Supplementary Material (ESI) for Chemical Communications

Supporting Material

Inhibition of *O*-GlcNAcase by a *gluco*-configured Nagstatin and a PUGNAc-imidazole Hybrid inhibitor.

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Synthesis of PUGNAc-Nagstatin hybrid

General: See reference[1] for details.

*(5*R*,6*R*,7*S*,8*S*)-6,7-Bis(benzyloxy)-5-[(benzyloxy)methyl]-2,3-diiodo-5,6,7,8-tetrahydro-8-[(triisopropylsilyl)oxy]-imidazo[1,2-*a*]pyridine* (**7**). Under Ar, a soln. of **6** [2] (316 mg, 0.5 mmol) in DMF (7 ml) was treated with *N*-iodosuccinimide (1.13 g, 5.0 mmol), heated to 80°, and stirred for 6 h. The brown mixture was diluted with Et_2O, washed successively with sat. NH_4Cl soln., a 5% aq. NaS_2O_3 soln., and H_2O, dried (Na_2SO_4), and filtered. Evaporation and FC (hexane/AcOEt 9:1) gave **7** (390 mg, 88%). Colourless viscous liquid. R_f (hexane/AcOEt 9:1) 0.63. $[\alpha]_D^{25} = -31.4$ (c = 1.125, $CHCl_3$). UV ($CHCl_3$): 242 (3.9). IR ($CHCl_3$): 3019*s*, 2944*m*, 2867*m*, 1496*m*, 1454*s*, 1390*m*, 1250*w*, 1213*s*, 1095*s*, 1029*m*, 882*m*, 699*s*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 1.04–1.23 (*m*, $(Me_2CH)_3Si$); 3.61 (*dd*, J = 4.6, 9.8, CH–C(5)); 3.67 (*t*, $J \approx$ 8.4, CH′–C(5)); 3.96 (*t*, $J \approx$ 3.8, irrad. at 5.00 → *d*, $J \approx$ 3.9, H–C(7)); 4.34 (*dd*, J = 1.6, 4.2, irrad. at 3.96 → *d*, $J \approx$ 1.5, H–C(6)); 4.41 (*d*, J = 11.3, PhC*H*); 4.39–4.42 (*m*, H–C(5)); 4.4.3 (*d*, J = 12.1, PhC*H*); 4.54 (*d*, J = 11.3, PhC*H*); 4.55 (*d*, J = 12.1, PhC*H*); 4.59 (*d*, J = 10.3, PhC*H*); 4.63 (*d*, J = 12.1, PhC*H*); 5.00 (*d*, J = 3.7, irrad. at 3.96 → *s*, H–C(8)); 7.16–7.22 (*m*, 4 arom. H), 7.27–7.33 (*m*, 11 arom. H). ^{13}C-NMR ($CDCl_3$, 75 MHz; assignment based on a HSQC spectrum): 12.69 (*d*, $(Me_2CH)_3Si$); 18.14, 18.20 (2*q*, $(Me_2CH)_3Si$); 60.46 (*d*, C(5)); 69.68 (*t*, CH_2–C(5)); 71.93, 72.94, 73.14 (3*t*, PhCH_2); 67.23 (*d*, C(8)); 72.92 (*d*, C(6)); 79.92 (*d*, C(7)); 80.20 (*s*, C(2)); 96.58 (*s*, C(3)); 127.62–128.46 (several *d*); 137.52, 137.59, 137.69 (3*s*); 150.66 (*s*, C(8a)). HR-MALDI-MS: 901.1386 (15, $[M + Na]^+$, $C_{38}H_{48}I_2N_2NaO_4Si^+$; calc. 901.1370), 880.1565 (28), 879.1531 (60, $[M + H]^+$, $C_{38}H_{49}I_2N_2O_4Si^+$; calc. 879.1551), 753.2592 (11, $[M - I + 2\ H]^+$, $C_{38}H_{50}IN_2O_4Si^+$; calc. 753.2585), 706.0161

(28), 705.0125 (87, [*M* – OTIPS]$^+$, $C_{29}H_{27}I_2N_2O_3^+$; calc. 705.0111), 578.1077 (100, [*M* – OTIPS – I]$^+$, $C_{29}H_{27}IN_2O_3^+$; calc. 578.1066). Anal. calc. for $C_{38}H_{48}I_2N_2O_4Si$ (878.70): C 51.94, H 5.51, N 3.19; found: C 52.18, H 5.64, N 3.04.

*(5*R*,6*R*,7*S*,8*S*)-6,7-Bis(benzyloxy)-5-[(benzyloxy)methyl]-2-iodo-5,6,7,8-tetrahydro-8-[(triisopropylsilyl)oxy]imidazo[1,2-*a*]pyridine* (**8**). A soln. of **7** (690 mg, 0.79 mmol) in THF (10 ml) was treated at 0° with a 1M soln. of EtMgBr in THF (1.2 ml, 1.2 mmol), stirred for 10 min, and treated with a sat. NH_4Cl soln. Extraction with AcOEt, work up, and FC (hexane/AcOEt) gave **8** (516 mg, 88%). Viscous liquid. R_f (hexane/AcOEt 4:1) 0.58. $[\alpha]_D^{25}$ = +22.1 (*c* = 1.2, $CHCl_3$). UV ($CHCl_3$): 241 (3.6), 232 (3.2). IR ($CHCl_3$): 3031*w*, 3015*m*, 2945*s*, 2867*s*, 1602*m*, 1496*m*, 1454*s*, 1424*m*, 1363*m*, 1363*m*, 1261*s*, 1219*s*, 1097*s*, 1015*s*, 882*m*, 809*s*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 0.98–1.20 (*m*, $(Me_2CH)_3Si$); 3.71 (*dd*, *J* = 3.1, 8.9, H–C(6)); 3.76 (*dd*, *J* = 5.9, 10.6, CH–C(5)); 3.85 (*dd*, *J* = 2.8, 10.9, CH′–C(5)); 4.07 (*t*, *J* = 3.4, H–C(7)); 4.32–4.38 (*m*, H–C(5)); 4.39 (*d*, *J* = 11.8, PhC*H*); 4.51 (*s*, PhCH_2); 4.55, 4.59, 4.71 (3*d*, *J* = 11.8, 3 PhC*H*); 5.09 (*d*, *J* = 3.1, H–C(8)); 7.10 (*s*, H–C(3)); 7.17–7.19 (*m*, 2 arom. H); 7.30–7.38 (*m*, 13 arom. H). ^{13}C-NMR ($CDCl_3$, 75 MHz; assignment based on HSQC spectrum): 14.76 (*d*, $(Me_2CH)_3Si$); 18.15, 18.33 (2*q*, $(Me_2CH)_3Si$); 57.26 (*d*, C(5)); 66.13 (*d*, C(8)); 67.95 (*t*, CH_2–C(5)); 72.44, 72.66, 73.72 (3*t*, 3 PhCH_2); 78.50 (*d*, C(6)); 80.90 (*s*, C(2)); 82.44 (*d*, C(7)); 123.13 (*d*, C(3)); 128.11–128.82 (several *d*); 137.41, 137.68, 137.73 (3*s*); 147.79 (*s*, C(8a)). HR-MALDI-MS: 775.2366 (7, [*M* + Na]$^+$, $C_{38}H_{49}IN_2NaO_4Si^+$; calc. 775.2404), 754.2595 (38), 753.2566 (70, [*M* + H]$^+$, $C_{38}H_{50}IN_2O_4Si^+$; calc. 753.2585), 580.1193 (39), 579.1151 (100, [*M* – OTIPS]$^+$, $C_{29}H_{28}IN_2O_3^+$; calc. 579.1145), 453.2153

(9), 452.2098 (20, [*M* – OTIPS – I]$^+$, $C_{29}H_{28}N_2O_3^+$; calc. 452.2100). Anal. calc. for $C_{38}H_{49}IN_2O_4Si$ (752.81): C 60.63, H 6.56, N 3.72; found: C 60.43, H 6.56, N 3.71.

*(5*R*,6*R*,7*S*,8*S*)-6,7-Bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydro*-N-*phenyl-8-[(triisopropylsilyl)oxy]imidazo[1,2-*a*]pyridine-2-carboxamide* (**9**). At 0°, a soln. of **8** (134 mg, 0.18 mmol) in THF (5 ml) was treated with a 1M soln. of EtMgBr in THF (0.26 ml), stirred for 10 min, treated with PhNCO (0.22 ml, 1.8 mmol), warmed to 23° within 30 min, and stirred for 2 h. The mixture was cooled to 0°, diluted with sat. NH_4Cl soln., and extracted with Et_2O. The org. layer was washed with sat. $NaHCO_3$ soln. and brine, dried ($NaSO_4$), and evaporated. FC (hexane/AcOEt 4:1) gave **9** (108 mg, 81%). Viscous liquid. R_f (hexane/AcOEt 7:3) 0.52. IR ($CHCl_3$): 3433*m*, 3373*m*, 3090*w*, 3066*m*, 3020*s*, 2945*s*, 2867*s*, 1672*s*, 1596*s*, 1556*s*, 1499*s*, 1443*m*, 1363*m*, 1316*m*, 1268*m*, 1095*s*, 1028*s*, 883*m*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 1.07–1.27 (*m*, $(Me_2CH)_3Si$); 3.73 (*dd*, *J* = 5.3, 10.6, CH–C(5)); 3.82–3.88 (*m*, CH′–C(5), H–C(6)); 4.03 (*t*, *J* = 5.0, H–C(7)); 4.27–4.31 (*m*, H–C(5)); 4.41 (*d*, *J* = 11.5, PhC*H*); 4.51 (*s*, PhCH_2); 4.67, 4.74 (2*d*, *J* = 11.2, 2 PhC*H*); 4.78 (*d*, *J* = 11.8, PhC*H*); 5.05 (*d*, *J* = 5.0, H–C(8)); 7.10–7.14 (*m*, 2 arom. H); 7.25–7.39 (*m*, 16 arom. H); 7.69 (*dd*, *J* = 1.2, 7.5, 2 arom. H); 7.75 (*s*, H–C(3)); 9.03 (*s*, NH). ^{13}C-NMR ($CDCl_3$, 75 MHz): 12.75 (*d*, $(Me_2CH)_3Si$); 18.18, 18.27 (2*q*, $(Me_2CH)_3Si$); 58.18 (*d*, C(5)); 67.60 (*d*, C(8)); 67.68 (*t*, CH_2–C(5)); 73.21, 73.35, 73.39 (3*t*, 3 PhCH_2); 76.91 (*d*, C(6)); 82.52 (*d*, C(7)); 119.34 (*d*, C(2) and C(6) of PhN); 120.71 (*d*, C(3)); 123.70 (*d*, C(4) of PhN); 127.80–128.96 (several *d*); 136.07, 136.97, 137.30, 137.55, 138.18 (5*s*); 145.47 (*s*, C(8a)); 160.72 (*s*, C=O). HR-MALDI-MS: 768.3746 (19, [*M* + Na]$^+$, $C_{45}H_{55}N_3NaO_5Si^+$; calc. 768.3809), 747.3999 (55), 746.3971 (89, [*M* + H]$^+$, $C_{45}H_{56}N_3O_5Si^+$; calc. 746.3989), 627.3579 (100, [*M* – CONHPh + 2 H]$^+$, $C_{38}H_{51}N_2O_4Si^+$;

calc. 627.3618), 572.2496 (28, [*M* – OTIPS]$^+$, $C_{36}H_{34}N_3O_4^+$; calc. 572.2549), 454.2208 (30, [*M* – OTIPS – CONHPh + 2 H]$^+$, $C_{29}H_{30}N_2O_3^+$; calc. 454.2256).

*(5*R*,6*R*,7*S*,8*S*)-6,7-Bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydro-8-hydroxy*-N-*phenylimidazo[1,2-*a*]pyridine-2-carboxamide* (**10**). A soln. of **9** (108 mg, 0.15 mmol) in THF (2 ml) was cooled to 0°, treated with 1M Bu_4NF in THF (0.3 ml, 0.3 mmol), stirred for 3 h, diluted with sat. NH_4Cl soln., and extracted with AcOEt. The combined org. layers were washed with sat. $NaHCO_3$ soln. and brine, dried (Na_2SO_4), and evaporated. FC (hexane/AcOEt 1:1) gave **10** (80 mg, 93%). White solid. M.p. 62–64°. R_f (hexane/AcOEt 1:1) 0.36. $[\alpha]_D^{25}$ = –25.0 (*c* = 0.98, $CHCl_3$). UV ($CHCl_3$): 268 (4.3). IR ($CHCl_3$): 3615*m*, 3496*m*, 3019*s*, 2975*m*, 2871*w*, 1672*s*, 1597*s*, 1561*s*, 1522*s*, 1498*m*, 1364*w*, 1327*m*, 1220*s*, 1099*m*, 1046*m*, 877*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 3.71 (*dd*, *J* = 5.6, 10.6, CH–C(5)); 3.77 (*dd*, *J* = 4.3 10.6, CH′–C(5)); 4.03 (*dd*, *J* = 4.8, 6.3, irrad. at 4.85 → *d*, *J* ≈ 6.3, H–C(7)); 4.07 (*dd*, *J* = 4.5, 6.3, H–C(6)); 4.32 (br. *q*, *J* ≈ 4.5, H–C(5)); 4.42 (*d*, *J* = 11.5, PhC*H*); 4.45–4.52 (br. *s*, exchange with D_2O, OH); 4.48 (*d*, *J* = 12.1, PhC*H*); 4.57, 4.70, 4.77, 4.79 (4*d*, *J* = 11.5, 4 PhC*H*); 4.85 (*d*, *J* = 5.1, irrad. at 4.03 → *s*, H–C(8)); 7.09 (br. *t*, *J* ≈ 7.2, 1 arom. H), 7.18–7.37 (*m*, 17 arom. H), 7.71 (*dd*, *J* = 1.0, 7.5, 2 arom. H); 7.78 (*s*, H–C(3)); 9.04 (*s*, exchange with D_2O, NH). ^{13}C-NMR ($CDCl_3$, 75 MHz): 59.31 (*d*, C(5)); 66.91 (*d*, C(8)); 69.37 (*t*, *C*H_2–C(5)); 73.69, 74.16, 74.20 (3*t*, 3 Ph*C*H_2); 76.90 (*d*, C(6)); 80.01 (*d*, C(7)); 119.34 (*d*, C(2) and C(6) of PhN); 121.91 (*d*, C(3)); 124.09 (*d*, C(4) of PhN); 127.80–128.96 (several *d*); 136.97, 137.29, 137.56, 137.61, 138.38 (5*s*); 145.24 (*s*, C(8a)); 160.54 (*s*, C=O). HR-MALDI-MS: 612.2463 (17, [*M* + Na]$^+$, $C_{36}H_{35}N_3NaO_5^+$; calc. 612.2474), 591.2668 (50), 590.2641 (100, [*M* + H]$^+$, $C_{36}H_{36}N_3O_5^+$; calc. 590.2655), 497.2068 (18, [*M* – NHPh]$^+$,

$C_{30}H_{29}N_2O_5^+$; calc. 497.2076). Anal. calc. for $C_{36}H_{35}N_3O_5$ (589.69): C 73.33, H 5.98, N 7.13; found: C 73.07, H 6.15, N 7.13.

*(5*R*,6*R*,7*S*,8*S*)- and (5*R*,6*R*,7*S*,8*R*)-8-Azido-6,7-bis(benzyloxy)-5-[(benzyl-oxy)methyl]-5,6,7,8-tetrahydro*-N-*phenylimidazo[1,2-*a*]pyridine-2-carboxamide* (**11** and **12**). A soln. of **10** (235 mg, 0.39 mmol) and diphenyl phosphoryl azide (DPPA; 0.43 ml, 1.99 mmol) in dry toluene was heated to 55°, treated dropwise within 15 min with a soln. of DBU (61 μl, 1.99 mmol) in toluene (1 ml), stirred for 6 h, cooled to r.t., diluted with sat. NH_4Cl soln., and extracted with Et_2O. The combined org. layers were washed with brine, dried (Na_2SO_4), and evaporated. FC (hexane/AcOEt 7:3) gave **11**/**12** 2:3 (209 mg, 85%). R_f (hexane/AcOEt 7:3) 0.32. IR ($CHCl_3$): 3379*s*, 3090*m*, 3013*s*, 2931*m*, 2870*s*, 2110*s*, 1673*s*, 1597*s*, 1552*s*, 1498*m*, 1363*m*, 1295*s*, 1203*s*, 1106*s*, 1028*m*, 990*m*. ^{1}H-NMR (C_6D_6, 300 MHz, **11**/**12** 2:3): 3.31 (*dd*, *J* = 5.8, 10.0, 0.4 H), 3.36 (*dd*, *J* = 4.1, 10.8, 0.6 H) (CH–C(5)); 3.42–3.47 (*m*, CH′–C(5)); 3.56 (*t*, *J* ≈ 7.9, 0.4 H–C(7)); 3.62–3.76 (*m*, 0.6 H–C(7), 0.6 H–C(5), H–C(6)); 3.90 (*q*, *J* ≈ 5.2, 0.4 H–C(5)); 3.98 (*d*, *J* = 12.0, 0.6 PhC*H*); 4.03 (*d*, *J* = 12.3, 0.6 PhC*H*); 4.08 (*d*, *J* = 13.1, 0.4 PhC*H*); 4.09 (*d*, *J* = 12.1, 0.6 PhC*H*); 4.25 (*d*, *J* = 12.3, 0.6 PhC*H*); 4.30 (*d*, *J* = 12.3, PhC*H*); 4.39 (*d*, *J* = 7.4, irrad. at 3.56 → *s*, 0.4 H–C(8)); 4.42 (*d*, *J* = 11.5, 0.4 PhC*H*); 4.48 (*d*, *J* = 11.5, 0.6 PhC*H*); 4.57 (*d*, *J* = 11.3, 0.4 PhC*H*); 4.64 (*d*, *J* = 11.0, 0.4 PhC*H*); 4.67 (*d*, *J* = 11.5, 0.4 PhC*H*); 4.78 (*d*, *J* = 3.9, irrad. at 3.68 → *s*, 0.6 H–C(8)); 6.83–6.89 (*m*, 1 arom. H); 7.05–7.28 (*m*, 17 arom. H); 7.74–7.78 (*m*, 2 arom. H); 7.85 (*s*, 0.4 H), 7.91 (*s*, 0.6 H) (H–C(3)); 9.31 (*s*, 0.6 H), 9.32 (*s*, 0.4 H) (N*H*). ^{13}C-NMR (C_6D_6, 75 MHz, **11**/**12** 2:3): Signals of **11**: 58.61 (*d*, C(8)); 59.19 (*d*, C(5)); 65.76 (*t*, CH_2–C(5)); 73.31, 74.56, 74.89 (3*t*, 3 $PhCH_2$); 74.76 (*d*, C(6)); 122.21 (*d*, C(3)); 141.08 (*s*, C(8a); signals of **12**: 55.14 (*d*, C(8)); 59.27 (*d*, C(5));

67.68 (*t*, CH_2–C(5)); 69.72, 72.50, 73.76 (3*t*, 3 $PhCH_2$); 77.37 (*d*, C(6)); 123.82 (*d*, C(3)); 140.34 (*s*, C(8a)); signals of **11** and **12**: 80.35 (*d*, C(7)); 119.61 (*d*, C(2) and C(6) of PhN); 121.19 (*d*, C(4) of PhN); 127.82–128.88 (several *d*); 136.63, 136.76, 136.89, 136.93, 136.95, 137.31, 137.60, 137.91 (8*s*); 160.06 (*s*, C=O).

Transformation of **11**/**12** *into* **13** *and* **4**. A soln. of **11/12** 3:2 (170 mg, 0.27 mmol) in AcOH (5 ml) was treated with 10% Pd/C, hydrogenated under 6 bar of H_2 for 18 h, and filtered over *Celite*. The filtrate was evaporated and co-evaporated with toluene. A soln. of the residue in pyridine (1 ml) was treated with Ac_2O (2 ml) and stirred for 24 h at r.t. Evaporation and co-evaporation with toluene gave a yellow oil (108 mg), which was dissolved in 3M NH_3 in MeOH and stirred at r.t. for 24 h. Evaporation and co-evaporation with toluene yielded crude **13**/**4** which was separated by reversed-phase preparative chromatography (*Lichrospher RP-18e*, 250 x 4 mm column; H_2O/MeCN 4:1) to give **13** (23 mg, 23%) and **4** (17 mg, 17%). Crystallisation from H_2O/MeCN 85:15 and drying at 10^{-4} Torr for 4 d gave pure samples of **13** and **4** for analysis.

*Data of (5*R*,6*R*,7*S*,8*S*)-8-Acetamido-5,6,7,8-tetrahydro-6,7-dihydroxy-5-(hydroxy-methyl)*-N-*phenylimidazo[1,2*-a*]pyridine-2-carboxamide* (**13**). R_f (reversed phase; H_2O/MeCN 4:1) 0.47. ^{1}H-NMR (CD_3OD, 300 MHz): 2.11 (*s*, AcN); 3.81 (*t*, *J* = 8.7, irrad. at 4.99 → *d*, *J* ≈ 9.0, H–C(7)); 3.91 (*t*, *J* = 8.7, H–C(6)); 3.96–4.02 (*m*, H–C(5), CH–C(5)); 4.22 (*dd*, *J* = 4.0, 13.5, CH′–C(5)); 4.99 (*d*, *J* = 9.0, irrad. at 3.81 → *s*, H–C(8)); 7.12 (*tt*, *J* ≈ 0.9, 7.0, H–C(4) of PhN); 7.33 (*tdd*, *J* = 1.8, 7.5, 8.4, H–C(3) and H–C(5) of PhN); 7.69 (br. *dd*, *J* = 0.9, 8.7, H–C(2) and H–C(6) of PhN); 7.99 (*s*, H–C(3)). ^{13}C-NMR (CD_3OD, 75 MHz): 22.69 (*q*, Me); 51.08 (*d*, C(8)); 61.22 (*t*, *C*H_2–C(5)); 62.98 (*d*, C(5)); 69.55 (*d*, C(6)); 73.64 (*d*, C(7)); 120.65 (*d*, C(2) and C(6) of PhN); 123.08 (*d*,

H–C(3)); 124.78 (*d*, C(4) of PhN); 129.41 (*d*, C(3) and C(5) of PhN); 137.12, 139.07 (2*s*, C(1) of PhN, C(2)); 146.03 (*s*, C(8a)); 162.27 (*s*, N*C*=O); 173.23 (*s*, Me*C*=O). HR-MALDI-MS: 383.1320 (52, $[M + Na]^+$, $C_{17}H_{20}N_4NaO_5^+$; calc. 383.1331), 361.1504 (37, $[M + H]^+$, $C_{17}H_{21}N_4O_5^+$; calc. 361.1512), 343.1398 (100, $[M - OH]^+$, $C_{17}H_{19}N_4O_4^+$; calc. 343.1406), 303.0193 (24), 301.1293 (33). Anal. calc. for $C_{17}H_{20}N_4O_5 \cdot 2\ H_2O$ (396.14): C 51.51, H 6.10, N 14.03; found: C 51.67, H 5.84, N 13.68.

*Data of (5*R*,6*R*,7*S*,8*R*)-8-Acetamido-5,6,7,8-tetrahydro-6,7-dihydroxy-5-(hydroxy-methyl)*-N-*phenylimidazo[1,2-*a*]pyridine-2-carboxamide* (**4**). R_f (reversed phase; H_2O/MeCN 4:1) 0.40. ^{1}H-NMR (CD_3OD, 300 MHz): 2.13 (*s*, AcN); 3.96 (*dd*, *J* = 6.0, 11.5, CH–C(5)); 4.02 (*dd*, *J* = 4.4, 11.8, CH′–C(5)); 4.14 (*dd*, *J* = 4.0, 6.0, irrad. at 5.53 → *d*, *J* ≈ 6.0, H–C(7)); 4.21–4.23 (*m*, H–C(5), H–C(6)); 5.53 (*d*, *J* = 3.7, irrad. at 4.14 → *s*, H–C(8)); 7.11 (*tt*, *J* ≈ 0.9, 7.5, H–C(4) of PhN); 7.33 (br. *tt*, *J* ≈ 2.1, 8.3, H–C(3) and H–C(5) of PhN); 7.70 (br. *dd*, *J* = 0.9, 8.7, H–C(2) and H–C(6) of PhN); 7.94 (*s*, H–C(3)). ^{13}C-NMR (CD_3OD, 75 MHz): 22.56 (*q*, *Me*C=O); 46.82 (*d*, C(8)); 63.95 (*t*, *C*H_2–C(5)); 65.04 (*d*, C(5)); 69.39 (*d*, C(6)); 69.79 (*d*, C(7)); 120.67 (*d*, C(2) and C(6) of PhN); 124.48 (*d*, C(3)); 124.79 (*d*, C(4) of PhN); 129.44 (*d*, C(3) and C(5) of PhN); 136.40, 139.12 (2*s*, C(1) of Ph, C(2)); 144.77 (*s*, C(8a)); 162.37 (*s*, N*C*=O); 173.00 (*s*, Me*C*=O).

Cloning and Over-Expression

The BtGH84 ORF was truncated to produce an N-terminally tagged recombinant protein beginning at residue N23 to omit the predicted signal peptide with subsequent gene expression and protein purification as described for the native enzyme previously [3].

Crystallization and Structure Solution

BtGH84 crystals were grown using hanging drop vapour diffusion from 0.6M Sodium Acetate, 13% (v/v) PEG 3500, 0.1M MES (pH 6.0) and 10% (v/v) glycerol. An additional 20% glycerol was included prior to flash freezing in liquid nitrogen. Crystals of *Bt*GH84 in complex with PUGNAc-Imidazole hybrid inhibitor were obtained by co-crystallization with 10mM ligand. X-ray data were collected from a single crystal at 100K at the European Synchrotron Radiation Facility on beamline ID 14-2. All X-ray data were processed with HKL2000, Table 1S. A calculated Matthews' coefficient of 2.4 $Å^3$ Da^{-1} and a theoretical solvent content of 49% are consistent with the asymmetric unit containing one *Bt*GH84 monomer. Structure solution was carried out using the molecular replacement technique as implemented in AMORE[4] using *Bt*GH84 (PDB code 2CHO) as the search model. COOT[5] was used to make manual corrections and augmentations to the model followed by refinement using REFMAC[6]. Solvent molecules were added using COOT and checked manually. The BtGH84 structure can be traced from residue 4 of the mature protein to residue 589, with two short disordered loops between residues 20-25 and 47-54. The C-terminal domain is, however, extremely mobile in the crystal and disordered in the electron density.

Coordinates have been deposited with the Protein Databank, PDB CODE 2j47.

Kinetic Analysis of Human O-GlcNAcase

All assays were carried out in triplicate at 37 °C for 45 minutes by using a stopped assay procedure in which the enzymatic reactions (80 μL) were quenched by the addition of a 4-fold excess (320 μL) of quenching buffer (200 mM glycine, pH 10.75). Assays were initiated by the addition, *via* syringe, of enzyme (10 μL), and in all cases the final pH of the resulting quenched solution was greater than 10. A time-dependent assay of the human *O*-GlcNAcase revealed that it was stable over the period of the assay: 50 mM NaH_2PO_4, 100 mM NaCl, 0.1% BSA, pH 7.4 and 50 mM citrate, 100 mM NaCl, 0.1% BSA, pH 4.25. The progress of the reaction at the end of 45 minutes was determined by measuring the extent of methyl umbelliferone liberated as determined by fluorescence measurements using a Varian CARY Eclipse Fluorescence Spectrophotometer 96-well plate system. Excitation and emission wavelengths of 368 and 450 nM were used, respectively, with 5 mm slit openings. Activity was defined as the absolute fluorescence detected for the 45 minute period. *O*-GlcNAcase was expressed fresh prior to use and was used in the inhibition assays at a concentration of 0.001 μg/μL. The inhibitor was tested at six concentrations ranging from 0.00 to 18 μM.

Kinetic Analysis of Bacterial O-GlcNAcase

Enzymatic reactions were carried out in PBS buffer (pH 7.4) and all assays were carried out by monitoring continuously at 37 ºC and 400 nM the release of 4-nitrophenol from the reaction mixture. The rate of release was measured using a Cary 3E UV-VIS spectrophotometer equipped with a Peltier temperature controller. Reactions (80 μL) were pre-heated in a 50 μl microcuvette (Varian) for approximately 2 min followed by addition of enzyme (10 μL) *via* syringe. Reaction velocities were determined by linear

regression of the linear region of the reaction progress curve between the first and second minute. The bacterial *O*-GlcNAcase was expressed fresh prior to use and was used in the inhibition assays at a concentration of 0.0008 μg/μL. The inhibitor was tested at six concentrations ranging from 0.00 to 20 μM.

Supplementary Material (ESI) for Chemical Communications

Table 1S

Data collection, phasing and refinement statistics for the structure solution of *B. thetaiotaomicron* GH84 *O*-GlcNAcase with PUGNAc-Imidazole hybrid Inhibitor

	*Bt*GH84 with PUGNAc-Imidazole
Data collection	
Space group	C2
Cell dimensions	
a, *b*, *c* (Å)	188.4,51.3,85.5
α, β, γ (°)	90.0,100.2,90.0
Wavelength	0.933
Resolution (Å)	50-1.97 (2.04-1.97)*
R_{sym} or R_{merge}	0.083 (0.491)
$I / \sigma I$	17 (3)
Completeness (%)	100 (99)
Redundancy	4.5 (4.4)
Refinement	
Resolution (Å)	1.97
No. reflections	53713
R_{work} / R_{free}	0.19/0.23
No. atoms	
Protein	4674
Ligand/ion	26
Water	319
B-factors	
Protein	26
Ligand/ion	16
Water	35
R.m.s deviations	
Bond lengths (Å)	0.016
Bond angles (°)	1.5

*Highest-resolution shell is shown in parentheses.

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