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Note

The synthesis of 2,3-didehydro-2,4-dideoxy-4-guanidinyl-*N*-acetylneuraminic acid: a potent influenza virus sialidase inhibitor

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The biochemistry and synthesis of sialidase inhibitors, in particular influenza virus sialidase inhibitors, has been of great interest to us [1–3]. The synthesis of a number of 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid (Neu5Ac2en, **1**) analogues has received considerable attention over the past decade [4–6]. These compounds are thought to be transition-state analogues of the enzyme reaction [1,2]. We have recently reported the design and biological evaluation of 5-acetamido-4-amino-2,6-anhydro-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid (4-amino-Neu5Ac2en, **2**) and 5-acetamido-2,6-anhydro-4-guanidino-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid (4-guanidino-Neu5Ac2en, **3**) as influenza virus sialidase inhibitors [7]. Both of these compounds, in particular **3**, have been shown, not only to be potent inhibitors of influenza virus sialidase and influenza virus replication, but also of the virus in a number of animal models [7].

The synthetic strategy that we adopted for the preparation of **3** required the introduction of nitrogen at C-4 of **1**. We have previously reported the preparation of **4** in good yield, and this was achieved by treatment of **1** with $BF_3 \cdot Et_2O$, followed by reaction with azide [8]. The synthesis of **3** was succesfully completed as detailed in Scheme 1. Thus, reduction of **4** was best achieved by hydrogenation at atmospheric pressure in the presence of Pd–C (10%) in toluene, methanol, and glacial acetic acid for 1 h, which afforded, following work-up and purification, methyl 5-acetamido-7,8,9-tri-*O*-acetyl-4-amino-2,6-anhydro-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (4-amino-Neu5,7,8,9$Ac_4$2en1Me, **5**) in 72% over-

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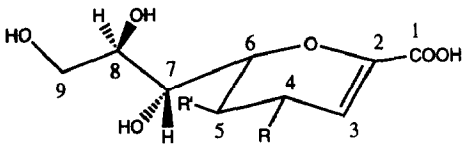
all yield. These reaction conditions for the reduction of the azide to **5** were found to be critical because of the possibility of unwanted side reactions, for example, acetate migration or even over-reduction to give the corresponding 4-acetamido and 2,3-dideoxy saturated analogues, respectively. 4-Amino-Neu5,7,8,9Ac$_4$2en1Me (**5**) was readily converted to **6** by sequential treatment with Amberlite-IRA 400 (OH^-) resin and aqueous sodium hydroxide, followed by neutralisation with Dowex-50W × 8 (H^+) resin and lyophilisation in an overall isolated yield of 66% from **4**. We have found that our strategy for the preparation of **6** was a significant improvement on a previously reported triphenylphosphine reduction method which gave the corresponding free acid **2** in a 31% overall isolated yield from **4** (ref 5). **6** was cleanly guanidinated by treatment with aminoiminomethanesulfonic acid [9] in the presence of aqueous potassium carbonate with stirring at 30–40°C for 8 h and subsequently at room temperature until the reaction was determined by ^{1}H NMR spectroscopy (D_2O) to be complete. After workup and purification, **3** was isolated in 57% yield.

Compound **3**, existing presumably as a zwitterion at physiological pH, has been determined to be a slow-binding inhibitor of influenza virus sialidase with a binding affinity of 10^{-10} M (refs. 3 and 7). Further studies, including clinical trials, are currently underway to determine the potential of **3** as an anti-influenza drug candidate.

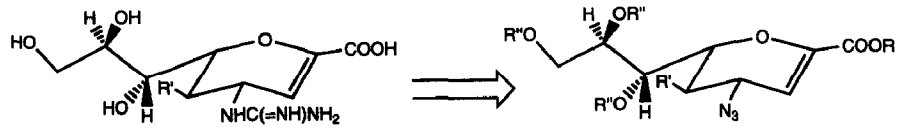


1 R = OH, R' = NHAc

2 R = NH_2, R' = NHAc

3 R = NH(C=NH)NH_2, R' = NHAc

10

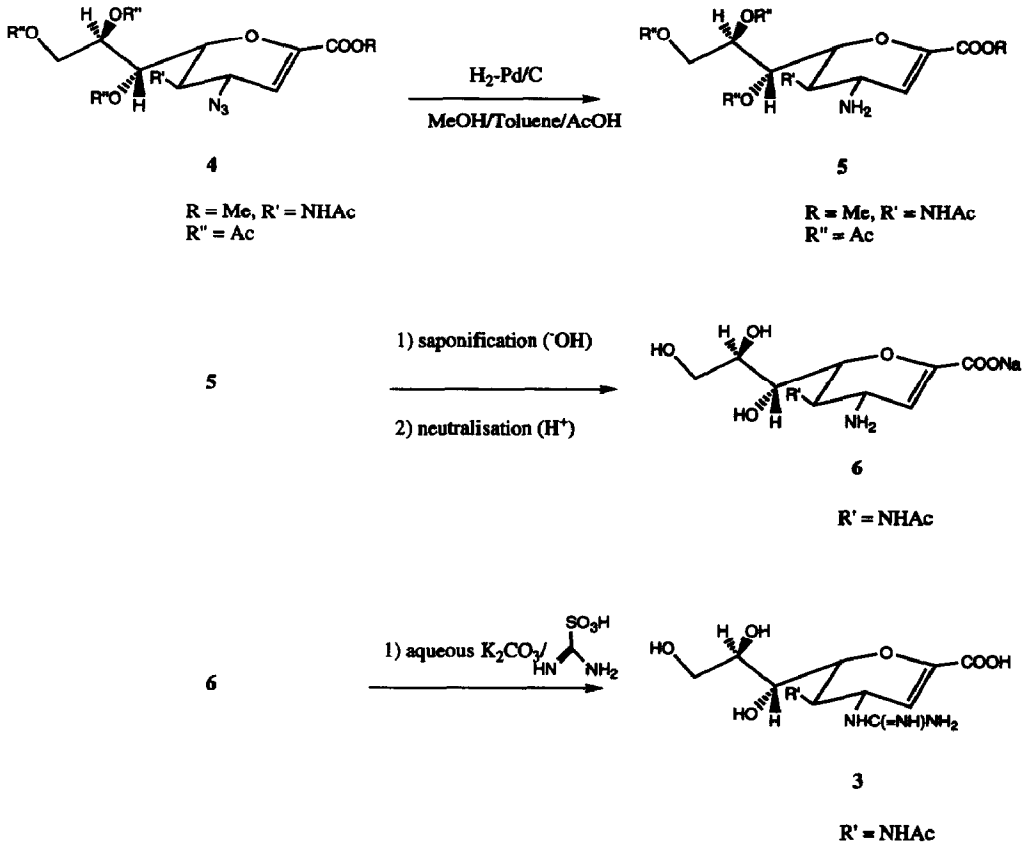


3

R' = NHAc

4

R = Me, R' = NHAc
R" = Ac



Scheme 1.

1. Experimental

*Methyl 5-acetamido-7,8,9-tri-*O*-acetyl-4-amino-2,6-anhydro-3,4,5-trideoxy-*D*-glycero-*D*-galacto-non-2-enonate* (**5**, *4-amino-Neu5,7,8,9Ac*$_4$*2en1Me*).—To a solution of **4** (1 g, 2.19 mmol) in MeOH (38 mL) was added toluene (23 mL), Pd–C (10%) (190 mg), and acetic acid (0.2 g, 3.33 mmol). This mixture was hydrogenated at atmospheric pressure for 1 h and then filtered. The filtrate was evaporated to dryness, and the residue was subjected to flash chromatography (silica gel, 5:2:1 EtOAc–2-propanol–water) to afford pure **5** (0.68 g, 72%); ν_{max} ($CHCl_3$) cm^{-1} 3400 (amine), 1740 (COOMe); ^1H NMR data (300 MHz, $CDCl_3 + CD_3OD$): δ 1.96, 2.06, 2.07, 2.10 (s, 12 H, 3 AcO, CH_3CONH), 3.81 (s, 3 H, CO_2CH_3), 3.92 (br t, 1 H, $J_{5,4}$ 10, $J_{5,6}$ 10 Hz, H-5), 4.17 (dd, 1 H, $J_{9',8}$ 7.2, $J_{9',9}$ 12.3 Hz, H-9'), 4.22 (br dd, 2 H, $J_{4,5}$ 10, $J_{4,3}$ 2.1, $J_{6,5}$ 10, $J_{6,7}$ 2.1 Hz, H-4,6), 4.71 (dd, 1 H, $J_{9,8}$ 2.6, $J_{9,9'}$ 12.3 Hz, H-9), 5.31 (m, 1 H, $J_{8,7}$ 4.9, $J_{8,9}$ 2.6, $J_{8,9'}$ 7.2 Hz, H-8), 5.45 (dd, 1 H, $J_{7,6}$ 2.1, $J_{7,8}$ 4.9 Hz, H-7), 5.97 (d, 1 H, $J_{3,4}$ 2.1 Hz, H-3); ^{13}C NMR data (300 MHz, $CDCl_3 + CD_3OD$): δ 22.2–22.3 (3 CH_3CO_2), 24.3 (CH_3CONH), 50.2 (C-5), 52.4

(C-4), 54.0 (CO_2CH_3), 64.1 (C-9), 69.8, 73.2 (C-7, C-8), 78.5 (C-6), 114.5 (C-3), 145.6 (C-2), 164.0 (C-1), 172.2, 172.4, 172.8, 174.2 (3 CH_3CO_2, CH_3CONH); FABMS: 431 $(M+1)^+$, 414 $(M^+ - NH_2)$. Anal. Calcd for $C_{18}H_{26}N_2O_{10}$: C, 50.2; H, 6.1; N 6.5. Found C, 50.0; H, 6.0; N, 6.4.

5-Acetamido-2,6-anhydro-4-guanidino-3,4,5-trideoxy-D-glycero-D-galacto-*non-2-enonic acid* (**3**, *4-guanidino-Neu5Ac2en*).—To a solution of **5** (1 g, 2.32 mmol) in anhyd MeOH (65 mL) was added dried Amberlite IRA-400 (OH^-) resin (3 g). The mixture was stirred for 3 h at room temperature and then filtered. The resin was washed with MeOH (2 × 30 mL), and the filtrate and washings were concentrated to dryness to afford a white solid. This material was then taken up in 0.15 M aq NaOH (15 mL), stirred at room temperature for 2 h, and finally the pH of the mixture was adjusted to 7.0–7.5 with Dowex-50W × 8 (H^+) resin. After filtration, the filtrate was lyophilised to afford compound **6** (0.67 g, 91.6%), which was very similar by ^1H NMR spectroscopy to the corresponding free acid **2** previously reported [5]; ν_{max} ($CHCl_3$) cm^{-1} 3416 (amine), 1628 (COO^-); ^1H NMR data (300 MHz, D_2O): δ 2.07 (s, 3 H, CH_3CONH), 3.59–3.70 (m, 2 H, H-7,9), 3.89 (dd, 1 H, $J_{9,8}$ 2.6, $J_{9,9'}$ 11.8 Hz, H-9), 3.95 (m, 1 H, H-8), 3.99 (br d, 1 H, $J_{4,5}$ 10.6 Hz, H-4), 4.21 (br t, 1 H, $J_{5,4}$ 10.6, $J_{5,6}$ 10.6 Hz, H-5), 4.29 (br d, 1 H, $J_{6,5}$ 10.6 Hz, H-6), 5.65 (d, 1 H, $J_{3,4}$ 1.9 Hz, H-3); ^{13}C NMR data (300 MHz, D_2O): δ 22.2 (C-11), 46.8 (C-5), 50.1 (C-4), 62.2 (C-9), 68.0 (C-8), 69.8 (C-7), 75.2 (C-6), 101.8 (C-3), 150.2 (C-2), 168.8, 174.9 (C-1,10); FABMS: 313 $(M+1)^+$.

To a well-stirred solution of compound **6** (6 g, 19.23 mmol) in water (100 mL) were added aminoiminomethanesulfonic acid [9] (8.3 g, 66.94 mmol) and potassium carbonate (9.38 g, 67.4 mmol) over a period of 8 h while maintaining a temperature of 30–40°C. The turbid mixture was then stirred at room temperature overnight until ^1H NMR (in D_2O) showed that the reaction was complete. The mixture was diluted with 100 mL of water and filtered. The filtrate was then treated with Amberlite IR-120 (H^+) resin (400 mL), and the resin was washed with water (4 L), then eluted with 0.6 M aq Et_3N. The isolated crude triethylammonium 4-guanidino-Neu5Ac2en was taken up in water and reconcentrated five times (200 mL/cycle), which afforded crude **3** (7.6 g). This material was then taken up in water (100 mL), heated to 40–50°C and filtered. The filtrate was diluted with 2-propanol and warmed with stirring upon which the mixture become turbid. The turbid suspension was then further stirred for 16 h at room temperature and 4-guanidino-Neu5Ac2en (**3**) was isolated as colourless crystals (4.04 g, 57%). $[\alpha]_D^{20}$ + 40.9° (*c* 0.9, H_2O); ^1H NMR data (300 MHz, D_2O): δ 1.92 (s, 3 H, AcO), 3.53 (dd, 1 H, $J_{9',8}$ 6.2, $J_{9',9}$ 11.9 Hz, H-9'), 3.56 (dd, 1 H, $J_{7,8}$ 9.0, $J_{7,6}$ 1.1 Hz, H-7), 3.77 (dd, 1 H, $J_{9,8}$ 2.7, $J_{9,9'}$ 11.9 Hz, H-9); 3.84 (ddd, 1 H, $J_{8,9}$ 2.7, $J_{8,9'}$ 6.2, $J_{8,7}$ 9.0 Hz, H-8), 4.11 (dd, 1 H, $J_{5,4}$ 9.2, $J_{5,6}$ 10.5 Hz, H-5), 4.27 (dd, 1 H, $J_{6,7}$ 1.1, $J_{6,5}$ 10.5 Hz, H-6), 4.34 (dd, 1 H, $J_{4,3}$ 2.3, $J_{4,5}$ 9.2 Hz, H-4), 5.51 (d, 1 H, $J_{3,4}$ 2.3 Hz, H-3); ^{13}C NMR (D_2O): δ 23.1 (CH_3CONH), 48.9 (C-5), 52.2 (C-4), 64.1 (C-9), 69.2 (C-7), 70.9 (C-8), 76.5 (C-6), 104.9 (C-3), 150.3 (C-2), 158.1 (C-10), 170.1, 175.9 (C-1, CH_3CONH); mp 256°C (dec); FABMS: 333 $(M+1)^+$; calculated 333.31. Anal. Calcd for $C_{12}H_{20}N_4O_7 \cdot 1.5H_2O$: C 40.11; H 6.45; N 15.59. Found: C 40.1; H 6.7; N 15.7.

Acknowledgments

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References

[1] A.K.J. Chong, M.S. Pegg, and M. von Itzstein, *Biochem. Int.*, 24 (1991) 165–171.
[2] A.K.J. Chong, M.S. Pegg, N.T. Taylor, and M. von Itzstein, *Eur. J. Biochem.*, 207 (1992) 335–343.
[3] C.T. Holzer, M. von Itzstein, B. Jin, M.S. Pegg, W.P. Stewart, and W-Y. Wu, *Glyconconj. J.*, 10 (1993) 40–44.
[4] E. Zbiral, H.H. Brandstetter, R. Christian, and R. Schauer, *Liebigs Ann. Chem.*, (1987) 781–786.
[5] E. Schreiner, E. Zbiral, R.G. Kleineidam, and R. Schauer, *Liebigs Ann. Chem.*, (1991) 129–134.
[6] B.I. Glänzer, Z. Györgydeák, B. Bernet, and A. Vasella, *Helv. Chim. Acta*, 74 (1991) 343–369.
[7] M. von Itzstein, W-Y. Wu, G.B. Kok, M.S. Pegg, J.C. Dyason, B. Jin, T. Van Phan, M.L. Smythe, H.F. White, S.W. Oliver, P.M. Colman, J.N. Varghese, D.M. Ryan, J.M. Woods, R.C. Bethell, V.J. Hotham, J.M. Cameron, C.R. Penn, *Nature*, 363 (1993) 418–423.
[8] M. von Itzstein, B. Jin, W-Y. Wu, M. Chandler, *Carbohydr. Res.*, 244 (1993) 181–185.
[9] K. Kim, Y-T. Lin, H.S. Mosher, *Tetrahedron Lett.*, 29 (1988) 3183–3186.