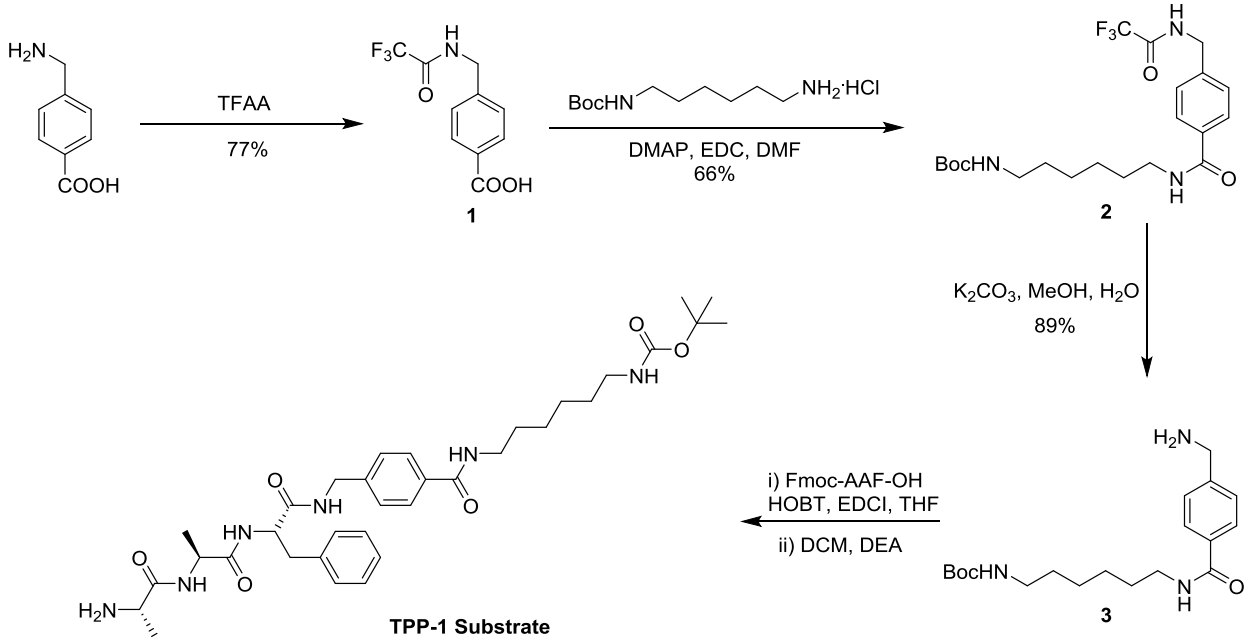
Supplementary Information

Contents

1. Synthetic Procedures

1.1. TPP1 Substrate and Internal Standard



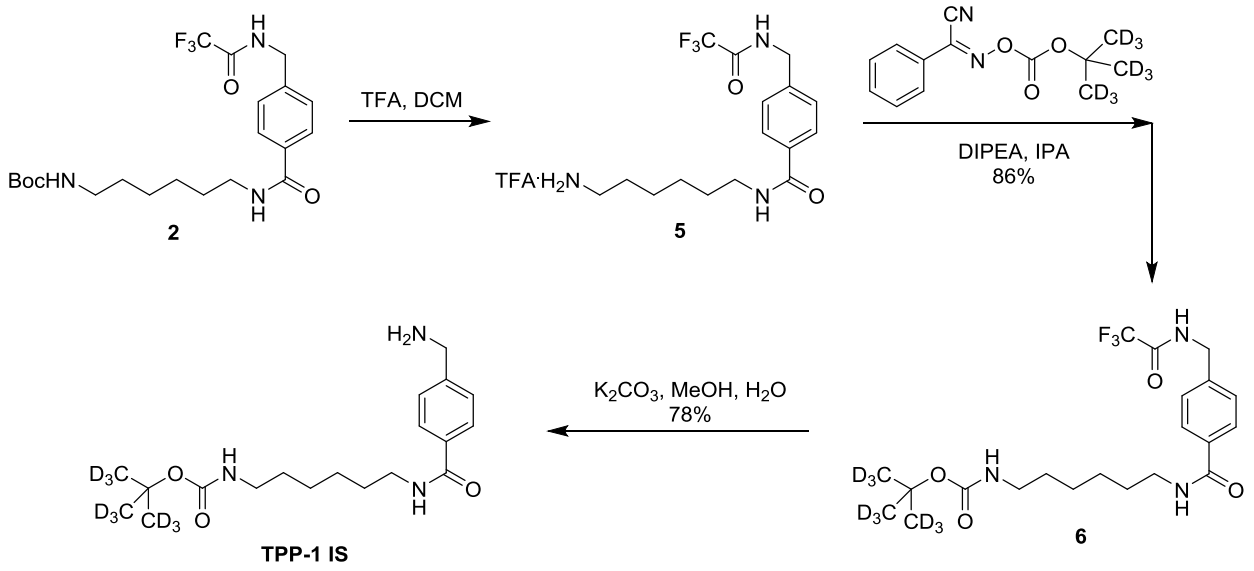
4-((2,2,2-trifluoroacetamido)methyl)benzoic acid (1).
Under nitrogen atmosphere, trifluoroacetic anhydride (2.98 mL, 21.1 mmol) was added dropwise to the solid 4-(aminomethyl)benzoic acid (1.26 g, 8.37 mmol) at 4°C and this neat mixture was let to stir at room temperature for 2 hours. To the reaction mixture crushed ice was added and the resultant precipitate was filtered and washed with cold water. The residue was dried under high vacuum to get **1** (1.62 g, 78%) as white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 8.2 Hz, 2H), 4.52 (s, 2H). MS (ESI^-) for $[M - H]^-$; calculated: 246.0, found: 246.6.

***t*-butyl (6-(4-((2,2,2-trifluoroacetamido)methyl)benzamido)hexyl)carbamate (2).**
To a solution of **1** (500 mg, 2.02 mmol) in DMF (6 mL), DMAP (246 mg, 1.99 mmol), EDC·HCl (581 mg, 3.03 mmol) and *N*-Boc-1,6-hexanediamine hydrochloride (767 mg, 3.04 mmol) were added and left to stir for 6 hours at room temperature. The reaction was then diluted with dichloromethane (DCM) and washed with 1N aqueous HCl solution. The organic layer was then

washed with 1:1 (water/brine) mixture and the then dried with anhydrous sodium sulfate. The resultant organic layer was concentrated to dryness under reduced pressure and subjected to flash silica column chromatography with 3% methanol in DCM as the eluent. The fractions with the desired compound were concentrated under reduced pressure to get compound **2** (590 mg, 66%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73 (d, *J* = 8.2 Hz, 2H), 7.32 (d, *J* = 8.2 Hz, 2H), 4.49 (s, 2H), 3.39 (t, *J* = 7.0 Hz, 2H), 3.07 (t, *J* = 6.9 Hz, 2H), 1.59 (q, *J* = 7.1 Hz, 2H), 1.49 – 1.43 (m, 2H), 1.41 (s, 9H), 1.39 – 1.30 (m, 4H). MS (ESI^+) for $[M+H]^+$; calculated: 446.2, found: 446.4.

***t*-butyl(6-(4-(((S)-2-((S)-2-((S)-2-aminopropanamido)propanamido)-3-phenylpropanamido)methyl)benzamido)hexyl)carbamate (TPP-1 Substrate).**

To a solution of **2** (386 mg, 0.867 mmol) in methanol (8 mL) and water (1 mL), potassium carbonate (1.00g, 7.23 mmol) was added and let to stir at room temperature for 16 hours. The reaction mixture was diluted with methanol (10 mL) and filtered, the filtrate was concentrated to dryness under reduced pressure. The residue was then subjected to flash silica column chromatography with 1% aqueous NH_4OH, 10% methanol in DCM as the eluent. After concentrating the fractions with the desired compound, the residue was dissolved in THF (20 mL) and to this solution (((9*H*-fluoren-9-yl)methoxy)carbonyl)-*L*-alanyl-*L*-alanyl-*L*-phenylalanine (purchased from Lifetein) (429 mg, 0.810 mmol), hydroxybenzotriazole (161 mg, 1.19 mmol) and EDC·HCl (202 mg, 1.05 mmol) were added at 0°C and warmed to room temperature and let to stir for 16 hours. The reaction was then concentrated and residue was extracted between aq. 1N HCl and 20% DMF in $CHCl_3$ solution, the organic layer was dried with anhydrous sodium sulfate and concentrated to dryness under reduced pressure. The resulting crude residue was purified by flash silica column chromatography with 1% DMF, 5% methanol in DCM as the eluent. After concentrating the fractions with the desired compound, the resulting residue was dissolved in DCM (8 mL). To this solution diethylamine (8 mL) was added dropwise and left to stir for 16 hours at room temperature. The reaction was concentrated to dryness and subjected to flash silica column chromatography with 1% aqueous NH_4OH, 2% DMF and 10% methanol in DCM as the eluent. The fractions with the desired compound were concentrated under reduced pressure to get **TPP-1 Substrate** (160 mg, 29%). ^{1}H NMR (300 MHz, MeOD) δ 7.72 (d, *J* = 8.2 Hz, 2H), 7.32 – 7.12 (m, 7H), 4.59 (t, *J* = 7.4 Hz, 1H), 4.43 – 4.22 (m, 3H), 3.44 – 3.34 (m, 2H), 3.16 – 2.96 (m, 5H), 2.86 (s, 1H), 1.69 – 1.55 (m, 2H), 1.53 – 1.35 (m, 13H), 1.29 (d, *J* = 7.2 Hz, 3H), 1.21 (d, *J* = 6.9 Hz, 3H). MS (ESI^+) for $[M+H]^+$; calculated: 639.4, found: 639.7.



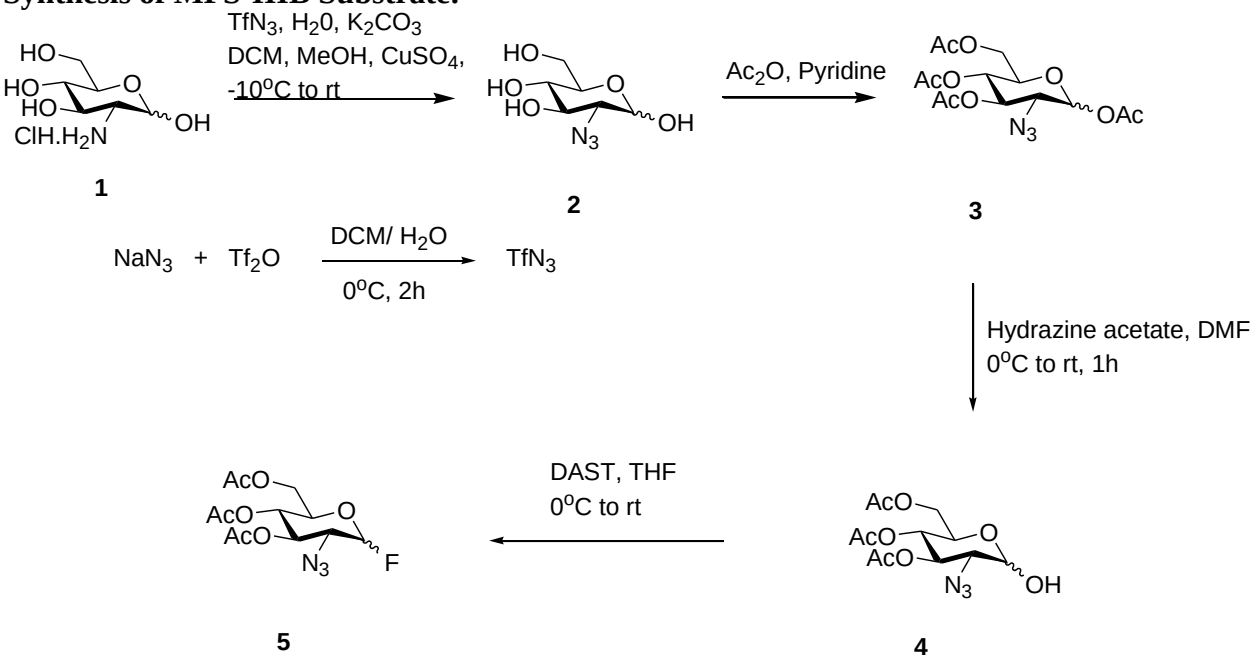
2-(methyl-d_3)propan-2-yl-1,1,1,3,3,3-d_6 (6-(4-((2,2,2-trifluoroacetamido)-methyl)benzamido) hexyl)carbamate (6).

To a solution of **2** (281 mg, 0.631 mmol) in DCM (7 mL), trifluoroacetic acid (7 mL) was added and left to stir at room temperature for 16 hours. the reaction mixture was concentrated to dryness under reduced pressure and further dried under high vacuum. The resulting residue was dissolved in isopropyl alcohol (5 mL), to this solution *N*,*N*-diisopropylethylamine (300 µL, 1.72 mmol) and Boc-ON-(*tert*-butyl-d_9) (purchased from Aldrich) (134 mg, 0.525 mmol) were added and left to stir for 3 hours at room temperature. The reaction was then diluted with dichloromethane (DCM) and washed with 1N aqueous HCl solution. The organic layer was then washed with 1:1 (water/brine) mixture and the then dried with anhydrous sodium sulfate. The resultant organic layer was concentrated to dryness under reduced pressure and subjected to flash silica column chromatography with 3% methanol in DCM as the eluent. The fractions with the desired compound were concentrated under reduced pressure to get compound **6** (205 mg, 86%). ^{1}H NMR (500 MHz, MeOD) δ 7.82 (d, *J* = 8.2 Hz, 2H), 7.42 (d, *J* = 8.2 Hz, 2H), 4.54 (s, 2H), 3.39 (t, *J* = 7.1 Hz, 2H), 3.06 (t, *J* = 6.8 Hz, 2H), 1.65 (dd, *J* = 14.2, 7.1 Hz, 2H), 1.50 (dd, *J* = 13.6, 6.8 Hz, 2H), 1.47 – 1.36 (m, 4H). MS (ESI$^+$) for $[M + H]^+$; calculated: 455.3, found: 455.3.

2-(methyl-d_3)propan-2-yl-1,1,1,3,3,3-d_6 (6-(4-(aminomethyl)benzamido)hexyl)carbamate (TPP-1 IS). To a solution of **6** (104 mg, 0.228 mmol) in methanol (4 mL) and water (1 mL), potassium carbonate (500 mg, 3.61 mmol) was added and let to stir at room temperature for 16 hours. The reaction mixture was diluted with methanol (5 mL) and filtered, the filtrate was concentrated to dryness under reduced pressure. The residue was then subjected to flash silica column chromatography with 1% aqueous NH_4OH, 10% methanol in DCM as the eluent. The fractions with the desired compound were concentrated under reduced pressure to get compound **TPP-1 IS** (64 mg, 78%). ^{1}H NMR (500 MHz, MeOD) δ 7.81 (d, *J* = 8.2 Hz, 2H), 7.45 (d, *J* = 8.2 Hz, 2H), 3.87 (s, 2H), 3.39 (t, *J* = 7.1 Hz, 2H), 3.06 (t, *J* = 7.0 Hz, 2H), 1.69 – 1.61 (m, 2H), 1.55 – 1.47 (m, 2H), 1.47 – 1.35 (m, 4H). MS (ESI$^+$) for $[M + H]^+$; calculated: 359.3, found: 359.3.

1.2. NAGLU (MPS IIIB) Substrate and Internal Standard

Synthesis of MPS-IIIB Substrate:



1, 3, 4, 6-Tetra-*O*-acetyl-2-azido-2-deoxy-α/β-D-glucopyranoside (3).

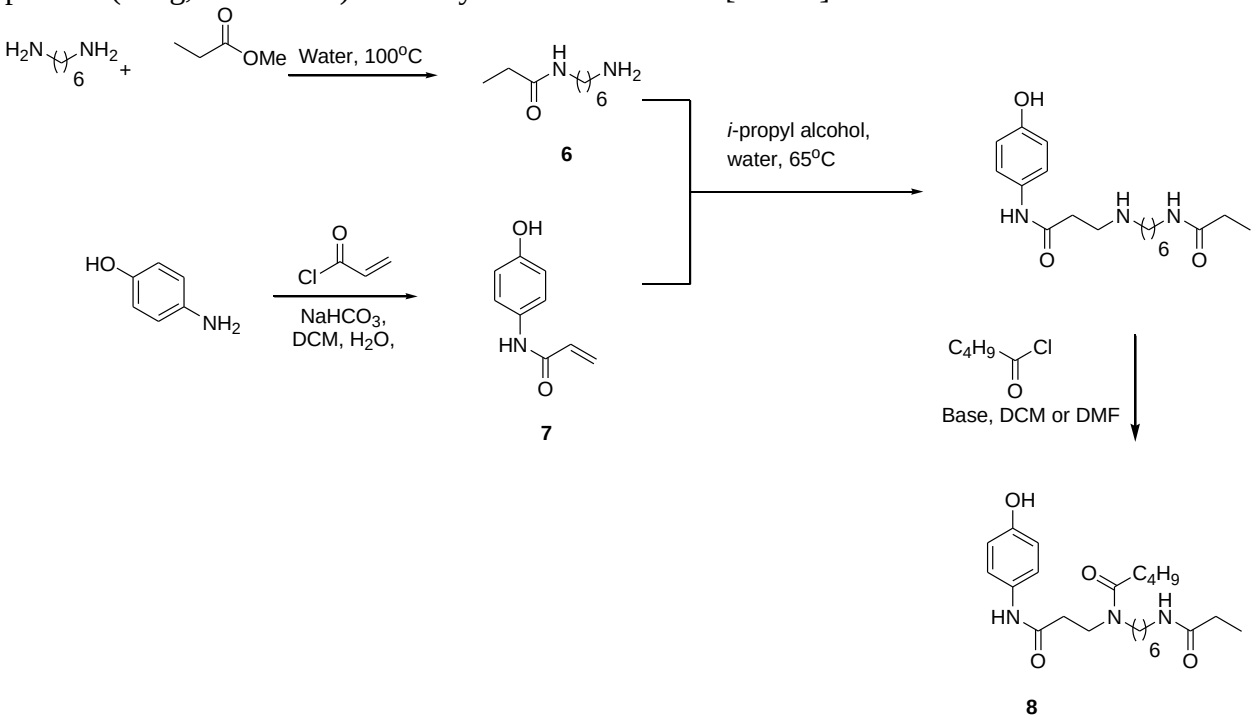
Preparation of TfN_3: To a solution of NaN_3 (23 g, 354 mmol) in H_2O (60 mL) was added 100 mL of DCM at 0 °C. The resulting biphasic mixture was stirred vigorously and treated with Tf_2O (12 mL, 70 mmol) over a period of about 10 min. The reaction mixture was stirred at ice bath temperature for 2 h. The organic phase was separated and the aqueous phase was extracted with DCM (50 mL x 2). The organic layer was washed with saturated $NaHCO_3$ solution and used for next step without further purification. (Caution: TfN_3 is explosive when not in organic solvent).

Glucosamine hydrochloride (7.64 g, 35.4 mmol) was dissolved in H_2O (20 mL) and treated with potassium carbonate (7.34 g, 276.4 mmol) and $CuSO_4$ hydrate (88 mg, 0.35 mmol). To the reaction mixture was added the above TfN_3 solution. Then, MeOH (200 mL) was added until the solution was homogeneous. The reaction was allowed to stir for 18 h at room temperature and the solvent was removed under reduced pressure. The residue was dissolved in pyridine (150 mL) then added catalytic amounts of DMAP and acetic anhydride (50 mL, 45.3 mmol) at 0°C and reaction mixture was stirred for 6 h at room temperature. After completion of reaction, MeOH (50 mL) was added and reaction mixture was stirred for 10 min. The solvent was evaporated at strictly below 40°C. The resulted residue was diluted with EtOAc (250 mL) and washed with water, 10% $CuSO_4$ in H_2O, saturated $NaHCO_3$ and saturated NaCl. The organic layer was dried over $NaSO_4$, filtered and concentrated by rotary evaporation. The residue was purified by silica gel column chromatography (1-15% EtOAC in Hexane) to afford product **3** (mixture of α/β) (9.6 g, 25.7 mmol) in 72.5 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) (β isomer): δ 5.54 (d, *J* = 8.6 Hz, 1H), 5.14 – 4.97 (m, 2H), 4.34 – 4.23 (m, 1H), 4.06 (d, *J* = 11.9 Hz, 1H), 3.79 (dd, *J* = 7.5, 2.0 Hz, 1H), 3.65 (t, *J* = 8.3 Hz, 1H) 2.18 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 1.97 (s, 3H). MS *m/z* 396.6 $[M+Na]^+$.

3, 4, 6-Tri-*O*-acetyl-2-azido-2-deoxy- α/β -D-glucopyranoside (4). To a solution of compound **3** (9.2 g, 24.6 mmol) in dry DMF (90 mL) under nitrogen atmosphere was added hydrazine acetate (3.7 g, 49.2 mmol) at 0°C. The reaction mixture was stirred for 45 min at room temperature. Then reaction was diluted with ethyl acetate and washed with saturated sodium bicarbonate, water and

brine. Afterwards, the mixture was dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (20-40% EtOAC in Hexane) to give product **4** (7.1 g, 21.4 mmol) in 87.1 % yield.

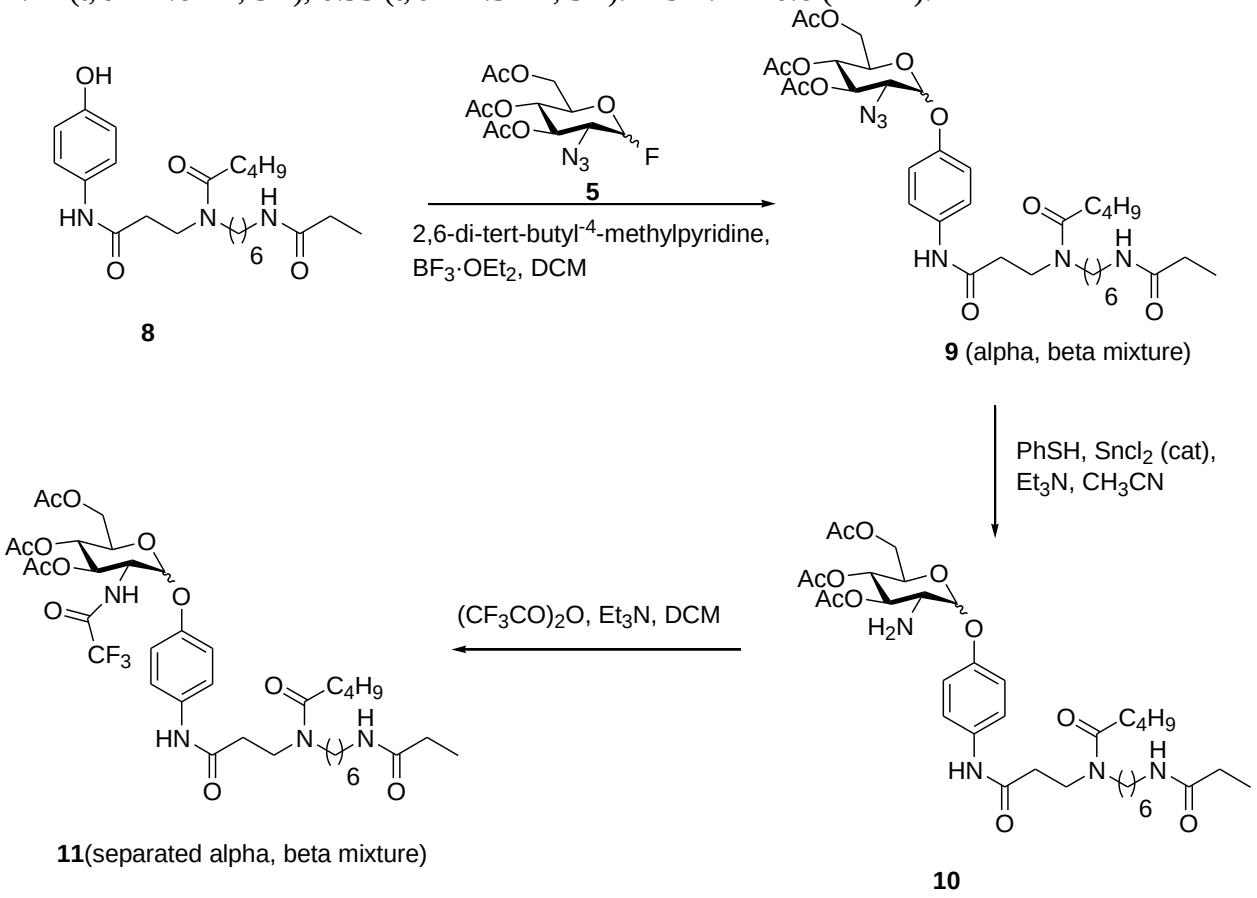
3, 4, 6-Tri-*O*-acetyl-2-azido-2-deoxy-1-fluoro- α/β -D-glucopyranoside (5). Compound **4** (5.3 g, 15.9 mmol) was dissolved in dry THF (30 mL) and Diethylaminosulfur trifluoride (DAST) (3.15 mL, 23.8 mmol) was added to the stirred solution at -30 ^{o}C under nitrogen atmosphere. After addition of DAST, the ice bath was removed and the solution was allowed to room temperature and stirred for overnight. The reaction was quenched with MeOH (10 mL) and concentrated under reduced pressure. Column chromatography on silica gel (10-30% EtOAC in Hexane) afforded product (4.1 g, 12.3 mmol) in 77% yield. MS *m/z* 356.1 $[M+Na]^{+}$.



N-(6-Amino-hexyl)-propionamide(6). To methyl propionate (11.8 mL, 122.4 mmol), hexane-1,6-diamine (14.2 g, 122.4 mmol) and water (6.2 mL) were added and the mixture was heated to 100°C for 24 hours under constant stirring. The reaction mixture was cooled to room temperature and directly loaded on to a short silica column. Upon elution with 10 to 20% of methanol (with 10% NH_4OH) in CH_2Cl_2 the desired mono-propionated product **6** was obtained (10.5 g, 60.9 mmol) in 49.7% yield. ^{1}H NMR (300 MHz, MeOD) δ 3.16 (t, *J* = 6.9 Hz, 2H), 2.63 (t, *J* = 7.0 Hz, 2H), 2.18 (q, *J* = 7.6 Hz, 2H), 1.58 – 1.26 (m, 8H), 1.12 (t, *J* = 7.6 Hz, 3H).

Pentanoic acid [2-(4-hydroxy-phenylcarbamoyl)-ethyl]-(6-propionylamino-hexyl)-amide (8). 4-Acrylamido-phenol **7** (9.46 g, 58 mmol) and mono-propionyl-1,6-hexanediamine **6** (10.5 g, 60.9 mmol) were dissolved in a solution of isopropanol (450 mL) and water (50 mL) and heated in an oil bath at 65^{o}C for 48 hrs. The reaction mixture was concentrated by rotary evaporation to afford the Michael addition product, which was used for the next step without further purification. To the residue from the above step was added CH_2Cl_2 (150 mL), DMF (15 mL) and 150 mL of saturated sodium bicarbonate in water. Pentanoyl chloride (7.96 mL, 67.1 mmol) was added dropwise at room temperature with stirring, and the mixture was stirred for an additional 6 h at room temperature. The organic layer was separated, and the water layer was extracted twice with 150

mL portions of 5% MeOH in CH_2Cl_2. The organic layers were combined and concentrated by rotary evaporation. The residue was purified by silica gel column chromatography (1-15% MeOH in CH_2Cl_2) to afford MPS –IIIB aglycone **8** (18.6 g, 44.3 mmol) in 76.4 % yield. ^{1}H NMR (300 MHz, MeOD) δ 7.31 (d, *J* = 8.9 Hz, 2H), 6.73(m, 2H), 3.76 – 3.61 (m, 2H), 3.36(m, 2H), 3.19 – 3.10 (m, 2H), 2.64 – 2.54 (m, 2H), 2.47 – 2.32(m, 2H), 2.22 – 2.12 (m, 2H), 1.68 – 1.23 (m, 12H), 1.11 (t, *J* = 7.6 Hz, 3H), 0.93 (t, *J* = 7.3 Hz, 3H). MS *m/z* 420.6 ($M+H^+$).



Acetic acid 3-acetoxy-2-acetoxymethyl-5-azido-6-(4-{3-[pentanoyl-(6-propionylamino-hexyl)-amino]-propionylamino}-phenoxy)-tetrahydro-pyran-4-yl ester (9). MPS-IIIB aglycone **8** (5.67 g, 13.5 mmol, 1.1eq), 3, 4, 6-Tri-*O*-acetyl-2-azido-2-deoxy-1-fluoro- α/β -D-glucopyranoside **5** (4.1 g, 12.3 mmol, 1 eq) and 2,6-di-*tert*-butyl-4-methylpyridine (5.05 g, 24.6 mmol, 2 eq) were dried for 1 hr under high vacuum (oil pump) and dissolved in dry CH_2Cl_2 (615 mL, 0.02 M). $BF_3.Et_2O$ (12.4 mL, 98.4 mmol, 8 eq) was added dropwise with stirring at room temperature under a nitrogen atmosphere. After the reaction mixture had been stirred for 2.5 h at room temperature, 350 mL of saturated aqueous $NaHCO_3$ was added. The aqueous layer was extracted with CH_2Cl_2 and the organic extracts were combined and washed with water, brine and dried over anhydrous Na_2SO_4. The solution was filtered and concentrated by rotary evaporation. The residue was purified by silica gel column chromatography (CH_2Cl_2, then 1-10% MeOH in CH_2Cl_2) to afford product **9** (3.5 g, 4.77 mmol) in 39% yield. MS *m/z* 733.5 ($M+H^+$).

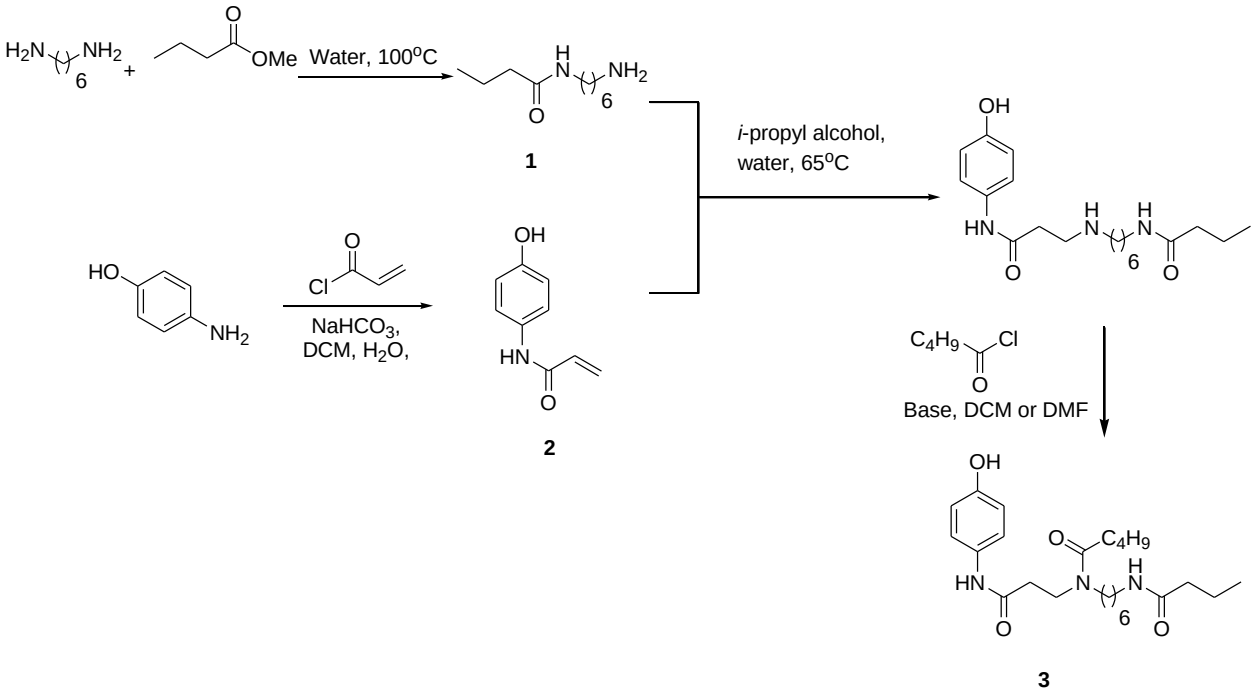
Acetic acid 4-acetoxy-2-acetoxymethyl-5-amino-6-(4-{3-[pentanoyl-(6-propionylamino-hexyl)-amino]-propionylamino}-phenoxy)-tetrahydro-pyran-3-yl ester (10). Compound **9** (3.5

g, 4.77 mmol, 1 eq) was dissolved in dry acetonitrile (24 mL) under nitrogen atmosphere and was cooled in an ice bath. To this solution was added a ice-cold solution of tin(II) chloride (181 mg, 0.95 mmol, 0.20 eq), triethylamine (2 mL, 14.3 mmol, 3eq) and thiophenol (1.95 mL, 19 mmol, 4 eq) in 15 mL of dry acetonitrile. The solution was stirred at 0°C for 2 hours and concentrated by rotary evaporation, diluted with dichloromethane then washed with cold 1N NaOH, water and brine. The organic layer was separated and concentrated by rotary evaporation, and the crude product was used for the next step without further purification.

Acetic acid 4-acetoxy-2-acetoxymethyl-6-(4-{3-[pentanoyl-(6-propionylamino-hexyl)-amino]-propionylamino}-phenoxy)-5-(2,2,2-trifluoro-acetylamino)-tetrahydro-pyran-3-yl ester (11). The crude product **10** was dried under high vacuum and dissolved in dry of dichloromethane (20 mL) and pyridine (3.84 mL, 47.7 mmol, 10 eq) was first added, followed by dropwise addition of trifluoroacetic anhydride (1 mL, 7.15 mmol, 1.5 eq). the resulted mixture was stirred for 4h at room temperature under nitrogen atmosphere. The solvent was removed by rotary evaporation, and the residue was purified using flash chromatography with 1-5% MeOH in DCM to give product **11** (α:β:1:1) (2.5 g, 3.1 mmol) in 65% yield. ^{1}H NMR (300 MHz, $CDCl_3$) (α isomer): δ 7.54 (d, *J* = 9.0 Hz, 2H), 7.02 (d, *J* = 9.0 Hz, 2H), 5.57 (d, *J* = 3.5 Hz, 1H), 5.49(t, *J* = 9.6 Hz, 1H), 5.22 (t, *J* = 9.7 Hz, 1H), 4.52 – 4.41 (m, 1H), 4.26 (dd, *J* = 12.1, 4.4 Hz, 1H), 4.16 – 4.02 (m, 2H), 3.68 (t, J = 6.4 Hz, 3H), 3.32 – 3.19 (m, 4H), 2.66 (t, *J* = 6.4 Hz, 2H), 2.34 – 2.27 (m, 2H), 2.24 – 2.14 (m, 2H), 2.10 – 2.01 (m, 9H), 1.68 – 1.28 (m, 12H), 1.15 (t, *J* = 7.6 Hz, 3H), 0.90 (t, *J* = 7.3 Hz, 3H). MS *m/z* 825.6 $[M+Na]^+$.

1.3. GUSB (MPS VII) Substrate and Internal Standard

Synthesis of MPS-VII Substrate:

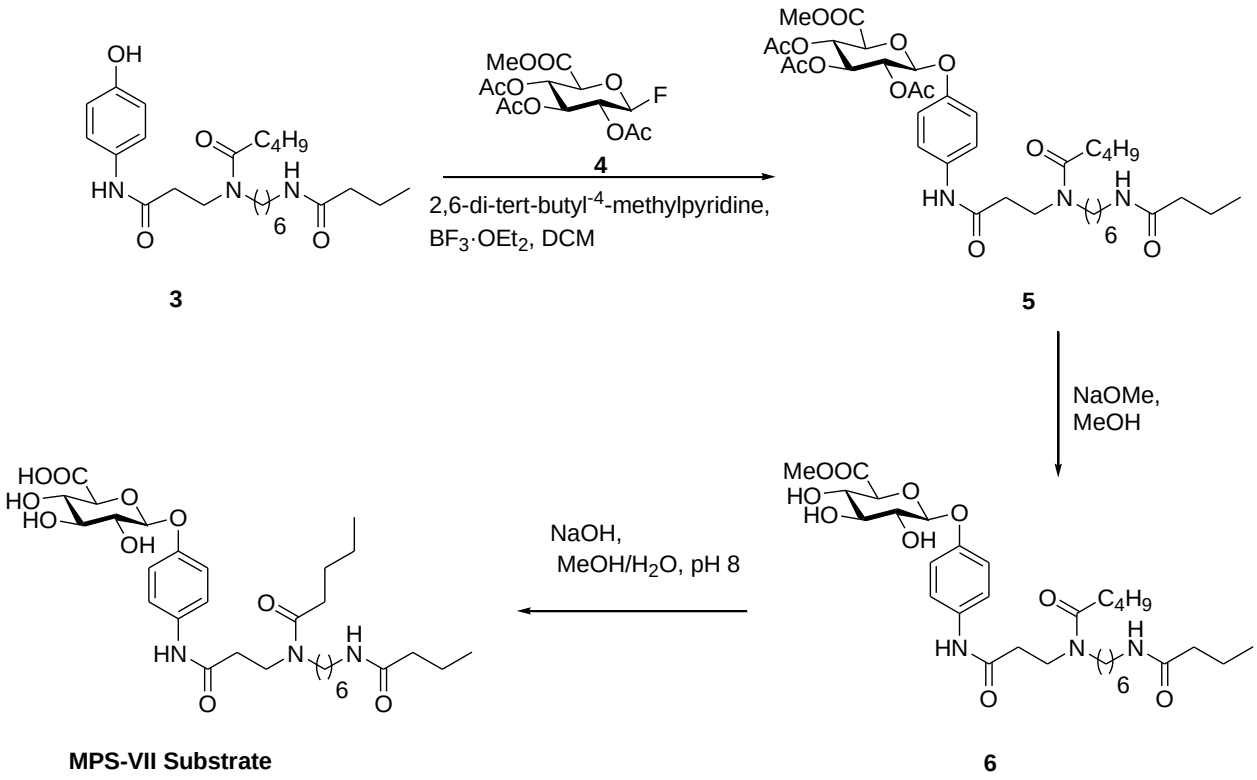


N-(6-Amino-hexyl)-butyramide (1). To methyl butanoate (6.9 mL, 61.2 mmol), hexane-1,6-diamine (7.1 g, 61.2 mmol) and water (3.1 mL) were added and the mixture was heated to 100°C

for 24 hours under constant stirring. The reaction mixture was cooled to room temperature and directly loaded on to a short silica column. Upon elution with 10 to 20% of methanol (with 10% NH_4OH) in CH_2Cl_2 the desired mono-butanoylated product was obtained (4.7 g, 25.1 mmol) in 41% yield. ^{1}H NMR (300 MHz, MeOD) δ 3.16 (t, *J* = 7.2 Hz, 2H), 2.65 (t, *J* = 7.2 Hz, 2H), 2.14 (t, *J* = 7.2 Hz, 2H), 1.67 – 1.47 (m, 6H), 1.39 – 1.33 (m, 4H), 0.94 (t, *J* = 7.8 Hz, 3H). MS *m/z* ($M+H^+$).

N-(4-hydroxyphenyl)acrylamide (2). A solution of 4-aminophenol (50 g, 458 mmole) in CH_2Cl_2 (400 mL) and saturated $NaHCO_3$ in water (400 mL) was stirred for 10 min at room temperature, then acryloyl chloride (40.9 mL, 503.8 mmole) was added dropwise and the reaction stirred for an additional 6 hr at room temperature. The resulting solid was collected by filtration, washed with water and dried under vacuum (oil pump) to afford 75 g of 4-acrylamido-phenol. ^{1}H NMR (300 MHz, MeOD) δ 7.43 (dd, *J* = 6.8, 2.2 Hz, 2H), 6.77 (dd, *J* = 6.8, 2.2 Hz, 2H), 6.48 – 6.27 (m, 2H), 5.74 (dd, *J* = 9.5, 2.5 Hz, 1H).

Pentanoic acid (6-butyrylamino-hexyl)-[2-(4-hydroxy-phenylcarbamoyl)-ethyl]-amide (3). 4-Acrylamido-phenol (4.08 g, 25.1 mmol) and mono-Butanoyl-1,6-hexanediamine (4.7 g, 25.1 mmol) were dissolved in a solution of isopropanol (180 mL) and water (20 mL) and heated in an oil bath at 65ºC for 48 hrs. The reaction mixture was concentrated by rotary evaporation to afford the Michael addition product, which was used for the next step without further purification. To the residue from the above step was added CH_2Cl_2 (50 mL), DMF (5 mL) and 50 mL of saturated sodium bicarbonate in water. Pentanoyl chloride (3.28 mL, 27.6 mmol) was added dropwise at room temperature with stirring, and the mixture was stirred for an additional 6 h at room temperature. The organic layer was separated, and the water layer was extracted twice with 50 mL portions of 5% MeOH in CH_2Cl_2. The organic layers were combined and concentrated by rotary evaporation. The residue was purified by silica gel column chromatography (1-5% MeOH in CH_2Cl_2) to afford MPS –VII aglycone (5.88 g, 13.6 mmol) in 54.1 % yield. ^{1}H NMR (300 MHz, MeOD) δ 7.31 (d, *J* = 9.0 Hz, 2H), 6.75 – 6.71 (m, 2H), 3.72 – 3.61 (m, 2H), 3.40 – 3.34 (m, 2H), 3.17 – 3.12 (m, 2H), 2.63 – 2.55 (m, 2H), 2.46 – 2.32 (m, 2H), 2.14 (t, *J* = 9.0 Hz, 2H), 1.64 – 1.31 (m, 14H), 0.95 – 0.89 (m, 6H). MS *m/z* 434.6 ($M+H^+$).

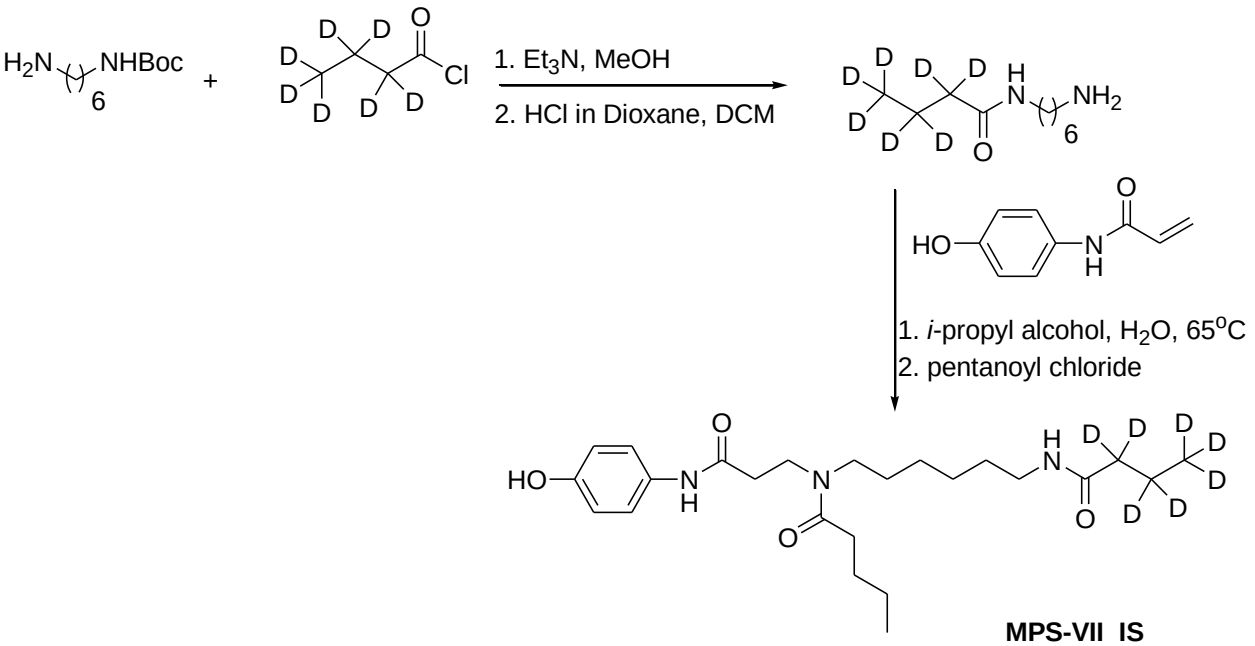


3,4,5-Triacetoxy-6-(4-{3-[(6-butyrylamino-hexyl)-pentanoyl-amino]-propionylamino}-phenoxy)-tetrahydro-pyran-2-carboxylic acid methyl ester (5). MPS-VII aglycone **3** (5.17 g, 11.9 mmol, 1eq), methyl (2,3,4-triacetoxy-beta-glucopyranosyl fluoride) uronate **4** (5.2 g, 15.5 mmol, 1.3 eq) and 2,6-di-*tert*-butyl-4-methylpyridine (7.33 g, 35.7 mmol, 3 eq) were dried for 1 hr under high vacuum (oil pump) and dissolved in dry CH_2Cl_2 (238 mL, 0.05 M). $BF_3.Et_2O$ (12 mL, 95.2 mmol) was added dropwise with stirring at room temperature under a nitrogen atmosphere. After the reaction mixture had been stirred for 2.5 h at room temperature, 250 mL of saturated aqueous $NaHCO_3$ was added. The aqueous layer was extracted with CH_2Cl_2 and the organic extracts were combined and washed with water, brine and dried over anhydrous Na_2SO_4. The solution was filtered and concentrated by rotary evaporation. The residue was purified by silica gel column chromatography (CH_2Cl_2, then 1-8% MeOH in CH_2Cl_2) to afford product (4.1 g, 5.46 mmol) in 46% yield. ^{1}H NMR (300 MHz, MeOD) δ 7.51 – 7.47 (m, 2H), 7.03 – 6.97 (m, 2H), 5.42 – 5.37 (m, 2H), 5.23 – 5.15 (m, 2H), 4.49 (d, *J* = 8.7 Hz, 1H), 3.76 – 3.64 (m, 5H), 3.41 – 3.36 (m, 2H), 3.21 – 3.12 (m, 2H), 2.65 – 2.61 (m, 2H), 2.48 – 2.35 (m, 2H), 2.16 (t, *J* = 7.2 Hz, 2H), 2.07 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 1.67 – 1.33 (m, 14H), 0.97 – 0.90 (m, 6H). MS *m/z* 750.7 ($M+H^+$).

6-(4-{3-[(6-Butyrylamino-hexyl)-pentanoyl-amino]-propionylamino}-phenoxy)-3,4,5-trihydroxy-tetrahydro-pyran-2-carboxylic acid methyl ester (6). To a solution of coupled product (4.1 g, 5.46 mmol, 1 eq) in 110 mL of dry methanol (Aldrich) was added 0.5 M sodium methoxide in methanol (4.4 mL, 2.18 mmol, 0.4 eq) dropwise at 0°C under a nitrogen atmosphere with stirring. The reaction mixture was stirred at 0°C for 3 h. The reaction mixture was neutralized with formic acid and concentrated by rotary evaporation. Column chromatography on silica gel (1-10% MeOH in CH_2Cl_2) afforded product (2.85 g, 4.57 mmol) in 83.6% yield. ^{1}H NMR (300 MHz, MeOD) δ 7.46 (d, *J* = 8.7 Hz, 2H), 7.06 – 7.02 (m, 2H), 4.03 (d, *J* = 9.6 Hz, 1H), 3.79 (s,

3H), 3.76 – 3.57 (m, 4H), 3.54 – 3.46 (m, 2H), 3.41 – 3.34 (m, 2H), 3.19 – 3.11 (m, 2H), 2.68 – 2.57 (m, 2H), 2.47 – 2.32 (m, 2H), 2.16 (t, *J* = 7.2 Hz, 2H), 1.69 – 1.27 (m, 14H), 0.96 – 0.88 (m, 6H). MS *m/z* 624.9 ($M+H^+$).

6-(4-{3-[(6-Butyrylamino-hexyl)-pentanoyl-amino]-propionylamino}-phenoxy)-3,4,5-trihydroxy-tetrahydro-pyran-2-carboxylic acid (MPS-VII-Substrate). Compound (2.31 g, 3.7 mmol, 1 eq) was dissolved in 180 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 (~1.5 eq NaOH added). The reaction mixture was stirred for overnight and concentrated by rotary evaporation. The residue was purified by column chromatography on silica (1-25% MeOH (with 5% H_2O) in CH_2Cl_2) to give product MPS-VII-S (2 g, 3.28 mmol) in 88% yield. Substrate (sodium salt) (~500 mg) dissolved in DI-water (10 mL) and MeOH (1 mL) was added dropwise to a solution of 5% formic acid in H_2O (100 mL). The resulted solution was loaded on a C18 cartridge by applying negative pressure. The cartridge was further washed with DI water (5x50 mL) and the substrate was eluted from the cartridge using MeOH (5x50 mL). The MeOH fraction was concentrated under reduced pressure to afford the pure substrate. ^{1}H NMR (300 MHz, MeOD) δ 7.49 – 7.42 (m, 2H), 7.12 – 7.04 (m, 2H), 3.81 (d, *J* = 8.7 Hz, 1H), 3.75 – 3.64 (m, 2H), 3.58 – 3.47 (m, 4H), 3.40 – 3.35 (m, 2H), 3.18 – 3.13 (m, 2H), 2.67 – 2.58 (m, 2H), 2.47 – 2.32 (m, 2H), 2.13 (t, *J* = 7.2 Hz, 2H), 1.67 – 1.31 (m, 14H), 0.97 – 0.91 (m, 6H). MS *m/z* 610.7 ($M+H^+$).



MPS-VII IS

Pentanoic acid (6-d_7-butyrylamino-hexyl)-[2-(4-hydroxy-phenylcarbamoyl)-ethyl]-amide (MPS-VII-Internal Standard). To a solution of Boc-1,6-diaminohexane (0.43 g, 2 mmol) and triethylamine (0.83 mL, 6 mmol) in methanol (8 mL), cooled on an ice bath, butanoyl chloride-d_7 (454 mg, 4 mmol) solution in anhydrous dichloromethane (4 mL) was added dropwise and warmed to room temperature. The reaction mixture was stirred for 3 hours and concentrated under reduced pressure. The residue was redissolved in 10% methanol in DCM and the resulting solution was washed with 1M aqueous NaOH solution and followed by brine-water (1:1) mixture. The organic

layer was concentrated to dryness under reduced pressure. The resultant residue was resuspended in dichloromethane (4 mL) and 4M HCl in dioxane (2 mL, 8 mmol) was added to it dropwise and let to stir at room temperature for 16 hours. The reaction mixture was concentrated to dryness under reduced pressure and redissolved in methanol (10 mL) and to it sodium bicarbonate powder (0.84 g) was added and let to stir for 15 minutes. The resultant slurry was filtered and the filtrate was concentrated under reduced pressure to yield mono-d_7-butanoyl-diamine and used for next step without further purification.

The residue was dissolved in 2-propanol (8 mL) and water (2 mL) mixture and to 4-Acrylamido-phenol (0.29 g, 1.82 mmol) was added and let to stir for 24 hours at 65°C. The reaction mixture was concentrated to dryness under reduced pressure and redissolved in CH_2Cl_2 (4 mL), DMF (1 mL) and 4 mL of saturated sodium bicarbonate in water. Pentanoyl chloride (0.26 mL, 2.2 mmol) was added dropwise at room temperature with stirring, and the mixture was stirred for an additional 3 h at room temperature. The layers were allowed to separate, and the CH_2Cl_2 layer was concentrated by rotary evaporation. The resulted residue was submitted to silica gel chromatography with 1-5% MeOH (with 2% formic acid) in CH_2Cl_2 to give 381 mg of pure product (43% overall yield). ^{1}H NMR (300 MHz, MeOD) 7.36 - 7.31 (m, 2H), 6.77 – 6.72 (m, 2H), 3.76 – 3.63 (m, 2H), 3.39 – 3.34 (m, 2H), 3.19 - 3.13 (m, 2H), 2.66 – 2.56 (m, 2H), 2.48 - 2.31 (m, 2H), 1.69 -1.25 (m, 12H), 0.97 – 0.91 (m, 3H). MS *m/z* 441.6 ($M+H^+$).

2. 1. Assay Protocol

Assay buffer: 50 mM ammonium acetate (Sigma, Cat. No. A1542-250G) to be stored at 4 °C in an air-tight vessel to minimize ammonia loss), 5 mM cerium(III)acetate (Sigma, Cat. No. Z374938), pH 5.0.

Assay cocktail: Make assay cocktail by adding stock solutions of substrates, internal standards (IS), and NAG-Thiazoline (TRC Chemicals, Cat. T293625) in methanol to an Eppendorf (polypropylene) tube or glass vial. Remove all solvent with a stream of oil-free nitrogen or in a centrifugal concentrator under vacuum.

Add assay buffer and mix on a vortex mixer well until the solution goes clear. Final assay cocktail has 0.2 mM TPP-1 substrate, 1 mM I2S (MPS-II) substrate, 0.5 mM NAGLU (MPS-IIIB) substrate, 1 mM GALNS (MPS-IVA) substrate, 1 mM ARSB (MPS-VI) substrate, and 0.5 mM GUSB (MPS-VII) substrate, 15 microM TPP-1-IS, 10 microM I2S-IS internal standard, 10 microM NAGLU-IS, 7.5 microM GALNS-IS, 10 microM ARSB-IS, and 10 microM GUSB-IS, and 0.100 mM NAG-Thiazoline.

Assay procedure: (all assay volume units are microliters)

(1) To each well in a 96-well assay plate (Fisher Scientific, Cat. No. 09-761-116) containing a single 3 mm DBS punch is added 30 μL of assay cocktail. The plate is sealed with adhesive please sealing film (Sigma, Cat. No. Z374938). The plate is shaken at 37 °C for 16 hr.
(2) After incubation, Quench assay by adding 100 μL of 50:50 methanol:ethyl acetate, mix up and down with Pipettor 4-5 times.
(3) Add 400 μL of ethyl acetate then 200 μL of 0.5 M NaCl in water (be sure to use reagent grade NaCl). Mix up and down ~10 times with pipet tip, Cover with silicone mat or adhesive film.

(4) Centrifuge 5 min at 3000 RPM at room temperature to separate the solvent layers
(5) Transfer 200 µL of top layer to new shallow well plate (Sigma, Cat. No. CLS3363) using a Pipettor
(6) Dry under oil-free nitrogen or air at room temperature long enough until all ethyl acetate is gone
(7) Add 100 µL of LC solvent (55/45 water/acetonitrile with 0.1% formic acid) Mix up and down ~10 times and Place the plate on the autosampler of the LC-MS/MS for isocratic LC-MS/MS

Note about ethyl acetate: Use reagent grade ethyl acetate from various vendors. Some batches contain small oxidant contaminants that react with the MPS-I, MPS-IIIB and MPS-VII products and internal standards. Add 3.6 g of reagent grade sodium sulfite (Na_2SO_3, not sodium sulfate) to 1 L bottle of ethyl acetate, cap and shake for ~30 sec. You can leave the Na_2SO_3 in the bottle, it will settle to the bottom, and if traces get into the samples, it is of no concern as it will remain in the water layer after ethyl acetate extraction. Note if you have verified that your bottles of ethyl acetate are oxidant free without addition of sodium sulfite you can skip the sodium sulfite treatment step. Oxidation will be apparent by a noticeable drop in the MS/MS signals for the MPS-I, MPS-IIIB, and MPS-VII internal standards due to oxidative damage to these analytes. A drop in product MS/MS signal for the quality control HIGH would also indicate problematic ethyl acetate.

2.2. HPLC Separation Conditions

The HPLC column is a XSelect CSH C18 (Waters, 130 Å, 3.5 µm, 2.1 mm x 50 mm, 1/pkg [Cat. #186005255]) with a XSelect CSH C18 Sentry Guard Cartridge, 130 Å, 3.5 µm, 2.1 mm x 10 mm. Mobile phase: 55/45 water/acetonitrile with 0.1% formic acid, flowrate 0.4 mL/min. The solvents are Fisher Scientific Optima™ LCMS. formulated for UHPLC-V.

Typically, we have been able to use the LC column for several thousand runs, we use it until peak separation is no longer adequate and/or the back pressure is too high. Column expense per sample is thus trivial, also the column can be changed in 10 min.

The weak needle wash was H_2O/acetonitrile 90:10 with 0.1% formic acid, and the strong needle wash was 100% acetonitrile with 0.1% formic acid.

Alternative monolith column:
Chromolith FastGradient RP18e 50-2mm
Sorbent Lot/column No.U11509/101
Mobile phase: 60/40 water/acetonitrile + 0.02% formic acid
Flow-rate: 0.4 mL/min

2.3. MSMS Data

Table S1. MSMS Acquisition Parameters.

Species[a]	Parent ion *m/z*	Daughter ion *m/z*	dwell time (s)	Cone Voltage (V)	Collision Energy (eV)
TPP 1 P	350.2	250.2	0.022	12	12
TPP 1 IS	359.3	251.2	0.022	12	12
MPS IIIB P	420.2	311.2	0.022	25	25
MPS IIIB IS	423.2	314.2	0.022	25	25
MPS VII P	434.3	325.3	0.022	25	15
MPS VII IS	441.3	332.3	0.022	25	15
MPS II P	644.3	359.2	0.022	34	23
MPII IS	649.3	364.3	0.022	34	23
MPS VI P	657.3	345.2	0.022	26	24
MPS VI IS	662.4	350.2	0.022	26	24
MPS IV P	685.4	373.2	0.022	26	25
MPS IV IS	690.4	378.3	0.022	26	25

[a]P = product, IS = internal standard

2.4. Electrospray Parameters

Capillary: 3.5 kV; Extractor: 3 V; Source temperature: 150 °C; Desolvation temperature: 500 °C; Cone gas flow: 30 L/h; Desolvation gas flow: 1000 L/h; Collision gas flow: 0.15 mL/min; LM 1 resolution: 2.9; HM 1 resolution: 15; Ion energy 1: 0; Gas nn MSMS mode entrance: 0.5; Gas on MSMS mode exit: 0.5; LM 2 resolution: 2.8; HM 2 resolution: 14.7; Ion energy 2: 0.6

3. Enzyme Activities

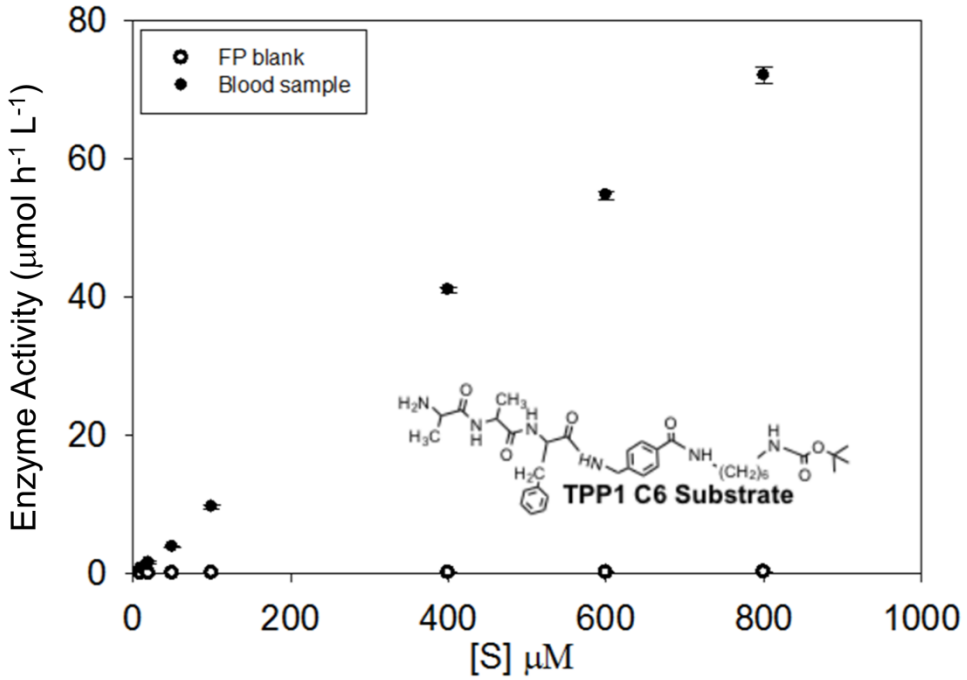


Figure S1. Activity vs. substrate concentration for TPP1-S. FP = filter paper blank

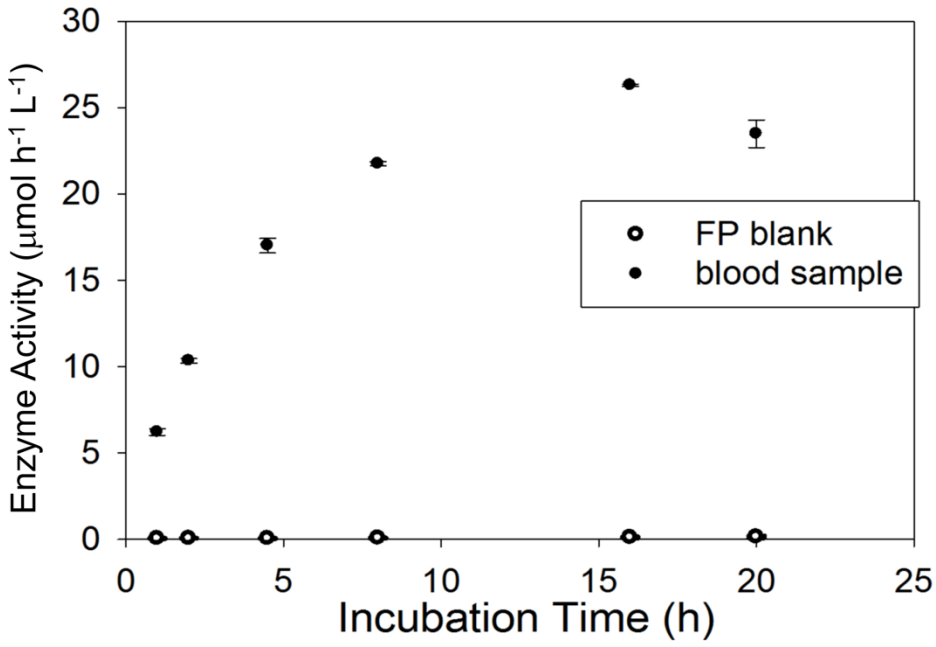


Figure S2. Activity vs. incubation time concentration for TPP1-S. FP = filter paper blank.

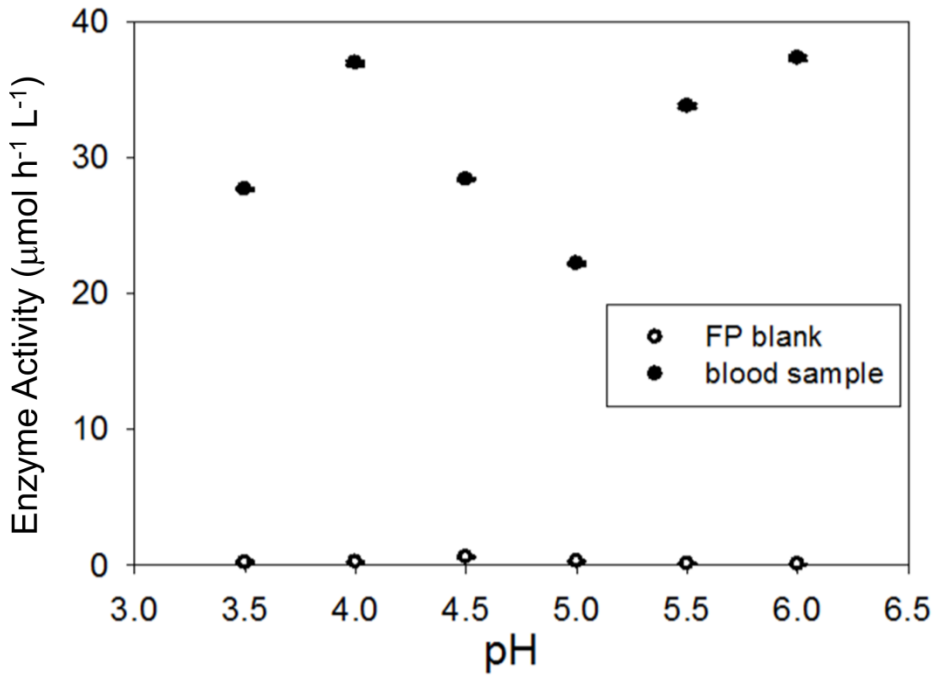


Figure S3. Activity vs. pH for TPP1-S. FP = filter paper blank.

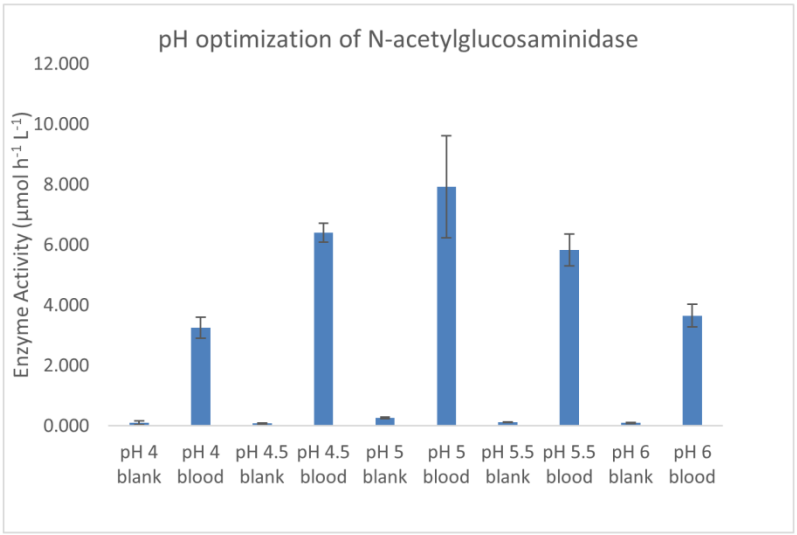


Figure S4. Activity vs. pH for NAGLU-S.

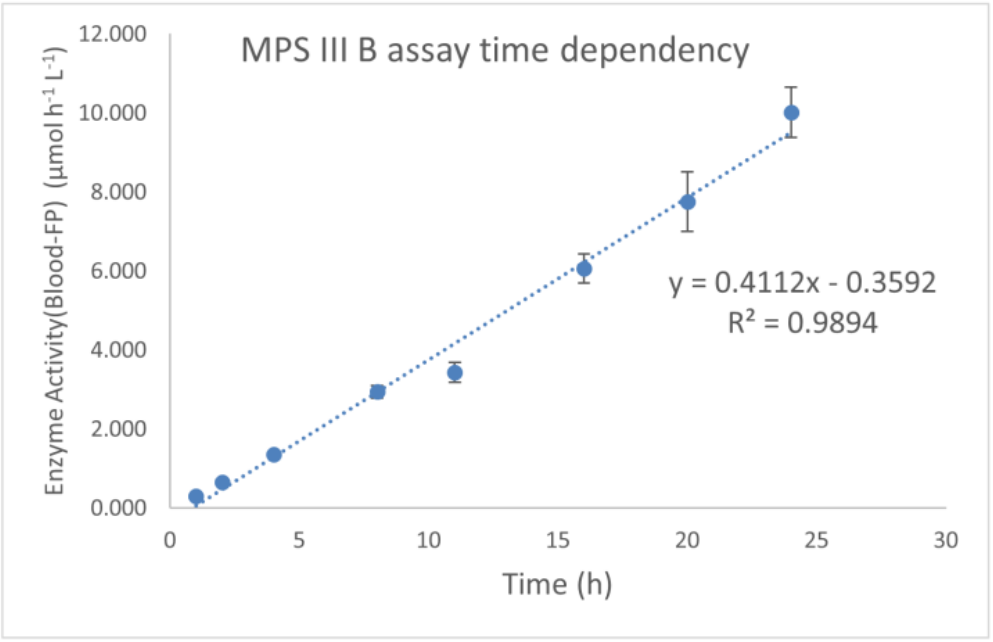


Figure S5. Activity vs. incubation time for NAGLU-S.

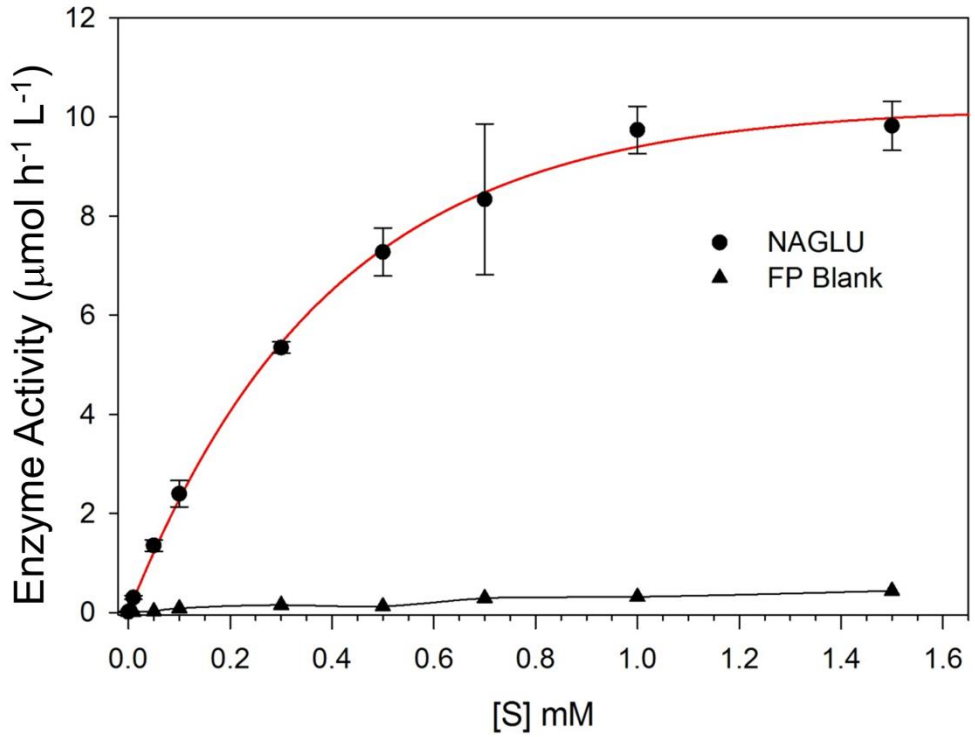


Figure S6. Activity vs. substrate concentration for NAGLU-S. The red curve is an exponential fit : Activity = $10.208 \times [1 - \exp(-2.5346 \times [S])]$. FP is filter paper blank

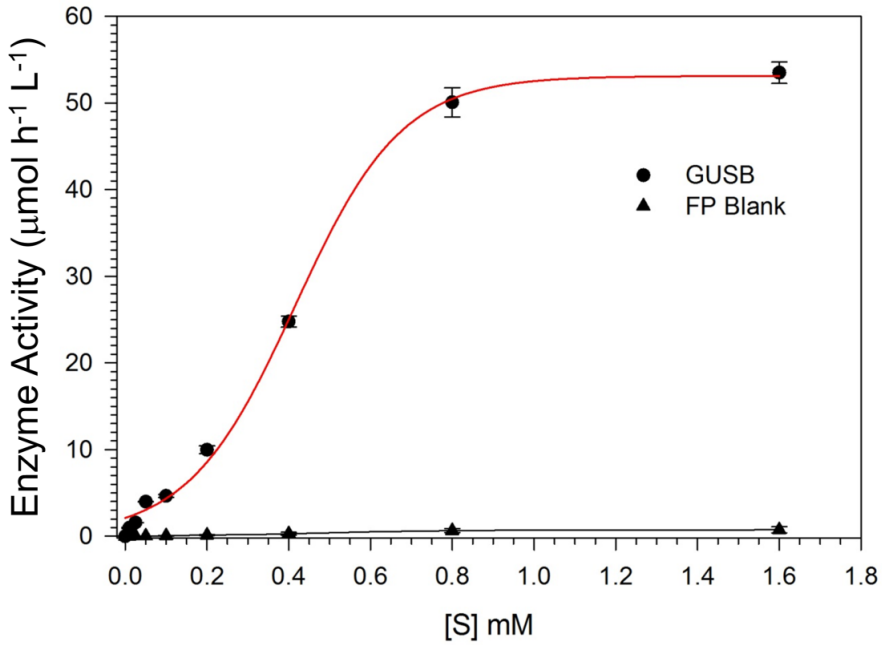


Figure S7. Activity vs. substrate concentration for GUSB-S. FP is filter paper blank. The red line is an exponential fit: Activity = 53.1072/{1 + exp[−([S] − 0.4151)/0.1305]}.

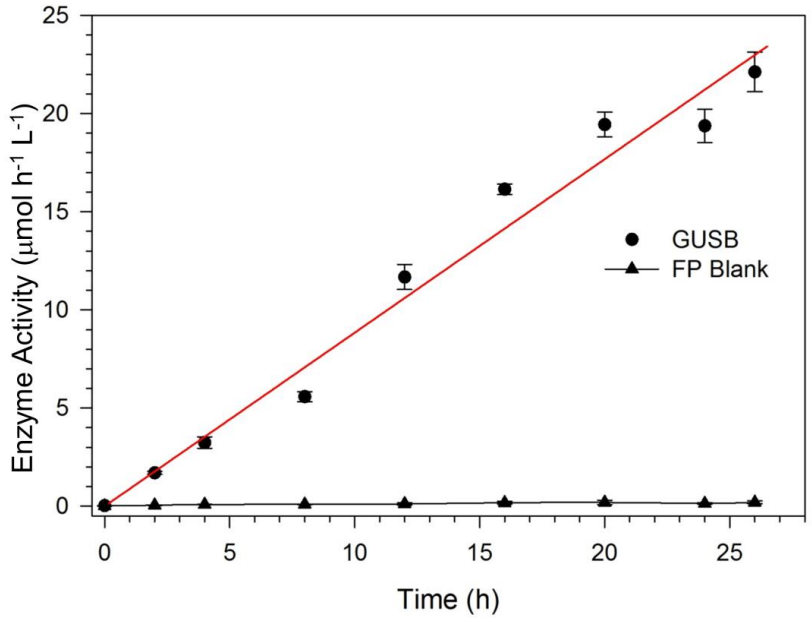


Figure S8. Activity vs. incubation time for GUSB-S. The red line is a linear regression fit: Activity = 0.88392 × time (r^2 = 0.991, n = 9). FP blank stands for filter-paper blank.

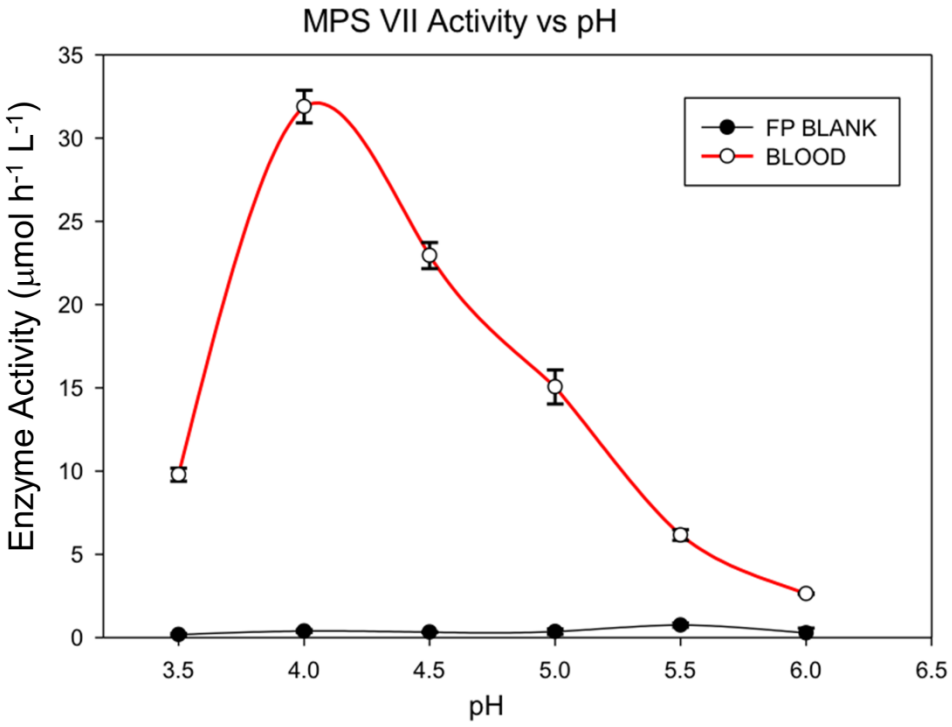
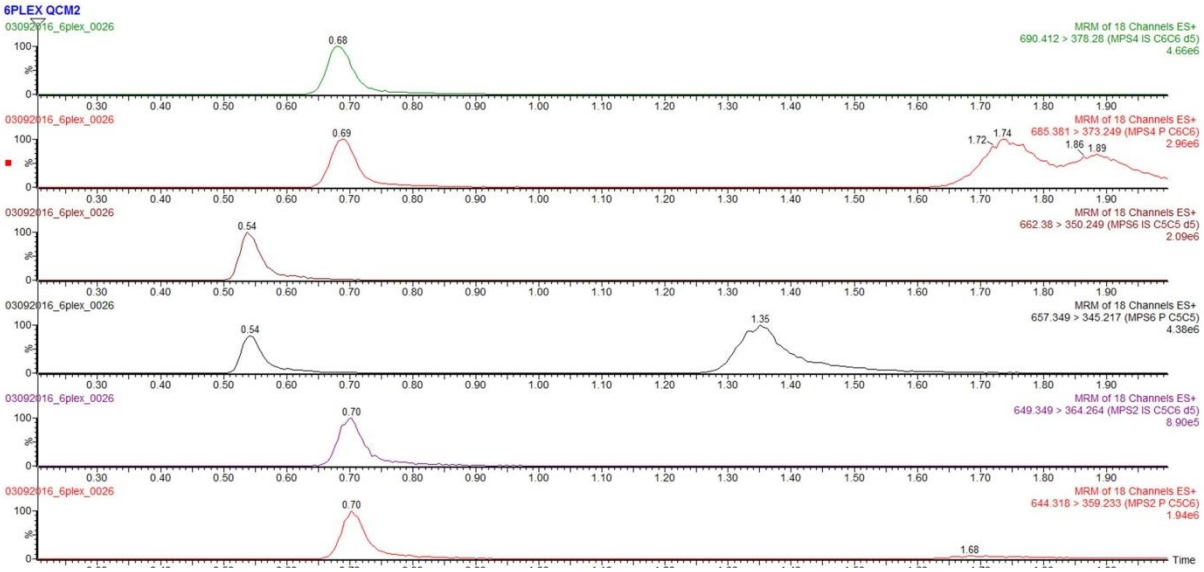


Figure S9. Activity vs. pH for GUSB-S. FP is filter paper blank.

- MPS IVA, VI, II



- MPS VII, IIIB, TPP1

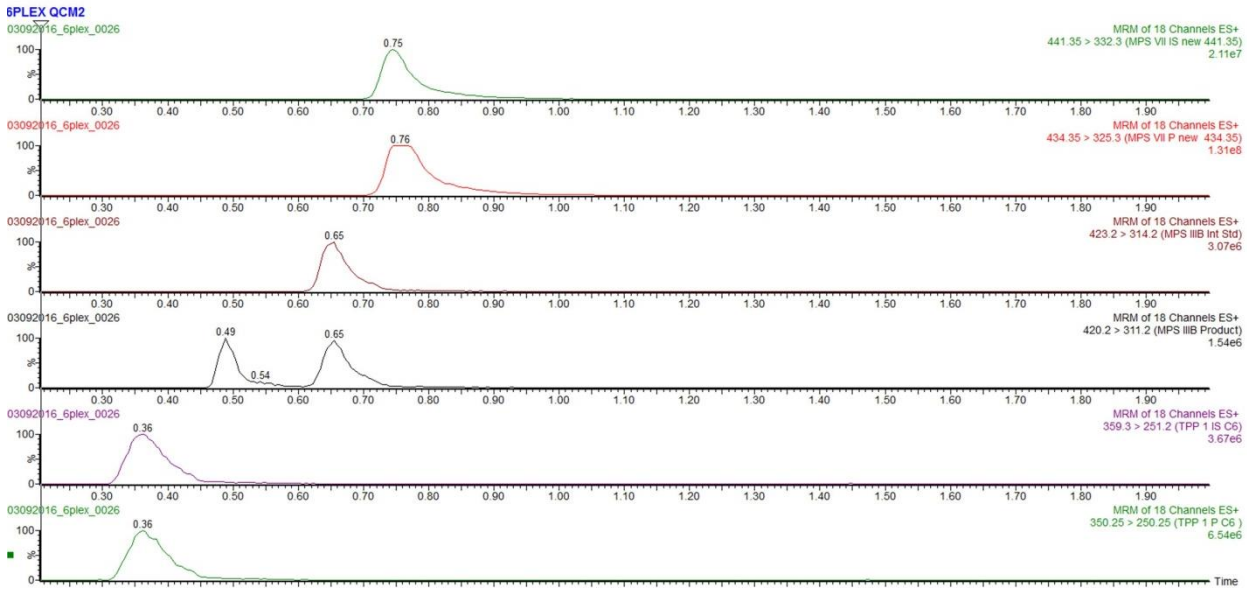


Figure S10. HPLC-MRM traces of internal standards on the XSelect CSH C18 column.

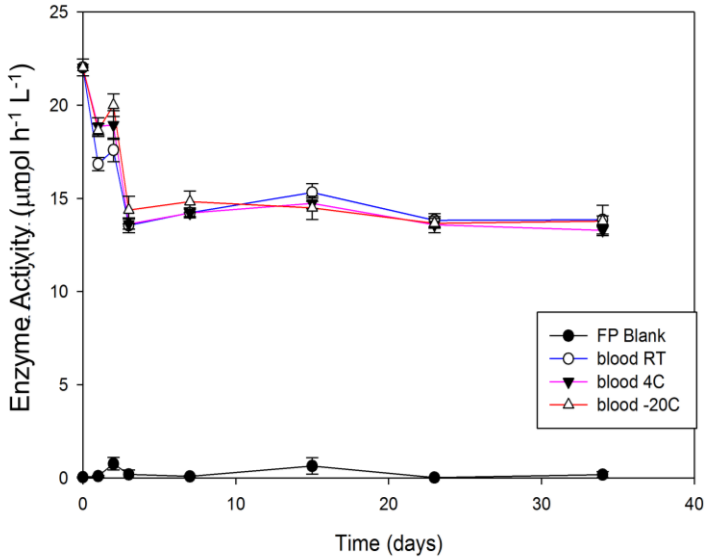


Figure S11. TPP1 activity in DBS as a function of storage time and temperature.

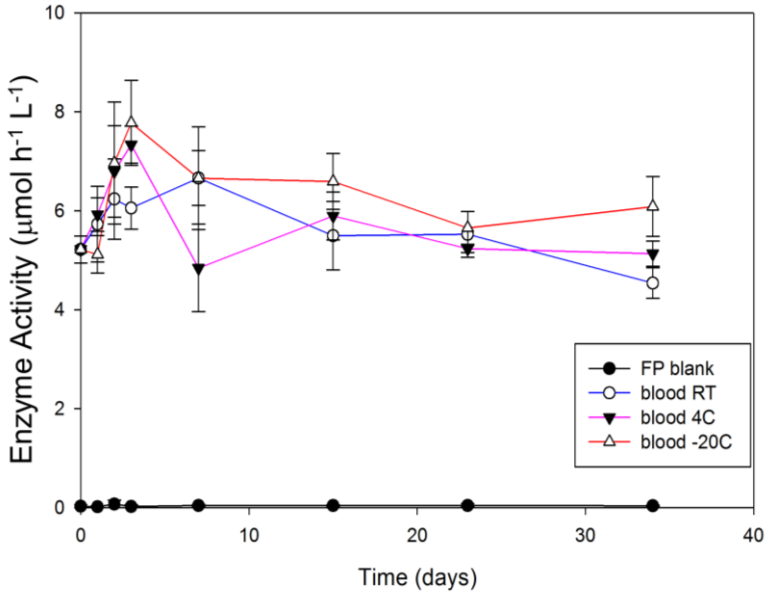


Figure S12. NAGLU activity in DBS as a function of storage time and temperature.

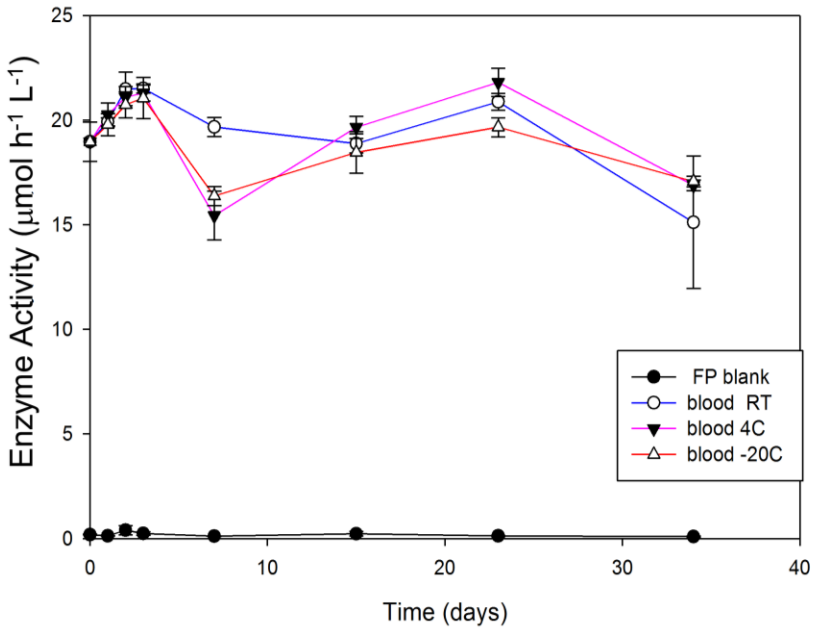


Figure S13. GUSB activity in DBS as a function of storage time and temperature.

Table S2. Enzyme activities in DBS.

	Activity[µmol/(L*h)]					
Sample Text	MPS VI	MPS II	MPS IVA	MPS IIIB	MPS VII	TPP 1
FP BLANK 1	0.09	0.18	0.02	0.01	0.05	0.04
FP BLANK 2	0.14	0.01	0.00	0.01	0.07	0.01
FP BLANK 3	0.01	0.04	0.00	0.01	0.05	0.02
FP BLANK 4	0.01	0.04	0.00	0.00	0.05	0.02
FP BLANK 5	0.01	0.02	0.00	0.01	0.06	0.03
FP BLANK 6	0.01	0.04	0.00	0.01	0.05	0.06
Mean blank	0.05	0.06	0.00	0.01	0.06	0.03
Mean Activity	4.37	16.11	0.67	1.56	28.46	35.88
Mean/Blank Ratio	95.28	289.39	152.08	199.17	511.34	1224.67
Min	1.96	8.23	0.20	0.63	17.02	21.35
Max	8.89	30.86	1.46	4.51	42.81	47.31
Min/Blank Ratio	42.77	147.89	45.00	81.00	305.68	728.70
St. Dev	1.41	3.33	0.28	0.67	6.67	5.42
nb 01	3.58	16.49	0.72	1.22	19.03	32.49
nb 02	5.12	13.83	0.63	0.82	22.66	29.51
nb 03	4.29	15.22	0.62	1.18	22.96	23.62
nb 04	2.23	13.42	0.25	1.23	17.02	40.41
nb 05	5.49	18.26	0.79	1.58	26.25	34.00
nb 06	4.84	19.81	0.80	1.04	32.02	40.00
nb 07	3.41	15.22	0.57	1.27	26.20	32.90
nb 08	3.38	16.11	0.49	1.22	30.88	30.12
nb 09	2.40	20.43	0.44	2.28	23.23	33.02
nb 10	5.12	21.97	0.71	1.28	28.86	32.96
nb 11	3.46	13.39	0.47	1.24	21.58	34.30
nb 12	4.37	18.09	1.20	2.05	30.43	38.03
nb 13	5.47	17.72	1.10	1.08	26.25	21.35
nb 14	3.70	13.75	0.48	0.63	20.68	34.32
nb 15	4.23	19.93	0.38	1.51	31.95	34.76
nb 16	2.70	14.70	0.50	0.97	22.69	26.83
nb 17	4.13	13.73	0.37	0.99	18.32	33.12
nb 18	3.56	30.86	0.33	2.51	27.47	37.51
nb 19	8.34	18.55	0.71	1.46	40.76	36.44
nb 20	5.38	14.10	0.73	1.15	35.63	31.19
nb 21	3.66	20.43	0.61	1.98	29.20	26.21

nb 22	4.70	18.83	0.73	1.55	30.33	34.07
nb 23	1.96	14.48	0.52	0.86	19.61	32.88
nb 24	4.58	17.55	0.60	1.57	31.65	41.12
nb 25	5.07	16.91	0.54	1.20	17.89	40.37
nb 26	4.80	16.33	0.94	3.52	31.19	40.17
nb 27	3.16	14.95	0.78	1.17	26.46	39.06
nb 28	2.34	14.88	0.51	2.45	19.34	47.31
nb 29	3.59	16.57	0.46	4.51	23.94	39.91
nb 30	5.82	20.66	0.77	1.33	38.54	29.93
nb 31	5.98	12.84	0.89	1.27	29.14	40.02
nb 32	3.35	12.60	0.60	1.39	21.59	47.18
nb 33	3.13	17.36	0.58	1.85	22.93	32.80
nb 34	4.01	20.39	0.74	1.30	28.72	41.12
nb 35	3.22	14.05	0.36	2.58	20.48	39.72
nb 36	3.13	14.78	0.52	1.58	26.48	42.45
nb 37	5.20	14.61	0.48	1.05	35.37	42.44
nb 38	6.48	14.23	0.94	1.37	35.79	38.00
nb 39	5.64	16.93	0.85	1.18	24.91	39.45
nb 40	4.35	18.39	0.61	1.27	27.11	38.26
nb 41	3.33	13.85	0.46	0.98	29.92	39.30
nb 42	5.40	8.23	0.59	1.07	28.65	41.74
nb 43	3.03	15.65	0.43	1.70	25.38	37.48
nb 44	3.05	12.76	0.35	2.12	17.87	39.26
nb 45	5.30	13.50	1.19	3.34	39.05	44.38
nb 46	6.41	10.68	0.38	2.26	41.34	28.94
nb 47	4.73	13.61	1.35	1.44	36.71	36.31
nb 48	3.24	19.00	0.47	1.53	24.01	39.83
nb 49	5.44	20.76	1.00	2.04	40.69	40.41
nb 50	8.89	17.92	1.46	1.19	42.43	42.92
nb 51	3.70	20.07	0.71	1.35	31.51	30.93
nb 52	5.71	16.14	1.40	1.89	42.81	33.29
nb 53	3.59	16.45	0.71	0.87	27.55	39.41
nb 54	2.63	14.92	0.78	1.37	27.80	28.48
nb 55	6.05	14.44	1.05	1.38	34.23	34.06
nb 56	3.64	14.47	0.55	1.46	24.21	34.84
nb 57	3.25	13.74	0.20	1.38	36.13	35.27
nb 58	4.21	16.22	0.58	1.66	29.13	34.46
nb 59	5.23	11.79	0.89	1.41	33.68	31.01
nb 60	6.55	15.77	0.59	1.34	26.29	36.37
nb 61	2.30	12.90	0.62	1.04	22.81	36.40
nb 62	6.15	14.40	0.76	1.26	34.34	25.81

nb 63	4.34	14.19	0.27	2.23	31.15	40.12
QC adult #1	1.28	20.94	1.21	4.47	19.64	19.30
QC adult #2	1.17	15.69	1.02	4.63	22.98	24.48
QC adult #3	1.04	15.12	1.09	3.13	17.55	20.78
QC adult #4	0.82	15.22	0.84	4.28	20.93	19.91
QC adult #5	1.49	21.02	1.13	6.24	22.24	17.83
QC adult #6	1.24	13.52	0.97	4.21	21.11	17.69
QCHigh 1	5.51	15.74	3.02	6.09	43.90	21.92
QCHigh 2	4.76	13.52	2.92	5.50	44.99	23.52
QCLow 1	0.47	1.05	0.19	0.25	13.49	4.96
QCLow 2	0.45	0.91	0.15	0.21	12.48	5.04
LINCL patient 1	2.91	24.18	2.52	1.76	32.41	0.96
LINCL patient 2	3.60	15.93	1.96	2.83	48.47	2.85
LINCL patient 3	1.89	15.61	1.83	5.87	24.91	2.23
LINCL patient 4	0.63	21.68	0.88	3.26	20.91	0.61
LINCL patient 5	0.61	10.02	0.30	4.34	17.35	0.15
LINCL patient 6	1.08	18.70	0.35	1.86	18.42	1.29
LINCL patient 7	1.57	13.71	0.64	1.95	26.56	1.25
MPS IIIB patient 1	2.38	21.14	0.85	0.02	21.04	22.03
MPS IIIB patient 2	1.49	14.84	0.88	0.01	15.62	23.07
MPS IIIB patient 3	1.58	22.31	0.73	0.01	17.40	18.67
MPS IIIB patient 4	1.18	18.39	0.85	0.00	16.72	20.85
MPS VII patient 1	1.71	18.39	0.91	2.90	0.07	23.80
MPS VII patient 2	2.25	23.23	1.23	2.79	0.09	21.33
MPS VII patient 3	1.56	36.60	0.85	3.97	0.10	15.92

6. Addition of IDUA (for MPS-I) to the multiplex assay.

The IDUA substrate, product and internal standard are as reported previously (ref. 32 of the main text). The assay is carried out exactly as described above with the addition of IDUA substrate (1 mM) and IDUA internal standard (9.7 μM). If MPS-VII is not included, D-saccharic acid-1,4-lactone monohydrate (Sigma Cat. S0375) should be added to the buffer at 0.042 mM. This reagent is an inhibitor GUSB, and its use minimizes the amount of aglycone coming from the action of GUSB on the trace amount of β-anomer present in the IDUA substrate. This aglycone is identical to the IDUA product. If MPS-VII is to be included, the GUSB inhibitor should be omitted, and in this case there may be a small increase in observed apparent activity of IDUA in DBS with very low IDUA activity.

LC-MS/MS is done as above but with gradient elution instead of isocratic elution. Solvent A is 100% water with 0.1% formic acid. Solvent B is 100% acetonitrile with 0.1% formic acid. The gradient starts at 35% B and goes in a linear fashion to reach 50% B at 0.4 min, then it goes linear again to reach 99% B at 0.8 min, and then holds at 99% B to reach 1.5 min, then it jumps back to 35% B to re-equilibrate the column, which is ready to inject at 2 min. The flow rate is constant at 0.4 ml/min. The additional MRM parameters are given in Table S3 below.

Table S3. MRM parameters for IDUA (MPS-I).

Compound	Precursor [m/z]	Product [m/z]	Cone [V]	Collision energy [eV]
MPS-I product	426.10	317.20	24	14
MPS-I int. std.	431.20	322.20	24	14

Shown in Figure S14 are typical LC-MSMS traces. The top peak for each pair is the trace for the internal standard MRM channel, and the bottom trace is the product channel. Note for the product channel there are two peaks, one for the enzymatically-generated product and one from ESI in-source breakdown of substrate to product. Only the product peak that has the same retention time as the internal standard is integrated. Enzymatic activities measured with a DBS punch from a healthy adult are given in Table S4.

Figure S24. LC-MRM chromatograms including IDUA (MPS-I).

Table S4. Listed in the table below are enzymatic activity values obtained using a 3 mm punch of a DBS made from a healthy adult.

MPS	Activity with Gelb DBS punch (umole/hr/L)[1]	Blank with filter paper punch (umole/hr/L)[1]	Analytical Range[2]
MPS-I	1.04 +/- 0.05	0.01 +/- 0.0008	104
MPS-II	24.39 +/- 3.00	0.09 +/- 0.01	271
MPS-IIIB	8.26 +/- 1.45	0.03 +/- 0.002	275
MPS-IVA	1.30 +/-0.05	0.02 +/- 0.002	65
MPS-VI	3.23 +/- 0.26	0.01 +/- 0.001	323
MPS-VII	19.52 +/- 0.76	0.07 +/- 0.004	279

[1]Activities are the mean and standard deviation of triplicate runs.
[2]The analytical range is the activity with Gelb DBS punch divided by blank activity with filter paper punch (no blood).