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Addendum and Corrigendum

Addendum and corrigendum to "Inhibition of *O*-GlcNAcase by PUGNAc is dependent upon the oxime stereochemistry" [Bioorg. Med. Chem. 14 (2006) 837–846]

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1. Confirmation of PUGNAc oxime stereochemistry and *O*-GlcNAcase inhibition

Based on the previous work of Vasella and co-workers,[1] the assignment of **3a** as *Z*-PUGNAc is confirmed via alignment of NMR signals supporting our conclusion that *Z*-PUGNAc is the active inhibitor of *O*-GlcNAcase (included are both proton and carbon spectroscopic data for compounds **6a** and **3a** with full assignments based upon COSY, HMQC, and HMBC experiments). Further, a recent crystallographic analysis of PUGNAc within the active site of a related hexosaminidase (*Cp*NagJ) confirms this conclusion by showing PUGNAc as the *Z* isomer in a binding position that would not accommodate the *E* stereochemistry.[2] However, recent results of ongoing studies of the presumptive PUGNAc stereoisomers prompt us to revise two of our conclusions.

2. Regarding the assignment of *Z*-PUGNAc (3a)

While the stability profiles for *Z*-PUGNAc and *E*-PUGNAc are conveniently explained by the Beckmann rearrangement, the observed mass analysis and proton NMR data for the products of the presumed Beckmann rearrangement are *more* consistent with the products that would arise from simple hydrolysis of **3a**. Full characterisation of such a hydrolysis product has *not* been achieved, and thus the exact nature of the mechanism by which **3a** breaks down should be considered tentative. However, the realisation that *Z*-PUGNAc likely breaks down via hydrolysis led to us question the apparent resistance of **3b** to a similar hydrolysis event and eventually led to us question the structure of **3b**.

3. Regarding the structural assignment of compound 3b

The spectroscopic data for **3b** potentially match the predicted values for *E*-PUGNAc; however, a more accurate match for the proton and carbon spectrum is the related 1,4-lactone species (a possible by-product of the synthesis of PUGNAc as previously stated by Vasella and co-workers[1]). A COSY correlation spectrum of **3b** in very dry DMSO-d_6 provides for the coupling between the hydroxyl protons and the sugar methine and methylene protons. This spectrum clearly shows the C5 methine proton coupled to a hydroxyl proton; no associated coupling between the C4 methine proton and any hydroxyl signal was noted. A HMBC correlation spectrum of **6b** subsequently confirmed the presence of the 1,4-lactone triacetate product via the three bond coupling of the C5 methine proton and the carbonyl carbon of an acetate; no associated coupling between an acetate carbonyl and the C4 methine proton was noted (the aforementioned COSY spectrum of **3b** and HMBC spectrum of **6b** are included here as Figs. 1 and 2). Thus, there is clear evidence for the existence of the 1,4-lactone product *rather* than *E*-PUGNAc, and compounds **6b** and **3b** should be listed as shown below (included are both proton and carbon spectroscopic data for compounds **6b** and **3b** with full assignments based upon COSY, HMQC, and HMBC experiments). Therefore, while the conclusion that *Z*-PUGNAc is the primary inhibitor remains valid, the activity assigned to compound **3b** should be associated with the 1,4-lactone product shown below and *not* *E*-PUGNAc.

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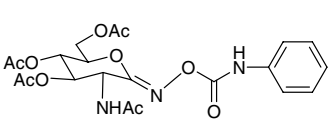
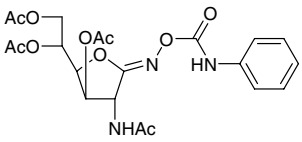
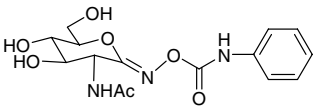


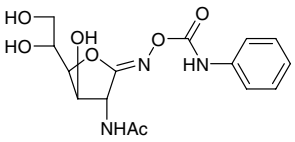
6a



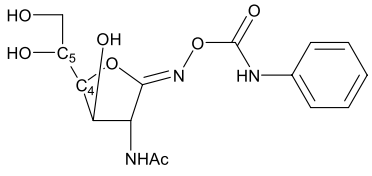
6b



3a



3b



3b

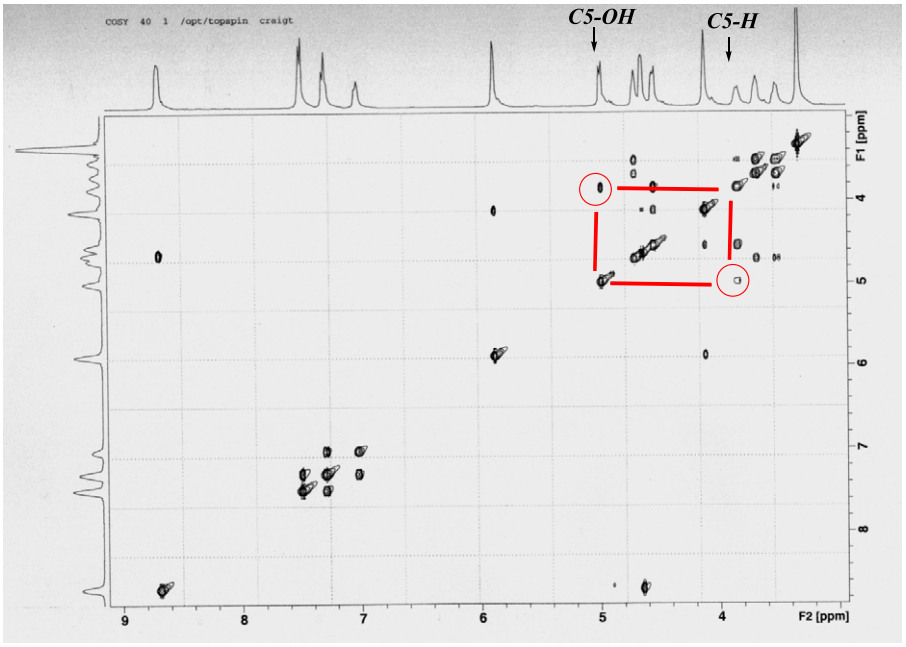
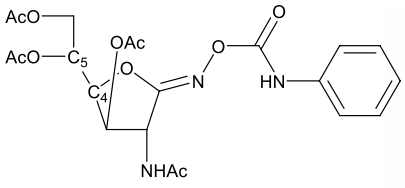


Figure 1. COSY spectrum of **3b**.



6b

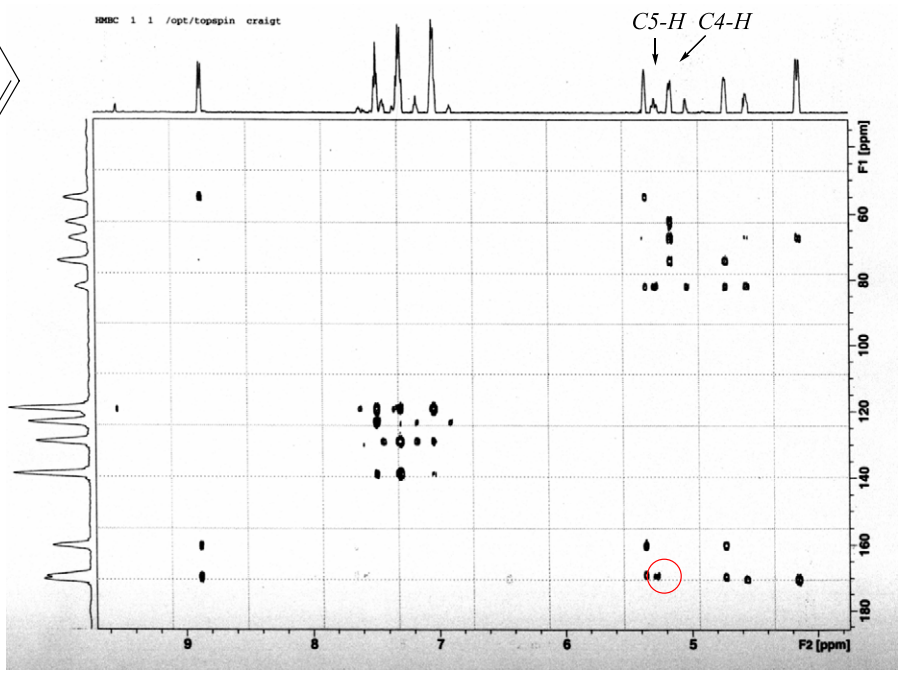


Figure 2. HMBC spectrum of **6b**.

4. *O*-(2-Acetamido-3,4,5-tri-*O*-acetyl-D-glucopyranosylidene)amino-*Z*-*N*-phenylcarbamoyl (6a)

^{1}H NMR (DMSO-d_6) δ 1.87 (s, 3H, Ac-CH_3), 2.00 (s, 3H, Ac-CH_3), 2.03 (s, 3H, Ac-CH_3), 2.05 (s, 3H, Ac-CH_3), 4.20 (dd, J_{HH} = 12.9, J_{HH} = 2.4 Hz, 1H, C6-H), 4.38 (dd, J_{HH} = 12.9, J_{HH} = 4.4 Hz, 1H, C6-H), 4.66 (ddd, J_{HH} = 9.4, J_{HH} = 4.3 Hz, J_{HH} = 2.4, 1H, C5-H), 4.79 (dd appears as *t*, J_{HH} = 7.9, J_{HH} = 7.9 Hz, 1H, C2-H), 5.24 (dd, J_{HH} = 9.4, J_{HH} = 8.0 Hz, 1H, C4-H), 5.34 (dd appears as *t*, J_{HH} = 8.0, J_{HH} = 8.0 Hz, 1H, C3-H), 7.02–7.05 (m, 1H, Ar-H), 7.29–7.32 (m, 2H, Ar-H), 7.48–7.49 (m, 2H, Ar-H), 8.61 (d, J_{HH} = 7.68 Hz, 1H, N*H*Ac), 9.74 (s, 1H, PhN*H*); ^{13}C NMR (DMSO-d_6) δ 18.8 (CO*C*H$_3$), 18.1 (CO*C*H$_3$), 18.9 (CO*C*H$_3$), 20.9 (CO*C*H$_3$), 47.1 (C2), 60.0 (C6), 65.5 (C4), 69.3 (C3), 74.2 (C5), 117.3, 121.4, 127.2, 136.8, 149.8 (O*C*ONH), 154.1 (C1), 167.5 (*C*OCH$_3$), 167.7 (*C*OCH$_3$), 168.0 (*C*OCH$_3$), 168.4 (*C*OCH$_3$).

5. (*Z*)-1-(4-Acetamido-3,5,6-triacetyl-5-(hydroxyimino)tetrahydrofuran-2-yl)ethane-1,2-diyl (6b)

^{1}H NMR (DMSO-d_6) δ 1.90 (s, 3H, Ac-CH_3), 1.99 (s, 3H, Ac-CH_3), 2.04 (s, 3H, Ac-CH_3), 2.05 (s, 3H, Ac-CH_3), 4.13 (dd, J_{HH} = 12.4, J_{HH} = 5.8 Hz, 1H, C6-H), 4.55 (dd, J_{HH} = 12.4, J_{HH} = 2.6 Hz, 1H, C6-H), 4.72 (dd, J_{HH} = 7.6, J_{HH} = 1.7 Hz, 1H, C2-H), 5.15 (dd, J_{HH} = 8.2, J_{HH} = 4.3 Hz, 1H, C4-H), 5.26 (ddd, J_{HH} = 8.3, J_{HH} = 5.6 Hz, J_{HH} = 2.6, 1H, C5-H), 5.34 (dd, J_{HH} = 4.3, J_{HH} = 1.8 Hz, 1H, C3-H), 7.02–7.04 (m, 1H, Ar-H), 7.28–7.31 (m, 2H, Ar-H), 7.47–7.48 (m, 2H, Ar-H), 8.90 (d, J_{HH} = 7.56 Hz, 1H, N*H*Ac), 9.84 (s, 1H, PhN*H*); ^{13}C NMR (DMSO-d_6) δ 18.5 (CO*C*H$_3$), 18.9 (CO*C*H$_3$), 19.0 (CO*C*H$_3$), 20.7 (CO*C*H$_3$), 53.1 (C2), 60.4 (C6), 65.3 (C5), 72.2 (C3), 80.0 (C4), 117.6, 121.4, 127.2, 136.9, 149.8 (O*C*ONH), 158.3 (C1), 167.3 (*C*OCH$_3$), 167.5 (*C*OCH$_3$), 167.7 (*C*OCH$_3$), 168.6 (*C*OCH$_3$).

6. *O*-(2-Acetamido-2-deoxy-D-glucopyranosylidene)-amino-*Z*-*N*-phenylcarbamoyl (3a)

^{1}H NMR (DMSO-d_6); δ 1.89 (s, 3H, Ac-CH_3), 3.58 (dd, J_{HH} = 8.5, J_{HH} = 7.8 Hz, 1H, C4-H), 3.64 (dd, J_{HH} = 8.2, J_{HH} = 8.0 Hz, 1H, C3-H), 3.65–3.70 (m, 1H, C6-H), 3.75–3.77 (m, 1H, C6-H), 3.92–3.95 (m, 1H, C5-H), 4.37 (dd appears as *t*, J_{HH} = 8.3, J_{HH} = 8.3 Hz, 1H, C2-H), 4.93 (br s, 1H, C6-OH), 5.55 (br s, 2H, C3-OH and C4-OH), 7.00–7.02 (m, 1H, Ar-H), 7.27–7.38 (m, 2H, Ar-H) 7.48–7.49 (m, 2H, Ar-H), 8.34 (d, J_{HH} = 8.1 Hz, 1H, N*H*Ac), 9.62 (s, 1H, PhN*H*); ^{13}C NMR (D_2O at 15 °C); δ 23.8 (CO*C*H$_3$), 51.8 (C2), 60.7 (C6), 69.2 (C4), 72.9 (C3), 83.1 (C5), 119.3, 123.5, 129.4, 139.2, 152.4 (O*C*ONH), 158.8 (C1), 169.9 (*C*OCH$_3$).

7. (*Z*)-*N*-(5-(1,2-Dihydroxyethyl)-4-hydroxy-2-(hydroxyimino)tetrahydrofuran-3-yl)acetamide (3b)

^{1}H NMR (DMSO-d_6); δ 1.87 (s, 3H, Ac-CH_3), 3.50–3.53 (m, 1H, C6-H), 3.60–3.61 (m, 1H, C6-H), 3.82–3.85 (m, 1H, C5-H), 4.11–4.12 (m, 1H, C3-H), 4.54 (dd, J_{HH} = 8.2, J_{HH} = 3.1 Hz, 1H, C4-H), 4.63 (d, J_{HH} = 7.7, 1H, C2-H), 4.69–4.71 (m, 1H, C6-OH), 4.97–4.99 (m, 1H, C5-OH), 5.82 (br s, 1H, C3-OH), 7.00–7.03 (m, 1H, Ar-H), 7.27–7.30 (m, 2H, Ar-H), 7.48–7.49 (m, 2H, Ar-H), 8.66 (d, J = 7.7 Hz, 1H, N*H*Ac), 9.73 (br s, 1H, PhN*H*); ^{13}C NMR (DMSO-d_6); δ 23.1 (CO*C*H$_3$), 57.4 (C2), 63.6 (C6), 68.8 (C5), 73.3 (C3), 86.0 (C4), 119.4, 123.5, 129.4, 139.2, 152.3 (O*C*ONH), 162.6 (C1), 169.5 (*C*OCH$_3$).

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References and notes

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