Supplementary Material

Detailed comparative analysis of the catalytic mechanisms of β-*N*-acetylglucosaminidases from families 3 and 20 of glycoside hydrolases.

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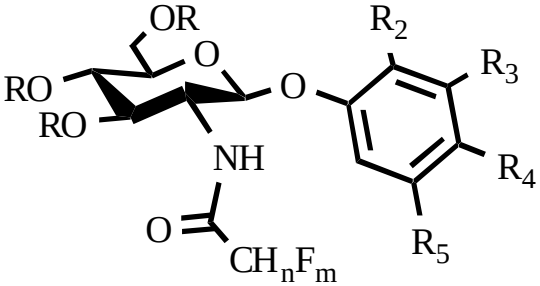
Substrate	Compound Number	Enzyme	[Enzyme] (nM)	Assay Wavelength (nm)	Δε ($mM^{-1}cm^{-1}$)
2,3DNPGlcNAc	**5**	SpHex	16.8	400	**0.544**
		ExoII	95	400	**2.67**
3,5DNPGlcNAc	**6**	SpHex	5.6	350	**1.25**
		ExoII	221	400	**1.49**
pNPGlcNAc	**1**	SpHex	1.6	360	**2.05**
		ExoII	189	400	**7.28**
MuGlcNAc	**8**	SpHex	8.4	360	**2.21**
		ExoII	189	360	**3.24**
mNPGlcNAc	**9**	SpHex	16.8	360	**0.318**
		ExoII	189	400	**0.39**
pNHAcGlcNAc	**10**	SpHex	5.6	288	**0.48**
		ExoII	ND	ND	**ND**
mNHAcGlcNAc	**11**	SpHex	5.6	288	**1.16**
		ExoII	4772	288	**1.02**
PGlcNAc	**12**	SpHex	16.8	277	**0.978**
		ExoII	9544	277	**0.778**
$pNPGlcNAcF_3$	**4**	SpHex	660	360	**2.05**
		ExoII	189	400	**7.28**
$pNPGlcNAcF_2$	**3**	SpHex	16.7	360	**2.05**
		ExoII	189	400	**7.28**
$pNPGlcNAcF_1$	**2**	SpHex	8.5	360	**2.05**
		ExoII	**189**	**400**	**7.28**



The chemical structure of the aryl 2-acetamido-2-deoxy-glucospyranosides used in this work. A table containing the list of R, m, and n, groups can be found on the subsequent page

Table 1 The series of aryl 2-acetamido-2-deoxy-β-D-glucopyranosides used in this study.

Compound Name	Compound Number	R	R_2	R_3	R_4	R_5	n	m	Melting Point and Elemental Analysis
2,3DNPGlcNAc-OAc	**5-Ac**	Ac	NO_2	NO_2	H	H	3	0	N/D
3,5DNPGlcNAc-OAc	**6-Ac**	Ac	H	NO_2	H	NO_2	3	0	N/D
pNPGlcNAc-OAc	**1-Ac**	Ac	H	H	NO_2	H	3	0	N/D
(^{2}H-1)pNPGlcNAc-OAc	**19-Ac**	Ac	H	H	NO_2	H	3	0	N/D
pNPGlcNAcF_3-OAc	**4-Ac**	Ac	H	H	NO_2	H	0	3	N/D
mNPGlcNAc-OAc	**9-Ac**	Ac	H	NO_2	H	H	3	0	N/D
pNAcGlcNAc-OAc	**10-Ac**	Ac	H	H	NHAc	H	3	0	N/D
mNAcGlcNAc-OAc	**11-Ac**	Ac	H	NHAc	H	NO_2	3	0	N/D
PGlcNAc-OAc	**12-Ac**	Ac	H	H	H	H	3	0	N/D
2,3DNPGlcNAc	**5**	H	NO_2	NO_2	H	H	3	0	mp 199-200 ºC, Calc + 0.5 H_2O: C, 42.42; H, 4.59; N, 10.60 Exp: C, 42.80; H, 4.50; N, 10.65
3,5DNPGlcNAc	**6**	H	H	NO_2	H	NO_2	3	0	mp 172-173 ºC, Calc: C, 43.42; H, 4.42; N, 10.85 Exp: C, 53.31; H, 4.55; N, 10.63
pNPGlcNAc	**1**	H	H	H	NO_2	H	3	0	mp 207-208 C, lit.: 210-212 ºC Calc: C, 49.12; H, 5.30; N, 8.18 Exp: C, 49.24; H, 5.22; N, 8.25
(^{2}H-1)pNPGlcNAc	**19**	H	H	H	NO_2	H	3	0	mp 208-209 ºC

									Calc: C, 48.98; H, 5.30; N, 8.16 Exp: C, 49.12; H, 5.12; N, 8.31
pNPGNAcF	**2**	H	H	H	NO_2	H	2	1	Dr. Tom Harvey mp 204-205 ºC, lit.: 217-218 ºC (dec)
pNPGNAcF_2	**3**	H	H	H	NO_2	H	1	2	mp 222-223 ºC (dec), lit.: 232-233 ºC (dec) Calc: C, 44.45; H, 4.26; N, 7.41 Exp: C, 44.48; H, 4.16; N, 7.30
pNPGNAcF_3	**4**	H	H	H	NO_2	H	0	3	mp 226-227 ºC (dec), lit.: 244-245 ºC (dec) Calc: C, 42.43; H, 3.82; N, 7.07 Exp: C, 42.45; H, 3.77; N, 6.98
mNPGlcNAc	**9**	H	H	NO_2	H	H	3	0	mp 187-188 ºC, Calc + 0.5 H_2O: C, 47.86; H, 5.46; N, 7.97 Exp: C, 48.11; H, 5.44; N, 8.05
pNAcGNAc	**10**	H	H	H	NHAc	H	3	0	mp 176-177 ºC, Calc: C, 54.23; H, 6.26; N, 7.91 Exp: C, 54.30; H, 6.20; N, 7.72
mNAcGNAc	**11**	H	H	NHAc	H	H	3	0	mp 194-195 ºC Calc: C, 54.23; H, 6.26; N, 7.91 Exp: C, 54.35; H, 6.18; N, 7.62
PGNAc	**12**	H	H	H	H	H	3	0	mp 227-228 ºC, Calc: C, 56.56; H, 6.44; N, 4.71 Exp: C, 56.40; H, 6.64; N, 4.60

Table 2 NMR data for the pyranose ring of the aryl 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucosides used in this study.

Compound	NH	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	Other Signals
3,5DNPGNAc	5.72 d $J_{NH,2}$ 8.6 Hz	5.59 d $J_{1,2}$ 8.6 Hz	4.03 ddd $J_{2,3}$ 9.5 Hz	5.50 dd $J_{3,4}$ 9.3 Hz	5.08 dd $J_{4,5}$ 9.2 Hz	4.06 ddd $J_{5,6a}$ 2.2 Hz $J_{5,6b}$ 6.4 Hz	4.23 dd $J_{6a,6b}$ 12.2 Hz	4.15 dd	2.08 s, 2.00 s, 2.06 s, OAc 1.96 s, NAc
2,3DNPGNAc	5.76 d $J_{NH,2}$ 7.8 Hz	5.55 d $J_{1,2}$ 8.1 Hz	3.80 ddd $J_{2,3}$ 10.4 Hz	5.59 dd $J_{3,4}$ 9.3 Hz	5.11 dd $J_{4,5}$ 9.5 Hz	3.90 ddd $J_{5,6a}$ 3.7 Hz $J_{5,6b}$ 3.7 Hz	4.24 d	4.24 d	2.09 s, 2.05 s, 2.04 s, OAc 2.00 s, NAc
mNPGNAc	5.84 d $J_{NH,2}$ 8.6 Hz	5.43 d $J_{1,2}$ 8.5 Hz	4.23 ddd $J_{2,3}$ 9.6 Hz	5.47 dd $J_{3,4}$ 9.6 Hz	5.10 dd $J_{4,5}$ 9.6 Hz	3.98 ddd $J_{5,6a}$ 4.4 Hz $J_{5,6b}$ 4.4 Hz	4.21 d	4.21 d	2.08 s, 2.06 s, 2.05 s, OAc 1.94 s, NAc
pNPGNAc	5.63 d $J_{NH,2}$ 8.5 Hz	5.46 d $J_{1,2}$ 8.1 Hz	4.08 ddd $J_{2,3}$ 10.4 Hz	5.45 dd $J_{3,4}$ 9.4 Hz	5.13 dd $J_{4,5}$ 9.4 Hz	3.93 ddd $J_{5,6a}$ 5.5 Hz $J_{5,6b}$ 2.4 Hz	4.28 dd $J_{6a,6b}$ 12.2 Hz	4.15 dd	2.07 s, 2 x OAc 2.05 s, OAc 1.95 s, NAc
mNAcGNAc CDCl3 (400)	6.25 d, RNH $J_{NH,2}$ 8.5 Hz 7.98 s, ArNH	5.18 d $J_{1,2}$ 8.3 Hz	4.14 ddd $J_{2,3}$ 9.7 Hz	5.27 dd $J_{3,4}$ 9.7 Hz	5.07 dd $J_{4,5}$ 9.5 Hz	3.72 ddd $J_{5,6a}$ 4.9 Hz $J_{5,6b}$ 2.4 Hz	4.21 dd $J_{6a,6b}$ 12.1 Hz	4.10 dd	2.02 s, 2.02 s, 2.00 s, OAc 1.92 s, NAc
pNAcGNAc CDCl3 (400)	5.71 d, RNH $J_{NH,2}$ 8.8 Hz 7.22 s, ArNH	5.15 d $J_{1,2}$ 8.4 Hz	4.11 ddd $J_{2,3}$ 9.8 Hz	5.35 dd $J_{3,4}$ 9.8 Hz	5.11 dd $J_{4,5}$ 9.8 Hz	3.80 ddd $J_{5,6a}$ 5.4 Hz $J_{5,6b}$ 1.8 Hz	4.26 dd $J_{6a,6b}$ 12.2 Hz	4.12 dd	2.05 s, 2.04 s, 2.03 s, OAc 1.94 s, NAc
PGNAc	5.57 d $J_{NH,2}$ 8.6 Hz	5.25 d $J_{1,2}$ 8.3 Hz	4.11 ddd $J_{2,3}$ 10.4 Hz	5.40 dd $J_{3,4}$ 9.4 Hz	5.13 dd $J_{4,5}$ 9.4 Hz	3.85 ddd $J_{5,6a}$ 5.2 Hz $J_{5,6b}$ 2.7 Hz	4.28 dd $J_{6a,6b}$ 12.1 Hz	4.14 dd	2.06 s, 2.05 s, 2.04 s, OAc 1.95 s, NAc
pNPGNAc (^{2}H-1)	5.63 d $J_{NH,2}$ 8.5 Hz	N/A	4.0 dd $J_{2,3}$ 10.4 Hz	5.45 dd $J_{3,4}$ 9.4 Hz	5.13 dd $J_{4,5}$ 9.4 Hz	3.93 ddd $J_{5,6a}$ 5.5 Hz $J_{5,6b}$ 2.4 Hz	4.28 dd $J_{6a,6b}$ 12.2 Hz	4.15 dd	2.07 s, 2 x OAc 2.05 s, OAc 1.95 s, NAc

PNPGNAcF$_3$	6.86 d $J_{NH,2}$ 8.6 Hz	5.36 d $J_{1,2}$ 8.1 Hz	4.33 ddd $J_{2,3}$ 10.5 Hz	5.40 dd $J_{3,4}$ 8.6 Hz	5.16 dd $J_{4,5}$ 9.6 Hz	3.97 ddd $J_{5,6a}$ 5.6 Hz $J_{5,6b}$ 2.7 Hz	4.30 dd $J_{6a,6b}$ 12.2 Hz	4.19 dd	2.08 s, 2.07 s, 2.05 s, OAc

Table 3 NMR data for the aryl ring of the aryl 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucosides used in this study.

Compound	H_{AR2}	H_{AR3}	H_{AR4}	H_{AR5}	H_{AR6}	Other Signals
3,5DNPGNAc	8.16 dd $J_{AR2,AR4}$ 1.9 Hz $J_{AR2,AR6}$ 1.9 Hz	N/A	8.73 dd $J_{AR4,AR6}$ 1.9 Hz	N/A	8.16 dd	N/A
2,3DNPGNAc	N/A	N/A	7.96 dd $J_{AR4,AR5}$ 8.3 Hz $J_{AR4,AR6}$ 1.4 Hz	7.76 dd $J_{AR5,AR6}$ 8.3 Hz	7.60 dd	N/A
mNPGlcNAc	7.86 dd $J_{AR2,AR4}$ 2.2 Hz $J_{AR2,AR6}$ 2.2 Hz	N/A	7.91 ddd $J_{AR4,AR5}$ 8.3 Hz $J_{AR4,AR6}$ 1.5 Hz	7.43 dd $J_{AR5,AR6}$ 8.1 Hz	7.30 ddd	N/A
mNAcGNAc CDCl3 (400)	7.32 s	N/A	7.05 d $J_{AR4,AR5}$ 8.0 Hz	7.14 dd $J_{AR5,AR6}$ 8.0 Hz	6.69 d	7.98 s, ArNH 2.07 s, ArNAc
pNAcGNAc CDCl3 (400)	6.91 $J_{AR2,AR3}$ 8.8 Hz	7.35 d	N/A	7.35 d	6.91 d	7.22 s, ArNH 2.14 s, ArNAc
PGNAc	7.00 dd $J_{AR2,AR3}$ 7.7 Hz $J_{AR2,AR4}$ 1.1 Hz	7.27 m	7.04 dd $J_{AR4,AR3}$ 7.2 Hz	7.27 m	7.00 dd	N/A
pNPGNAc	7.05 d $J_{AR2,AR3}$ 9.3 Hz	8.18 d	N/A	8.18 d	7.05 d	N/A
pNPGNAc (^{2}H-1)	7.05 d $J_{AR2,AR3}$ 9.3 Hz	8.18 d	N/A	8.18 d	7.05 d	N/A

pNPGNAcF_3	7.06 d $J_{AR2,AR3}$ 9.3 Hz	8.18 d	N/A	8.18 d	7.06 d	N/A

Table 4 NMR data for the pyranose ring of the aryl 2-acetamido-2-deoxy-β-D-glucosides used in this study

Compound	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	Other Signals
3,5DNPGNAc	5.29 d $J_{1,2}$ 8.4 Hz	4.00 dd $J_{2,3}$ 10.3 Hz	3.60 dd $J_{3,4}$ 8.8 Hz	3.40 dd $J_{4,5}$ 9.6 Hz	3.54 m	3.91 dd $J_{6a,6b}$ 12.1 Hz $J_{6a,5}$ 2.0 Hz	4.15 dd $J_{6b,5}$ 6.2 Hz	1.98 s, NAc
2,3DNPGNAc	5.16 d $J_{1,2}$ 8.6 Hz	3.84 dd $J_{2,3}$ 10.0 Hz	3.55 dd $J_{3,4}$ 8.8 Hz	3.38 dd $J_{4,5}$ 9.3 Hz	3.50 m	3.94 dd $J_{6a,6b}$ 12.1 Hz $J_{6a,5}$ 1.2 Hz	3.71 dd $J_{6b,5}$ 6.1 Hz	1.99 s, NAc
mNPGNAc	5.14 d $J_{1,2}$ 8.4 Hz	3.94-3.88 m	3.58 dd $J_{3,2}$ 10.0 Hz $J_{3,4}$ 8.8 Hz	3.41 dd $J_{4,5}$ 9.2 Hz	3.48 m	3.94-3.88 m	3.70 dd $J_{6b,6a}$ 12.0 Hz $J_{6b,5}$ 5.8 Hz	1.97 s, NAc
pNPGNAc	5.19 d $J_{1,2}$ 8.4 Hz	3.93 dd $J_{2,3}$ 10.4 Hz	3.57 dd $J_{3,4}$ 8.6 Hz	3.41 dd $J_{4,5}$ 9.7 Hz	3.49 m	3.90 dd $J_{6a,6b}$ 12.2 Hz $J_{5,6a}$ 2.3 Hz	3.70 dd $J_{6b,5}$ 5.8 Hz	1.96 s, NAc
mNAcGNAc CDCl3 (400)	5.01 d $J_{1,2}$ 8.4 Hz	3.93-3.85 m	3.45-3.39 m	3.45-3.39	3.54 m	3.93-3.85 m	3.66 dd $J_{6b,6a}$ 11.6 Hz $J_{6b,5}$ 3.5 Hz	1.96 s, NAc
pNAcGNAc CDCl3 (400)	4.97 d $J_{1,2}$ 8.4 Hz	3.92-3.84 m	3.42-3.37 m	3.42-3.37 m	3.53 m	3.92-3.84 m	3.70 dd $J_{6b,6a}$ 12.0 Hz $J_{6b,5}$ 4.8 Hz	1.96 s, NAc
PGNAc	5.02 d $J_{1,2}$ 8.4 Hz	3.91-3.85 m	3.55 dd $J_{3,2}$ 10.2 Hz $J_{3,4}$ 8.3 Hz	3.46-3.36 m	3.46-3.36 m	3.91-3.85 m	4.28 dd $J_{6b,6a}$ 11.9 Hz $J_{6b,5}$ 5.1 Hz	1.96 s, NAc

pNPGNAc (^{2}H-1)	N/A	4.08 d $J_{2,3}$ 10.4 Hz	5.45 dd $J_{3,4}$ 9.4 Hz	5.13 dd $J_{4,5}$ 9.4 Hz	3.93 ddd $J_{5,6a}$ 5.5 Hz $J_{5,6b}$ 2.4 Hz	4.28 dd $J_{6a,6b}$ 12.2 Hz	4.15 dd	1.96 s, NAc
pNPGNAcF	5.29 d $J_{1,2}$ 8.4 Hz	4.06 dd $J_{2,3}$ 10.4 Hz	3.66 dd $J_{3,4}$ 8.6 Hz	3.43 dd $J_{4,5}$ 9.8 Hz	3.51 m	3.91 dd $J_{6a,6b}$ 12.2 Hz $J_{6a,5}$ 2.2 Hz	3.70 dd $J_{6b,5}$ 5.6 Hz	4.82 d, CH_2F $J_{H,F}$ 47.1 Hz ^{19}F nmr: –151.8 dd, CH_2F
pNPGNAcF_2	5.25 d $J_{1,2}$ 8.4 Hz	4.01 dd $J_{2,3}$ 10.4 Hz	3.65 dd $J_{3,4}$ 8.7 Hz	3.43 dd $J_{4,5}$ 9.7 Hz	3.51 m	3.92 dd $J_{6a,6b}$ 12.2 Hz $J_{5,6a}$ 1.9 Hz	3.66 dd $J_{5,6b}$ 5.7 Hz	6.04 dd, CHF_2 $J_{H,F}$ 54.0 Hz ^{19}F nmr: AB quartet –51.8 dd, CHF_2
PNPGNAcF_3	5.25 d $J_{1,2}$ 8.1 Hz	4.00 dd $J_{2,3}$ 10.3 Hz	3.64 dd $J_{3,4}$ 8.7 Hz	3.44 dd $J_{4,5}$ 9.6 Hz	3.51 m	3.90 dd $J_{6a,5}$ 1.9 Hz $J_{6a,6b}$ 12.2 Hz	3.72 dd $J_{6b,5}$ 5.7 Hz	^{19}F nmr: –0.74 dd, CF_3

Table 5 NMR data for the aryl ring of the aryl 2-acetamido-2-deoxy-β-D-glucosides used in this study.

Compound	H_{AR2}	H_{AR3}	H_{AR4}	H_{AR5}	H_{AR6}	Other Signals
3,5DNPGNAc	8.22 dd $J_{AR2,AR4}$ 1.9 Hz $J_{AR2,AR6}$ 1.9 Hz	N/A	8.64 dd $J_{AR4,AR6}$ 1.9 Hz	N/A	8.16 dd	N/A
2,3DNPGNAc	N/A	N/A	7.93 dd $J_{AR4,AR5}$ 8.3 Hz $J_{AR4,AR6}$ 1.4 Hz	7.72 dd $J_{AR5,AR6}$ 8.3 Hz	7.90 dd	N/A
mNPGlcNAc	7.88-7.84 m	N/A	7.88-7.84 m	7.51 dd $J_{AR5,AR6}$ 8.1 Hz	7.50 m	N/A
mNAcGNAc	7.31 dd $J_{AR2,AR4}$ 2.2 Hz $J_{AR2,AR6}$ 2.2 Hz	N/A	7.19-7.12 m	7.19-7.12 m	6.75 ddd $J_{AR6,AR5}$ 7.3 Hz $J_{AR6,AR4}$ 2.2 Hz	2.08 s, ArNAc
pNAcGNAc	6.96 $J_{AR2,AR3}$ 9.0 Hz	7.40 d	N/A	7.40 d	6.96 d	2.07 s, ArNAc
PGNAc	7.11-6.94 m	7.24 dd J 8.1Hz J 7.7 Hz	7.11-6.94 m	7.11	6.94 m	N/A
pNPGNAc	7.15 d $J_{AR2,AR3}$ 9.3 Hz	8.18 d	N/A	8.18 d	7.15 d	N/A
pNPGNAc (^{2}H-1)	7.15 d $J_{AR2,AR3}$ 9.3 Hz	8.18 d	N/A	8.18 d	7.15 d	N/A
pNPGNAcF	7.16 d $J_{AR2,AR3}$ 9.3 Hz	8.18 d	N/A	8.18 d	7.16 d	N/A
$PNPGNAcF_2$	7.16 d $J_{AR2,AR3}$ 9.2 Hz	8.19	N/A	8.19 d	7.16 d	N/A

pNPGNAcF_3	7.20 d $J_{AR2,AR3}$ 9.2 Hz	8.20 d	N/A	8.20 d	7.20 d	N/A