatic NBn), 112.6 (C(CH3)2), 83.2 (C-3), 78.4 (C-4), 74.3 (C-1), 65.4 (C-6), 63.9 (C-2), 59.9 (N-CH2-Ph), 47.1 (C-5), 27.3, 25.4 (C(CH3)2), 23.6 (NHCOCH3).

After extended storage, a compound sample provided small crystals which could be employed for X-ray structure determination (CCDC 1826203).

MS (EI): Calc for [C18H23N2O4]+: m/z 332.1736 [M]+; Found [M]+ 332.1737.

3.6. (3aS,4R,5R,6S,6aR)-5-Amino-tetrahydro-6-acetamido-2,2-dimethyl-4H-cyclopenta-1,3-dioxole-4-methanol or 1-L-(1,2,4,5/3)-1-amino-2-acetamido-2-deoxy-β-D-galacto-cyclopentane 19

A 5% solution of aceticamide 18 (422 mg, 1.27 mmol) in methanol was stirred with Pearlman’s catalyst (Pd(OH)2/C, 20%) under an atmosphere of H2 at ambient pressure. After completed conversion (1 hour), the catalyst was filtered off, the filtrate was concentrated under reduced pressure, and the residue was chromatographically purified (chloroform/methanol/NH4OH (25%) 14:1:0.01 v/v/v) to obtain intermediate 19 as a pale yellow syrup (253 mg, 1.04 mmol, 81.6%). [α]D20 +7.5 (c = 0.85, CHCl3); 1H-NMR (300 MHz, CDCl3) δ = 7.29 (d, 1H, NHCOCH3), 4.68 (dd, 1H, J4,5 = 6.0 Hz, J4,5 = 5.7 Hz, H-4), 4.47 (d, 1H, H-3), 4.05 (d, 1H, J1,2 = 6.5 Hz, H-4), 3.90 (m, 2H, H-6a, H-6b), 3.25 (d, 1H, J1,5 = 1 Hz, H-1), 3.00 (bs, 3H, 6-OH, 1-NH2), 2.41 (m, 1H, H-5), 1.95 (s, 3H, NHCOCH3), 1.45, 1.25 (2s, 3H each, C(CH3)2). 13C-NMR (75.5 MHz, CDCl3): δ = 170.7 (NHCOCH3), 111.0 (C(CH3)2), 85.4 (C-3), 80.8 (C-4), 63.2 (C-2), 59.3 (C-1), 58.1 (C-6), 47.2 (C-5), 26.9, 23.2 (C(CH3)2), 23.1 (NHCOCH3).

MS (MALDI): Calc for [C11H20N2O4H]+: m/z 245.1501 [M + H]+; Found [M + H]+ 245.1506.

3.7. (1S,2R,3S,4R,5R)-3-Acetamido-4-amino-5-hydroxymethylcyclopentanetriol or “1-amino-2-acetamido-2-deoxy-β-D-galacto-cyclopentane” 20

A solution of compound 19 (34.8 mg, 0.142 mmol) in methanol (1 mL) was treated with HCl (12 M 100 µL). After completed deprotection, the solvent was removed under reduced pressure, and the remaining residue was purified by silica gel chromatography (chloroform/methanol/NH4OH (25%) 8:4:1 v/v/v) to furnish aminopolyol 20 as the free base (22.4 mg, 0.110 mmol, 77.0%). Treatment with HCl in methanol in the presence of small amounts of ethyl acetate as co-solvent afforded the corresponding hydrochloride (20 HCl) as a white solid. [α]D20 +57.6 (c = 0.90, H2O) (hydrochloride); 1H-NMR (500 MHz, D2O) (free base): δ = 4.21 (dd, 1H, J1,3 = J4,5 = 3.7 Hz, H-4), 4.12 (dd, 1H, J1,2 = 5.7 Hz, J2,3 = 9.1 Hz, H-2), 4.03 (dd, 1H, J3,4 = 3.8 Hz, H-3), 3.96 (dd, 1H, J5,6 = 7.4 Hz, J6a,6b = 11.2 Hz, H-6a), 3.90 (dd, 1H, J5,6b = 7.7 Hz, H-6b), 3.44 (dd, 1H, J1,5 = 8.7 Hz, H-1), 2.48 (dddd, 1H, J4,5 = 3.9 Hz, H-5),
2.10 (s, 3H, NHCOCH₃). ¹³C-NMR (125.9 MHz, D₂O) (free base): δ = 174.9 (NHCOCH₃), 75.7 (C-3), 72.2 (C-4), 62.0 (C-2), 57.0 (C-6), 55.6 (C-1), 42.9 (C-5), 21.9 (NHCOCH₃).

MS (MALDI): Calcd for [C₈H₁₄N₂O₄H]: m/z 205.1188 [M + H]+; Found [M + H]+ 2051184.

3.8. (1S,2R,3S,4R,5R)-N-(1-Hexyl)-3-acetamido-4-amino-5-hydroxymethylcyclopentanetriol or “2-Acetamido-2-deoxy-1-(hexyl)amino-β-D-galacto-cyclopentane” ²¹

Amine ⁹ (32.2 mg, 0.132 mmol) was dissolved in DMF (1 mL) and treated with 1-bromohexane (22.1 µL, 0.158 mmol) in the presence of NaHCO₃ (53.2 mg, 0.633 mmol) at 60 °C. After completed consumption of the starting material, the mixture was concentrated under reduced pressure. The residue was diluted with methanol and treated with HCl (100 µL, 12 M) and stirred for one hour. After evaporation of the solvents, the remaining precipitate was purified by chromatography on silica gel (chloroform/methanol/NH₄OH (25%) 8:1:0.01 v/v/v) to yield N-hexyl carbacycle ²¹ (25.4 mg, 881 µmol, 66.8% over two steps). [α]²⁰D: +49.8 (c = 0.97, MeOH); ¹H-NMR (500 MHz, CD₂OD): δ = 4.16 (dd, 1H, J₂,₃ = 5.0 Hz, J₂,₃ = 7.7 Hz, H-2), 4.08 (dd, 1H, J₃,₄ = J₄,₅ = 4.1 Hz, H-4), 3.96 (dd, 1H, J₅,₆b = 7.4 Hz, H₆a,₆b = 11.2 Hz, H-6a), 3.91 (m, 2H, H-3, H-6b), 3.21 (dd, 1H, J₁,₅ = 8.2 Hz, H-1), 2.94 (m, 1H, H-1′a), 2.73 (m, 1H, H-1′b), 2.44 (dd, 1H, H-5), 2.04 (s, 3H, NHCOCH₃), 1.58 (m, 2H, H-2′), 1.47–1.33 (m, 6H, H₃, H-4′, H-5′), 0.97 (t, 3H, H-6′). ¹³C-NMR (125.9 MHz, CD₂OD): δ = 173.3 (NHCOCH₃), 78.9 (C-3), 74.0 (C-4), 64.6 (C-1), 63.0 (C-2), 59.1 (C-6), 47.9 (C-1′), 45.6 (C-5), 32.7, 29.6, 27.8, 23.6 (C-2′, C-3′, C-4′, C-5′) 22.8 (NHCOCH₃), 14.3 (C-6′).

MS (MALDI): Calcd for [C₁₄H₂₈N₂O₄H]: m/z 289.2127 [M + H]+; Found [M + H]+ 2892126.

3.9. (1S,2R,3S,4R,5R)-N-(Methoxycarbonyl)pentyl-3-acetamido-4-amino-5-hydroxymethyl-cyclopentanetriol or “2-Acetamido-2-deoxy-1-(methoxycarbonyl)hexylamino-β-D-galacto-cyclopentane” ²²

Amine ⁹ (25.7 mg, 0.105 mmol) was dissolved in DMF (1 mL) and NaHCO₃ (42.4 mg, 0.505 mmol) followed by methyl 6-iodohexanoate (20.8 mg, 0.505 mmol) were added. The reaction mixture was heated to 60 °C until completed consumption of the starting material was observed (tLC). The mixture was then concentrated under reduced pressure, and MeOH was added to obtain a ca. 50% solution. This was added to a mixture of methanol (5 mL) and acetic chloride (100 µL) at 0 °C, and the mixture was stirred for 15 min. Removal of solvent in vacuo and followed by chromatography of the residue (chloroform/methanol/NH₄OH (25%) 8:1:0.01 v/v/v) gave free methyl ester ²² (23.8 mg, 71.6 µmol, 68.1% over two steps) as a colourless syrup. [α]²⁰D: +41.0 (c = 0.99, MeOH); ¹H-NMR (300 MHz, CD₂OD): δ = 4.07 (dd, 1H, J₁,₂ = 5.0 Hz, J₂,₃ = 7.4 Hz, H-2), 4.02 (dd, 1H, J₃,₄ = J₄,₅ = 4.1 Hz, H-4), 3.97–3.80 (m, 3H, H-3, H-6), 3.66 (s, 3H, H-1′), 3.06 (dd, 1H, J₁,₅ = 8.1 Hz, H-1), 2.83 (m, 1H, H-1′a), 2.56 (m, 1H, H-1′b), 2.42–2.28 (m, 3H, H-5, H-5′), 1.98 (s, 3H, NHCOCH₃), 1.72–1.28 (m, 6H, H-2′, H-3′, H-4′). ¹³C-NMR (75.5 MHz, CD₂OD): δ = 175.8 (COOME), 173.1 (NHCOCH₃), 79.1 (C-3), 74.0 (C-4), 64.5 (C-1), 63.6 (C-2), 59.1 (C-6), 52.0 (C-1′), 47.9 (C-1′), 46.0 (C-5), 34.7, 29.9, 27.7, 25.8 (C-2′, C-3′, C-4′, C-5′) 22.8 (NHCOCH₃).

MS (MALDI): Calcd for [C₁₅H₂₈N₂O₄H]: m/z 333.2024 [M + H]+; Found [M + H]+ 3332024.

3.10. (1S,2R,3S,4R,5R)-N-(6-Amino)hexyl-3-acetamido-4-amino-5-hydroxymethyl-cyclopentanetriol or “2-Acetamido-2-deoxy-1-(6-amino)hexylamino-β-D-galacto-cyclopentane” ²⁴

Amine ⁹ (68.2 mg, 0.279 mmol) was dissolved in DMF (3 mL) and treated with 6-bromohexanoic nitrile (44.4 µL, 0.335 mmol) in the presence of NaHCO₃ (93.8 mg, 1.34 mmol). The reaction mixture was heated to 60 °C until completed consumption of the starting material was observed. The solvents were removed under reduced pressure. The residue was diluted with methanol, 2 M HCl (100 µL) was added, and the mixture was stirred for one hour. After evaporation of the solvents, the residue was purified by column chromatography (chloroform/methanol/NH₄OH (25%) 8:1:0.01 v/v/v) to yield nitrile ²³ (30.9 mg, 0.102 mmol, 37.0% from compound ⁹), which was directly used in the next step. [α]²⁰D: +24.3 (c = 0.975, MeOH); ¹H-NMR (300 MHz, CD₂OD): δ = 4.32 (dd, 1H, J₁,₂ = 5.2 Hz, J₂,₃ = 7.2 Hz, H-2), 4.10–3.94 (m, 4H, H-3, H-4, H-6a, H-6b), 3.64 (dd, 1H, J₁,₅ = 7.8 Hz, H-1), 3.23 (m, 2H,
H-1'), 2.61 (m, 1H, H-5), 2.51 (t, 2H, H-5'), 2.05 (s, 3H, NHCOCH3), 1.84–1.45 (m, 6H, H-2', H-3', H-4').

$^{13}$C-NMR (75.5 MHz, CDCl3): $\delta$ = 174.1 (NHCOCH3), 121.0 (CN), 77.8 (C-3), 73.8 (C-4), 64.5 (C-1), 60.3 (C-2), 58.5 (C-6), 48.0 (C-1'), 44.5 (C-5), 26.6, 26.0, 25.9, (C-2', C-3', C-4') 22.8 (NHCOCH3), 17.2 (C-5').

A 10% solution of nitrile 23 (30.9 mg, 0.102 mmol) in methanol was stirred with small amounts of Raney-Ni under an atmosphere of H2 at ambient temperature. After full conversion of the starting material (20 min), the catalyst was filtered off, and the filtrate was concentrated under reduced pressure.

Chromatographic purification (chloroform/methanol/NH4OH (25%) 8:4.1 v/v/v) afforded amine 24 as pale yellow syrup (30.9 mg, 0.102 mmol, 77.4%). Treatment with HCl provided the corresponding dihydrochloride 24·HCl as a white solid. $[\alpha]_{D}^{20}$: +38.5 (c = 1.115, H2O) (hydrochloride); $^{1}$H-NMR (300 MHz, CD3OD) (free base): $\delta$ = 4.14–4.03 (m, 2H, H-2, H-4), 3.86 (dd, 1H, $J_{3,4}$ = 8.5 Hz, $J_{3,4}$ = 4.2 Hz, H-3), 3.81 (d, 2H, H-6), 3.08 (dd, 1H, $J_{1,5}$ = 5.6 Hz, H-5), 2.76–2.56 (m, 3H, H-6'a, H-6'b, H-1'a), 2.47–2.34 (m, 2H, H-5, H-1') 2.00 (s, 3H, NHCO CH3), 1.57–1.21 (m, 8H, H-2, H-3', H-4', H-5'). $^{13}$C-NMR (75.5 MHz, D2O) (free base): $\delta$ = 173.2 (NHCOCH3), 77.1 (C-3), 72.0 (C-4), 61.5 (C-2), 60.8 (C-1), 57.4 (C-6), 46.5 (C-1'), 43.8 (C-5), 40.2, 30.3, 28.0, 26.2, 25.7 (C-2', C-3', C-4', C-5', C-6') 22.2 (NHCOCH3).

MS (MALDI): Calcd for [C14H29NO3H4]: m/z 304.2236 [M + H]+; Found [M + H]+ 304.2234.

3.11. (1S,2R,3S,4R,5R)-N-(6-Dansylamino)hexyl-3-acetamido-4-amino-5-hydroxymethyl-cyclopentanetriol or "2-Acetamido-2-deoxy-1-(6-dansylamino)hexyl-β-D-galacto-cyclopentanetriol" 25

A solution of amine 24 (23.2 mg, 61.6 µmol) in methanol (1mL) was treated with Et3N (38.5 µL, 277 mmol) and dansyl chloride (18.3 mg, 67.8 µmol). After completed conversion of the starting material (30 min), the solvent was removed under reduced pressure. Purification on silica gel (chloroform/methanol/NH4OH (25%) 8:1:0.1 v/v/v) provided compound 25 (16.1 mg, 48.6 µmol, 78.9%) as light yellow, fluorescent syrup. $[\alpha]_{D}^{20}$: +23.7 (c = 0.960, MeOH); $^{1}$H-NMR (300 MHz, CD3OD): $\delta$ = 4.07 (dd, 1H, $J_{1,2}$ = 4.9 Hz, $J_{2,3}$ = 7.6 Hz, H-2), 4.01 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 4.0 Hz, H-4), 3.93–3.79 (m, 3H, H-3, H-6a, H-6b), 3.09 (dd, 1H, $J_{1,5}$ = 8.1 Hz, H-1), 2.90–2.71 (m, 3H, H-1'a, H-6'a, H-6'b), 2.53 (m, 1H, H-1'b), 2.35 (dd, 1H, $J_{5,6a} = J_{5,6b}$ = 7.2 Hz), 1.97 (s, 3H, NHCOCH3), 1.40–1.05 (m, 8H, H-2, H-3, H-4, H-5, C-5'). $^{13}$C-NMR (75.5 MHz, CD3OD): $\delta$ = 173.2 (NHCOCH3), 78.9 (C-3), 74.0 (C-4), 64.6 (C-1), 63.1 (C-2), 59.0 (C-6), 47.9 (C-1'), 45.6 (C-5), 43.7, 30.4, 29.5, 27.5, 27.2 (C-2', C-3', C-4', C-5', C-6') 22.8 (NHCOCH3).

MS (MALDI): Calcd for [C26H44N4O6SH]: m/z 537.2747 [M+H]+; Found [M+H]+ 537.2750.

Supplementary Materials: NMR spectra of compounds are available online.

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Sample Availability: Samples of compounds are available from the authors.