Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 339 (2004) 1531–1536
Carbohydrate RESEARCH

Chemical synthesis of UDP-4-*O*-methyl-GlcNAc, a potential chain terminator of chitin synthesis

Robert Chang, Philip Moquist and Nathaniel S. Finney*

Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA 92093-0358, USA

Received 23 September 2003; accepted 5 March 2004

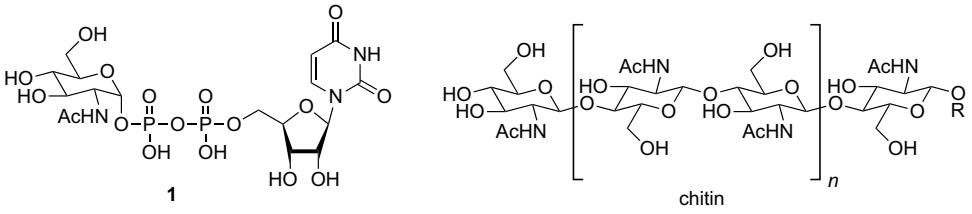
Abstract—Chitin synthase converts uridine diphosphoryl-*N*-acetylglucosamine (UDP-GlcNAc) to chitin (poly-β-(1→4)-GlcNAc). During polymerization, elongation occurs at the 4-OH (nonreducing) terminus of the growing chitin chain. Blockage of the 4-OH via incorporation of UDP-*N*-acetyl-4-*O*-methylglucosamine (UDP-4-OMe-GlcNAc, **3**) can potentially terminate chitin polymerization, and represents a novel strategy for chitin synthase inhibition. The chemical synthesis of **3** and preliminary evaluation of its possible incorporation by chitin synthase are reported herein.

Keywords: Chitin synthase; Glucosamine; 4-*O*-Methyl-GlcNAc; UDP-sugar analog; Synthesis

1. Introduction

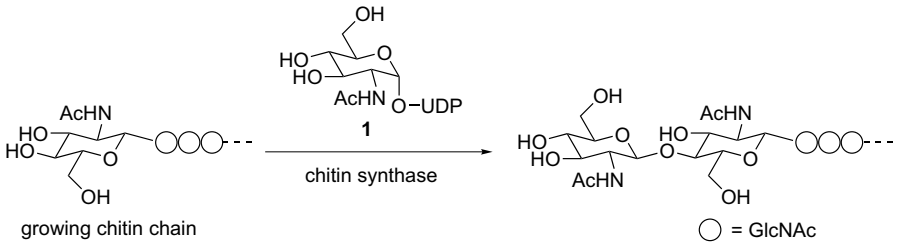
Chitin synthase (CS) is the enzyme that converts uridine diphosphoryl-*N*-acetylglucosamine (UDP-GlcNAc) **1** to chitin,[1–5] the β-(1→4)-linked polymer of *N*-acetylglucosamine. It is a member of a class of enzymes known as polymerizing, or processive, glycosyltransferases, which are also responsible for the synthesis of other important biopolymers such as cellulose and hyaluronan.[6] Absent in mammals, chitin is an essential component of the fungal cell wall. Consequently, the inhibition of CS has been recognized as a promising strategy for the development of antifungal therapeutics. To date, the development of chitin synthase inhibitors has primarily involved the synthesis of substrate analogs or structure-based design.[7–12] As part of our ongoing research program on chitin synthase,[13–15] we present a novel approach to the manipulation of CS based on termination of the polymerization reaction.

It is generally accepted that chitin is elongated at the nonreducing terminus[16]—the sugar donor UDP-GlcNAc **1** delivers a sugar residue to the 4-hydroxyl group of the growing chitin chain (Scheme 1). We envisioned that if the 4-OMe UDP-GlcNAc analog **3** could serve as a substrate for CS, then the resulting product **2** could not undergo subsequent elongation (Scheme 2), thus terminating chitin synthesis.[17] This blocking strategy could also be applicable to other polymerizing

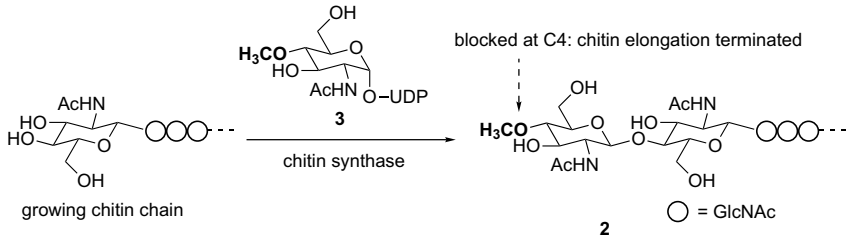


* Corresponding author. Tel.: +1-858-822-1293; fax: +1-858-822-0386; e-mail: nfinney@chem.ucsd.edu

0008-6215/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2004.03.014



Scheme 1.



Scheme 2.

glycosyltransferases, such as cellulose synthase and hyaluronan synthase. In addition, this compound could be valuable for studying other UDP-GlcNAc transferase, such as those responsible for the ubiquitous mono-GlcNac glycosylation of proteins.[6] We report here the chemical synthesis of **3** (Scheme 3), as well as the results of preliminary evaluation of **3** as a substrate for chitin synthase.

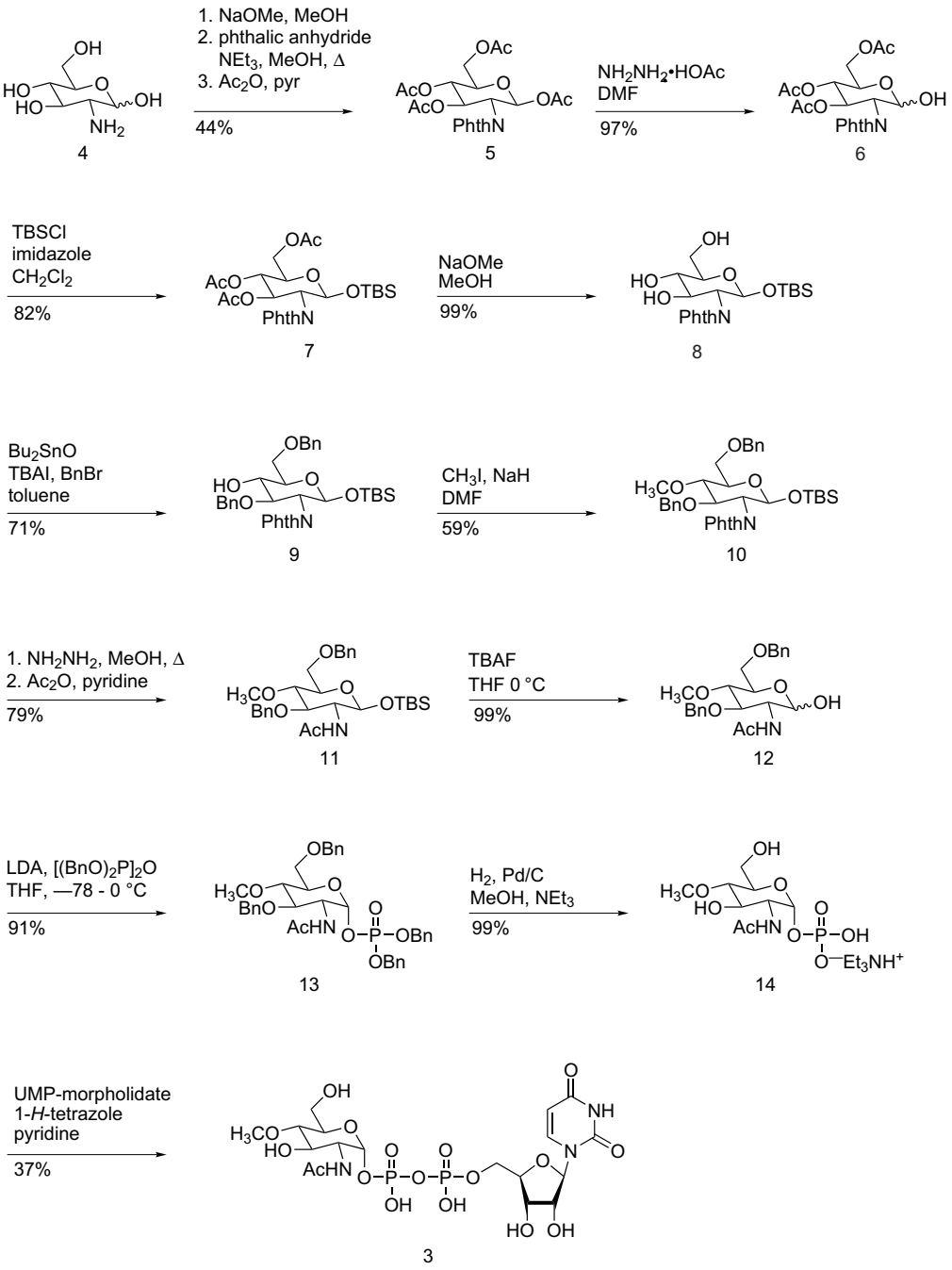
2. Results and discussion

The synthesis of **3** began with commercially available glucosamine **4**, which is easily converted to the known triol **8**[17] in four steps (35% overall yield from **4**). Regioselective O-benzylation of **8** by treatment with Bu_2SnO and benzyl bromide in the presence of TBAI afforded **9** in 71% yield.[18–21] Subsequent O-methylation of the 4-hydroxyl group of **9** with NaH and CH_3I proceeded slowly, providing **10** in modest yield after 3 days.

Removal of the *N*-phthalimido protecting group by hydrazinolysis, acylation of the resulting amine with acetic anhydride, and TBS removal with TBAF proceeded smoothly, furnishing **12** in three steps (78% overall yield from **10**). Phosphorylation with tetrabenzyl pyrophosphate gave the benzyl-protected anomeric phosphate **13** as the desired α anomer in high yield.[22] Global debenzylation was achieved by hydrogenation over palladium catalyst to provide **14** as the ammonium salt. Finally, coupling of the phosphate **14** with UMP-morpholidate provided **3** in 37% yield,[23,24] representing a 4% overall yield from glucosamine **4**.

Chitin synthase assays were carried out by an established method[25] based on precipitation and quantification of precipitated radioactive chitin at fixed time (1 h, which is below the cutoff for linear time dependence) with fixed concentration of **3** (0 or 3 mM) in the presence of varied concentration of UDP-GlcNAc (Fig. 1). Our initial studies have led to three interesting observations. First, preliminary measurements suggest that **3** does not function as a competitive inhibitor. While this could mean it is a noncompetitive inhibitor, it is also consistent with **3** serving as a noninhibitory terminating substrate. (The kinetic profile for such a scenario is complex and will require much further study.) Second, we observe a linear decrease in precipitated chitin as a function of the mole fraction of **3** incorporated in the assay solution (data not shown), which is consistent with **3** terminating growing chitin chains in a statistical manner. Finally, enzyme activity is constant over time—chitin production remains linear out to $\geqslant$2 h in the presence of inhibitor (as it does in the absence of inhibitor).

Taken together, these data are consistent with a scenario in which incorporation of **3** leads to termination of the growing chain, after which the 4-OMe-GlcNAc terminated chitooligomers are released, regenerating active enzyme. It is important to emphasize that these are only preliminary results and that two significant challenges must be overcome to clarify the behavior of **3**. Chitin synthase is an integral membrane protein assayed as a part of a particulate membrane preparation. This makes obtaining accurate kinetic data (as opposed to the single time point/variable substrate concentration data described here) very difficult. Given that the kinetics of chain termination are complex—we are not aware of an appropriate established kinetic model—this is a significant hurdle. Similarly, isolation



Scheme 3.

and identification of the putative 4-OMe-GlcNAc terminated oligomers is not a trivial objective, since it requires separation of small quantities of short chitin chains from a complex reaction mixture—something that has not previously been accomplished—followed by appropriate characterization. Again, while not insurmountable, this is a significant obstacle. Once these issues have been addressed, we anticipate that application of this chain-termination strategy should also have application to other important polymerizing glycosyltransferases.

3. Experimental

3.1. General methods

All reactions were carried out in oven or flame-dried glassware, under an atmosphere of N_2, unless otherwise noted. THF and CH_2Cl_2 were dried by passage through an activated column of alumina, and pyridine was distilled from CaH_2. All other reagents were used as received unless otherwise stated. Thin-layer chromatography (TLC) was performed on Silica Gel 60 plates

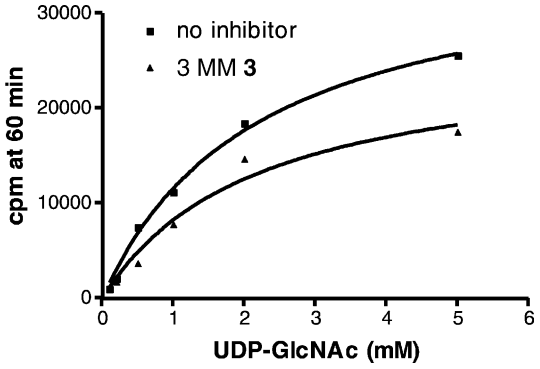


Figure 1. Preliminary evaluation of **3** as a substrate for chitin synthase.

(F_{254}, EM Science) that were visualized with UV light or stained with $KMnO_4$, ninhydrin, or phosphomolybdic acid. Flash column chromatography was performed using silica gel (Selecto Scientific, 32–63 μm) or reversed-phase silica gel (EM Science, Silica Gel 60, RP-18) as indicated. IR spectra were recorded using a Nicolet 550 spectrometer. ^{1}H NMR data were acquired on a Varian Mercury-400 (400 MHz) spectrometer and are reported in ppm downfield from Me_4Si relative to residual monoprotium solvent ($CHCl_3$ at 7.26 ppm, CHD_2OD at 3.30 ppm, HOD at 4.67 ppm). Proton-decoupled ^{13}C NMR spectra were obtained on a Varian Mercury-400 (100 MHz) spectrometer and are reported in ppm relative to solvent as internal standard ($CDCl_3$ at 77.0 ppm, CD_3OD at 49.0 ppm, added CH_3OH at 49.5 ppm for D_2O). High resolution mass spectra were obtained on an Ionspec Ultima FTMS operating in the MALDI mode (MALDI-FTMS) at the Scripps Research Institute, La Jolla, CA.

3.2. 3,6-Di-*O*-benzyl-1-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranose (9)

In a vessel equipped with a Dean–Stark trap, a suspension of dibutyltin oxide (1.10 g, 4.40 mmol, 2.2 equiv) and **8** (0.85 g, 2.00 mmol, 1 equiv) was refluxed in toluene (40 mL) for 12 h. TBAI (1.63 g, 4.40 mmol, 2.2 equiv) and BnBr (0.52 mL, 4.40 mmol, 2.2 equiv) were added, and the reaction was stirred for an additional 4 h. The reaction mixture was then concentrated under reduced pressure, and the residue was suspended in EtOAc. Insoluble material was removed by filtration, and the filtrate was concentrated under reduced pressure. Purification by silica gel chromatography (1:9 EtOAc–hexanes) afforded **9** as a yellow oil (0.86 g, 71%). ^{1}H NMR ($CDCl_3$): δ −0.08 (s, 3H), 0.06 (s, 3H), 0.68 (s, 9H), 3.11 (br s, 1H), 3.68 (m, 1H), 3.82 (m, 3H), 4.14 (dd, 1H, *J* 8.4 Hz, 11.2 Hz), 4.29 (dd, 1H, *J* 8.4 Hz, 10.8 Hz), 4.53–4.78 (m, 4H), 5.38 (d, 1H, *J* 8.0 Hz), 6.97 (m, 5H), 7.37 (m, 10H); ^{13}C NMR ($CDCl_3$): δ −5.5, −4.1, 17.6, 25.3, 57.5, 70.7, 73.6, 73.7, 74.1, 74.2, 78.3, 93.2, 122.8, 123.1, 127.2, 127.5, 127.6, 127.7, 127.9, 128.3, 133.6, 137.5, 138.0, 167.4; MALDI-HRMS: Calcd for $C_{34}H_{41}NO_7Si$ (M+Na$^+$) *m*/*z* 626.2544; found *m*/*z* 626.2552.

3.3. 3,6-Di-*O*-benzyl-1-*O*-*tert*-butyldimethylsilyl-2-deoxy-4-*O*-methyl-2-phthalimido-β-D-glucopyranose (10)

NaH (0.23 g, 5.70 mmol, 5.0 equiv) was added to a solution of **9** (0.69 g, 1.14 mmol, 1 equiv), iodomethane (7.1 mL, 114.00 mmol, 100.0 equiv), and DMF (11.4 mL) at 0 °C. The reaction was allowed to warm to room temperature and was stirred for 3 days. The solution was then poured into ice-cold brine (50 mL), extracted with EtOAc (50 mL), washed with brine (3 × 50 mL), dried over Na_2SO_4, and concentrated under reduced pressure to afford an oily residue. Purification by silica chromatography (5:95 EtOAc–hexanes) afforded **10** as a yellow solid (0.37 g, 59%). ^{1}H NMR ($CDCl_3$): δ −0.11 (s, 3H), 0.04 (s, 3H), 0.65 (s, 9H), 3.45 (dd, 1H, *J* 8.4 Hz, 9.6 Hz), 3.54 (m, 1H), 3.57 (s, 3H), 3.77 (m, 2H), 4.08 (dd, 1H, *J* 8.4 Hz, 11.2 Hz), 4.23 (dd, 1H, *J* 8.4 Hz, 11.2 Hz), 4.45–4.81 (m, 4H), 4.32 (d, 1H, *J* 8.0 Hz), 6.90 (m, 4H), 7.37 (m, 10H); ^{13}C NMR ($CDCl_3$): δ −5.5, −4.1, 17.6, 25.4, 57.8, 60.5, 68.8, 73.3, 74.2, 75.0, 78.4, 81.6, 93.1, 122.8, 123.0, 127.1, 127.3, 127.4, 127.7, 127.8, 128.1, 133.5, 137.9, 138.1, 167.4; MALDI-HRMS: Calcd for $C_{35}H_{43}NO_7Si$ (M+Na$^+$) *m*/*z* 640.2701; found *m*/*z* 640.2718.

3.4. 2-Acetamido-3,6-di-*O*-benzyl-1-*O*-*tert*-butyldimethylsilyl-2-deoxy-4-*O*-methyl-β-D-glucopyranose (11)

A suspension of **10** (0.23 g, 0.36 mmol, 1 equiv) in hydrazine (1.13 mL, 36.00 mmol, 100.0 equiv) and MeOH (3.6 mL) was heated to 55 °C for 24 h and concentrated under reduced pressure. The residue was diluted with EtOAc (50 mL), washed with brine (50 mL), dried over Na_2SO_4, and concentrated to obtain an oily residue. Purification by silica chromatography (1:1 EtOAc–hexanes) afforded the free amine, which was subsequently dissolved in 1:1 pyridine–Ac_2O (2 mL) and stirred overnight. The solution was concentrated and chromatographed on silica gel (3:7 EtOAc–hexanes) to afford **11** (0.15 g, 79%) as a clear oil. ^{1}H NMR ($CDCl_3$): δ 0.08 (s, 3H), 0.11 (s, 3H), 0.87 (s, 9H), 1.84 (s, 3H), 3.33 (m, 1H), 3.46 (m, 1H), 3.51 (s, 3H), 3.69 (m, 2H), 3.97 (dd, 1H, *J* 8.4 Hz, 10.0 Hz), 4.56–4.84 (m, 4H), 4.97 (d, 1H, *J* 7.2 Hz), 5.37 (d, 1H, *J* 8.0 Hz), 7.34 (m, 10H); ^{13}C NMR ($CDCl_3$): δ −5.2, −4.0, 18.0, 23.6, 25.7, 58.8, 60.3, 69.1, 73.3, 74.2, 74.8, 80.1, 80.7, 94.9, 127.3, 127.4, 127.5, 127.9, 128.1, 128.2, 138.2, 138.4, 169.7; MALDI-HRMS: Calcd for $C_{29}H_{43}NO_6Si$ (M+Na$^+$) *m*/*z* 552.2752; found *m*/*z* 552.2736.

3.5. 2-Acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-methyl-D-glucopyranose (12)

TBAF (0.30 mL, 1.0 M solution in THF, 0.30 mmol, 1.2 equiv) was added to a solution of **11** (0.13 g, 0.25 mmol, 1 equiv) in THF (2.5 mL) at 0 °C. After 1 h, the solution was concentrated and chromatographed on silica (2:98 MeOH–CH_2Cl_2) to afford **12** (0.10 g, 99%) as a white solid. ^{1}H NMR ($CDCl_3$): δ 1.83 (s, 3H), 3.36 (dd, 1H, *J* 8.5 Hz, 10.0 Hz), 3.51 (s, 3H), 3.69 (m, 3H), 3.95 (m, 1H), 4.09 (m, 1H), 4.55–4.88 (m, 4H), 5.22 (app t, 1H, *J* 3.2 Hz), 5.31 (d, 1H, *J* 8.8 Hz), 7.35 (m, 10H); ^{13}C NMR ($CDCl_3$): δ 23.5, 52.9, 60.6, 69.0, 70.8, 73.7, 74.8, 79.1, 80.7, 92.0, 127.5, 127.6, 127.7, 128.1, 128.2, 128.4, 138.0, 138.3, 170.0; MALDI-HRMS: Calcd for $C_{23}H_{29}NO_6$ (M+Na^+) *m*/*z* 438.1887; found *m*/*z* 438.1888.

3.6. 2-Acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-methyl-α-D-glucopyranose 1-dibenzylphosphate (13)

A solution of **12** (0.053 g, 0.13 mmol, 1 equiv) in THF (2.6 mL) was cooled to −78 °C under N_2 and LDA (0.070 mL, 2.0 M in 4:2:1.5 THF–heptane–ethylbenzene, 0.14 mmol, 1.1 equiv) was added dropwise. After 15 min, a solution of tetrabenzyl pyrophosphate (0.086 g, 0.16 mmol, 1.3 equiv) in THF (0.800 mL) was added. The reaction was warmed to 0 °C and stirred for 3 h. The solution was subsequently diluted with CH_2Cl_2 (20 mL), washed with satd aq $NaHCO_3$ (1 × 20 mL), brine (1 × 20 mL), dried over Na_2SO_4, and concentrated to obtain a yellow oil. Chromatography on silica gel (5:95 MeOH–CH_2Cl_2) afforded **13** (0.080 g, 91%) as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 1.63 (s, 3H), 3.50 (s, 4H), 3.65 (dd, 1H, *J* 4.0 Hz, 11.6 Hz), 3.81 (m, 1H), 4.22 (m, 2H), 4.45–4.84 (m, 5 H), 4.97–5.09 (m, 4H), 5.19 (d, 1H, *J* 8.8 Hz), 5.64 (dd, 1H, *J* 3.3 Hz, 5.4 Hz), 7.31 (m, 20H); ^{13}C NMR ($CDCl_3$): δ 23.1, 52.3, 60.7, 67.9, 69.6, 73.1, 73.4, 74.6, 78.9, 79.4, 97.5, 127.4, 127.5, 127.7, 127.9, 128.2, 128.3, 128.4, 128.5, 128.6, 135.2, 137.7, 138.0, 169.7. MALDI-HRMS: Calcd for $C_{37}H_{42}NO_9P$ (M+Na^+) *m*/*z* 698.2489; found *m*/*z* 698.2495.

3.7. 2-Acetamido-2-deoxy-4-*O*-methyl-α-D-glucopyranose-1-phosphate, monotriethylammonium salt (14)

A solution of **13** (0.074 g, 0.11 mmol, 1 equiv) in MeOH was placed under an H_2 atmosphere H_2 (1 atm) in presence of Pd–C (0.015 g, 5% w/w). After 6 h, the reaction was filtered through a pad of Celite. Et_3N (0.017 mL, 0.12 mmol, 1.1 equiv) was added to the filtrate, and the mixture was concentrated to afford **14** (0.046 g, quant) as a white solid. ^{1}H NMR (CD_3OD): δ 1.30 (t, 9H, *J* 7.6 Hz), 2.21 (s, 3H), 3.16 (q, 6H, *J* 7.6 Hz), 3.45 (m, 1H), 3.56 (s, 3H), 3.66 (dd, 1H, *J* 4.4 Hz, 11.6 Hz), 3.80 (m, 3H), 3.95 (dt, 1H, *J* 2.8 Hz, 10.4 Hz), 4.27 (d, 1H, *J* 8.4 Hz), 5.42 (dd, 1H, *J* 3.2 Hz, 6.8 Hz); ^{13}C NMR ($CDCl_3$): δ 9.3, 22.9, 47.4, 55.6, 60.9, 62.2, 73.3, 73.5, 81.3, 95.0, 173.6; MALDI-HRMS: Calcd for $C_9H_{18}NO_9P$ (M+Na^+) *m*/*z* 338.0611; found *m*/*z* 338.0612.

3.8. Uridinediphosphoryl 4-*O*-methyl-*N*-acetyl-glucosamine, ammonium salt (3)

A solution of **14** (0.044 g, 0.11 mmol, 1 equiv) in dry pyridine (0.550 mL) was charged to a solution of 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium uridine 5′-monophosphomorpholidate (0.151 g, 0.22 mmol, 2.0 equiv) and 1-*H*-tetrazole (0.023 g, 0.33 mmol, 3.0 equiv) in dry pyridine (0.550 mL) and stirred for 5 days at room temperature. The solution was then concentrated under reduced pressure and applied to a size-exclusion gel (Bio-Rad, Bio-Gel, P-2 Fine, 2.5 × 100 cm), eluted with 0.25 M NH_4HCO_3 and lyophilized to obtain **3** (0.027 g, 37%) as a fluffy white solid. ^{1}H NMR (D_2O): δ 1.93 (s, 3H), 3.21 (dd, 1H, *J* 9.2 Hz, 10 Hz), 3.43 (s, 3H), 3.63 (dd, 1H, *J* 4.0 Hz, 12.8 Hz), 3.73 (m, 3H), 3.82 (dt, 1H, *J* 3.6 Hz, 10.4 Hz), 4.12 (m, 3H), 4.22 (m, 2H), 5.35 (dd, 1H, *J* 3.2 Hz, 7.2 Hz), 5.83 (m, 2H), 7.82 (d, 1H, *J* 8.0 Hz); ^{13}C NMR (D_2O): δ 22.8, 54.4, 60.8, 65.5, 70.2, 71.3, 72.6, 74.4, 79.6, 83.7, 88.8, 89.1, 95.0, 103.1, 142.0, 152.2, 166.6, 175.1; MALDI-HRMS: Calcd for $C_{18}H_{29}N_3O_{17}P_2$ (M+H^-) *m*/*z* 620.0899; found *m*/*z* 620.0910.

3.9. Chitin synthase assay

The assay protocol used is based on the procedure of Orlean,[25] modified after helpful discussions with Prof. Peter Orlean (University of IL) and Dr. Enrico Cabib (NIH). *S. cerevisiae* strains (PP-1D, wild type) were kindly provided by Prof. Orlean and were stored at −70 °C on freezer stabs. Active yeast cultures were temporarily maintained on agar plates and stored at 4 °C. Cells were 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 satd medium was transferred to 400 mL of YEPG medium to give an optical density of 0.15–0.20 and allowed to grow to an optical density of 0.65–0.70. The cells were 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 was typically ~1 g; this weight was used to determine the volume of buffer in which the final pellet was suspended (see below).

The cells were suspended in 2 mL of TM buffer in a 50-mL plastic centrifuge tube, and glass beads (0.45 mm) were added until the volume of beads reaches about 3 mm below the liquid's surface. The tube was then vortexed 20 × 30 s, with 30 s of cooling on ice between each vortex; vortexing was performed in a 4 °C cold

room. The broken cells were removed from the bottom of the tube with a glass Pasteur pipet, and the glass beads were rinsed 5–7 × 1.5 mL with TM buffer. The pooled rinsings were centrifuged at 2000*g* for 4 min, the supernatant was removed, and the remaining cell-wall precipitate was washed once more with TM buffer. The cell-wall free supernatants were combined and centrifuged at 60,000*g* for 1 h.

The enzyme pellet was suspended in 1.6 mL × (gram wet weight of cells) TM buffer and homogenized thoroughly with a glass Dounce homogenizer. The membranes are pretreated with trypsin (quantified by weight, 10 min, 30 °C) and then treated with 3.0 × (mass of trypsin) trypsin inhibitor. Typically, four different concentrations of trypsin/trypsin inhibitor were tested for each new membrane preparation, and the combination with the highest activity at 30 min was used in the assay. Concentrations of trypsin typically tested were 0.5, 1.0, 2.0, and 4.0 mg/mL, and trypsin was added to the membrane preparation at a concentration of 1 mL of trypsin solution for every 5 mL of membrane preparation. Individual assays were performed in 1.5-mL Eppendorf centrifuge tubes.

The assay solution used contained UDP-GlcNAc (1.0 mM), GlcNAc (40 mM), 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 tube as a solution that was then evaporated to dryness under vacuum) in 40 mL assay solution (containing either 0 or 3.0 mM **3**) was transferred to each tube. Trypsin-treated membrane (20 mL) was then added, and the mixture was incubated for 1 h at 30 °C. The reaction was stopped by the addition of 1 mL of cold (0 °C) aq trichloroacetic acid (10% v/v) and filtered onto glass fiber filter disks (Whatman GF/C, 25 mm), rinsed with 7:3 EtOH–1 M HOAc (4 × 1 mL), and the remaining radioactivity on the filter paper was measured by scintillation counting.

Enzyme activity (in the absence of inhibitor) was typically 25–30,000 cpm/h, with a time-independent background of 500–600 cpm. Under these conditions, enzyme activity was linear (based on plots of incorporated radioactivity vs time) to at least 3 h. Control reactions run in the presence of 0.1 mM polyoxin D or nikkomycin Z, both of which are known competitive inhibitors ($K_i \approx 10\,\mu M$) of chitin synthase, always showed ⩾99% inhibition.

Acknowledgements

We thank the NIH (GM60875), the Hellman Foundation and the UC Systemwide Biotechnology Program for direct support and the NIH and NSF for infrastructure support (GM62116, GM61894, CHE-9709183). We thank Prof. Peter Orlean (U of IL) and Dr. Enrico Cabib (NIH) for assay advice, Prof. Orlean for donation of yeast strains, Prof. Scott Singleton (UNC Chapel Hill) for helpful discussion and Prof. Jack Kyte and Mr. Steven Adams (UCSD) for technical assistance.

References

1. Munro, C. A.; Gow, N. A. R. *Med. Mycol.* **2001**, *39*, 41–53.
2. Valdivieso, M. H.; Duran, A.; Roncero, C. *EXS* **1999**, *87*, 55–69.
3. Merz, R. A.; Horsch, M.; Nyhlen, L. E.; Rast, D. M. *EXS* **1999**, *87*, 9–37.
4. Bulawa, C. E. *Annu. Rev. Microbiol.* **1993**, *47*, 505–534.
5. Cabib, E. *Adv. Enzymol.* **1987**, *59*, 59–101.
6. Varki, A.; Cummings, R.; Esko, J.; Freeze, H.; Hart, G.; Marth, J. *Essentials of Glycobiology*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York, 1999.
7. Behr, J. B.; Gourlain, T.; Helimi, A.; Guillerm, G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1713–1716.
8. Xie, J.; Thellend, A.; Becker, H.; Vidal-Cros, A. *Carbohydr. Res.* **2001**, *334*, 177–182.
9. Behr, J.-B.; Gautier-Lefebvre, I.; Mvondo-Evina, C.; Guillerm, G.; Ryder, N. S. *J. Enzyme Inhib.* **2001**, *16*, 107–112.
10. Grugier, J.; Xie, J.; Duarte, I.; Valery, J.-M. *J. Org. Chem.* **2000**, *65*, 979–984.
11. Schafer, A.; Thiem, J. *J. Org. Chem.* **2000**, *65*, 24–29.
12. Obi, K.; Uda, J.-I.; Iwase, K.; Sugimoto, O.; Ebisu, H.; Matsuda, A. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1451–1454.
13. More, J. D.; Finney, N. S. *Org. Lett.* **2002**, *4*, 3001.
14. Chang, R.; Yeager, A. R.; Finney, N. S. *Org. Biomol. Chem.* **2003**, *1*, 39–41.
15. More, J. D.; Finney, N. S. *Synlett* **2003**, *9*, 1307.
16. Sugiyama, J.; Boisset, C.; Hashimoto, M.; Watanabe, T. *J. Mol. Biol.* **1999**, *286*, 247–255.
17. For a previous synthesis of 2-, 3-, and 4-deoxy-UDP-GlcNAc, see: (a) Srivastava, G.; Alton, G.; Hindsgaul, O. *Carbohydr. Res.* **1990**, *207*, 259–276; For a review of modified sugar nucleotides, see: (b) Elhalabi, J. M.; Rice, K. G. *Curr. Med. Chem.* **1999**, *6*, 93–116.
18. Mohamed, R. E. A.; Castro-Palomino, J. C.; Ibrahim, E. I.; El-Ashry, E. H.; Schmidt, R. R. *Eur. J. Org. Chem.* **1998**, *11*, 2305–2316.
19. *Preparative Carbohydrate Chemistry*; Hanessian, S., Ed.; Marcel Dekker: New York, 1997.
20. Park, T. K.; Kim, I. J.; Hu, S.; Bilodeau, J.; Randolph, J. T.; Kwon, O.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1996**, *118*, 11488–11500.
21. Simas, A. B. C.; Pais, K. C.; da Silva, A. A. T. *J. Org. Chem.* **2003**, *68*, 5426–5428.
22. Khorana, H. G.; Todd, A. R. *J. Chem. Soc.* **1953**, 2257–2260.
23. Wong, C. H.; Wittman, V. *J. Org. Chem.* **1997**, *57*, 2144–2147.
24. Moffatt, J. G. *Methods Enzymol.* **1966**, *8*, 136–142.
25. Orlean, P. *J. Biol. Chem.* **1987**, *262*, 5732–5739.