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Fluorescent glycosidase inhibiting 1,5-dideoxy-1,5-iminoalditols

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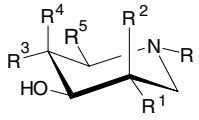
Abstract—1,5-Dideoxy-1,5-iminoalditols of various configurations as well as isofagomine were N-alkylated with non-polar straight chain spacer-arms by a set of simple standard procedures. The spacer-arms' terminal functional groups, primary amines, were employed to introduce fluorescent tags such as dansyl and dapoxyl moieties. Resulting derivatives in the D-*xylo*, D-*gluco*, D-*galacto* as well as GlcNAc series showed distinctly improved glycosidase inhibitory activities compared to parent compounds and are designed to be useful analytical tools.

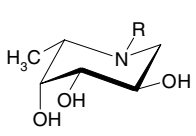
Iminosugars including iminoalditols and related bicyclic alkaloids are well-known, (usually) competitive, glycosidase inhibitors.[1] Quite a few representatives of this class of compounds have found important roles as diagnostic compounds such as in the investigation of glycoprotein trimming glycosidases[2] or as pharmaceutical substances such as in the treatment of diabetes type II symptoms.[3] Other biological activities associated with their glycosidase inhibitory properties are anti-viral, anti-cancer and anti-metastatic, anti-infective as well as insect anti-feedant and plant growth-regulating effects.[4]

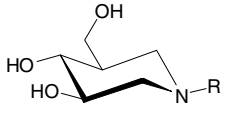
Recently, we have found that some fluorescently labeled derivatives of the glucosidase inhibitor 2,5-dideoxy-2,5-imino-D-mannitol (or DMDP) are powerful inhibitors exceeding the parent compound's activity by two orders of magnitude.[5] Such labeled inhibitors are deemed to be highly useful diagnostic tools for activity-based high-throughput analyses as well as on-gel-staining. With the aim to evaluate a more general means of tagging of iminosugars than was operative in the mentioned structurally quite restricted case, we resorted to the connection of fluorophores to the iminosugars via non-polar medium length spacer-arms. These moieties were conveniently attached to the ring nitrogen of the inhibitors **1**–**7** according to Scheme 1.[6] Starting from the respective free inhibitor (Scheme 2), the ring nitrogen was alkylated with 5-bromohexanoic nitrile to give



R = H

1 (Glu): R^1 = OH, R^2 = H, R^3 = OH, R^4 = H, R^5 = CH_2OH
2 (Man): R^1 = H, R^2 = OH, R^3 = OH, R^4 = H, R^5 = CH_2OH
3 (Gal): R^1 = OH, R^2 = H, R^3 = H, R^4 = OH, R^5 = CH_2OH
4 (GlcNAc): R^1 = NHAc, R^2 = H, R^3 = OH, R^4 = H, R^5 = CH_2OH
5 (Xyl): R^1 = OH, R^2 = H, R^3 = OH, R^4 = H, R^5 = H





R = H **6** **7**

1a - **7a**: R = $(CH_2)_5CN$
1b – **7b**: R = $(CH_2)_6NH_2$
1c – **7c**: R = $(CH_2)_6$NHDansyl
1d, **2d**: R = $(CH_2)_6$NHDapoxyl
1e: R = $(CH_2)_5CONH(CH_2)_2$NHDansyl

Scheme 1. Inhibitors and intermediates.

Keywords: Glycosidase inhibitor; Iminoalditol; Fluorescent.
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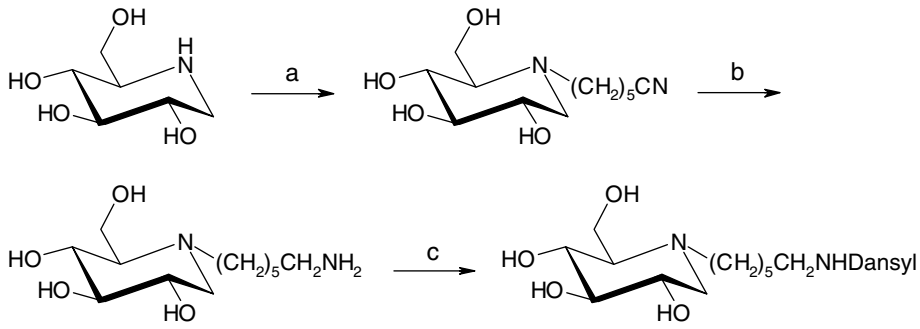

Scheme 2. General reaction sequence example (D-*gluco* series). Reagents and conditions: (a) $Br(CH_2)_5CN$, Na_2CO_3, DMF, 40 °C; (b) Raney-Ni, H_2, MeOH, 24 h; (c) dansylCl or dapoxylCl, NEt_3, DMF.

compounds **1a**–**7a** followed by reduction of the nitriles to the corresponding primary amines **1b**–**7b** which, in turn, were reacted with the dansyl or dapoxyl fluorophors to yield compounds **1c**–**7c** as well as **1d** and **2d**. Introducing a variation of the spacer-arm, compounds **1** were transformed into N-alkylated derivative **1e** (Scheme 1).

Compounds investigated are reversible inhibitors of the enzymes probed in this study.[7] In the D-*gluco* (**1c**–**e**), D-*galacto* (**3c**), as well as *N*-acetyl-D-glucosamine (**4c**) series, practically independent of the fluorophores employed in this study, improvements over the parent compounds were observed (Table 1). Comparing dansyl (**1c** and **2c**), and dapoxyl (**1d** and **2d**) labeled compounds of the same configuration, the respective dapoxyl derivative was found to be slightly less active than its corresponding dansyl counterpart. In the D-*manno* series, the *N*-substituent in **2c** led to an acceptable four- to five-fold reduction of inhibitory activity.

Table 1. K_i values of new compounds in comparison with parent inhibitors

Compound	K_i (μM)
1	12[a]
1c	0.32[a]
1d	1.0[a]
1e	0.14[a]
2	18[b]
2c	78[b]
2d	65[b]
3	12.5[c]
3c	0.95[c]
4	80[d]
4c	1.8[d]
5	206[e]
5c	7.6[e]
6	0.01[f]
6c	0.5[f]
7	0.007[a]
7c	9.0[a]

[a] β-Glucosidase *Agrobacterium* sp.
[b] α-Mannosidase jack beans.
[c] β-Galactosidase *Agrobacterium* sp.
[d] β-D-Hexosaminidase *Streptomyces plicatur*.
[e] β-Xylosidase *Thermoanaerobact. Sacch.*
[f] α-L-Fucosidase human liver.

Contrasting these gratifying results, iminofucitol **6c** showed markedly decreased potency. The same was true for tagged isofagomine **7c**, which lost activity by three orders of magnitude compared to parent compound **7**, in keeping with previous findings[8] that N-alkylation of this powerful β-glucosidase inhibitor results in significant losses of inhibitory power.

Acknowledgments

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6. Typical procedures: a 3% solution of the respective iminoalditol in dry DMF was stirred with ω-bromohexanoic nitrile (2 equiv) in the presence of Na_2CO_3 (1.3 equiv) at 40 °C for 72 h. Removal of the solvent and chromatographic purification ($CHCl_3$/MeOH, 1:1), gave products

1a–**7a** in yields ranging between 45% and 90%. Standard hydrogenation of a 5% solution of the respective hexanoic nitrile under an atmosphere of H_2 (50 bar) in MeOH in the presence of excess Raney-Ni for 24 h, followed by chromatography ($CHCl_3$/MeOH/concd NH_4OH, 50:50:1) gave primary amines **1b**–**7b** in yields between 20% and 60%. Treatment of primary amines **1b**–**7b**, with 3% solution in dry DMF with the respective fluorophor (dansyl or dapoxylchloride, 1.2 equiv) in the presence of Et_3N (2 equiv) gave the corresponding fluorescently tagged inhibitors **1c**–**7c**, **1d**, and **2d** in yields ranging between 30% and 60%. Chromatography: Silica gel 60 (Merck), $CHCl_3$/MeOH/NH_4OH (300:100:1 or 500:100:1). Electrospray mass spectra were recorded with a HP 1100 series MSD, Hewlett Packard. Samples were dissolved in acetonitrile/MeOH mixtures. The scan mode for negative ions (mass range 100–1000 D) was employed varying the fragmentation voltage from 30 to 130 V. NMR spectra were recorded at 200 as well as 500 MHz (^{1}H), and at 50 and 125 MHz (^{13}C) from samples in MeOH-d_4 unless stated otherwise. Chemical shifts are listed in δ employing residual, not deuterated, solvent as the internal standard. The signals of aromatic groups were found in the expected regions and are not listed explicitly.

Compound **1a**: ^{13}C NMR: δ 120.0 (C-6′), 78.9, 70.3, 69.0, 66.3, 57.6, 56.0 (C-1, C-2, C-3, C-4, C-5, C-6), 52.3 (C-1′), 26.3, 25.1, 23.2, 16.1 (C-2′, C-3′, C-4′, C-5′). ^{1}H NMR (D_2O): δ 3.74 (m, 2H, H-6a, H-6b), 3.44 (ddd, 1H, $J_{1a,2}$ 10.1 Hz, $J_{1e,2}$ 4.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 3.27 (dd, 1H, $J_{3,4}$ 9.2 Hz, H-3), 3.14 (dd, 1H, $J_{4,5}$ 8.8 Hz, H-4), 2.96 (dd, 1H, $J_{1a,1e}$ 11.9 Hz, H-1e), 2.80–2.50 (m, 2H, H-1′), 2.40–2.22 (m, 4H, H-1a, H-5, H-5′), 1.62–1.20 (m, 6H, H-2′, H-3′, H-4′). MS (API-ES): Calcd for [$C_{12}H_{22}N_2O_4$]: *m*/*z* 258.3201. Found: *m*/*z* 257.30 [M−H].

Compound **1c**: ^{13}C NMR: δ 78.6, 69.5, 68.5, 66.1, 58.3, 56.7 (C-1, C-2, C-3, C-4, C-5, C-6), 49.1 (C-1′), 44.6 (Me_2N-aryl), 42.5 (C-6′), 29.2, 26.2, 26.0, 23.5 (C-2′, C-3′, C-4′, C-5′). ^{1}H NMR: δ 3.91 (br d, 1H, $J_{6a,6b}$ 12.0 Hz, H-6a), 3.83 (dd, 1H, $J_{5,6b}$ 2.4 Hz, H-6b), 3.53 (m, 1H, H-2), 3.42 (dd, 1H, $J_{3,4} = J_{4,5}$ 9 Hz, H-4), 3.20 (dd, 1H, $J_{2,3}$ 9 Hz, H-3), 3.08 (m, 1H, H-1e), 2.90 (m, 7H, H-1′a, Me_2N-aryl), 2.66 (m, 1H, H-1′b), 2.38 (m, 2H, H-1a, H-5), 1.42–1.05 (m, 8H, H-2′, H-3′, H-4′, H-5′). MS (API-ES): Calcd for [$C_{24}H_{37}N_3SO_6$]: *m*/*z* 495.6430. Found: *m*/*z* 494.60 [M−H].

Compound **1d**: ^{13}C NMR: δ 79.4, 70.9, 69.6, 66.2, 58.3, 56.2 (C-1, C-2, C-3, C-4, C-5, C-6), 52.5 (C-1′), 42.8 (C-6′), 39.2 (Me_2N-aryl), 29.5, 26.9, 26.3, 24.0 (C-2′, C-3′, C-4′, C-5′); ^{1}H NMR: δ 3.85 (dd, 1H, $J_{5,6a}$ 2 Hz, $J_{6a,6b}$ 12.7 Hz, H-6a), 3.81 (dd, 1H, $J_{5,6b}$ 3 Hz, H-6b), 3.45 (ddd, 1H, $J_{1a,2} = J_{2,3}$ 9.8 Hz, $J_{1e,2}$ 4.9 Hz, H-2), 3.33 (dd, 1H, $J_{3,4}$ 8.8 Hz, H-4), 3.20 (dd, 1H, H-3), 2.96 (dd, 1H, $J_{1a,1e}$ 10.8 Hz, H-1e), 2.90 (t, 2H, H-1′), 2.76 (m, 1H, H-6′a), 2.50 (m, 1H, H-6′b), 2.14 (dd, 1H, H-1a), 2.08 (m, 1H, H-5), 1.50–1.17 (m, 8H, H-2′, H-3′, H-4′, H-5′). MS (API-ES): Calcd for [$C_{29}H_{40}N_4O_7S$]: *m*/*z* 588.7288. Found: *m*/*z* 587.70 [M−H].

Compound **1e**: ^{13}C NMR: δ 175.3 (C=O), 79.2 (C-3), 70.6 (C-4), 69.3 (C-2), 66.2 (C-5), 57.9 (C-6), 56.3 (C-1), 52.4 (C-1′), 44.7 (Me_2N-aryl), 42.1 (C-5′), 39.1 (C-7′), 35.6 (C-8′), 26.8, 25.4, 23.7 (C-2′, C-3′, C-4′). ^{1}H NMR: δ 3.86 (m, 2H, H-6a, H-6b), 3.49 (ddd, 1H, H-2), 3.37 (dd, 1H, $J_{3,4}$ 10.3 Hz, H-4), 3.15 (dd, 1H, $J_{2,3}$ 8.8 Hz, H-3), 3.17 (t, 2H, H-6′), 3.02 (dd, 1H, $J_{1a,1e}$ 10.9 Hz, $J_{1e,2}$ 4.9 Hz, H-1e), 2.92 (t, 2H, H-7′), 2.88 (s, 6 H, Me_2N-aryl), 2.85–2.79 (m, 1H, H-1′), 2.63–2.58 (m, 1H, H-1′), 2.23 (dd, 1H, $J_{1a,2}$ 11.2 Hz, H-1a), 2.19 (m, 1H, H-5), 2.01 (t, 2H, H-5′), 1.56–1.45 (m, 4H, H-3′, H-4′), 1.30–1.20 (m, 2H, H-2′). MS (API-ES): Calcd for [$C_{26}H_{40}N_4SO_7$]: *m*/*z* 552.6953. Found: *m*/*z* 552.60 [M−H].

Compound **2a**: ^{13}C NMR: δ 119.9 (C-6′), 73.0, 66.4, 66.3, 66.2, 55.5, 54.5 (C-1, C-2, C-3, C-4, C-5, C-6), 52.8 (C-1′), 25.6, 24.9, 22.3, 16.0 (C-2′, C-3′, C-4′, C-5′). ^{1}H NMR: δ 3.78 (m, 1H, H-2), 3.73–3.55 (m, 2H, H-6a, H-6b), 3.54 (dd, 1H, $J_{3,4} = J_{4,5}$ 9 Hz, H-4), 3.24 (dd, 1H, $J_{2,3}$ 2.6 Hz, H-3), 3.10 (dd, 1H, $J_{1a,1e}$ 12.8 Hz, $J_{1e,2}$ 4 Hz, H-1e), 3.10–2.80 (m, 3H, H-1′, H-5′), 2.68 (m, 1H, H-5′), 2.20 (m, 2H, H-1a, H-5), 1.60–1.10 (m, 6H, H-2′, H-3′, H-4′). MS (API-ES): Calcd for [$C_{12}H_{22}N_2O_4$]: *m*/*z* 258.3201. Found: *m*/*z* 257.20 [M−H].

Compound **2b**: ^{13}C NMR: δ 75.3, 68.4, 68.3, 66.0, 57.8, 55.2 (C-1, C-2, C-3, C-4, C-5, C-6), 52.3 (C-1′), 39.8 (C-6′), 28.2, 26.6, 25.9, 23.9 (C-2′, C-3′, C-4′, C-5′). MS (API-ES): Calcd for [$C_{12}H_{26}N_2O_4$]: *m*/*z* 262.3520. Found: *m*/*z* 261.30 [M−H].

Compound **2c**: ^{13}C NMR: δ 74.7, 67.9, 67.8, 66.0, 57.2, 55.0 (C-1, C-2, C-3, C-4, C-5, C-6), 52.7 (C-1′), 44.7 (Me_2N-aryl), 42.5 (C-6′), 29.2, 26.5, 26.0, 23.6 (C-2′, C-3′, C-4′, C-5′). ^{1}H NMR: δ 3.91 (m, 2H, H-2, H-6a), 3.84 (dd, 1H, $J_{5,6b}$ 2 Hz, $J_{6a,6b}$ 11.7 Hz, H-6b), 3.73 (dd, 1H, $J_{3,4}$ 8.8 Hz, $J_{4,5}$ 9.3 Hz, H-4), 3.37 (dd, 1H, $J_{2,3}$ 2.4 Hz, H-3), 3.04 (br s, 1H, H-1e), 2.90 (s, 6H, Me_2N-aryl), 2.86 (t, 2H, H-1′), 2.76 (m, 1H, H-6′a), 2.44 (m, 2H, H-1a, H-6′b), 2.32 (br s, 1H, H-5), 1.40–1.10 (m, 8H, H-2′, H-3′, H-4′, H-5′). MS (API-ES): Calcd for [$C_{24}H_{37}N_3SO_6$]: *m*/*z* 495.6430. Found: *m*/*z* 494.60 [M−H].

Compound **2d**: δ 75.4, 68.5, 68.4, 65.8, 58.0, 55.3 (C-1, C-2, C-3, C-4, C-5, C-6), 52.5 (C-1′), 42.8 (C-6′), 39.2 (Me_2N-aryl), 29.4, 26.9, 26.3, 24.0 (C-2′, C-3′, C-4′, C-5′). ^{1}H NMR: δ 3.87 (dd, 1H, $J_{5,6a}$ 2.4 Hz, $J_{6a,6b}$ 11.7 Hz, H-6a), 3.83 (dd, 1H, $J_{5,6b}$ 2.4 Hz, H-6b), 3.79 (m, 1H, H-2), 3.64 (dd, 1H, $J_{3,4}$ 9.3 Hz, $J_{4,5}$ 8.8 Hz, H-4), 3.28 (dd, 1H, $J_{2,3}$ 3.3 Hz, H-3), 3.01 (s, 6H, Me_2N-aryl), 2.92 (m, 3H, H-1e, H-1′), 2.71 (m, 1H, H-6′a), 2.51 (m, 1H, H-6′b), 2.40 (dd, 1H, $J_{1a,2}$ 1.5 Hz, $J_{1a,1e}$ 12.2 Hz, H-1a), 2.07 (ddd, 1H, H-5), 1.50–1.16 (m, 8H, H-2′, H-3′, H-4′, H-5′). MS (API-ES): Calcd for [$C_{29}H_{40}N_4O_7S$]: *m*/*z* 588.7288. Found: *m*/*z* 587.70 [M−H].

Compound **3a**: ^{13}C NMR: δ 120.1 (C-6′), 75.6, 71.7, 67.6, 63.5, 61.1, 56.5, 52.5 (C-1, C-2, C-3, C-4, C-5, C-6, C-1′), 26.4, 25.2, 23.1, 16.2 (C-2′, C-3′, C-4′, C-5′). MS (API-ES): Calcd for [$C_{12}H_{22}N_2O_4$]: *m*/*z* 258.3201. Found: *m*/*z* 257.30 [M−H].

Compound **3b**: ^{13}C NMR: δ 76.1, 71.1, 67.8, 64.2, 61.3, 56.9, 52.7 (C-1, C-2, C-3, C-4, C-5, C-6, C-1′), 40.2 (C-6′), 29.5, 27.0, 26.3, 23.9 (C-2′, C-3′, C-4′, C-5′). ^{1}H NMR: δ 3.98 (dd, 1H, $J_{3,4}$ 3 Hz, $J_{4,5}$ 1.5 Hz, H-4), 3.84–3.77 (m, 3H, H-2, H-6a, H-6b), 3.22 (dd, 1H, $J_{2,3}$ 9.5 Hz, H-3), 2.99 (dd, 1H, $J_{1a,1e}$ 11.0 Hz, $J_{1e,2}$ 4.9 Hz, H-1e), 2.81 (t, 2H, H-1′), 2.79 (m, 1H, H-6′a), 2.50 (m, 1H, H-6′b), 2.38 (ddd, 1H, H-5), 2.11 (dd, 1H, $J_{1a,2}$ 10.5 Hz, H-1a), 1.65–1.26 (m, 8H, H-2′, H-3′, H-4′, H-5′). MS (API-ES): Calcd for [$C_{12}H_{26}N_2O_4$]: *m*/*z* 262.3520. Found: *m*/*z* 261.35 [M−H].

Compound **3c**: ^{13}C NMR: δ 74.6, 70.3, 66.4, 64.7, 60.2, 54.9, 52.9 (C-1, C-2, C-3, C-4, C-5, C-6, C-1′), 44.6 (Me_2N-aryl), 42.4 (C-6′), 29.2, 26.3, 25.9, 23.1 (C-2′, C-3′, C-4′, C-5′). ^{1}H NMR: δ 3.97 (dd, 1H, H-4), 3.78 (ddd, 1H, H-2), 3.74 (m, 2H, H-6a, H-6b), 3.20 (dd, 1H, $J_{2,3}$ 9.3 Hz, $J_{3,4}$ 3.4 Hz, H-3), 2.91 (dd, 1H, $J_{1a,1e}$ 11.3 Hz, $J_{1e,2}$ 4.9 Hz, H-1e), 2.90 (s, 6H, Me_2N-aryl), 2.85 (t, 2H, H-1′), 2.58 (m, 1H, H-6′a), 2.37 (m, 1H, H-6′b), 2.52 (m, 1H, H-5), 2.06 (dd, 1H, $J_{1a,2}$ 10.3 Hz, H-1a), 1.35–0.97 (m, 8H, H-2′, H-3′, H-4′, H-5′). MS (API-ES): Calcd for [$C_{24}H_{37}N_3SO_6$]: *m*/*z* 495.6430. Found: *m*/*z* 494.40 [M−H].

Compound **4a**: ^{13}C NMR: δ 172.5 (CO), 120.0 (C-6′), 76.3, 71.2, 66.2, 58.0, 54.1, 52.0 (C-1, C-2, C-3, C-4, C-5, C-6), 50.4 (C-1′), 26.4, 25.2, 23.6, 21.6 (C-2′, C-3′, C-4′, C-5′), 16.1 (CO*Me*). MS (API-ES): Calcd for [$C_{14}H_{25}N_3O_4$]: *m*/*z* 299.3731. Found: *m*/*z* 298.20 [M−H].

Compound **4b**: ^{13}C NMR: δ 172.4 (CO), 76.2, 71.6, 66.4, 58.6, 54.5, 52.2 (C-1, C-2, C-3, C-4, C-5, C-6), 50.8 (C-1′), 40.8 (C-6′), 31.1, 27.1, 26.5, 24.4 (C-2′, C-3′, C-4′, C-5′), 21.6 (CO*Me*). ^{1}H NMR: δ 3.90 (dd, 1H, $J_{5,6a}$ 2.5 Hz, $J_{6a,6b}$

11.7 Hz, H-6a), 3.83 (dd, 1H, $J_{5,6b}$ 3.2 Hz, H-6b), 3.81 (m, 1H, H-2), 3.40 (dd, 1H, $J_{3,4}$ 9.3 Hz, $J_{4,5}$ 9.0 Hz, H-4), 3.21 (dd, 1H, $J_{2,3}$ 10.0 Hz, H-3), 3.03 (dd, 1H, $J_{1e,2}$ 4.6 Hz, $J_{1a,1e}$ 11.2 Hz, H-1e), 2.83 (m, 1H, H-1′a), 2.72 (t, 2H, H-6′), 2.54 (m, 1H, H-1′b), 2.13 (m, 1H, H-5), 2.09 (dd, 1H, $J_{1a,2}$ 11 Hz, H-1a), 1.97 (s, 3H, CO*Me*), 1.57–1.26 (m, 8H, H-2′, H-3′, H-4′, H-5′). MS (API-ES): Calcd for [$C_{14}H_{29}N_3O_4$]: *m/z* 303.4049. Found: *m/z* 302.3 [M−H].

Compound **4c**: ^{13}C NMR: δ 172.4 (CO), 76.5, 71.5, 66.2, 58.4, 54.4, 52.2 (C-1, C-2, C-3, C-4, C-5, C-6), 50.7 (C-1′), 44.7 (Me_2N-aryl), 42.6 (C-6′), 29.3, 26.7, 26.1, 24.1 (C-2′, C-3′, C-4′, C-5′), 21.6 (CO*Me*). ^{1}H NMR: δ 3.86–3.78 (m, 3H, H-2, H-6a, H-6b), 3.39 (dd, 1H, $J_{3,4} = J_{4,5}$ 9.3 Hz, H-4), 3.21 (dd, 1H, $J_{2,3}$ 9.8 Hz, H-3), 2.97 (dd, 1H, $J_{1e,2}$ 4.4 Hz, $J_{1a,1e}$ 11.2 Hz, H-1e), 2.89 (s, 6H, Me_2N-aryl), 2.84 (t, 2H, H-1′), 2.68 (m, 1H, H-6′a), 2.43 (m, 1H, H-6′b), 2.12 (m, 1H, H-5), 2.07 (dd, 1H, $J_{1a,2}$ 11.2 Hz, H-1a), 1.98 (s, 3H, CO*Me*), 1.34–0.65 (m, 8H, H-2′, H-3′, H-4′, H-5′). MS (API-ES): Calcd for [$C_{26}H_{40}N_4O_6S$]: *m/z* 536.696. Found: *m/z* 535.5 [M−H].For compounds **5a** and **b**, see Häusler, H.; Rupitz, K.; Stütz, A. E.; Withers, S. G. *Chem. Mon.* **2002**, *133*, 555; compound **5c**: ^{13}C NMR: δ 79.2 (C-3), 70.2 (C-2, C-4), 58.3 (C-1, C-5), 57.6 (C-1′), 44.7 (Me_2N-aryl), 42.5 (C-6′), 29.2, 26.7, 26.3, 26.1 (C-2′, C-3′, C-4′, C-5′). ^{1}H NMR: δ 3.23 (ddd, 2H, $J_{2,3} = J_{3,4}$ 8.8 Hz, H-2, H-4), 2.84 (dd, 1H, H-3), 2.68 (m, 2H, $J_{1e,2} = J_{4,5e}$ 4.7 Hz, H-1e, H-5e), 2.64 (s, 6H, Me_2N-aryl), 2.60 (dd, 2H, $J_{1a,2} = J_{4,5a}$ 10.3 Hz, $J_{1a,1e} = J_{5a,5e}$ 11.0 Hz, H-1a, H-5a), 2.01 (t, 2H, H-1′), 1.70–0.77 (m, 8H, H-2′, H-3′, H-4′, H-5′). MS (API-ES): Calcd for [$C_{12}H_{22}N_2O_3$]: *m/z* 242.3207. Found: *m/z* 241.3 [M−H].

Compound **6a**: ^{13}C NMR: δ 120.0 (C-6′), 73.9, 71.8, 65.7, 63.0 60.5 (C-1, C-2, C-3, C-4, C-5), 52.5 (C-1′), 25.7, 24.9 (C-2′, C-3′, C-4′, C-5′), 16.1 (C-6). ^{1}H NMR: δ 4.01 (ddd, 1H, $J_{1a,2} = J_{2,3}$ 10 Hz, $J_{1e,2}$ 4.6 Hz, H-2), 3.90 (br s, 1H, H-4), 3.53 (m, 1H, H-3), 3.40–3.32 (m, 2H, H-1e, H-5), 3.14 (m, 1H, H-6′a), 3.05 (m, 1H, H-6′b), 2.83 (dd, 1H, H-1a), 2.51 (m, 2H, H-1′), 1.80–1.46 (m, 8H, H-2′, H-3′, H-4′, H-5′), 1.40 (d, 3H, $J_{5,6}$ 6.6 Hz, H-6). MS (API-ES): Calcd for [$C_{12}H_{22}N_2O_3$]: *m/z* 242.3207. Found: *m/z* 241.20 [M−H].

Compound **6c**: ^{13}C NMR: δ 76.4, 73.9, 68.1, 58.3, 56.9, 52.6 (C-1, C-2, C-3, C-4, C-5, C-1′), 42.5 (C-6′), 29.6, 26.9, 26.2, 23.3 (C-2′, C-3′, C-4′, C-5′), 15.6 (C-6). ^{1}H NMR: δ 3.75 (ddd, 1H, $J_{1a,2} = J_{2,3}$ 10.3 Hz, $J_{1e,2}$ 4.9 Hz, H-2), 3.60 (dd, 1H, $J_{3,4}$ 2.9 Hz, $J_{4,5}$ 1.4 Hz, H-4), 3.20 (dd, 1H, H-3), 2.89 (m, 1H, H-1e), 2.88 (s, 6H, Me_2N-aryl), 2.85 (t, 2H, H-1′), 2.49 (m, 1H, H-6′a), 2.35–2.24 (m, 2H, H-5, H-6′b), 1.99 (dd, 1H, $J_{1a,1e}$ 10.7 Hz, H-1a), 1.34–0.94 (m, 8H, H-2′, H-3′, H-4′, H-5′), 1.12 (d, 3H, H-6). MS (API-ES): Calcd for [$C_{24}H_{37}N_3SO_5$]: *m/z* 479.6436. Found: *m/z* 478.4 [M−H].

Compound **7a**: ^{13}C NMR: δ 119.9 (C-6″), 71.8, 69.3, (C-3, C-4) 59.7, 56.8, 55.5, 53.6, 41.9 (C-2, C-6, C-5, C-5′, C-1″), 25.6, 24.8, 23.7, 16.0 (C-2″, C-3″, C-4″, C-5″). ^{1}H NMR: δ 3.83 (dd, 1H, $J_{5,5'a}$6.6 Hz, $J_{5'a,5'b}$11.2 Hz, H-5′a), 3.76 (ddd, 1H, H-3), 3.70 (dd, 1H, $J_{5,5'b}$3.4 Hz, H-5′b), 3.57–3.45 (m, 2H, H-2e, H-6e), 3.41 (dd, 1H, $J_{3,4}$ 9.5 Hz, $J_{4,5}$ 9.2 Hz, H-4), 3.11 (m, 2H, H-1″), 2.87 (dd, 1H, $J_{5,6a}$ 11.7 Hz, $J_{6a,6e}$ 12.7 Hz, H-6a), 2.75 (dd, 1H, $J_{2a,2e} = J_{2a,3}$ 11.5 Hz, H-2a), 2.52 (t, 2H, H-5″), 1.99 (m, 1H, H-5), 1.86–1.45 (m, 6H, H-2″, H-3″, H-4″). MS (API-ES): Calcd for [$C_{12}H_{22}N_2O_3$]: *m/z* 242.3207. Found: *m/z* 241.2 [8M−H].

Compound **7b**: ^{13}C NMR: δ 74.9, 71.9 (C-3, C-4), 61.5, 58.6, 58.2, 55.3, 43.8 (C-2, C-5, C-5′, C-6, C-1″), 41.0 (C-6″), 27.3, 26.6, 26.5, 26.4 (C-2″, C-3″, C-4″, C-5″). ^{1}H NMR: δ 3.83 (dd, 1H, $J_{5,5'b}$3.9 Hz, $J_{5'a,5'b}$11.2 Hz, H-5′b), 3.53 (dd, 1H, $J_{5,5'a}$7 Hz, H-5′a), 3.52 (m, 1H, H-3), 3.09 (dd, 1H, $J_{3,4}$ 9.2 Hz, $J_{4,5}$ 9.8 Hz, H-4), 3.04 (m, 2H, H-2a, H-6e), 2.68 (br s, 2H, H-1″), 2.39 (m, 2H, H-2a, H-6a), 1.84 (m, 2H, H-6″), 1.75 (m, 1H, H-5), 1.58–1.30 (m, 8H, H-2″, H-3″, H-4″, H-5″). MS (API-ES): Calcd for [$C_{12}H_{26}N_2O_3$]: *m/z* 246.3526. Found: *m/z* 245.3 [M−H].Compound **7c**: ^{13}C NMR: δ 74.9, 71.9 (C-3, C-4), 61.5, 58.8, 58.0, 55.4 (C-2, C-5′, C-6, C-1″), 44.7 (Me_2N-aryl), 43.8, 42.5 (C-5, C-6″), 29.2, 26.8, 26.7, 24.1 (C-2″, C-3″, C-4″, C-5″). ^{1}H NMR: δ 3.82 (dd, 1H, $J_{5,5'b}$3.7 Hz, $J_{5'a,5'b}$10.7 Hz, H-5′b), 3.52 (dd, 1H, $J_{5,5'a}$7.0 Hz, H-5′a), 3.49 (m, 1H, H-3), 3.08 (dd, 1H, $J_{3,4}$ 9.0 Hz, $J_{4,5}$ 10.0 Hz, H-4), 2.97 (m, 2H, H-2e, H-6e), 2.90 (s, 6H, Me_2N-aryl), 2.86 (t, 2H, H-1″), 2.23 (m, 2H, H-2a, H-6a), 1.97 (m, 2H, H-6″), 1.73 (m, 1H, H-5), 1.36–1.02 (m, 8H, H-2″, H-3″, H-4″, H-5″). MS (API-ES): Calcd for [$C_{24}H_{37}N_3SO_5$]: *m/z* 479.6436. Found: *m/z* 478.5 [M−H].

7. Assays were conducted as previously published: β-glucosidase: Namchuk, M. N.; Withers, S. G. *Biochemistry* **1995**, *34*, 16194; α-mannosidase: Withers, S. G.; Rupitz, K.; Street, I. P. *J. Biol. Chem.* **1988**, *263*, 7929; β-*N*-acetylhexosaminidase: Mark, B. L.; Vocadlo, D. J.; Zhao, D.; Knapp, S.; Withers, S. G.; James, M. N. G. *J. Biol. Chem.* **2001**, *276*, 42131; α-L-fucosidase: Sulzenbacher, G.; Bignon, C.; Nishimura, T.; Tarling, C. A.; Withers, S. G.; Henrissat, B.; Bourne, Y. *J. Biol. Chem.* **2004**, *279*, 13119; β-xylosidase: Häusler, H.; Rupitz, K.; Stütz, A. E.; Withers, S. G. *Chem. Mon.* **2002**, *133*, 555.

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