ELSEVIER

Carbohydrate Research 291 (1996) 183–187

CARBOHYDRATE RESEARCH

Note

Practical route to the anomeric methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosyl)onate azides

Zoltán Györgydeák [a,*], László Szilágyi [a], Zoltán Dinya [b], József Jekő [c]

[a] *Department of Organic Chemistry, L. Kossuth University, P.O. Box 20, H-4010 Debrecen, Hungary*
[b] *Research Group for Antibiotics of the Hungarian Academy of Sciences, P.O. Box 70, H-4010 Debrecen, Hungary*
[c] *Department of Research, Alkaloida Chemical Company, P.O. Box 1, H-4440 Tiszavasvári, Hungary*

Received 6 December 1995; accepted 30 April 1996

Keywords: Neuraminyl azides; Anomeric azides; ^{1}H and ^{13}C spectra; Mass spectra

Glycosyl azides are not only versatile starting materials for the synthesis of various saccharide derivatives (amines, phosphinimines, 1,2,3-triazoles, etc.) [1] but they also show glycosidase inhibitor activity in some cases. 5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid azide (*N*-acetyl-α-neuraminyl azide) is an excellent substrate for sialidases [2–4].

In view of the biological importance of this compound we have explored the possibility of using stereoselective procedures for the synthesis of α- and β-azido *N*-acetylneuraminic acids **6** and **4**. We have previously reported a simple and reliable procedure for obtaining 1,2-*trans*-glycosyl azides [5] whereby peracetylated aldoses reacted with trimethylsilyl azide in the presence of Lewis acid catalysts such as stannic chloride (1 h for pentopyranoses, 3–4 h for hexopyranoses [5], and 6–8 h for disaccharides [6]). This method has since been applied [7–9] to substrates already described by us; others have used rare-earth-based catalysts [10] in place of stannic chloride. The reaction of furanosides with trimethylsilyl azide in the presence of

* Corresponding author.

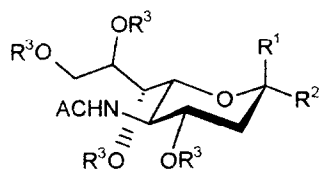
PII S0008-6215(96)00136-X

trimethylsilyl triflate requires extended reaction times and leads to anomerized products [11,12].

The acetylated neuraminic acid methyl ester **3** required for the azidolysis reaction is usually obtained by using the dangerous diazomethane for esterification [13–15]. We have applied instead a one-pot procedure whereby commercially available *N*-acetylneuraminic acid (**1**) was acetylated using acetic anhydride in pyridine, and the resulting acid (**2**) was converted without purification into a 1,8-diazabicylo[5.4.0]undec-7-ene(1,5-5) (DBU) salt which, in turn, could be conveniently converted into methyl ester **3** using methyl iodide [16,17]. The anomeric composition of the ester was in accordance with literature data [15].

Due to the quaternary anomeric center in **3** the stereochemical course of the azidolysis reaction (trimethylsilyl azide–$SnCl_4$) might, in principle, be different from those seen with aldose derivatives [1]. The stannic chloride catalyzed reaction of **3** with trimethylsilyl azide resulted, however, in the formation of a single product, which has been shown to be the β anomer (**4**, see below).

The α-azide **6** has been synthesized [18,19] from the β-chloro-neuraminic acid derivative **5** [20,25] using sodium azide under phase-transfer conditions. Treatment of **5** with lithium azide in hexamethylphosphoric triamide (HMPA) [1] also afforded **6** in 50% yield after chromatographic purification. No elimination product was observed.



1 R^1 ,R^2 = OH, COOH; R^3 = H

2 R^1 ,R^2 = OAc, COOH; R^3 = Ac

3 R^1 ,R^2 = OAc, $COOCH_3$; R^3 = Ac

4 $R^1 = N_3$; $R^2 = COOCH_3$; R^3 = Ac

5 R^1 = Cl; $R^2 = COOCH_3$; R^3 = Ac

6 $R^1 = COOCH_3$; $R^2 = N_3$; R^3 = Ac

Several NMR criteria have previously been proposed for establishing the anomeric configuration in sialic acid derivatives. However, chemical shifts for H-3eq [15,18], H-4, H-7 [21], or C-1 [22] in **4** display little or negligible differences with respect to those

published for the α-anomeric azide **6**. On the other hand, the three-bond couplings of H-3ax to both C-1 and C-2 were found to be significantly larger in **6** than in **4** and this allowed unequivocal assignment of the anomeric configurations in accordance with literature data [22,23].

No informative mass spectra could be obtained for **4** and **6** under EI conditions. Thermospray spectra, on the other hand, contained intense $M^+ + 1$ peaks for both compounds. While no further peaks were seen for the β anomer **4**, characteristic fragment ions appeared in the spectrum of **6**. The molecular ion of **4** appears to be more stable in the FAB spectrum as well. The fragment ions, identified by high-resolution measurements, display characteristic intensity differences between the two anomers. The ions $m/z = 474$, 414, 413, and 307 are of higher intensity for **4** than for the α anomer **6**. It appears that the stability of the molecular ions is determined by the mass, rather than the electronic properties of the substituents at position 1: the equatorial CO_2Me group confers more stability to the β anomer **4** than the equatorial N_3 does for the α anomer **6**. This is in agreement with FAB fragmentation data described [24,26] for carbohydrates with similar structures.

In conclusion, a mild and simple procedure has been devised for the completely stereoselective syntheses of the important azido-neuraminic acid anomers **4** and **6**.

1. Experimental

Fast-atom bombardment (FAB) mass spectrometry was carried out using a VG 7070 HS double focusing mass spectrometer (VG Analytical Ltd., Manchester), which was separated in the low- ($\Delta m/m = 1000$) and high-resolution (peak matching) mode ($\Delta m/m = 8000$). Xenon was used as the bombarding gas and the Ion Tech saddle-field FAB gun was operated at 8 kV, 1 μA. Samples were dissolved in MeOH (5–10 μg mL^{-1}) and 1-μL aliquots were added to a thin smear of *m*-nitrobenzyl alcohol on the stainless steel target. Spectra were recorded 2 min after introduction of the sample in the ion source at 10 s/decade scan rate. Thermospray spectra were recorded on a VG TRIO-2 (VG MassLab, Manchester) quadrupole spectrometer equipped with a thermospray–plasmaspray interface. The high-performance liquid chromatograph was a Waters MS-600 unit and samples were analyzed in the flow-injection mode. Volumes of 2–3 μL of the sample solution in MeOH were introduced through a U6K injector. The mobile phase was 1:1 MeOH–water containing 0.1 M NH_4OAc. The spectra were acquired at a rate of 1 s per scan, the mass spectrometer being operated in filament-off mode and without discharge ionization. The optimum values of the capillary tip temperature and of the repeller voltage were 240–250 °C, respectively. The source temperature was 210 °C.

The NMR spectra were recorded at 200/50 MHz ($^1H/^{13}C$) using a Bruker WP 200SY spectrometer. Chemical shifts are referenced to Me_4Si (^1H) or to the residual solvent signal [^{13}C: 77.00 ppm for $CDCl_3$]. Assignments are based on H,H COSY and C,H COSY measurements. The carbon–proton coupling constants were obtained from proton-coupled ^{13}C spectra, the assignments being confirmed by single frequency (CW) on-resonance proton-decoupling experiments.

Methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-{glycero}}-β-D-galacto-non-2-ulopyranosyl)onate azide (**4**).—To a mixture of dry pyridine (11.9 mL) and Ac_2O (13.9 mL) was added **1** (0.92 g, 3 mmol) under stirring at −20 °C. The resulting homogenous solution was kept at room temperature for 20 h and then evaporated under reduced pressure. After repeated codistillation with toluene (3 × 40 mL), the pyridine-free residue was dissolved in DMF (13.5 mL) and DBU (0.44 mL) was added; then after stirring for 1 min, MeI (0.185 mL) was added. After 20 h, the volatiles were removed in vacuo, and the residue was dissolved in water and extracted with EtOAc (4 × 30 mL). The pooled organic phase was washed with water (2 × 15 mL), dried ($MgSO_4$), and evaporated. The crude product containing minor impurities (TLC, 3:1 toluene–EtOH) was purified by flash-chromatography. The methyl ester **2** (1.06 g, 2 mmol) was dissolved in CH_2Cl_2 (10 mL), and trimethylsilyl azide (0.32 mL) and $SnCl_4$ (0.12 mL) were added. After 7 h the mixture was diluted with CH_2Cl_2 (~ 25 mL), then washed with saturated aq $NaHCO_3$ and water. The dried ($MgSO_4$) organic phase contained a single component (TLC, 40:1 EtOAc–EtOH). Concentration yielded **4** (0.89 g, 86.1%) which crystallized from $CHCl_3$ upon addition of a small amount of hexane; mp 179–180 °C; $[\alpha]_D^{23}$ −73.9° (*c* 0.35, $CHCl_3$); IR (KBr): ν 2122 cm^{-1}; ^1H NMR (200 MHz, C_6D_6): δ 1.96 (dd, 1 H, $J_{3ax,3eq}$ 13.4, $J_{3ax,4}$ 9.8 Hz, H-3ax), 2.15 (dd, 1 H, $J_{3eq,4}$ 5.2 Hz, H-3eq), 3.91 (dd, 1 H, $J_{6,7}$ 2.2, $J_{5,6}$ 10.2 Hz, H-6), 4.31 (dd, 1 H, $J_{9a,9b}$ 12.2, $J_{8,9a}$ 6.6 Hz, H-9a), 4.40 (q, 1 H, $J_{4,5} = J_{5,6} = J_{5,NH}$ = 10.3 Hz, H-5), 4.46 (d, 1 H, NH), 4.98 (dd, 1 H, $J_{8,9b}$ 2.2 Hz, H-9b), 5.03 (ddd, 1 H, H-4), 5.53–5.64 (m, 2 H, H-7,8); ^{13}C NMR [50 MHz, $(CD_3)_2CO$]: δ 166.01 (C-1), 90.35 (C-2), 35.50 (C-3), 68.54 (C-4), 48.75 (C-5), 72.96 (C-6), 67.51 (C-7), 70.91 (C-8), 61.93 (C-9), 53.22 (OMe), 20.46 (3 × *Me*CO), 20.77 (*Me*CO), 22.76 (*Me*CO), 169.73 (*C*OMe), 170.00 (*C*OMe), 170.28 (2 × *C*OMe), 170.48 (*C*OMe); $J_{C\text{-}1,H\text{-}3ax} < 1$, $J_{C\text{-}1,H\text{-}3eq} < 1$, $J_{C\text{-}2,H\text{-}3ax}$ 4, $J_{C\text{-}2,H\text{-}3eq} < 4$ Hz. FABMS: *m/z* (%) 517(17), $[M^+ + H]$; 489(3), $[M^+ + H - N_2]$; 474(4), $[M^+ + H - HN_3]$, A_1 [1]; 457(4), $[M^+ + H - HCOOMe]$, A_1; 429(1), $[M^+ + H - HCOOMe - N_2]$; 414(5), $[A_1 - HN_3]^+$, $[A_1 - HCOOMe]^+$; 413(20), $[A_1 - HN_3 - H]$, $[A_1 - HCOOMe - H]^+$; 398(1); 397(1); 372(2), $[M^+ + H - (CHOAc–CH_2OAc)]$; 354(3); 307(2); 217(2), $[(CHOAc)_2–CH_2OAc]^+$; 129(10), $[O{=}C(N_3)COOMe]^{+\cdot}$. Anal. Calcd for $C_{20}H_{28}N_4O_{12}$ (516.6): C, 46.51; H, 5.46; N, 10.85. Found: C, 46.31; H, 5.49; N, 10.78.

Methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosyl)onate azide (**6**).—Compound **5** (0.83 g, 1.62 mmol) was dissolved in HMPA (9.2 mL) and LiN_3 (0.33 g) added under stirring. After 2 h at room temperature the solution was diluted with water (40 mL) and extracted with EtOAc (3 × 50 mL). After washing with water (2 × 10 mL), the organic phase was evaporated and the residue flash-chromatographed to give 0.5 g (59.7%) of a syrup which became crystalline when kept in a vacuum desiccator; mp 67–68 °C; $[\alpha]_D^{21}$ −32° (*c* 1, $CDCl_3$); lit. [18,19] mp 84 °C, $[\alpha]_D^{21}$ −26.5° (*c* 0.6, $CHCl_3$); IR (KBr): ν 2122 cm^{-1}; ^1H NMR (200 MHz, C_6D_6): δ 1.82 (dd, 1 H, $J_{3ax,3eq}$ 3.0, $J_{3ax,4}$ 11.2 Hz, H-3ax), 2.59 (dd, 1 H, $J_{3eq,4}$ 4.9 Hz, H-3eq), 3.97 (dd, 1 H, $J_{6,7}$ 3.2 Hz, H-6), 4.38 (dd, 1 H, $J_{9a,9b}$ 12.5 Hz, H-9a), 4.43 (q, 1 H, $J_{5,6}$ 10.5 Hz, H-5), 4.72 (dd, 1 H, $J_{8,9b}$ 2.8 Hz, H-9b), 5.10 (ddd, 1 H, $J_{4,5}$

[1] For A_1 nomenclature, see ref. [24].

10.1 Hz, H-4), 5.28 (d, 1 H, $J_{NH,5}$ 10.2 Hz, NH), 5.57 (m, 1 H, $J_{7,8}$ 6.2 Hz, H-7), 5.70 (dd, 1 H, $J_{8,9a}$ 6.2 Hz, H-8); ^{13}C NMR (50 MHz, $CDCl_3$): δ 167.50 (C-1), 88.92 (C-2), 36.39 (C-3), 68.78 (C-4), 48.90 (C-5), 73.93 (C-6), 67.44 (C-7), 79.72 (C-8), 62.08 (C-9), 53.26 (OMe), 20.53 (3x), 20.81, 23.00 (*Me*CO), 170.61, 170.46, 170.21, 170.00, 169.86 (Me*C*O); $J_{C-1,H-3ax}$ 8, $J_{C-1,H-3eq} < 1$, $J_{C-2,H-3ax}$ 7, $J_{C-2,H-3eq}$ 4 Hz. FABMS: *m/z* (%) 517(25), $[M^+ + H]$; 489(5), $[M^+ + H - N_2]$; 474(14), $[M^+ + H - HN_3]$, A_1; 457(2), $[M^+ + H - HCOOMe]$, A_1; 429(2), $[M^+ + H - HCOOMe - N_2]$; 414(15), $[A_1 - HN_3]^+$, $[A_1 - HCOOMe]^+$; 413(60), $[A_1 - HN_3 - H]$, $[A_1 - HCOOMe - H]^+$; 398(3); 397(2); 372(4), $[M^+ + H - (CHOAc–CH_2OAc)]$; 354(6); 307(20); 217(2), $[(CHOAc)_2–CH_2OAc]^+$; 129(14), $[O=C(N_3)COOMe]^{+\cdot}$.

Acknowledgements

This work was supported by the Hungarian National Science Fund (OTKA) through Grant Nos. T-013999 and T-4023, and by the Ministry of Education, Grant No. MKM K + F 175/95. Professor H. Paulsen is thanked for his interest and advice.

References

[1] Z. Györgydeák, L. Szilágyi, and H. Paulsen, *J. Carbohydr. Chem.*, 12 (1993) 139–163.
[2] H. Friebolin, W. Baumann, R. Brossmer, G. Keilich, M. Supp, O. Ziegler, and H. von Nicolai, *Biochem. Int.*, 3 (1981) 321–326.
[3] M. Supp, U. Rose, and R. Brossmer, *Hoppe-Seyler's Z. Physiol. Chem.*, 350 (1969) 1088–1093.
[4] H. Mack and R. Brossmer, *Tetrahedron Lett.*, 28 (1987) 191–194.
[5] H. Paulsen, Z. Györgydeák, and M. Friedmann, *Chem. Ber.*, 107 (1974) 1568–1578.
[6] Cs. Pető, Gy. Batta, Z. Györgydeák, and F. Sztaricskai, *Liebigs Ann. Chem.*, (1991) 505–507.
[7] S. Maity, S.K. Dutta, A.K. Banerjee, B. Achari, and M. Singh, *Tetrahedron*, 50 (1994) 6965–6974.
[8] A.W. Harrison, J.D. Fisher, D.M. Guido, S.J. Couch, J.A. Lawson, D.M. Sutter, M.V. Williams, G.L. DeGraaf, J.E. Rogers, D.T. Pals, and D.W. Du Charme, *Bioorg. Med. Chem.*, 2 (1994) 1339–1361.
[9] K. Matsubara and T. Mukaiyama, *Chem. Lett.*, (1994) 247–250.
[10] W. Schörkhuber and E. Zbiral, *Liebigs Ann. Chem.*, (1980) 1455–1469.
[11] J. Hiebl and E. Zbiral, *Liebigs Ann. Chem.*, (1988) 765–774.
[12] F. Hammerschmidt, J.P. Polsterer, and E. Zbiral, *Synthesis*, (1995) 415–418.
[13] R. Kuhn, P. Lutz, and D.L. MacDonald, *Chem. Ber.*, 99 (1966) 611–617.
[14] A. Marra and P. Sinaÿ, *Carbohydr. Res.*, 190 (1989) 317–322, and references cited therein.
[15] A. Hasegawa, H. Ohki, T. Nagahama, H. Ishida, and M. Kiso, *Carbohydr. Res.*, 212 (1991) 277–281.
[16] B.I. Glänzer, Z. Györgydeák, B. Bernet, and A. Vasella, *Helv. Chim. Acta*, 74 (1991) 343–369.
[17] Z. Györgydeák and J. Thiem, *Carbohydr. Res.*, 268 (1995) 85–92.
[18] F.D. Tropper, F.O. Anderson, S. Braun, and R. Roy, *Synthesis*, (1992) 618–620.
[19] J. Rothermel, B. Weber, and H. Faillard, *Liebigs Ann. Chem.*, (1992) 799–802.
[20] H. Paulsen and H. Tietz, *Carbohydr. Res.*, 125 (1984) 47–64.
[21] H. Paulsen and H. von Deesen, *Carbohydr. Res.*, 146 (1986) 147–153.
[22] S. Prytulla, J. Lambert, J. Lauterwein, M. Klessinger, and J. Thiem, *Magn. Reson. Chem.*, 28 (1990) 888–901.
[23] S. Prytulla, J. Lauterwein, M. Klessinger, and J. Thiem, *Carbohydr. Res.*, 215 (1991) 345–349.
[24] N.K. Kochetkov and O.S. Chizhov, *Adv. Carbohydr. Chem.*, 21 (1966) 39–93.
[25] M.N. Sharma and R. Eby, *Carbohydr. Res.*, 127 (1984) 201–210.
[26] J.R.J. Paré, K. Jankowski, and J.W. ApSimon, *Adv. Heterocycl. Chem.*, 42 (1987) 335–410.