Supplemental Data

Synthesis of GLA-S, GLA-IS, GAA-S and GAA-IS

The synthetic scheme is shown in the figure below.

General Methods. Thin layer chromatography (TLC) was carried out on silica plates (Merck, $60F_{254}$), and flash column chromatography was carried out with silica gel (Merck, 230-400 Mesh). Preparative HPLC was carried out on a C18 reverse phase column (Vydac 218TP1022, 2.2 cm x 25 cm). Elution of compounds was monitored with a UV detector (λ = 254 nm). Dry CH_2Cl_2 was obtained by distillation from CaH_2 under Ar, and other dry solvents were obtained from Aldrich (Sure-Seal). As noted below, reactions were carried out in a round bottom flask (RBF) or in a vial with a Teflon septum-lined screw cap. ^{1}H-NMR spectra were obtained on a Bruker DPX200 spectrometer (200 MHz) unless otherwise noted.

Acetic acid 4-nitro-phenyl ester (**1**): Acetic anhydride (50 ml) was added to a solution of 4-nitrophenol (5.56 g, 40 mmol) in dry pyridine (50 ml). The solution was stirred at ambient temperature for 2 hr and then at 70 °C overnight with a reflux condenser under Ar. The mixture was poured onto ice, and a white precipitate formed after standing for several hours. Water (400 ml) was added, and the white solid was collected by vacuum filtration and dried *in vacuo* to yield a white solid (5.1 g, 70%). ESI-MS $(M+H)^+$: 182.2. ^{1}H-NMR ($CDCl_3$) δ 8.30 (2H, d, J = 9.0 Hz, $NO_2CC\mathit{H}$), 7.31 (2H, d, J = 9.0 Hz, OCC*H*), 2.25 (3H, s, $C\mathit{H}_3$).

Acetic acid 4-acryloylamino-phenyl ester (**2**): H_2 was bubbled through a solution of **1** (280 mg, 1.54 mmol) and 10 mg of 10% Pd on carbon in 20 ml of MeOH for 1 hr. The catalyst was removed by filtration. Triethylamine (410 μl, 3.08 mmol) was added to the

filtrate which was chilled on ice, then acryloyl chloride (250 μl, 3.08 mmol, Aldrich) in 10 ml of dry CH_2Cl_2 was added dropwise with stirring over 0.5 hr under Ar. The reaction was allowed to return to ambient temperature, followed by 2 hr of stirring. Anion exchange resin (Bio-Rad, AG-MP1, OH^-) (4 equivalents based on acryloyl chloride) was added, the mixture was filtered, and the filtrate was treated with sufficient cation exchange resin (Dowex, 50W X 8, H^+) to bring the mixture to neutrality (moist pH paper). The resin was removed by filtration, and the solvent was removed by rotary evaporation to yield an off-white solid (268 mg, 85%). ESI-MS $(M+H)^+$: 206.1. ^{1}H-NMR (acetone-d_6) δ 9.15 (1H, br, N*H*), 7.78 (2H, d, J = 9.0 Hz, NHCC*H*), 7.08 (2H, d, J = 9.0 Hz, NHCCHC*H*), 6.55~6.37 (2H, m, COC*H*C*H*H (anti to each other)), 5.75 (1H, dd, J = 9.8 and 2.2 Hz, COCHCH*H* (syn to COC*H*)), 2.25 (3H, s, CH_3).

N-(4-Hydroxy-phenyl)-acrylamide (**3**). To **2** (200 mg, 0.98 mmol) in 1.5 ml of MeOH in a 5 ml screw-capped vial was added 1.0 ml of 0.5 M of sodium methoxide in MeOH. The mixture was stirred at ambient temperature, and the reaction was complete in 10 min. The mixture was neutralized by addition of cation exchange resin (Dowex, 50W X 8, H^+) (moist pH paper). The resin was removed by filtration and washed with MeOH. The combined filtrate and wash was concentrated by rotary evaporation to yield an off-white solid (152 mg, 95%), ESI-MS $(M+H)^+$: 164.2. ^{1}H-NMR (acetone-d_6), δ 9.15 (1H, br, N*H*), 7.59 (2H, d, J = 9.0 Hz, NHCC*H*), 6.82 (2H, d, J = 9.0 Hz, NHCCHC*H*), 6.52~6.35 (2H, m, COC*H*C*H*H (anti to each other)), 5.70 (1H, dd, J = 9.8 and 2.2 Hz, COCHCH*H* (syn to COC*H*)).

4-Acrylaminophenyl α-D-galactopyranoside (**4**): The compound was prepared as described for **2** using 1 g of 4-nitrophenyl α-D-galactopyranoside (Sigma) to obtain 0.94

g (87%) of **4**; ESI-MS $(M+H)^+$: 326.3. ^{1}H-NMR (D_2O) δ 7.43 (2H, d, J = 9.0 Hz, NHCC*H*), 7.16 (2H, d, J = 9.0 Hz, NHCCHC*H*), 6.47~6.24 (2H, m, COC*H*C*H*H (anti to each other)), 5.81 (1H, dd, J = 9.8 and 2.2 Hz, COCHCH*H* (syn to COC*H*)), 5.52 (1H, d, J= 3.4 Hz, H-1), 4.01~3.86 (4H, m, H-2,3,4,5), 3.63~3.60 (2H, d, J = 6.2 Hz, H-6, 6').

4-Acrylaminophenyl α-D-glucopyranoside (**5**): The compound was prepared as described for **4**, using 1 g of 4-nitrophenyl α-D-glcucopyranoside (Sigma) to obtain 0.97 g (90%) of **5**; ESI-MS $(M+H)^+$: 326.3. ^{1}H-NMR (D_2O) δ 7.43 (2H, d, J = 9.0 Hz, NHCC*H*), 7.16 (2H, d, J = 9.0 Hz, NHCCHC*H*), 6.47~6.24 (2H, m, COC*H*C*H*H (anti to each other)), 5.81 (1H, dd, J = 9.8 and 2.2 Hz, COCHCH*H* (syn to COC*H*)), 5.60 (1H, d, J = 3.6 Hz, 1-H), 3.94~3.66 (5H, m, H-2,3,5,6,6'), 3.48 (1H, t, J = 9.2 Hz, H-4).

N-(6-Amino-hexyl)-benzamide (**6**): To a stirred solution of 1,6-diaminohexane (10.0 g, 86.3 mmol, Aldrich) in 30 ml dry CH_2Cl_2 was added benzoyl chloride (1 ml, 8.6 mmol) in 300 ml dry CH_2Cl_2 dropwise at ambient temperature under Ar. A white precipitate formed as the reaction proceeded, and the mixture was stirred at ambient temperature for 5 hr after the addition was completed. Aqueous NaOH (3 ml of 4 N) was added to dissolve the precipitate. The reaction mixture was washed with water (3 x 60 ml), dried over Na_2SO_4 and solvent was removed by rotary evaporation. The oil was purified by flash chromatograph on silica eluting (Merck 230-400 Mesh) with 30:1 acetone/concentrated ammonium hydroxide to yield product as a yellowish oil (0.75 g, 32%). R_f = 0.43 (TLC, same solvent). ESI-MS $(M+H)^+$: 221.3. ^{1}H-NMR (acetone-d_6) δ 7.82~7.75 (2H, m, COCC*H*), 7.55~7.35 (3H, m, COCHC*H*C*H*), 6.35 (1H, br, N*H*), 3.42 (2H, dt, J = 5.8 and 6.8 Hz, CONHCH_2), 3.20 (1H, t, J = 6.8 Hz, NH_2CH_2), 1.90-1.32 (8H, m, $NHCH_2(CH_2)_4$).

N-(6-Amino-hexyl)-d_5-benzamide (**7**): The compound was prepared as for **6** using d_5-benzoyl chloride (Cambridge Isotope Inc.). ESI-MS $(M+H)^+$: 226.3. ^{1}H-NMR (acetone-d_6) δ 6.35 (1H, br, N*H*), 3.42 (2H, dt, J = 5.8 and 6.8 Hz, CONHCH_2), 3.20 (1H, t, J = 6.8 Hz, NH_2CH_2), 1.90-1.32 (8H, m, $NHCH_2(CH_2)_4$).

N-(7-Amino-heptyl)-benzamide (**8**): The compound was prepared as for **6** using 1,7-diaminoheptane (Aldrich). ESI-MS $(M+H)^+$: 235.3. ^{1}H-NMR (acetone-d_6) δ 7.82~7.75 (2H, m, COCC*H*), 7.55~7.35 (3H, m, COCHC*H*C*H*), 6.35 (1H, br, N*H*), 3.42 (2H, dt, J = 5.8 and 6.8 Hz, CONHCH_2), 3.20 (1H, t, J = 6.8 Hz, NH_2CH_2), 1.90-1.32 (10H, m, $NHCH_2(CH_2)_5$).

N-(7-Amino-heptyl)-d_5-benzamide (**9**): The compound was prepared as for **6** using d_5-benzoyl chloride. ESI-MS $(M+H)^+$: 240.3. ^{1}H-NMR (acetone-d_6) δ 6.35 (1H, br, N*H*), 3.42 (2H, dt, J = 5.8 and 6.8 Hz, CONHCH_2), 3.20 (1H, t, J = 6.8 Hz, NH_2CH_2), 1.90-1.32 (10H, m, $NHCH_2(CH_2)_5$).

(6-Benzoylamino-hexyl)-{2-[4-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxy)-phenylcarbamoyl]-ethyl}-carbamic acid tert-butyl ester (**GLA-S**): Compound **4** (0.88 g, 2.7 mmol) and **6** (0.71 g, 3.2 mmol) in a solution of isopropanol (30 ml) and H_2O (4 ml) was stirred at 65 °C (oil bath) in a capped 100 ml RBF for 48 hrs. TLC on silica showed that at least 85% of **4** was converted to the Michael addition product ($R_f = 0$, 30:1 acetone-concentrated ammonium hydroxide). The reaction was allowed to cool to ambient temperature, followed by the addition of powdered K_2CO_3 (0.44 g, 3.2 mmol) and di-*tert*-butylcarbonate (0.84 mg, 3.8 mmol, Aldrich). The mixture was stirred at ambient temperature for 3 hr. TLC showed at least 80% of Michael addition product was converted to the desired product ($R_f = 0.17$, 10:1 acetone-concentrated ammonium

hydroxide). The solid was collected by vacuum filtration and was washed with 30 ml of MeOH. The filtrates were combined, and solvent was removed by rotary evaporation to give an oily residue. MeOH (6.5 ml) was added to dissolve the residue, and the pH was adjusted to ~3-4 (moist pH paper) by addition of trifluoroacetic acid with chilling on ice. The desired product was purified by 10 runs of preparative HPLC: 50% MeOH in H_20, at a flow rate of 6 ml/min; t_R = 27 min. Product fractions were pooled, and most of the solvent was removed by rotary evaporation at ambient temperature. The remaining solvent was removed by lyophilization, and the resulting residue was dissolved in 20 ml of MeOH. Solvent was removed by rotary evaporation, and the oily residue was dried in vacuum to give a white solid (1.1 g, 63%). ESI-MS $(M+H)^+$: 646.6; ^{1}H-NMR (1:2.5 D_2O/acetone-d_6) δ 7.80~7.75 (2H, m, COCC*H*), 7.55~7.35 (5H, m, COCHC*H*C*H* and NHCC*H*), 7.05 (2H, d, J = 9.0 Hz, NHCCHC*H*), 5.39 (1H, d, J = 3.4 Hz, H-1), 4.00~3.57 (6H, m, H-2,3,4,5,6,6'), 3.51 (2H, t, J = 6.8 Hz, $COCH_2CH_2$), 3.30 (2H, t, J = 7.0 Hz, $CONHCH_2$), 3.16 (2H, t, J = 7.0 Hz, $CONH(CH_2)_5CH_2$), 2.55 (2H, t, J = 6.4 Hz, $COCH_2$), 1.70-1.20 (17H, m, O-*tert*-C_4H_9 and $NHCH_2(CH_2)_4$).

(7-Benzoylamino-heptyl)-{2-[4-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxy)-phenylcarbamoyl]-ethyl}-carbamic acid tert-butyl ester (**GAA-S**): The compound was prepared as for **GLA-S** starting from 0.63 g of **5** and 0.55 g of **8**. HPLC t_R = 40 min. Yield 60%. ESI-MS $(M+H)^+$: 660.6. ^{1}H-NMR (1:2.5 D_2O/acetone-d_6) δ 7.80~7.75 (2H, m, COCC*H*), 7.55~7.35 (5H, m, COCHC*H*C*H* and NHCC*H*), 7.05 (2H, d, J = 9.0 Hz, NHCCHC*H*), 5.39 (1H, d, J = 3.6 Hz, H-1), 3.90~3.57 (5H, m, H-2,3 ,5,6,6'), 3.51 (2H, t, J = 6.8 Hz, $COCH_2CH_2$), 3.45 (1H, t, j = 9.6 Hz, H-4), 3.30 (2H, t, J = 7.0 Hz,

CONHCH_2), 3.20 (2H, t, J = 7.0 Hz, CONH(CH_2)$_5$CH_2), 2.65 (2H, t, J = 6.4 Hz, COCH_2), 1.70-1.20 (19H, m, O-*tert*-C_4H_9 and NHCH$_2$(CH_2)$_5$).

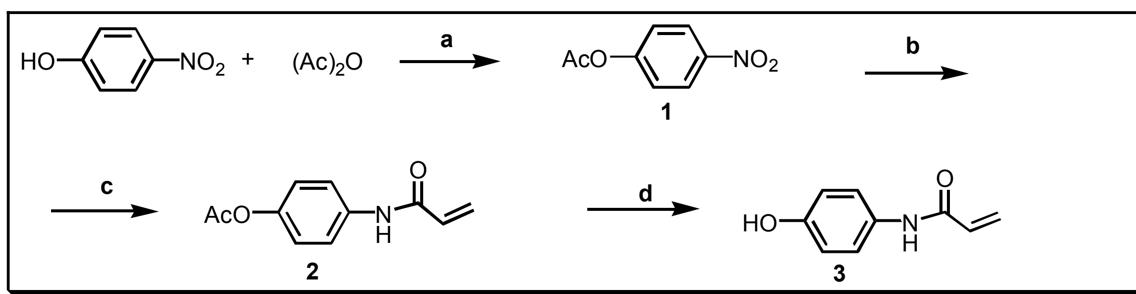
(6-d_5-Benzoylamino-hexyl)-[2-(4-hydroxy-phenylcarbamoyl)-ethyl]-carbamic acid tert-butyl ester (**GLA-IS**): Compound **3**, 10 mg, 0.06 mmol) and **7** (21 mg, 0.09 mmol) were dissolved in 1.5 ml of isopropanol in a screw capped vial. The mixture was stirred at 65 °C overnight. TLC showed that more than 85% of **3** had been converted into the Michael addition product (R_f = 0.22, 30:1 acetone/ concentrated ammonium hydroxide solution). After the reaction was cooled to ambient temperature, K_2CO_3 (10 mg, 0.07 mmol) and di-*tert*-butylcarbonate (16 mg, 0.07 mmol) were added, and the mixture stirred for 2 hr at the same temperature. TLC showed that all the Michael addition product had been converted into the desired product (R_f = 0.93, 30:1 acetone/concentrated ammonium hydroxide solution). The final product was purified by HPLC (solvent A, H_2O; solvent B, MeOH; Gradient 0-30 min, 30-60% B; 30-70 min, 60-85%; flow rate 6 ml/min; t_R = 45.4 min) to yield 22 mg of desired product (yield 75%). ESI-MS $(M+H)^+$: 489.5. ^{1}H-NMR($CDCl_3$) δ 8.78 and 8.48 (2H, br, NH), 7.35 (2H, d, J = 9.0 Hz, NHCCH), 6.91 (1H, br, OH), 6.77 (2H, d, J = 9.0 Hz, HOCCH), 3.47 (2H, t, J = 6.2 Hz, COCH$_2$CH_2), 3.34 (2H, dt, J = 5.8, 6.8 Hz, CONHCH_2), 3.09 (2H, t, J = 6.8 Hz, CONH(CH_2)$_5$CH_2), 2.55 (2H, t, J = 6.2 Hz, COCH_2), 1.70-1.10 (17H, m, O-*tert*-C_4H_9 and NHCH$_2$(CH_2)$_4$).

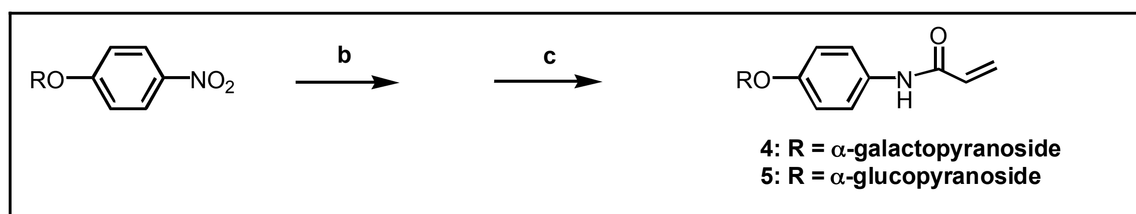
(6-Benzoylamino-hexyl)-[2-(4-hydroxy-phenylcarbamoyl)-ethyl]-carbamic acid tert-butyl ester (**GLA-P**): The compound was prepared as for **GLA-IS** using 10.4 mg of **6**. HPLC t_R = 45.3 min. Yield 72.1%. ESI-MS $(M+H)^+$: 484.5. ^{1}H-NMR($CDCl_3$) δ 8.78 and 8.48 (2H, br, NH), 7.84~7.79 (2H, m, COCCH), 7.55~7.35 (5H, m, NHCCH, COCHCHCH), 6.91 (1H, br, OH), 6.82 (2H, d, J = 9.0 Hz, HOCCH), 3.57 (2H, t, J = 6.2

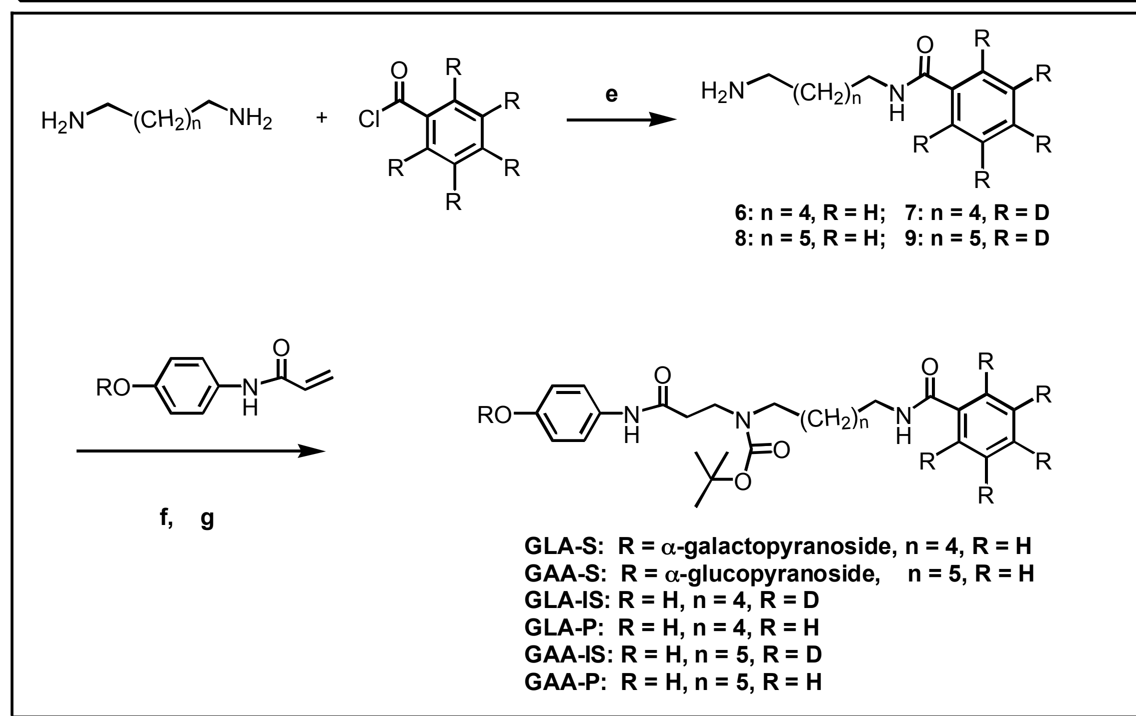
Hz, $COCH_2CH_2$), 3.42 (2H, dt, J = 5.8, 6.8 Hz, CONHCH_2), 3.20 (2H, t, J = 6.8 Hz, CONH$(CH_2)_5$CH_2), 2.64 (2H, t, J = 6.2 Hz, $COCH_2$), 1.70-1.10 (17H, m, O-*tert*-C_4H_9 and $NHCH_2(CH_2)_4$).

(7-d_5-Benzoylamino-heptyl)-[2-(4-hydroxy-phenylcarbamoyl)-ethyl]-carbamic acid tert-butyl ester (**GAA-IS**): The compound was prepared as for **GLA-IS** using 22 mg of **9**. HPLC t_R = 47.0 min. Yield 70.5%. ESI-MS $(M+H)^+$: 503.5. ^{1}H-NMR($CDCl_3$) δ 8.78 and 8.48 (2H, br, N*H*), 7.35 (2H, d, J = 9.0 Hz, NHCC*H*), 6.91 (1H, br, O*H*), 6.77 (2H, d, J = 9.0 Hz, HOCC*H*), 3.47 (2H, t, J = 6.2 Hz, $COCH_2CH_2$), 3.34 (2H, dt, J = 5.8, 6.8 Hz, CONHCH_2), 3.09 (2H, t, J = 6.8 Hz, CONH$(CH_2)_6$CH_2), 2.55 (2H, t, J = 6.2 Hz, COCH_2), 1.70-1.20 (19H, m, O-*tert*-C_4H_9 and $NHCH_2(CH_2)_5$).

(7-Benzoylamino-heptyl)-[2-(4-hydroxy-phenylcarbamoyl)-ethyl]-carbamic acid tert-butyl ester (**GAA-P**): The compound was prepared as for **GAA-IS** using 11 mg of **8**. HPLC t_R = 46.8 min. Yield 75.5%. ESI-MS $(M+H)^+$: 498.5. ^{1}H-NMR($CDCl_3$) δ 8.78 and 8.48 (2H, br, N*H*), 7.84~7.79 (2H, m, COCC*H*), 7.55~7.35 (5H, m, NHCC*H*, COCHC*H*C*H*), 6.91 (1H, br, O*H*), 6.82 (2H, d, J = 9.0 Hz, HOCC*H*), 3.57 (2H, t, J = 6.2 Hz, $COCH_2CH_2$), 3.42 (2H, dt, J = 5.8, 6.8 Hz, CONHCH_2), 3.20 (2H, t, J = 6.8 Hz, CONH$(CH_2)_5$CH_2), 2.64 (2H, t, J = 6.2 Hz, $COCH_2$), 1.70-1.10 (19H, m, O-*tert*-C_4H_9 and $NHCH_2(CH_2)_5$).







Synthesis of GLA-S, GLA-P, GLA-IS, GAA-S, GAA-P and GAA-IS using the following reagents: a, pyridine, reflux; b, H_2, Pd-C, MeOH, rt; c, CH_2=CH-COCl, Et_3N, MeOH, rt; d, NaOMe, MeOH, rt; e, CH_2Cl_2, rt; f, isopropanol, H_2O, 65 °C; g, K_2CO_3, (*t*-BuOCO)$_2$O, rt.

Loading the Filter Plate with Silica Gel.

This is facilitated by use of a plate loader similar to those available from Millipore (Cat. MACL 096). A homemade plate loader was used. The loader was prepared by boring wells into an aluminum plate such that each well holds 100 mg of silica gel when filled. The loader was used to fill the multiwell filter plate as described by Millipore.

Analysis of Additional alpha-Glucosidase Inhibitors.

We analyzed additional compounds for inhibition of acid alpha-glucosidases using the fluorimetric assay with 1.4 mM 4-methylumbelliferyl-alpha-glucopyranoside in citrate-phosphate buffer, pH 4.0 (Umapathysivam, K., Hopwood, J. J. and Meikle, P. J. (2001) Clin. Chem. 47, 1378-1383). The alpha-glucosidase inhibitors tested were: 1) Blintol (Johnston, B. D., Ghavami, A., Jensen, M. T., Svensson, B. and Pinto, B. M. (2002) J. Am. Chem. Soc., 124, 8245-8250); 2) Castanospermine (Sigma); 3) Miglitol (purified on a cation exchange resin from the drug Glyset); 4) Salacinol (Ghavami, A., Johnston, B. D. and Pinto, B. M. (2001) J. Org. Chem., 66, 2312 -2317). The following IC_{50} values were obtained using recombinant GAA and PMN lysate as the source of acid alpha-glucosidase, respectively: Blintol (0.6 μM, 6 μM), Castanospermine (0.6 μM, 0.2 μM), Miglitol (26 μM, 53 μM) and Salacinol (0.9 μM, 8 μM). These data show that these additional inhibitors are not sufficiently selective to block RAAG for the selective detection of GAA.

Regression Analysis of the Callibration Curves Shown in Supporting Data Figure 1.

Assay	n	m	b	r^2	s_y	s_m	s_b	s_x
GALC	21	0.969	0.017	0.998	0.049	0.011	0.016	0.053
ASM	21	1.107	-0.003	0.998	0.066	0.014	0.022	0.062
ABG	21	1.293	0.0004	0.999	0.049	0.011	0.016	0.040

n = number of data points in calibration
m = slope
b = intercept
r^2 = correlation coefficient (squared)
s_y = standard deviation of measured product/internal standard ratio
s_m = standard deviation of slope
s_b = standard deviation of intercept
s_x = standard deviation of product/internal standard ratio, as read from the least-squares calibration line.

Supplemental Data Table 1. Instrument settings for positive ion mode, electrospray ionization tandem mass spectrometry.

<table>
<tr><td colspan="2">ISV (ion spray vltg): 4300 V</td><td colspan="2">IN (interface plate vltg): 650 V</td></tr>
<tr><td colspan="2">OR (orifice plate vltg): 45 V</td><td colspan="2">R0 (R0 rod offset vltg): 30 V</td></tr>
<tr><td colspan="2">Nebulizer gas: 35 psi</td><td colspan="2">Curtain gas: 1.2 L/min</td></tr>
<tr><td colspan="2">RE1 (Q1 resolution): 110.0</td><td colspan="2">RE3 (Q3 resolution): 105</td></tr>
<tr><td colspan="4">Collision energy: 25 eV</td></tr>
<tr><td colspan="4">CGT (collision gas thickness) ~150 x 10^{12} molecules/cm^3 (argon)</td></tr>
<tr><td colspan="4"></td></tr>
<tr><th colspan="2">Parent Ion Scanning</th><th colspan="2">Neutral Loss scanning</th></tr>
<tr><td colspan="2">Parent ion scan of 264</td><td colspan="2">Neutral loss scan of 100</td></tr>
<tr><td colspan="2">Dwell time: 3.0 ms</td><td colspan="2">Dwell time: 5.0 ms</td></tr>
<tr><td colspan="2">Pause time: 0.052 ms</td><td colspan="2">Pause time: 0.052 ms</td></tr>
<tr><td colspan="2">Step size: 0.2 amu</td><td colspan="2">Step size: 1.0 amu</td></tr>
<tr><td colspan="2">Scans: 200</td><td colspan="2">Scans: 200</td></tr>
<tr><td colspan="2">Duration: 0.87 min</td><td colspan="2">Duration: 0.42 min</td></tr>
<tr><td rowspan="6">Q1 Scanning Range (m/z)</td><td>369.4 – 372.4 (ASM-IS)</td><td rowspan="6">Q1 Scanning Range (m/z)</td><td rowspan="6">482.0-506.5
(GLA-P, GLA-IS
GAA-P and GAA-IS)</td></tr>
<tr><td>397.4 - 400.4 (ASM-P)</td></tr>
<tr><td>425.4 – 428.4 (GALC-P)</td></tr>
<tr><td>453.4 - 456.4 (GALC-IS)</td></tr>
<tr><td>481.4 – 484.4 (ABG-P)</td></tr>
<tr><td>509.4 – 512.4 (ABG-IS)</td></tr>
</table>

Supplemental Data Table 2.

Informaton on DBS from Affected Patients and Heterozygote Carriers

Enzyme	Patient number[1]	Patient gender	Patient Age when DBS was made (yrs)	Date DBS was made (month/day /year)	Storage of DBS[2]	Enzyme activity 5 mm DBS extract (µmol/hr/ L blood)[3]	Enzyme activity 2 mm DBS per reaction (µmol/hr/ L blood)[3]
ABG	GD1	F	13	9/19/2003	4°C	0.02	0
ABG	GD2	M	56	10/15/2001	4°C	0.18	0.47
ABG	GD3	F	1	2/1/2002	4°C	0.02	0.03
ABG	GD4	M	4	4/19/2002	4°C	0.18	0.45
ABG	GD5	M	20	4/19/2002	4°C	0.12	0.18
ABG	GD6	F	7	12/26/2000	4°C	0.02	nd[4]
ABG	GC1	F	62	5/30/2000	4°C	1.07	1.78
ABG	GC2	F	52	5/30/2000	4°C	1.59	2.97
ABG	GC3	F	51	8/6/2001	4°C	1.87	4.13
ABG	GC4	F	42	9/23/2002	4°C	1.14	2.37
ABG	GC5	F	36	10/19/2001	4°C	0.81	1.29
ASM	NPD1	M	17	8/11/2000	4°C	0	0.16
ASM	NPD2	M	1	5/22/2001	4°C	0	0.02
ASM	NPD3	M	3	6/19/2002	4°C	0.3	0.15
ASM	NPD4	M	16	9/27/2000	4°C	0.02	0.21
ASM	NPD5	F	10 months	8/23/2003	4°C	0	0.09
ASM	NPC1	F	adult	6/19/2002	4°C	1.21	3.02
ASM	NPC2	F	adult	5/22/2001	4°C	0.48	1.69
ASM	NPC3	F	adult	10/18/2000	4°C	0.4	0.89
ASM	NPC4	F	adult	6/27/2001	4°C	0.47	1.61
ASM	NPC5	F	adult	11/28/2003	4°C	0.46	0.67
GALC	KD1	F	5	8/15/2003	-20°C	0.07	0.047
GALC	KD2	F	14	8/15/2003	-20°C	0.07	0.078
GALC	KD3	F	26	8/15/2003	-20°C	0.2	0.227
GALC	KD4	F	51	8/19/2003	-20°C	0.05	0.076
GALC	KD5	nd	15	9/12/2003	-20°C	0.11	0.074
GALC	KD6	nd	5	10/27/2003	-20°C	0	0.126
GALC	KD7	M	26	10/18/2003	-20°C	0.109	0.199
GALC	KD8	nd	5	10/17/2003	-20°C	0.123	0.09
GALC	KD9	nd	15 months	2/10/2003	-20°C	0.091	0.097
GALC	KD10	nd	14	7/14/2000	4°C	nd	0.109
GALC	KD11	nd	3 months	11/26/2002	4°C	nd	0.139
GALC	KD12	nd	21	3/18/2001	4°C	nd	0.171

GALC	KC1	nd	45	11/26/2002	4°C	nd	1.27
GALC	KC2	nd	38	11/28/2001	4°C	nd	0.88
GALC	KC3	nd	28	7/13/2000	4°C	nd	0.99
GALC	KC4	nd	23	7/14/2000	4°C	nd	1.14

GAA	PD1	M	5 months	2/13/2002	4°C	0.16	0.03
GAA	PD2	F	46	2/4/2002	4°C	0.24	0.17
GAA	PD3	M	40	2/4/2002	4°C	0.16	0.07
GAA	PD4	F	29	4/25/2003	4°C	0.33	0
GAA	PD5	F	51	9/5/2003	4°C	0.09	0.15
GAA	PD6	nd	nd	11/6/2003	4°C	nd	0
GAA	PD7	nd	nd	11/13/2003	4°C	nd	0
GAA	PD8	nd	nd	11/25/2003	4°C	nd	0.05
GAA	PD9	nd	nd	12/17/2003	4°C	nd	0.01
GAA	PD10	nd	nd	2/10/2003	4°C	nd	0.01
GAA	PD11	nd	nd	9/10/2003	4°C	nd	0
GAA	PC1	F	33	2/13/2002	4°C	1.53	1.5
GAA	PC2	M	64	1/8/2003	4°C	0.89	1.26
GAA	PC3	F	69	12/2/2003	4°C	2.76	2.02
GAA	PC4	M	63	12/2/2003	4°C	1.52	0.59
GAA	PC5	F	35	12/17/2003	4°C	1.16	1.03
GAA	PC6	M	adult	2/10/2003	4°C	nd	1.62
GAA	PC7	F	adult	2/10/2003	4°C	nd	3.07
GAA	PC8	M	adult	9/10/2003	4°C	nd	2.03
GAA	PC9	F	adult	9/10/2003	4°C	nd	1.73

GLA	FD1	M	6	10/16/2001	4°C	0.08	0
GLA	FD2	M	8	6/12/2002	4°C	0.08	0.11
GLA	FD3	M	12	1/24/2002	4°C	0.13	0
GLA	FD4	M	38	4/8/2002	4°C	0.01	0
GLA	FD5	M	20	5/20/2003	4°C	0.17	0.25
GLA	FC1	F	32	11/7/2001	4°C	1.51	5.17
GLA	FC2	F	68	2/28/2002	4°C	0.34	0.59
GLA	FC3	F	44	2/28/2002	4°C	0.97	4.02
GLA	FC4	F	35	6/12/2002	4°C	0.68	1.26
GLA	FC5	F	45	5/26/2003	4°C	0.58	1.03

[1]GD: GD1 is Gaucher disease affected patients number 1, GC1 is Gaucher disease heterozygote carrier number 1. Likewise for other patients.

[2]DBS were stored at the indicated temperature in zip-lock plastic bags (1 closed bag inside a second closed bag). DBS were kept at ambient temperature for < 10 days (during shipment).

[3]For ABG, ASM, GAA, and GLA, all of the above DBS were used to obtain the data in Figures 6 and 7 of the main text. For GALC, only DBS obtained in 2003 were used to

obtain the data in Figures 6 and 7 of the main text. All enzyme activity measurements were made in March and April, 2004.

[4]nd means data is not available.

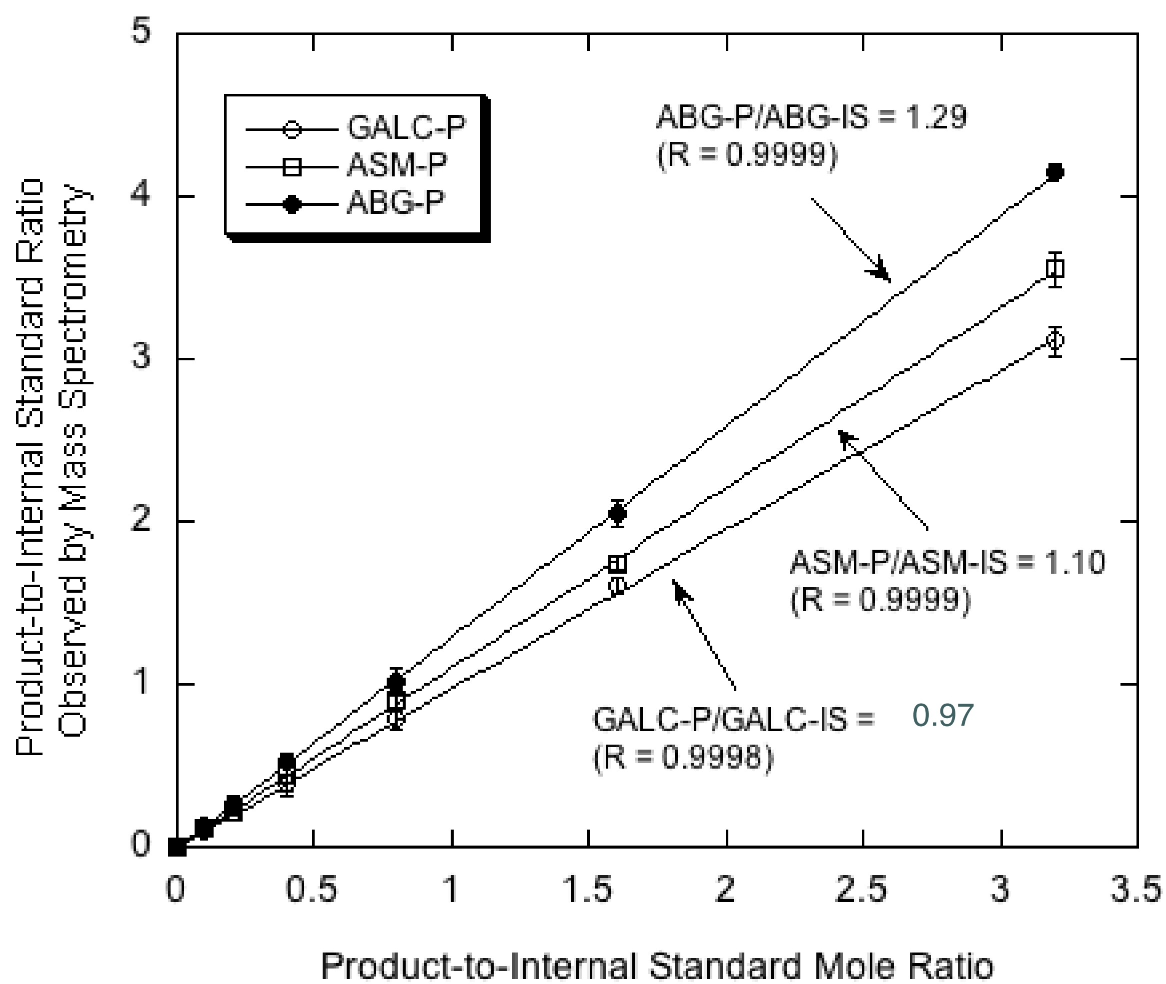
Supplemental Data Table 3. Reagent and supply costs for the 5 lysosomal enzyme assay[1].

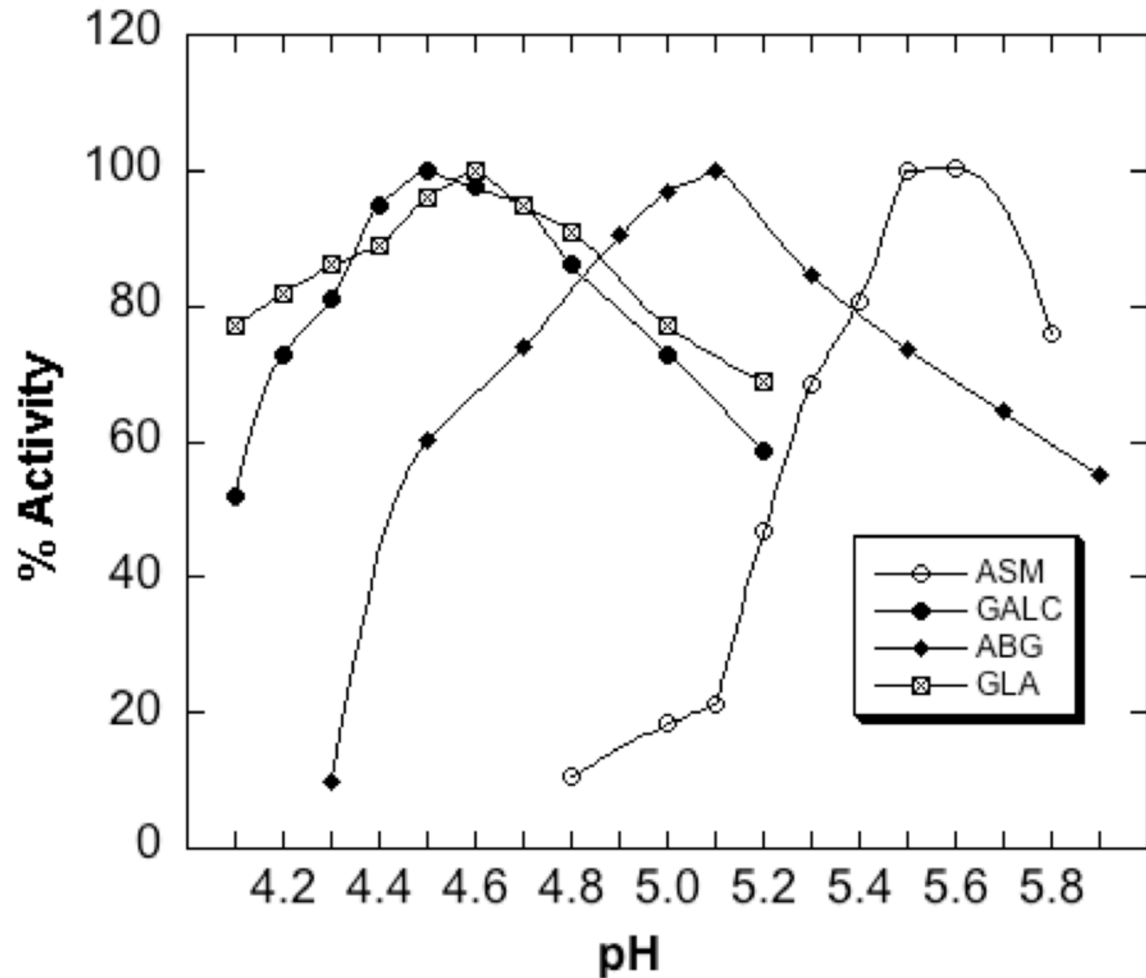
Assay	Cost of Substrate per Assay ($)	Cost of Internal Standard per Assay ($)	Cost of Buffer Salts & Detergent per Assay ($)
ABG	0.026	0.0004	0.0051
ASM	0.053	0.00012	0.00037
GALC	0.023	0.000023	0.0051
GAA	0.066	0.00018	0.000075
GLA	0.161	0.00017	0.00062
		Total	**$ 0.34**

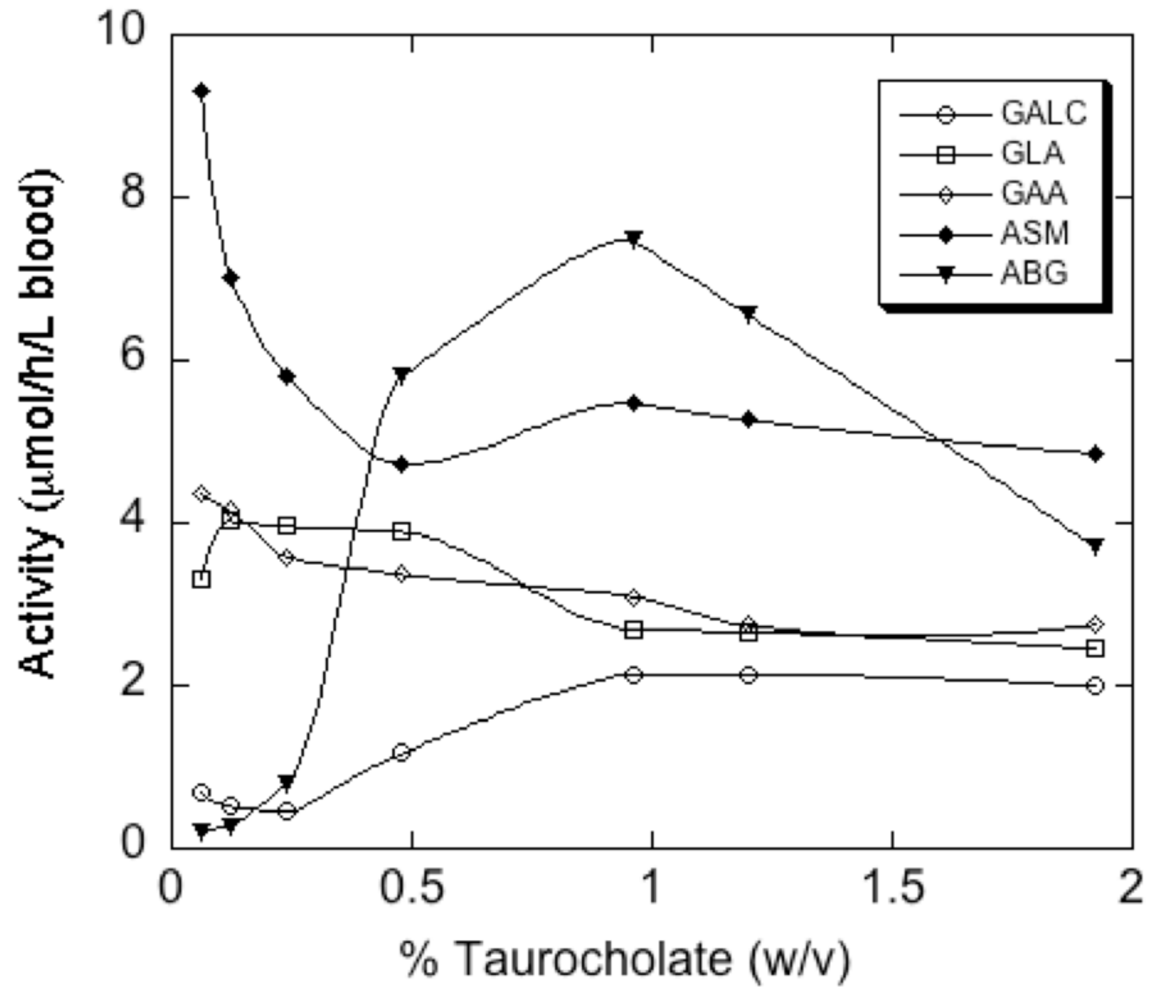
Cost of Work Up Reagents per Assay ($)	Cost of Pipettor Tips per Assay ($)	Cost of 96-well Plates per Assay ($)	Cost of 96-Well Filter Plate per Assay ($)	Cost of Ammonium Formate per Assay ($)
0.0065	0.10	0.17	0.10	0.0000024
			Total	**$ 0.38**

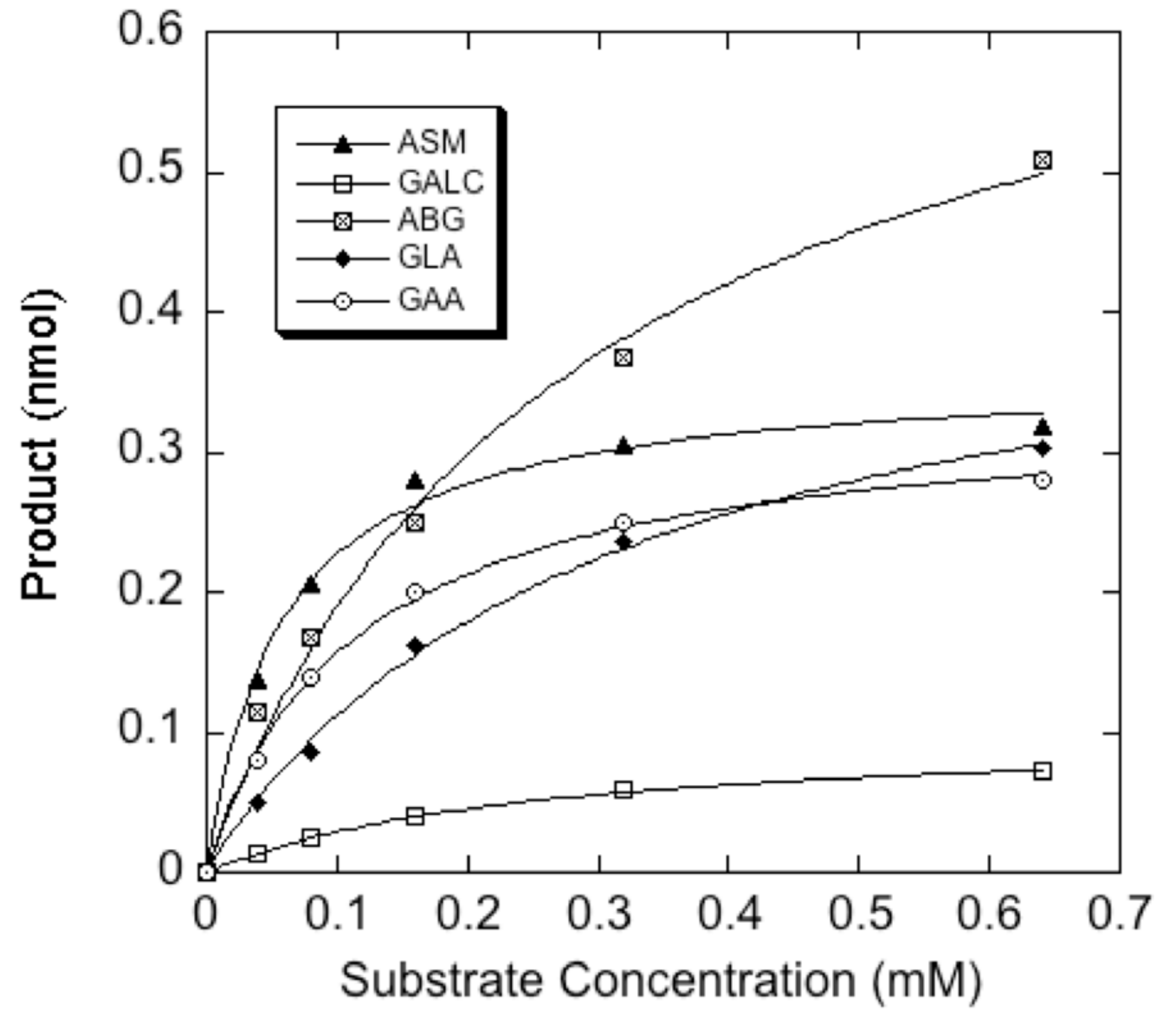
Grand Total $ 0.72

[1]Substrate and internal standard reagent costs for ABG, ASM, and GALC are based on current commercial prices as our all buffer salts, detergents and plasticware. Substrate and internal standard reagent costs for GAA and GLA are based on reasonable estimates.


5
4
3
2
1
0
0
0.5
1
1.5
2
2.5
3
3.5
Product-to-Internal Standard Ratio
Observed by Mass Spectrometry
Product-to-Internal Standard Mole Ratio
GALC-P
ASM-P
ABG-P
ABG-P/ABG-IS = 1.29
(R = 0.9999)
ASM-P/ASM-IS = 1.10
(R = 0.9999)
GALC-P/GALC-IS = 0.97
(R = 0.9998)


% Activity
120
100
80
60
40
20
0
4.2
4.4
4.6
4.8
5.0
5.2
5.4
5.6
5.8
pH
ASM
GALC
ABG
GLA


Activity (μmol/h/L blood)
% Taurocholate (w/v)
0
2
4
6
8
10
0
0.5
1
1.5
2
GALC
GLA
GAA
ASM
ABG



Product (nmol)
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Substrate Concentration (mM)
ASM
GALC
ABG
GLA
GAA

Figure Legends for Supplemental Data

Figure 1. Standards curves used for the ASM, GALC and ABG assays. The Product/Internal-Standard ion ratio (observed by parent ion scan electrospray ionization tandem mass spectrometry) versus the Product/Internal-Standard mole ratio (based on the amount of material added to the assay mixture). Assay samples contained all components except substrate. For each enzyme, the assay contained a fixed amount of internal standard (ASM-IS, 0.2 nmol; GALC-IS, 0.1 nmol; ABG-IS, 0.2 nmol) and various amounts of product. The solid lines are the linear regression fit to the mean of triplicate assays, and the error bars show the standard deviation. The summary of the full statistical analysis is given in the Supplemental Data text section. The product-to-internal standard response factors are 1.29, 1.10, and 0.97 for ABG, ASM and GALC assays, respectively. The standard curves were found to be stable over a 2 month period.

Figure 2. pH-Rate profiles. Each point is the average of duplicate assays carried out with 2 mm DBS from a healthy adult per reaction tube using the assays described in Supporting Information except that 25 μl instead of 12.5 μl of assay cocktail was used for the GLA assay and the pH was varied as indicated. The GAA assay was not included because of interference from neutral alpha-glucosidases at increasing pH values. All assay data has been normalized to 100% activity at the pH optimum.

Figure 3. Dependence of enzyme activity on detergent concentration. Each point is a single assay using a 2 mm DBS per reaction tube using the conditions described in Supporting Information except for the use of a variable amount of detergent as indicated. The detergent used for GAA is Triton X-100 instead of dosium taurocholate.

Figure 4. Amount of enzymatic reaction product versus the substrate concentration. Each point is the average of a duplicate assay, each carried out using a 2 mm DBS from a healthy adult per reaction tube using the assays described in Supporting Information except that 25 µl instead of 12.5 µl of assay cocktail was used for the GLA assay and the substrate concentration was varied as indicated. The actual substrate concentrations for the GLA and GAA assays are 10 times the values indicated on the plot. Values of K_M were obtained by fitting the data to the Michaelis-Menten equation as shown by the solid lines.