Supporting Information for:

Probing the mechanism of a fungal glycosyltransferase essential for cell wall biosynthesis. UDP-Chitobiose is not a substrate for chitin synthase

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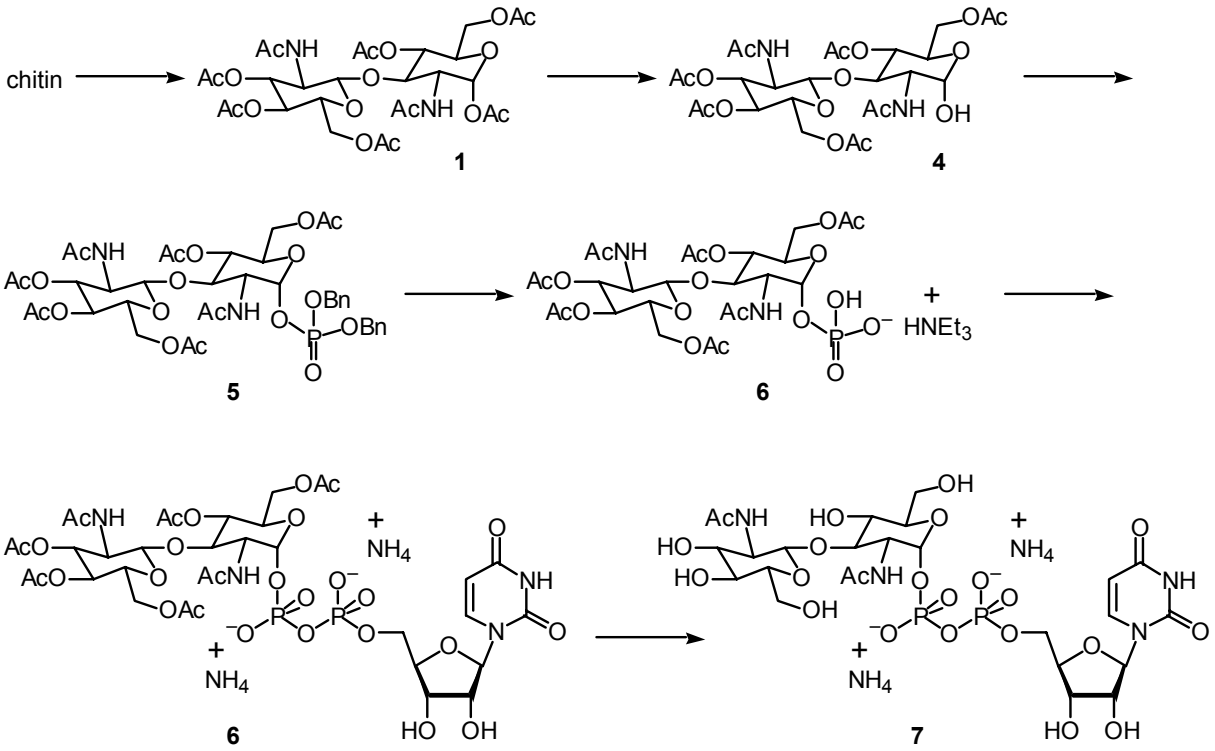
Section A: General Experimental Details

^{1}H NMR spectra were obtained on Varian HG–300 (300 MHz), HG–400 (400 MHz) or HG–500 (500 MHz) spectrometers. Chemical shifts (δ) are reported in parts per million (ppm) relative to residual solvent ($CDCl_3$, CD_3OD or D_2O). Multiplicities are given as: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), dt (doublet of triplets), or m (multiplet). Proton-decoupled ^{13}C NMR spectra were obtained on HG-400 (100 MHz) or HG-500 (125 MHz) spectrometers. ^{13}C chemical shifts are reported relative to residual solvent (CD_3OD) or added CH_3OH (D_2O). IR stretches are given in cm^{-1}; spectra were obtained on a Nicolet Magna-IR 550 Series II spectrometer. Mass spectroscopic analyses were provided by the facility at UCSD. Chromatographic purifications were performed by flash chromatography with silica gel (Selecto, 32-63 μm) packed in glass columns; eluting solvent for each purification was determined by thin layer chromatography (TLC). Analytical TLC was performed on aluminum plates coated with 0.25 mm sensitized silica gel using UV light.

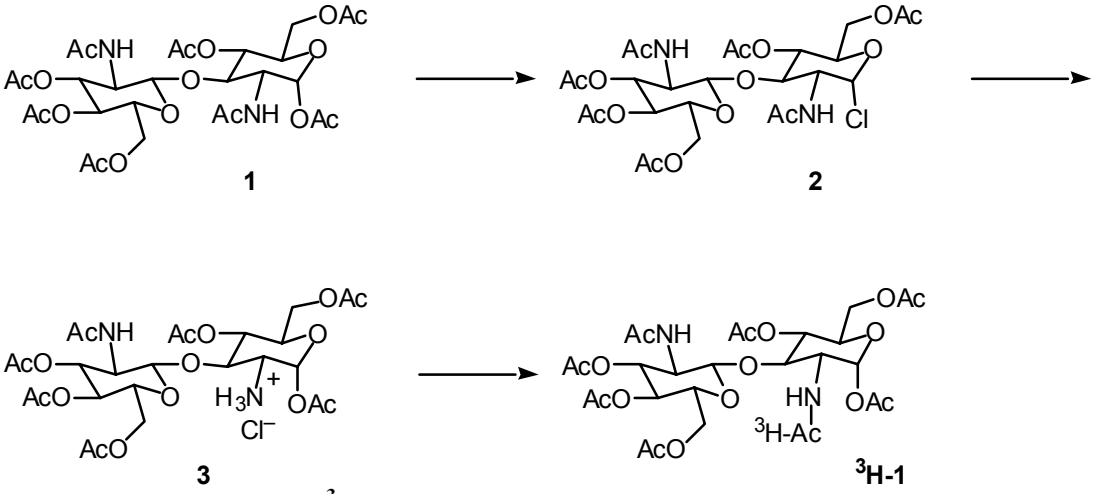
Synthetic procedures were carried out under an inert atmosphere using standard Schlenk line techniques. Where noted, solutions were degassed by evacuating and backfilling with N_2 several times. CH_3CN was distilled from CaH_2. Tetrahydrofuran (THF) and benzene were dried by passage through a column of activated alumina.[1] All other reagents and solvents were used as received unless otherwise specified.

(1) Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organomet.* **1996**, *15*, 1518-1520.

Section B: Synthesis of UDP-chitobiose (UDP-Chi).



Scheme S1. Synthesis of UDP-Chi.



Scheme S2. Synthesis of ^{3}H-1.

2-Acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-1,3,6-tri-O-acetyl-2-deoxy-α-D-glucopyranose (1):
Powdered chitin (50 g) was sonicated in 450 mL concentrated HCl for 1 hour. The resulting suspension was poured onto 2.5 L ice-water and stored at 4 °C overnight. The solid was isolated by Buchner filtration and washed with water (500 mL), 1M NaOH (500 mL), water (500 mL), acetone (500 mL), and diethyl ether (500 mL) to obtain colloidal chitin (40 g). Colloidal chitin (20 g) was added to an ice-cold solution of H_2SO_4 (20 mL) and acetic anhydride (200mL). The mixture was warmed to 55 °C and stirred for 6 hours, poured onto ice water, neutralized with NaOAc (40 g), and filtered. The filtrate was extracted with $CHCl_3$ (4×250 mL). The combined extracts were washed with water (1×1L), saturated $NaHCO_3$ (1×1L), and water (1×1L), dried over Na_2SO_4, and concentrated under reduced pressure to obtain an orange solid, which was recrystallized from MeOH to obtain the title compound as a white solid (2.3 g, 11.5% from colloidal chitin). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.10 (d, 1H, J = 3.6 Hz), 5.92 (d, 1H, J = 8.8 Hz), 5.62 (d, 1H, J = 9.2 Hz), 5.22 (dd, 1H, J = 9.2 Hz, 11.2 Hz), 5.13 (app t, 1H, J = 9.6 Hz), 5.05 (app t, 1H, J = 9.6 Hz), 4.40 (m, 4H), 4.18 (dd, 1H, J = 1.6 Hz, 12 Hz), 4.02 (dd, 1H, J = 1.6 Hz, 12 Hz), 3.92 (m, 2H), 3.73 (app t, J = 9.2 Hz), 3.62 (m, 1H), 2.18-1.92 (8×s, 24H).

2-Acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (2):
A solution of acetic acid and acetic anhydride (1:1, 10 mL) was saturated with anhydrous HCl at 0 °C. **1** (0.66 g, 0.97 mmol) was subsequently added and stirred slowly at room temperature for 3 days. The reaction mixture was then diluted with 50 mL $CHCl_3$, washed with H_2O (1×50 mL), saturated $NaHCO_3$ (1×50 mL), H_2O (1×50 mL), dried over Na_2SO_4, and concentrated under reduced pressure to obtain a yellow solid, which was triturated with diethyl ether to afford pure **2** as a white solid (0.39 g, 62%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.11 (d, 1H, J = 3.6 Hz), 5.92 (d, 1H, J = 8.4 Hz), 5.83 (d, 1H, J = 8.7 Hz), 5.33-5.20 (m, 2H), 5.05 (app t, 1H, J = 9.8 Hz), 4.63 (d, 1H, J = 8.1 Hz), 4.47-4.30 (m, 4H), 4.19 (m, 1H), 4.02 (dd, 1H, J = 2.0 Hz, 12.5 Hz), 3.88-3.76 (m, 2H), 3.68-3.63 (m, 1H), 2.16-1.95 (7×s, 21H).

2-Acetamido-4-*O*-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-1,3,6-tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranose hydrochloride (3):
2 (0.33 g, 0.50 mmol) was dissolved in acetone (10 mL) and H_2O (0.01 mL, 0.50 mmol, 1.0 eq) and stirred at 50 °C for 2 days. The mixture was then cooled to 0 °C and stirred for 2 hours. The resulting suspension was filtered to obtain a white solid, which was washed with cold acetone to afford pure **3** (0.24 g, 72%). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 8.45 (m, 2H), 8.02 (d, 1H, J = 9.6 Hz), 6.08 (d, 1H, J = 3.3 Hz), 5.15-5.07 (m, 2H), 4.83 (app t, 1H, J = 9.6 Hz), 4.63 (d, 1H, J = 8.1 Hz), 4.38-4.29 (m, 2H), 4.04-3.80 (m, 5H), 3.72-3.69 (m, 1H), 3.60-3.51 (m, 1H), 2.16-1.73 (7×s, 21H); HRMS (MALDI) calculated for $C_{26}H_{38}N_2O_{16}$ (MNa^+) 657.2113 found 657.2115.

2-Acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-D-glucopyranose (4):
1 (1.00 g, 1.78 mmol, 1 eq) and hydrazine acetate (0.20 g, 2.14 mmol, 1.2 eq) was added to DMF (10 mL) and stirred at room temperature under N_2 for 4 hours. The reaction mixture was then concentrated with toluene co-evaporations and chromatographed on silica gel with 2-5%

MeOH/$CHCl_3$ to afford **4** (0.94 g, 83%, α-anomer only) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.18 (d, 1H, J = 10.4 Hz), 6.22 (d, 1H, J = 8 Hz), 5.65 (dd, 1H, 9.3 Hz, 11.1 Hz), 6.22 (m, 1H), 5.09 (app t, 1H, J = 9.6 Hz), 4.98 (m, 1H), 4.43 (dd, 1H, J = 4.2 Hz, 12.6 Hz), 4.30 (m, 2H), 4.08 (m, 5H), 3.64 (app t, 1H, J = 9.3 Hz), 3.56 (m, 1H), 2.14-1.95 (7×s, 21H).

2-Acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl dibenzyl phosphate (5):
4 (0.23 g, 0.36 mmol, 1 eq) was dissolved in dry THF (20 mL) in a flame-dried flask and cooled to -78 °C under N_2. A solution of LDA (270 mL, 2.0 M in 4:2:1.5 THF:heptane:ethylbenzene, 0.54 mmol, 1.10 eq) was added dropwise via syringe. After stirring for 15 minutes, a solution of tetrabenzyl pyrophosphate (0.24 g, 0.45 mmol, 1.25 eq) in dry THF (5 mL) was added via syringe. The reaction solution was subsequently warmed to 0 °C, stirred for 2 hours, and concentrated under reduced pressure. Flash chromatography on silica gel with 2% MeOH/$CHCl_3$ afforded **4** (0.25 g, 77%) exclusively as the α-anomer. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.36 (m, 10H), 6.15 (d, 1H, J = 9 Hz), 5.75 (d, 1H, J = 9 Hz), 5.62 (dd, 1H, J = 3.3 Hz, 5.8 Hz), 5.21 (app t, 1H, J = 10.3 Hz), 5.14 (m, 1H), 5.10-5.01 (m, 4H), 4.56 (d, 1H, J = 8 Hz), 4.37 (dd, 1H, J = 4.3 Hz, 12.3 Hz), 4.30-4.23 (m, 3H), 4.09-4.07 (m, 1H), 3.99 (dd, 1H, J = 2 Hz, 12.5 Hz), 3.96 – 3.94 (m, 1H), 3.83 (app q, 1H, J = 10.5 Hz), 3.72 (app t, 1H, J = 9.8 Hz), 3.63-3.60 (m, 1H), 2.06-1.69 (7×s, 21H).

2-Acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl-1-phosphate monotriethylammonium salt (6):
5 (0.25 g, 0.28 mmol, 1 eq) was dissolved in MeOH (5 mL) and stirred under positive H_2 pressure in the presence of Pd on activated carbon (0,03 g). After 6 hours, the reaction mixture was filtered through celite. Triethylamine (0.04 mL, 0.31 mmol, 1.10 eq) was added to the filtrate. The filtrate was subsequently concentrated under reduced pressure to afford **6** (0.23 g, 99%) exclusively as the α-anomer, which was used in the next step without further purification.
^{1}H NMR (500 MHz, CD_3OD) δ (ppm): 5.42 (dd, 1H, J = 3.2 Hz, 6.7 Hz), 5.34 (app t, 1H, J = 9.9 Hz), 5.24 (app t, 1H, J = 9.9 Hz), 4.94 (app t, 1H, J = 9.8 Hz), 4.81 (d, 1H, J = 8.5 Hz), 4.55 (m, 1H), 4.43 (dd, 1H, J = 4 Hz, 12.5 Hz), 4.18-4.15 (m, 2H), 4.06-4.00 (m, 2H), 3.89 (app t, 1H, J = 9.5 Hz), 3.81-3.71 (m, 1H), 3.63-3.56 (m, 1H), 3.17 (q, 6H, J = 7.6), 2.10-1.89 (7×s, 21H), 1.30 (t, 9H, J = 7.6 Hz).

2-Acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl uridine diphosphate monoammonium salt (7):
A solution of **6** (0.26 g, 0.32 mmol, 1 eq) in dry pyridine (1 mL) was charged to a solution of 4-morpholine-N,N'-dicyclohexylcarboxamidinium uridine 5'-monophosphomorpholidate (0.44 g, 0.64 mmol, 2.00 eq), 1-*H* tetrazole (0.07 g, 0.96 mmol, 3.00 eq), and dry pyridine (4 mL) and stirred at room temperature under N_2 for 3 days. The solution was then concentrated under reduced pressure and applied to size exclusion gel (Bio-Rad Bio-Gel P-2 Fine, 2.5×100 cm), eluted with 0.250 M NH_4HCO_3, and lyophilized to obtain **7** (0.18 g, 55 %) as a fluffy white solid. ^{1}H NMR (500 MHz, CD_3OD) δ (ppm): 8.04 (d, 1H, J = 8.5 Hz), 5.93 (d, 1H, J = 4.5 Hz), 5.78 (1H, d, J = 8 Hz), 5.51 (dd, 1H, J = 3.0 Hz, 7.0 Hz), 5.36 (app t, 1H, J = 9.5 Hz), 5.17 (app t, 1H, J = 10.0 Hz), 4.79 (d, 1H, J = 8.5 Hz), 4.59 (m, 1H), 4.43 (dd, 1H, J = 4.0 Hz, 12.5 Hz), 4.35-4.21 (m, 7H), 4.13 (m, 1H), 4.07-3.99 (m, 2H), 3.91 (app t, 1H, J = 9.5 Hz), 3.79 (m, 1H),

3.58 (app t, 1H, J = 9.5 Hz), 2.10-1.89 (7×s, 21H); ^{13}C NMR (125 MHz, CD_3OD) δ (ppm): 174.2, 173.0, 173.7, 173.0, 172.8, 172.4, 172.0, 166.5, 152.8, 142.9, 103.1, 101.9, 95.8, 94.9, 90.5, 84.8, 76.8, 75.7, 73.6, 73.2, 71.1, 70.0, 69.9, 66.3, 63.1, 61.1, 56.4, 53.3, 23.2, 23.1, 21.3, 21.1, 21.0, 20.8, 20.7; IR (KBr pellet) ν (cm^{-1}): 3257, 1755, 1557, 1442, 1376, 1244, 1046, 922, 806; HRMS (MALDI) calculated for $C_{35}H_{50}N_4O_{27}P_2$ (MH^-) 1019.2065, found 1019.2073.

UDP-Chitobiose monoammonium salt(8):
7 (0.15 g, 0.14 mmol) was dissolved in 1 mL MeOH/NEt_3/H_2O (7:1:3) and stirred at 55 °C for 3 days. The solution was then concentrated under reduced pressure and applied to size exclusion gel (Bio-Rad Bio-Gel P-2 Fine, 2.5×100 cm), eluted with 0.250 M NH_4HCO_3, and lyophilized to obtain **8** (0.10 g, 85%) as a fluffy white solid. ^{1}H NMR (500 MHz, D_2O) δ 7.97 (d, 1H, J = 8.0 Hz), 5.92 (m, 2H), 5.48 (dd. 1H, J = 3.5 Hz, 7.5 Hz), 4.59 (d, 1H, J = 8.5 Hz), 4.33 (m, 2H), 4.25 (m, 2H), 4.17 (m, 1H), 3.99 (dt, 1H, J = 3.0 Hz, 6.0 Hz, 11.0 Hz), 3.89 (m, 2H), 3.82-3.63 (m, 6H), 3.56 (m, 1H), 3.48 (m, 2H), 2.05 (m, 6H); ^{13}C NMR (100 MHz, D_2O) δ 175.0, 174.8, 166.6, 152.0, 141.9, 102.9, 102.0, 94.8, 89.5, 83.5, 79.9, 76.5, 74.6, 74.2, 71.2, 70.4, 70.2, 69.6, 65.3, 61.2, 60.3, 56.2, 53.7, 22.9, 22.8; IR ν (cm^{-1}): 3241, 1673, 1549, 1442, 1390, 1210, 1062, 922; HRMS (MALDI) calculated for $C_{25}H_{40}N_4O_{22}P_2$ (MH^-) 809.1537, found 809.1522.

Tritiated UDP-chitobiose (^{3}H-1):
A solution of tritated acetic acid (0.01 mL, 0.08 mmol, 0.19 eq, 4.20 mCi) in pyridine (0.34 mL) was charged to a solution of **3** (0.30 g, 0.45 mmol, 1 eq) in 10 mL dry pyridine and stirred under N_2 for 2 hours, at which time TLC analysis revealed partial formation of peracetyl chitobiose. Subequently, Ac_2O (0.03 mL, 0.45 mmol, 1.00 eq) was added via syringe and the mixture was allowed to stir overnight. The solution was then concentrated under reduced pressure, dissolved in choloroform (50 mL), washed with saturated $NaHCO_3$ (1×50mL), brine (1×50 mL), dried over Na_2SO_4, and concentrated to obtain ^{3}H-**1** (0.25 g, 83%). ^{1}H NMR spectral data were identical to those reported for **1**. ^{3}H-**1** was subsequently subjected to the procedure of Scheme S1 to obtain tritiated UDP-chitobiose (^{3}H-UDP-Chi) , in similar yields. The radioactivity of tritiated UDP-Chitobiose was determined to be 2.2 x 10^4 μCi/mmol.

Section C: Procedure for chitin synthase assay.

The assay protocol used is closely based on the procedure of Orlean,[2] modified with helpful suggestions from Prof. Peter Orlean (University of IL) and Dr. Enrico Cabib (NIH).[3] Yeast (*S. cerevisiae*) strains (PP-1D, wild type) were kindly provided by Professor Orlean and are stored at –70 °C on freezer stabs. Active yeast cultures are temporarily maintained on agar plates, stored at 4 °C. Cells are cultured in 200 mL YEPG (1% yeast extract, 2% bactopeptone, 2% glucose) medium at 30 °C and allowed to grow to saturation. An aliquot (10-12 mL) of the saturated medium is transferred to 400 mL of YEPG medium to give an optical density of 0.15-0.20 (measured with a Pharmacia Biotech Ultraspec 2000 spectrometer) and allowed to grow to an optical density of 0.65-0.70. The cells are washed with cold water and TM buffer (50 mM tris•HCl, 2.5 mM $MgCl_2$, pH 7.5) by suspension and centrifugation (15 min, 2000×g). The wet weight of the cells at this point is typically around 1 gram; this weight is used to determine the volume of buffer in which the final pellet is suspended (*vida infra*). The cells are suspended in 2 mL of TM buffer in a 50 mL plastic centrifuge tube, and glass beads (0.45 mm) are added until the volume of beads reaches about 3 mm below the liquid's surface. The tube is then vortexed 20×30 seconds, with 30 seconds of cooling on ice between each vortex (vortexing is performed in a 4 °C cold room). The broken cells are removed from the bottom of the tube with a glass Pasteur pipet and the glass beads are rinsed 5-7×1.5 mL with TM buffer. The pooled rinsings are centrifuged at 2000×g for four minutes, the supernatant is removed, and the remaining cell wall precipitate is washed once more with TM buffer. The cell-wall free supernatants are combined and centrifuged at 60,000×g for one hour. The enzyme pellet is suspended in 1.6 mL×{*gram wet weight of cells*} TM buffer and homogenized throughly with a glass Dounce homogenizer. The membranes are pre-treated with trypsin (quantified by weight, 10 minutes, 30 °C) and then treated with 1.5×{*mass of trypsin*} trypsin inhibitor . Typically, four different concentrations of trypsin/trypsin inhibitor are tested for each new membrane preparation, and the combination with the highest activity at 30 minutes in used in the assay. Concentrations of trypsin typically tested are 0.5, 1.0, 2.0, and 4.0 mg/mL, and trypsin is added to the membrane preparation at a concentration of 1 μL of trypsin solution for every 5 μL of membrane preparation. Individual assay are performed in 1.5 mL eppendorf centrifuge tubes. The assay solution used contains UDP-GlcNAc (1.0 mM), GlcNAc (40mM), and digitonin (0.2% w/v) dissolved in pH 7.5 tris buffer (50 mM) containing $MgCl_2$ (5.0 mM). Radioactive substrate (typically 0.125 μCi, transferred to the eppendorf as a solution which was then evaporated to dryness under vacuum) in 40 μL assay solution (containing necessary inhibitor) are transferred to each tube. Trypsin-treated membrane (20 μL) is then added and the mixture is incubated for one hour at 30 °C. The reaction is stopped by the addition of 1 mL cold (0 °C) trichloroacetic acid (10% v/v) and filtered onto glass fiber filter disks (Whatman GF/C, 25 mm), rinsed with 95% EtOH:1 M acetic acid (7:3), (4×1mL) and the remaining radioactivity on the filter paper is measured by scintillation counting (Beckman Instruments model LSI 1701 scintillation counter, 5 mL "Econo-Safe" scintillation fluid, Research Products International).

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[2] Orlean, P. *J. Biol. Chem.* **1987**, *262*, 5732-5739.
[3] P. Orlean and E. Cabib, personal correspondence.

Assays employing cold UDP-Chi were carried out in a manner identical to that above; assays employing ^{3}H-UDP-Chi were carried out as above, save that ^{3}H-UDP-GlcNAc was not used. Enzyme activity (in the absence of inhibitor) was typically 12-13,000 cpm/hr, with a time-independent background of 250-300 cpm; background radioactivity for ^{3}H-UDP-Chi assays was slightly higher due to higher initial radioactivity. Under these conditions, enzyme activity was linear (incorporated radioactivity vs. time) to at least 3 hours. All assay sets included a control reaction run in in the presence of 1 mM polyoxin D or nikkomycin Z (Sigma), both of which are known competitive inhibitors ($K_i \approx 10$ μM) of chitin synthase; these controls always showed ≥99% inhibition.

Section D: Data from UDP-Chi assays.

Table S1. Evaluation of UDP-Chi as a chitin synthase substrate.

	UDP-GlcNAc	UDP-Chi	^{3}H-UDP-GlcNAc[a]	^{3}H-UDP-Chi[a]	Relative activity
1	0.75 mM	–	+	–	100 %
2	–	0.75 mM	–	+	≤2%
3	–	3.75 mM	–	+	≤2%
4	–	–	–	+	≤2%
5	0.75 mM	0.75 mM	+	–	83%
6	–	0.75 mM	+	–	≤2%
7	–	–	+	–	≤2%

[a][^{3}H-substrate]≈0.01 mM.

Table S2. Total retained radioactivity (cpm) for experiments in Entries 1-4 of Table S1.

	Entry 1[a]	Entry 2	Entry 3	Entry 4
Run 1	32421	150	1834	595
Run 2	33975	189	1354	414
Run 3	34609	241	974	
Run 4	33412	134	545	
Run 5		299	443	
Run 6		263	1113	
Ave.	33604	213	1044	505

[a] Maximum enzyme activity differs from that in Table S3 due to variations in trypsin activation.

Table S3. Total retained radioactivity (cpm) for experiments in Entries 5-7 of Table S1.

	Entry 1A[a]	Entry 5	Entry 6	Entry 7
Run 1	9739	7974	376	123
Run 2	10172	8603	234	151
Run 3	12916	11491	234	346
Run 4	16117	11677	277	216
Run 5	12567	10978	360	380
Run 6	14140	12128	344	311
Ave.	12609	10475	304	255

[a] Maximum enzyme activity differs from that in Table S2 due to variations in trypsin activation.

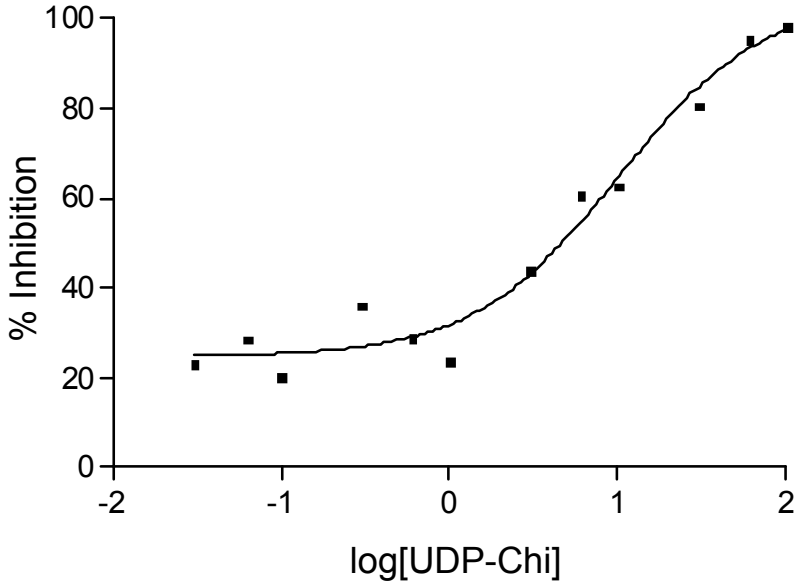
Section E: K_i determination.

Table S4. Total retained radioactivity (cpm) from UDP-Chi K_i determination.[a]

[UDP-Chi] (mM)	Run 1	Run 2	average	% inhibition
0	16543	14587	15565	0
0.01	14483	14636	14560	7
0.03	11828	12052	11940	23
0.06	10401	11815	11108	29
0.10	12938	11859	12399	20
0.30	7871	12040	9956	36
0.60	11456	10622	11039	29
1.0	11133	12682	11908	24
3.0	7544	9934	8739	44
6.0	6091	6185	6138	61
10	5167	6486	5827	63
30	2384	3649	3017	81
60	896	513	705	95
1×10^2	250	259	255	98

[a] Assays performed as described in Section C, with varying amounts of UDP-Chi added.

Figure S1. Graphical representation of fitted data, analyzed in Prism3.[4]



[4] Prism3; GraphPad Software, San Diego, CA 92121.

Table S5. Data analysis from nonlinear least-squares fit in Prism3.

Best-fit values	
BOTTOM	105.0
TOP	24.70
LOGIC50	1.004
IC50	10.09
Std. Error	
BOTTOM	5.511
TOP	2.453
LOGIC50	0.1161
95% Confidence Interval	
BOTTOM	92.49 to 117.4
TOP	19.15 to 30.25
LOGIC50	0.7413 to 1.266
IC50	5.512 to 18.47
Goodness of Fit	
Degrees of Freedom	9
R squared	0.97
Absolute Sum of Squares	255
Sy.x	5.32

K_i values were extracted using the relationship $K_i = IC_{50}/(1+[substrate]/K_m)$. As assays were performed at [UDP-GlcNAc] = K_m = 1 mM, this reduces to $K_i = IC_{50}/2$.