

Supplementary Data

ESI-MSMS analyses. ESI-MSMS was performed on a Waters ACQUITY tandem triple quadrupole instrument operating in positive-ion mode.

Ion scanning was carried out with MassLynx 4.1 software with the following settings: capillary voltage, 4500 V; cone voltage, 19 V; extractor, 3 V; RF, 0.4 V; source temperature, 80 °C; desolvation temperature, 250 °C; cone gas flow, 0 L/h; desolvation gas flow, 500 L/h; collision gas flow, 0.20 mL/min; LM 1 resolution, 13.2; HM 1 resolution, 14.6; ion energy 1, 0; MSMS mode entrance, 1; MSMS collision energy, 10 eV (IdA-P) and 9 eV (IdA-IS); MSMS mode exit, 0.5; LM 2 resolution, 13.0; HM 2 resolution, 14.6; ion energy 2, 0.7; collision cell pressure, $\sim 3.7 \times 10^{-3}$ mbar; collision gas, argon. Multiple-reaction-monitoring mode was used for m/z 391.2 \rightarrow 291.1 and 377.2 \rightarrow 277.1 transitions with the following settings: dwell time, 0.1 s; delay, 0.02 s.

The sample (20 μ L of the 70 μ L sample in 5 mmol/L ammonium formate in methanol, see main text) was injected into the mass spectrometer with a Waters autoinjector system. After sample injection, 0.2 % formic acid in methanol was infused for 4 min at 0.2 mL/min to deliver the sample to the mass spectrometer and clean the delivery line prior to the next injection. MSMS data was collected in the period 0-3 min after sample injection [The sample was analyzed within the first 45 seconds of infusion, and after 1 min, the MSMS signal has returned to the background level; in newborn screening laboratories, the analysis could be reduced to 1-2 min per sample to minimize the time spent on a given sample.]

The amount of product was calculated from the ion abundance ratio of product (IdA-P) to internal standard (IdA-IS), minus that from a blank control (blood extract and substrate in buffer pH 3.4 incubated separately), multiplied by the amount of added internal standard and divided by the response factor ratio of product to internal standard (Supplementary Data Fig. 5). Enzymatic activity was calculated from the amount of product which was divided by the incubation time and the volume of blood (3.6 μ L of blood in a 3-mm DBS punch).

Synthesis of IdA-S. Given below is a small scale (milligrams amount) synthesis of IdA-S (Supplementary Data Fig. 6 for a synthetic scheme).

Methyl (2,3,4-tri-*O*-acetyl- β -D-glucopyranosylfluoride) uronate (**2**). Methyl 1,2,3,4-tetra-*O*-acetyl- α,β -D-glucopyranosyluronate **1** (4.98 g, 13.25 mmol, 1 eq) was suspended at 0 °C in 67 mL of 33 % hydrobromic acid in acetic acid under nitrogen. After stirring for 15 min at 0 °C, the reaction mixture was allowed to warm up to room temperature and stirred for 2 h. The reaction mixture was then diluted with toluene and concentrated under vacuum. The residue was diluted with 250 mL of ethyl acetate and washed with 150 mL of cold saturated sodium bicarbonate and 150 mL of cold brine. The organic layer was dried over MgSO₄ and concentrated under vacuum to yield the crude bromide derivative used directly in the next step. The bromide intermediate was dissolved in 167 mL of anhydrous acetonitrile under nitrogen at room temperature. Silver fluoride (3.36 g, 26.49 mmol, 2 eq) was then added. The reaction mixture was stirred for a total of 21 h in the dark. The reaction mixture was filtered through Celite

and the filtrate concentrated under vacuum. Column chromatography on silica gel (hexane:EtOAc, 4:1 to 2:1) afforded product **2** (3.3 g, 74 %): Spectral data were in good agreement with those reported.

Methyl (5-bromo-2,3,4-tri-*O*-acetyl- β -D-glucopyranosylfluoride) uronate (**3**). A suspension of **2** (3.3 g, 9.8 mmol, 1 eq) and *N*-bromosuccinimide (3.32 g, 18.65 mmol, 1.9 eq) in anhydrous carbon tetrachloride was stirred under nitrogen and under reflux with irradiation for a total of 6 h. *N*-bromosuccinimide (3.32 g, 18.65 mmol, 1.9 eq) was added after 2 h and 4 h reaction. The reaction mixture was cooled to room temperature and filtered through glass wool. The solvent was removed under vacuum. Column chromatography on silica gel (hexane:EtOAc, 3:1) afforded product **3** (3.12 g, 77 %): Spectral data were in good agreement with those reported.

Methyl (2,3,4-tri-*O*-acetyl- α -L-idopyranosylfluoride) uronate (**4**). Bromide **3** (3.16 g, 7.61 mmol, 1 eq) was dissolved in 50 mL of anhydrous benzene and stirred under nitrogen. Tributyltin hydride (3.1 mL, 11.4 mmol, 1.5 eq) was added, and the reaction mixture was refluxed for 40 min. The mixture was cooled to room temperature, and the solvent was removed under vacuum. Column chromatography on silica gel (toluene:EtOAc, 8:1 to 6:1) afforded product **4** (1.67 g, 65 %): Spectral data were in good agreement with those reported.

(2',2',2'-Trichloroethyl) 7-acetoxycoumarin-4-acetate (**6a**). To a suspension of 7-acetoxycoumarin-4-acetic acid **5** (945 mg, 3.6 mmol, 1 eq) in 47 mL of anhydrous dichloromethane at room temperature under nitrogen was added 2,2,2-trichloroethanol (431 μ L, 4.5 mmol, 1.25 eq). A solution of *N,N'*-dicyclohexylcarbodiimide (818 mg, 4

mmol, 1.1 eq) in 10 mL of anhydrous dichloromethane was added. The reaction mixture was stirred for 15 min, after which it was diluted with dichloromethane and filtered. The filtrate was concentrated under vacuum. Column chromatography on silica gel (CH₂Cl₂ then CH₂Cl₂:EtOAc, 10:1) afforded product **6a** (1.37 g, 96 %): *R_f* 0.78 (CH₂Cl₂:EtOAc, 5:1); ¹H NMR (300 MHz, CDCl₃): δ 7.61 (d, 1 H, *J*_{5,6} 8.7 Hz, H-5), 7.15 (d, 1 H, *J*_{6,8} 2.1 Hz, H-8), 7.07 (dd, 1 H, *J*_{6,8} 2.3, *J*_{5,6} 8.7 Hz, H-6), 6.42 (s, 1 H, H-3), 4.77 (s, 2 H, CH₂CCl₃), 3.91 (2 s, 2 H, CH₂CO₂), 2.33 (s, 3 H, OAc); ¹³C NMR (75 MHz, CDCl₃): δ 168.6, 167.0, 154.5, 153.5, 146.5, 125.5, 118.5, 117.0, 116.5, 110.9, 74.6, 37.7, 21.2; ESI-MS: *m/z* 393 [M+H]⁺

(2',2',2'-Trichloroethyl) 7-hydroxycoumarin-4-acetate (**6b**). A solution of **6a** (1.08 g, 2.74 mmol, 1 eq) in 108 mL of anhydrous tetrahydrofuran was prepared under nitrogen at room temperature. A solution of 2 M ammonia in 2-propanol (6.8 mL, 13.7 mmol, 5 eq) was added dropwise. The reaction mixture was stirred at room temperature in a tightly sealed flask for 18 h. The reaction mixture was concentrated under vacuum. Purification by column chromatography on silica gel (CH₂Cl₂, then CH₂Cl₂:EtOAc, 10:1 to 5:1) afforded product **6b** (753 mg, 78 %): *R_f* 0.6 (CH₂Cl₂:EtOAc, 5:1); ¹H NMR (300 MHz, *d*₆-DMSO): δ 7.55 (d, 1 H, *J*_{5,6} 8.7 Hz, H-5), 6.79 (dd, 1 H, *J*_{6,8} 2.3, *J*_{5,6} 8.7 Hz, H-6), 6.74 (d, 1 H, *J*_{6,8} 2.3 Hz, H-8), 6.31 (s, 1 H, H-3), 4.94 (s, 2 H, CH₂CCl₃), 4.14 (2 s, 2 H, CH₂CO₂); ¹³C NMR (75 MHz, *d*₆-DMSO): δ 168.4, 161.8, 160.5, 155.5, 149.2, 127.3, 113.5, 112.9, 111.5, 102.8, 95.5, 74.0, 36.9; ESI-MS: *m/z* 351 [M+H]⁺

(2'',2'',2''-trichloroethyl) 7-*O*-(methyl 2',3',4'-tri-*O*-acetyl- α -L-idopyranosyluronate)coumarin-4-acetate (**7**). A suspension of **6b** (703 mg, 2 mmol, 1.26 eq) and LiClO₄ / SiO₂ (200 mg) in 2 mL of anhydrous dichloromethane was stirred at room temperature under nitrogen. 1,1,1,3,3,3-Hexamethyldisilazane (835 μ L, 4 mmol, 2.52 eq) was added dropwise. The reaction mixture was stirred for 35 min. The reaction mixture was diluted with dichloromethane and filtered. The filtrate was concentrated by rotary evaporation to afford (2',2',2'-trichloroethyl) 7-*O*-trimethylsilylcoumarin-4-acetate **6c**, which was used in the next step without further purification. A solution of glycosyl donor **4** (534 mg, 1.6 mmol, 1 eq) and previously prepared glycosyl acceptor **6c** in 10 mL of anhydrous dichloromethane under nitrogen was cooled down to 0 °C. Boron trifluoride diethyl etherate (196 μ L, 1.6 mmol, 1 eq) was added dropwise, after which the reaction mixture was allowed to warm to room temperature. The reaction flask was tightly sealed, and the reaction mixture was stirred for 1.5 h, and then concentrated under vacuum. The residue was dissolved in acetic anhydride (10 mL), and boron trifluoride diethyl etherate (88 μ L) was added. After stirring for 20 min, the reaction was diluted with 200 mL of dichloromethane and washed with 100 mL of water, 100 mL of saturated sodium bicarbonate and 100 mL of brine. The organic layer was dried over MgSO₄ and concentrated under vacuum with additional co-evaporations with toluene. Column chromatography on silica gel (CH₂Cl₂, then CH₂Cl₂:EtOAc, 10:1 to 5:1) afforded product **7** (934 mg, 88 %): [α]_D -80° (c 1, CHCl₃); *R*_f 0.5 (CH₂Cl₂:EtOAc, 5:1); ¹H NMR (300 MHz, CDCl₃): δ 7.53 (d, 1 H, *J*_{5,6} 8.7 Hz, H-5), 7.06 (d, 1 H, *J*_{6,8} 2.5 Hz, H-8), 7.01 (dd, 1 H, *J*_{6,8} 2.5, *J*_{5,6} 8.9 Hz, H-6), 6.33 (s,

1 H, H-3), 5.84 (d, 1 H, $J_{1,2}$: 2.5 Hz, H-1'), 5.20 (m, 2 H, H-3', H-4'), 5.05 (m, 1 H, H-2'), 4.89 (m, 1 H, H-5'), 4.77 (2 s, 2 H, CH_2CCl_3), 3.87 (s, 2 H, CH_2CO_2), 3.77 (s, 3 H, CO_2Me), 2.16-2.09 (3 s, 9 H, 3 OAc); ^{13}C NMR (75 MHz, CDCl_3) δ 169.5, 169.4, 169.0, 167.9, 167.2, 160.2, 158.9, 155.3, 146.7, 126.0, 115.7, 114.1, 113.2, 104.9, 95.7, 94.5, 74.6, 67.8, 67.0, 66.8, 52.9, 37.7, 20.9, 20.9, 20.7; ESI-MS: m/z 667 $[\text{M}+\text{H}]^+$

(*N*-[4''-(*tert*-butoxycarbonylamino)-butyl]) 7-*O*-(methyl 2',3',4'-tri-*O*-acetyl- α -L-idopyranosyluronate)coumarin-4-acetamide (**9a**). Glycoside **7** (831 mg, 1.2 mmol, 1 eq) was dissolved in 41 mL of anhydrous tetrahydrofuran at room temperature. The solution was cooled to 0 °C, and 90 % aqueous acetic acid (5.5 mL) was added. Finally, copper chloride (167 mg, 1.2 mmol, 1 eq) and zinc dust (813 mg, 12.4 mmol, 10 eq) were added. The reaction mixture was stirred at 0 °C for a total of 39 h, during which zinc dust (813 mg, 12.4 mmol, 10 eq) was added after 15 h and 25 h reaction. The reaction mixture was filtered through Celite, and the filtrate was concentrated under vacuum. The residue was solubilized in 200 mL of dichloromethane and washed with 150 mL of water (twice) and 150 mL brine. The organic layer was dried over MgSO_4 and concentrated under vacuum. Column chromatography on silica gel (CH_2Cl_2 , then CH_2Cl_2 :EtOAc, 5:1 to 2:1; all solvents with 1 % acetic acid) afforded product **8** (634 mg, 95 %). A solution of acid **8** (627 mg, 1.2 mmol, 1 eq) in 20 mL of anhydrous tetrahydrofuran was cooled to 0 °C. *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (245 mg, 1.28 mmol, 1.1 eq) and 1-hydroxybenzotriazole (196 mg, 1.28 mmol, 1.1 eq) were added, and the suspension was stirred for 30 min at 0 °C. A solution of *N*-Boc-1,4-diaminobutane (223 μL , 1.2 mmol, 1 eq) in 2 mL of anhydrous

N,N-dimethylformamide was slowly added to the suspension. The reaction mixture was allowed to warm to room temperature and stirred for 3 h. The reaction mixture was concentrated under vacuum. The residue was taken up in 250 mL of ethyl acetate and washed with 150 mL of 1 M HCl, 150 mL of water and 150 mL of brine. The organic layer was dried over MgSO₄ and concentrated under vacuum. Column chromatography on silica gel (toluene:acetone, 3:1 to 2:1) afforded product **9a** (528 mg, 65 %): [α]_D -70° (*c* 0.85, CHCl₃); *R_f* 0.38 (CH₂Cl₂:MeOH, 95:5); ¹H NMR (300 MHz, CDCl₃): δ 7.67 (d, 1 H, *J*_{5,6} 8.7 Hz, H-5), 7.03 (d, 1 H, *J*_{6,8} 2.3 Hz, H-8), 6.99 (dd, 1 H, *J*_{6,8} 2.5, *J*_{5,6} 8.7 Hz, H-6), 6.29 (s, 1 H, H-3), 5.84 (d, 1 H, *J*_{1',2'} 2.1 Hz, H-1), 5.20 (m, 2 H, H-3', H-4'), 5.04 (m, 1 H, H-2'), 4.89 (d, 1 H, *J*_{4',5'} 2.1 Hz, H-5'), 3.77 (s, 3 H, CO₂Me), 3.65 (s, 2 H, CH₂CONH), 3.26 (m, 2 H, CH₂NHCO), 3.09 (m, 2 H, CH₂NHCO), 2.17-2.09 (3 s, 9 H, 3 OAc), 1.49 (m, 4 H, CH₂-CH₂), 1.42 (s, 9 H, CMe₃); ¹³C NMR (75 MHz, CDCl₃) δ 169.4, 169.4, 169.0, 167.8, 167.6, 160.7, 158.8, 156.4, 155.2, 149.7, 126.7, 114.5, 114.5, 113.2, 104.5, 95.6, 79.4, 67.7, 66.9, 66.6, 52.8, 39.8, 28.5, 20.8, 20.8, 20.6; ESI-MS: *m/z* 707 [M+H]⁺

(*N*-[4''-(*tert*-butoxycarbonylamino)-butyl])

7-*O*-(α-L-idopyranosyluronic

acid)coumarin-4-acetamide (**IdA-S**). A solution of **9a** (98 mg, 0.165 mmol, 1 eq) in 16 mL of methanol was cooled to 0 °C. A solution of 0.5 M sodium methoxide in methanol (140 μL, 0.07 mmol, 0.4 eq) was added dropwise. The reaction mixture was stirred at 0 °C for 1.5 h. The reaction mixture was neutralized with Amberlite IR-120 (H⁺) and filtered. The filtrate was concentrated under vacuum. Column chromatography on silica gel (CH₂Cl₂ then CH₂Cl₂:MeOH, 9:1) afforded product **9b** (69 mg, 86 %): ¹H NMR (300

MHz, CD₃OD): δ 7.69 (d, 1 H, $J_{5,6}$ 9.7 Hz, H-5), 7.14 (d, 1 H, $J_{6,8}$ 2.3 Hz, H-8), 7.13 (dd, 1 H, H-6), 6.28 (s, 1 H, H-3), 5.76 (d, 1 H, $J_{1',2'}$ 3.9 Hz, H-1'), 4.75 (d, 1 H, $J_{4',5'}$ 3.5 Hz, H-5'), 3.97-3.89 (m, 2 H, H-2', H-3'), 3.77 (s, 3 H, CO₂Me), 3.74 (s, 2 H, CH₂CONH), 3.73 (m, 1 H, H-4'), 3.21 (m, 2 H, CH₂NHCO), 3.03 (m, 2 H, CH₂NHCO), 1.49 (m, 4 H, CH₂-CH₂), 1.42 (s, 9 H, CMe₃); ESI-MS: m/z 581 [M+H]⁺. Compound **9b** (21 mg, 0.036 mmol, 1 eq) was dissolved in 2 mL of water/methanol (1:1) at room temperature. An aqueous solution of sodium hydroxide 0.1 M was added in increments of 0.1 eq of NaOH until the pH of the solution reached approximately 8 (pH paper). The pH was maintained by incremental additions of the 0.1 M NaOH solution as the reaction proceeded. The reaction mixture was stirred for 5.5 h. The reaction mixture was neutralized with Amberlite IR-120 (H⁺) and filtered. The filtrate was concentrated under vacuum and then lyophilized to yield product **IdA-S** (20 mg, quant.): $[\alpha]_D$ -29° (*c* 0.66, MeOH); ¹H NMR (300 MHz, CD₃OD): δ 7.68 (d, 1 H, $J_{5,6}$ 8.9 Hz, H-5), 7.54 (dd, 1 H, $J_{6,8}$ 2.5, $J_{5,6}$ 8.9 Hz, H-6), 7.46 (d, 1 H, $J_{6,8}$ 2.3 Hz, H-8), 6.25 (s, 1 H, H-3), 5.58 (d, 1 H, $J_{1',2'}$ 6.8 Hz, H-1'), 4.36 (d, 1 H, $J_{4',5'}$ 5.6 Hz, H-5'), 3.73 (s, 2 H, CH₂CONH), 3.69-3.48 (m, 3 H, H-2', H-3', H-4'), 3.21 (m, 2 H, CH₂NHCO), 3.03 (m, 2 H, CH₂NHCO), 1.49 (m, 4 H, CH₂-CH₂), 1.42 (s, 9 H, CMe₃); ESI-MS: m/z 567 [M+H]⁺

Synthesis of IdA-IS. Given below is a small scale (milligrams amount) synthesis of IdA-IS (Supplementary Data Fig. 7 for a synthetic scheme).

(*N*-[3'-(*tert*-butoxycarbonylamino)-propyl]) 7-acetoxycoumarin-4-acetamide (**10**).

A solution of **5** (302 mg, 1.15 mmol, 1 eq) in 20 mL of anhydrous tetrahydrofuran was

cooled to 0 °C. *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (243 mg, 1.27 mmol, 1.1 eq) and 1-hydroxybenzotriazole (194 mg, 1.27 mmol, 1.1 eq) were added, and the suspension was stirred for 30 min at 0 °C. A solution of *N*-Boc-1,3-diaminopropane (201 μ L, 1.15 mmol, 1 eq) in 1 mL of anhydrous *N,N*-dimethylformamide was slowly added to the suspension. The reaction mixture was allowed to warm to room temperature and stirred for 6 h. The reaction mixture was concentrated under vacuum. The residue was taken up in 200 mL of ethyl acetate and washed with 60 mL of 1 M HCl, 60 mL of water and 60 mL of brine. The organic layer was dried over MgSO₄ and concentrated under vacuum. Column chromatography on silica gel (CH₂Cl₂, then CH₂Cl₂:MeOH, 98:2 to 97:3) afforded product **10** (296 mg, 61 %): *R_f* 0.6 (CH₂Cl₂:MeOH, 95:5); ¹H NMR (300 MHz, CD₃OD): δ 7.78 (d, 1 H, *J*_{5,6} 8.7 Hz, H-5), 7.21 (d, 1 H, *J*_{6,8} 2.3 Hz, H-8), 7.15 (dd, 1 H, *J*_{6,8} 2.3, *J*_{5,6} 8.7 Hz, H-6), 6.41 (s, 1 H, H-3), 3.79 (s, 2 H, CH₂CONH), 3.22 (t, 2 H, *J* 6.8 Hz, CH₂NHCO), 3.02 (t, 2 H, *J* 6.8 Hz, CH₂NHCO), 2.31 (s, 3 H, OAc), 1.63 (m, 2 H, CH₂-CH₂-CH₂), 1.41 (s, 9 H, CMe₃); ¹³C NMR (75 MHz, *d*₆-DMSO): δ 168.7, 167.3, 159.5, 152.8, 150.5, 126.4, 118.4, 116.9, 115.5, 110.2, 77.4, 38.8, 37.5, 36.5, 29.4, 28.2, 20.8; ESI-MS: *m/z* 419 [M+H]⁺

(*N*-[3'-(*tert*-butoxycarbonylamino)-propyl]) 7-hydroxycoumarin-4-acetamide (**IdA-IS**). Compound **10** (160 mg, 0.38 mmol, 1 eq) was dissolved in 21 mL of methanol/dichloromethane (6:1), and the solution was cooled to 0 °C. A solution of 0.5 M sodium methoxide in methanol (383 μ L, 0.19 mmol, 0.5 eq) was added dropwise. After stirring for 30 min at 0 °C, the reaction mixture was allowed to warm to room temperature and stirred for 18 h. The reaction mixture was then neutralized with

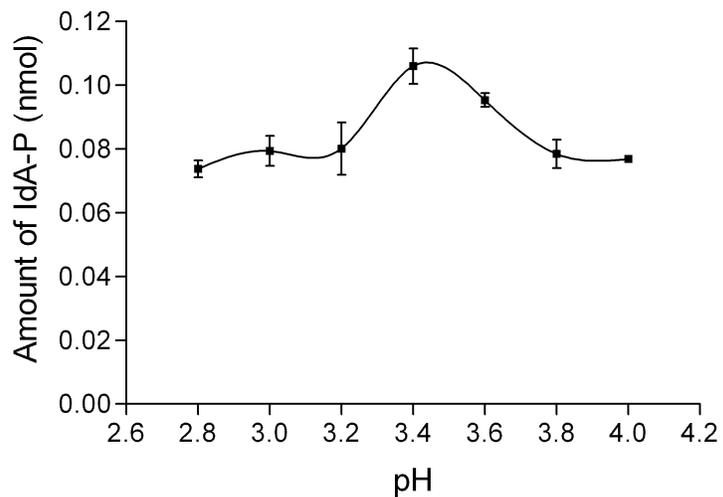
Amberlite IR-120 (H⁺) and filtered. The filtrate was concentrated under vacuum. Purification by column chromatography on silica gel (CH₂Cl₂, then CH₂Cl₂:MeOH, 95:5 to 90:10) afforded product **IdA-IS** (132 mg, 92 %): *R_f* 0.24 (CH₂Cl₂:MeOH, 95:5); ¹H NMR (300 MHz, *d*₆-DMSO): δ 10.57 (bs, 1 H, OH), 8.17 (t, 1 H, *J* 5.4 Hz, NH), 7.62 (d, 1 H, *J*_{5,6} 8.7 Hz, H-5), 6.82 (dd, 1 H, *J*_{6,8} 2.3, *J*_{5,6} 8.7 Hz, H-6), 6.79 (m, 1 H, NH), 6.75 (d, 1 H, *J*_{6,8} 2.5 Hz, H-8), 6.19 (s, 1 H, H-3), 3.66 (s, 2 H, CH₂CONH), 3.08 (m, 2 H, CH₂NHCO), 2.94 (m, 2 H, CH₂NHCO), 1.54 (m, 2 H, CH₂-CH₂-CH₂), 1.40 (s, 9 H, CMe₃); ¹³C NMR (75 MHz, *d*₆-DMSO): δ 167.5, 161.1, 160.2, 155.0, 151.1, 126.6, 112.9, 111.8, 111.5, 102.3, 77.5, 37.5, 36.6, 29.5, 28.2; ESI-MS: *m/z* 377 [M+H]⁺

Supplementary Data Table 1. IdA activities for individual DBS

IdA activity ($\mu\text{mol/h/L}$ blood)			
MPS I patients	MPS I carriers	Normal newborns	
-0.05	5.66	14.4	11.5
-0.15	2.55	11.2	11.1
-0.04	2.35	23.4	18.0
0.268	1.45	7.8	7.4
-0.67	2.60	7.4	10.6

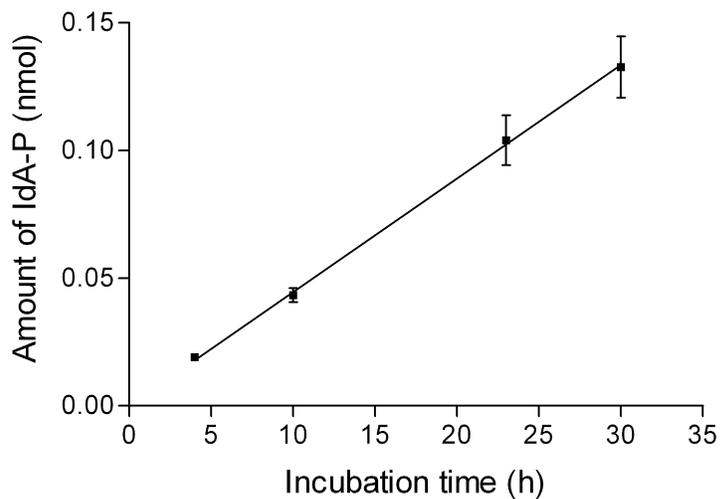
The blank value [mean (SD), 2.67 (0.26) $\mu\text{mol/h/L}$ blood; calculated from 15 independent assays], obtained from an identical assay in which the blood extract and the substrate in buffer pH 3.4 were incubated separately (see main text), was subtracted to give the values.

Supplementary Data Figure 1



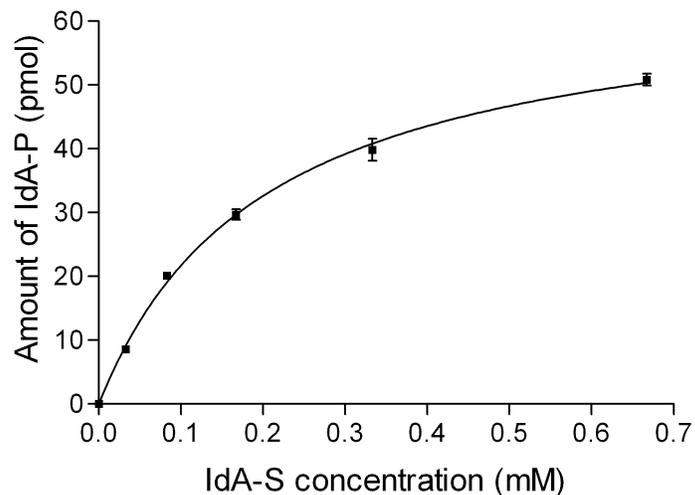
Amount of IdA-generated product measured in DBS as a function of the pH of the enzymatic reaction during incubation. Reactions were carried out at 37 °C for 20 h using the standard assay given in the main text. Error bars are shown for triplicate analyses.

Supplementary Data Figure 2



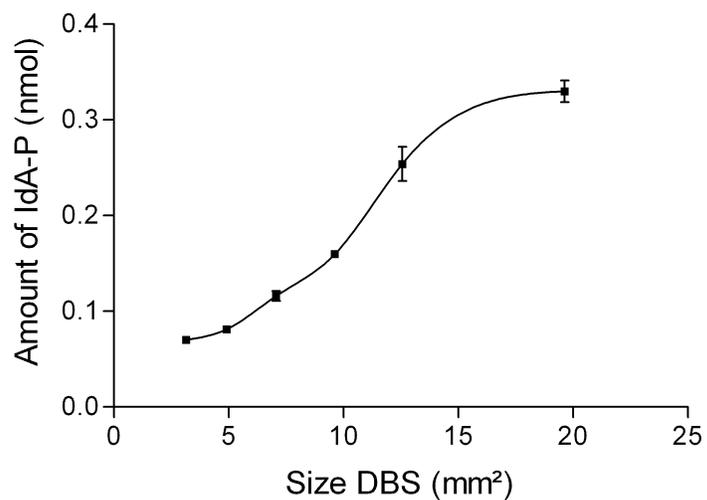
Amount of IdA-generated product measured in DBS as a function of the incubation time. Reactions were carried out at 37 °C using 0.5 mmol/L IdA-S and the conditions given in the main text. Error bars are shown for triplicate analyses. The solid line shows the linear regression fit of the data.

Supplementary Data Figure 3



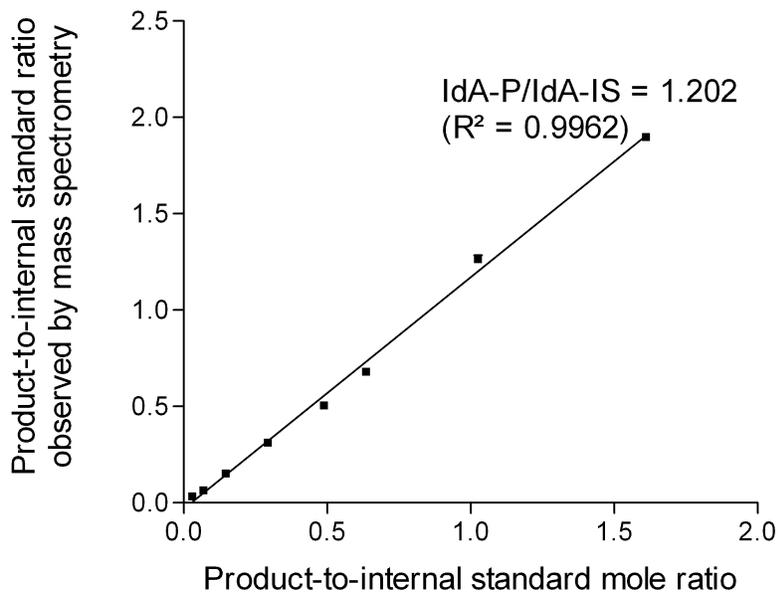
Amount of IdA-generated product measured in DBS as a function of the concentration of IdA-S. Reactions were carried out at 37 °C for 20 h using the standard assay given in the main text. Error bars are shown for triplicate analyses. The solid line shows the regression fit of the data to the Michaelis-Menten equation.

Supplementary Data Figure 4



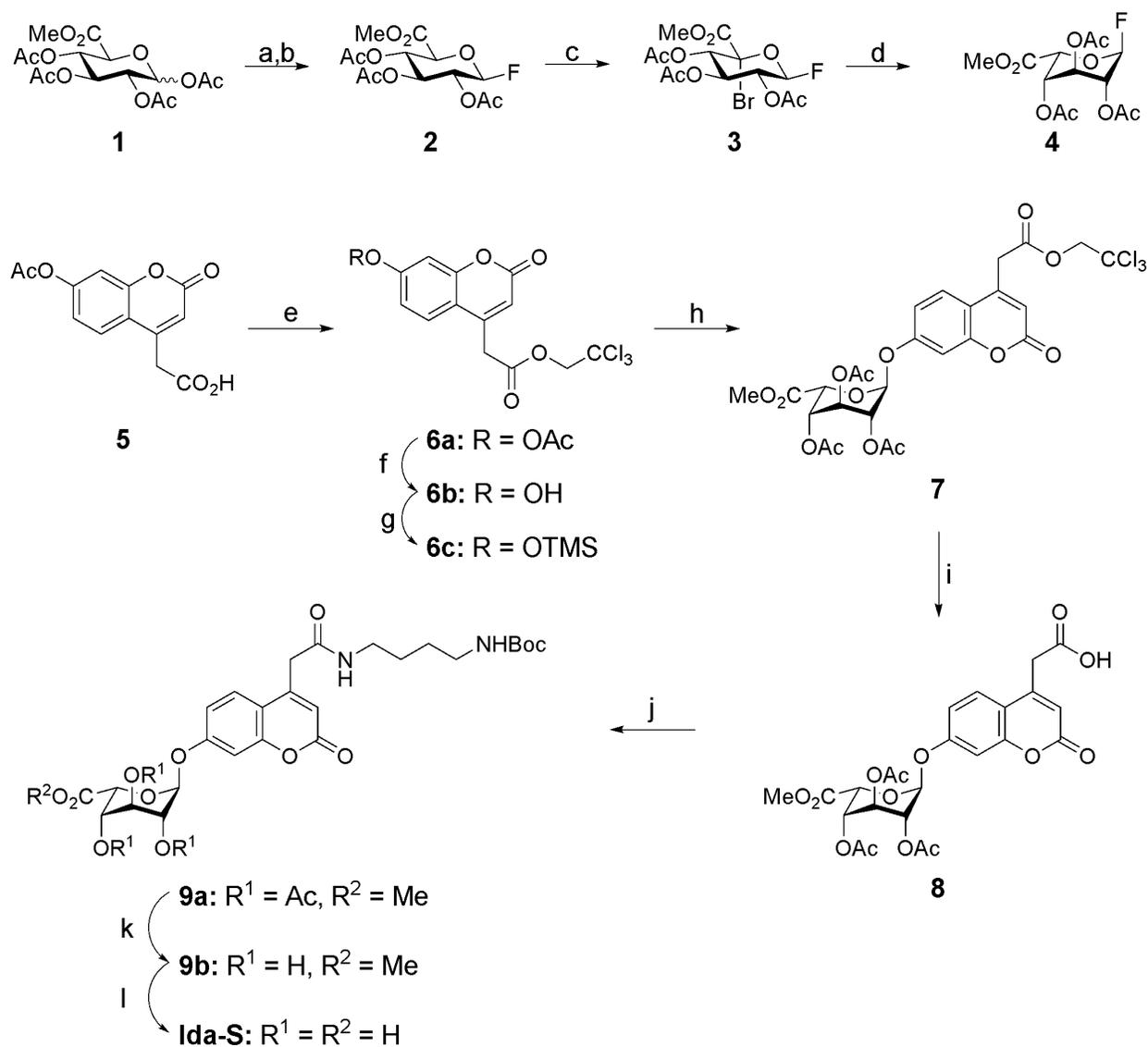
Amount of IdA-generated product measured in DBS as a function of the size of the DBS punch. Reactions were carried out at 37 °C for 20 h using the standard assay given in the main text. Error bars are shown for triplicate analyses.

Supplementary Data Figure 5



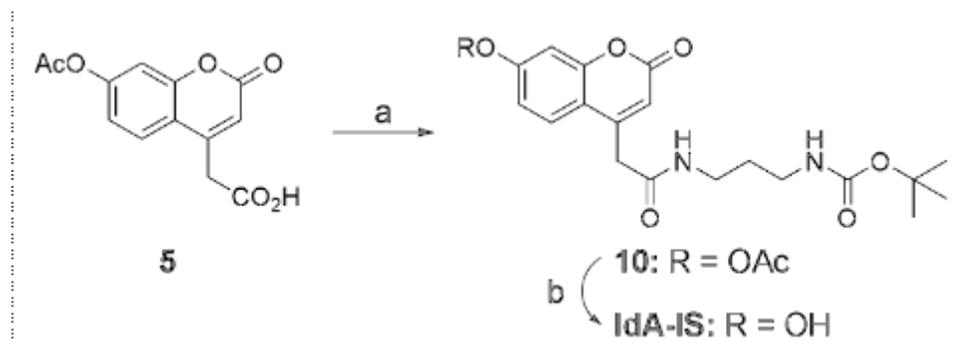
IdA-P/IdA-IS ion ratio observed by ESI-MSMS as a function of the relative amount of IdA-P and IdA-IS added to the IdA assay. Samples contained all assay components except substrate, a fixed amount of IdA-IS (0.2 nmol) and various amounts of IdA-P. Error bars are shown for triplicate analyses. The solid line shows the linear regression fit of the data.

Supplementary Data Figure 6



Synthesis of IdA-S. Reagents and conditions: (a) HBr/AcOH; (b) AgF, MeCN, 74 % (2 steps); (c) NBS, CCl₄, hv, reflux, 77 %; (d) Bu₃SnH, benzene, reflux, 65 %; (e) HOCH₂CCl₃, DCC, CH₂Cl₂, 96 %; (f) 2 M NH₃ in 2-propanol, THF, 78 %; (g) (Me₃Si)₂NH, LiClO₄/SiO₂, CH₂Cl₂; (h) *i.* **4**, BF₃.OEt₂, CH₂Cl₂; *ii.* BF₃.OEt₂, Ac₂O, 88 % (2 steps); (i) Zn dust, CuCl₂, 90 % aq. AcOH, THF, 0 °C, 95 %; (j) H₂N(CH₂)₄NHBoc, EDC, HOBT, THF, 65 %; (k) NaOMe, MeOH, 86 %; (l) NaOH, MeOH/H₂O (1:1), quant.

Supplementary Data Figure 7



Synthesis of internal standard IdA-IS. Reagents and conditions: (a) $\text{H}_2\text{N}(\text{CH}_2)_3\text{NHBoc}$, EDC, HOBT, THF, 61 %; (b) NaOMe, MeOH/ CH_2Cl_2 (6:1), 92 %.