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Note

A convenient method for the introduction of nitrogen and sulfur at C-4 on a sialic acid analogue

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Sialic acids and the chemistry surrounding this class of biologically important carbohydrates have received much attention over the past five years[1–4]. The biological functions ascribed to these sugars are vast and extend from cell-recognition processes through to involvement in immunologic events. These aspects are reviewed elsewhere[5,6].

One of our recent interests in sialic acids has been towards the synthesis of C-4 substituted sialic acid analogues. Recently, Schreiner et al., have reported the synthesis of 5-acetamido-2,6-anhydro-4-azido-3,4,5-trideoxy-D-*galacto*-D-*glycero*-non-2-enonic acid (4-azido-4-deoxy-Neu5Ac2en, **1**) via a Mitsunobu reaction on the protected precursor, methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*talo*-non-2-enonate (4-*epi*-Neu5,7,8,9Ac$_4$2en1Me, **2**) (ref 7).

In the course of our work we have developed a facile stereoselective methodology for the introduction of nitrogen and sulfur at the C-4 position on 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*galacto*-D-*glycero*-non-2-enonic acid (Neu5Ac2en, **3**) via the known reactive intermediate **5** (ref 8). Compound **5** was prepared in high purified yields by treatment of compound **4**, methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enonate (Neu4,5,7,8,9Ac$_5$2en1Me), with $BF_3 \cdot Et_2O$. An alternative synthesis of **5** has been reported[7]. We have found that this activated allylic oxazoline group is vulnerable towards stereoselective nucleophilic attack by azide and thiolacetate at the C-4 position on the sialic acid template.

Thus treatment of **5** with lithium azide in the presence of Dowex-50W × 8 (H^+) resin in anhydrous *N,N*-dimethylformamide at 75°C gave a residue which contained the desired 4-azido-substituted compound **6** in greater than 90% yield (Scheme 1). Similarly we have found that treatment of **5** with thiolacetic acid in

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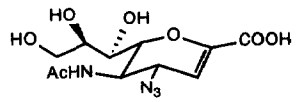

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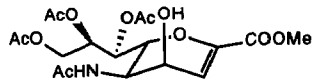

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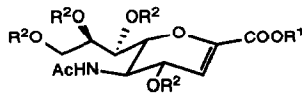
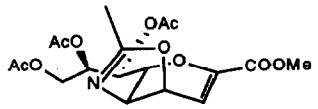
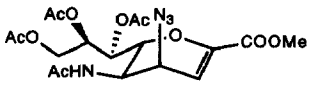


3 $R^1 = R^2 = H$
4 $R^1 = Me$, $R^2 = Ac$



5

anhydrous *N,N*-dimethylformamide resulted in the formation of methyl 5-acetamido-7,8,9-tri-*O*-acetyl-4-*S*-acetyl-2,6-anhydro-3,4,5-trideoxy-4-thio-D-*glycero*-D-*galacto*-non-2-enonate (**7**) in an isolated yield of greater than 60% (Scheme 1). Although no NMR evidence for the formation of the 4-*epi* isosteres was found, upon further purification of the crude reaction mixtures, $< 2\%$ of these compounds, in both cases, were identified, indicating that the expected inversion about C-4 was indeed the predominant reaction pathway. These trace contaminants could possibly come from an acid-facilitated opening of the oxazoline ring with subsequent S_N1 attack on either face of the C-4 cation. This is in direct contrast to the use of the activated triphenylphosphonium intermediate in the Mitsunobu reaction[7], where support for an S_N2' process followed by a [3,3] sigmatropic rearrangement was found, leading to the formation of significant amounts of 4-*epi*-azido-4-deoxy-Neu5,7,8,9Ac$_4$2en1Me (**8**). Presumably in the present work the S_N2 ring opening of the oxazoline moiety is promoted by protonation of the oxazoline ring nitrogen (Scheme 1).



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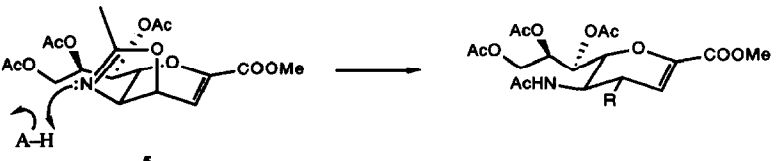

5

6 R = N_3
7 R = SAc

Scheme 1.

We have also prepared **6** on a large scale from **5** using azidotrimethylsilane (Me_3Si-azide) as a substitute for LiN_3. Thus, treatment of **5** with Me_3Si-azide at 80°C over 4 h gave **6** in 82% yield.

We find that this method provides, with good stereoselective control, a very useful entry into 4-nitrogen or sulfur sialic acid analogues using the easily prepared intermediate **5**, which, in comparison to other reported methods[7], holds considerable advantage in overall yields as well as in general ease of use. Importantly, scale-up of these reactions to multigram level presents no problems, with no loss in overall yield observed. We are currently investigating further extensions of this work on other model systems.

EXPERIMENTAL

*Preparation of 2-methyl-(methyl 7,8,9-tri-*O*-acetyl-2,6-anhydro-3,5-dideoxy-*D*-glycero-*D*-talo-non-2-enonate)-[4,5-*d*]-2-oxazoline* (*5*, *4,5-oxazolino-Neu7,8,9Ac$_3$-2en1Me*).—To a solution of methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-*D*-*glycero*-*D*-*galacto*-non-2-enonate (Neu4,5,7,8,9Ac$_5$2en1Me, **4**) (1.00 g, 2.11 mmol) in CH_2Cl_2 (15 mL) were added MeOH (67 mg, 2.12 mmol) and $BF_3 \cdot Et_2O$ (2.5 mL, 20.2 mmol). This mixture was stirred for 16 h at 25–30°C. The mixture was then slowly poured onto a stirring ice (3 g)–water (9 mL) mixture containing EtOAc (50 mL) and Na_2CO_3 (2.90 g, 27.4 mmol). The aqueous layer was extracted with fresh EtOAc (40 mL), and the organic extracts were combined, washed with a satd NaCl solution (3 × 5 mL), and subsequently dried over anhyd Na_2SO_4. Evaporation of the organic filtrate afforded the title compound **5** as a foam (0.838 g, 96%), which was virtually identical by ^1H NMR spectroscopy to that previously reported[7,8]; ^1H NMR data (300 MHz; $CDCl_3$): δ 2.00, 2.05, 2.06, 2.10, (s, 12 H, 4 Ac), 3.42 (dd, 1 H, $J_{6,5}$ 10, $J_{6,7}$ 2.6 Hz, H-6), 3.81 (s, 3 H, $-CO_2CH_3$), 3.95 (br t, 1 H, $J_{5,4}$ 8.7, $J_{5,6}$ 10 Hz, H-5), 4.22 (dd, 1 H, $J_{9',8}$ 6.4, $J_{9',9}$ 12.3 Hz, H-9′), 4.60 (dd, 1 H, $J_{9,8}$ 2.6, $J_{9,9'}$ 12.3 Hz, H-9), 4.82 (dd, 1 H, $J_{4,3}$ 3.9, $J_{4,5}$ 8.7 Hz, H-4), 5.44 (ddd, 1 H, $J_{8,7}$ 5.9, $J_{8,9}$ 2.6, $J_{8,9'}$ 6.4 Hz, H-8), 5.83 (dd, 1 H, $J_{7,6}$ 2.6, $J_{7,8}$ 5.9 Hz, H-7), 6.38 (d, 1 H, $J_{3,4}$ 3.9 Hz, H-3); FABMS: 414 $(M + 1)^+$.

*Preparation of methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-4-azido-3,4,5-trideoxy-*D*-glycero-*D*-galacto-non-2-enonate* (*6*, *4-azido-4-deoxy-Neu5,7,8,9Ac$_4$-2en1Me*).—A mixture of **5** (1.00 g, 2.42 mmol), lithium azide (232 mg, 4.70 mmol), and dried Dowex-50W × 8 (H^+) resin (200–400 mesh, 1 g) in anhyd DMF (8 mL) was stirred at ~ 80°C for 16 h under Ar. The mixture was filtered and the resin was washed with MeOH (2 × 4 mL). The filtrate and washings were combined and vacuum evaporated to dryness. The residue was then partitioned between EtOAc (50 mL) and water (20 mL), and the organic layer was successively washed with 0.01 M HCl (10 mL) and water (3 × 10 mL); the solvent was evaporated to give crude 4-azido-4-deoxy-Neu5,7,8,9Ac$_4$2en1Me (**6**) (1.09 g, 98.7%), which was subjected to flash column chromatography (silica gel, 2:1 EtOAc–hexane) to afford

pure **6** (0.8 g, 72.5%) that was identical by ^{1}H NMR and IR spectroscopies to that previously reported[7]; ν_{max} ($CHCl_3$) cm^{-1} 2100 (N_3), 1748 (OAc and CO_2Me); ^{1}H NMR data (300 MHz, $CDCl_3$): δ 2.04, 2.05, 2.06, 2.12 (s, 12 H, 4 Ac), 3.79 (s, 3 H, CO_2CH_3), 3.91 (ddd, 1 H, $J_{5,NH}$ 8.4, $J_{5,4}$ 8.8, $J_{5,6}$ 9.9Hz, H-5), 4.17 (dd, 1 H, $J_{9',8}$ 6.8, $J_{9',9}$ 12.5 Hz, H-9′), 4.42 (dd, 1 H, $J_{4,3}$ 2.9, $J_{4,5}$ 8.8 Hz, H-4), 4.48 (dd, 1 H, $J_{6,7}$ 2.3, $J_{6,5}$ 9.9 Hz, H-6), 4.64 (dd, 1 H, $J_{9,8}$ 2.7, $J_{9,9'}$ 12.5 Hz, H-9), 5.31 (m, 1 H, $J_{8,7}$ 5.2, $J_{8,9}$ 2.7, $J_{8,9'}$ 6.8 Hz, H-8), 5.45 (dd, 1 H, $J_{7,6}$ 2.3, $J_{7,8}$ 5.2 Hz, H-7), 5.96 (d, 1 H, $J_{3,4}$ 2.9 Hz, H-3), 6.13 (d, 1 H, $J_{NH,5}$ 8.4 Hz, –CONH–); ^{13}C NMR data (300 MHz, $CDCl_3$) δ 20.7 ($3 \times CH_3CO_2$), 23.2 (CH_3CONH), 48.3 (C-5), 52.6 (CO_2CH_3), 57.8 (C-4), 62.1 (C-9), 67.7, 70.9, (C-7, C-8), 75.9 (C-6), 107.6 (C-3), 145.1 (C-2), 161.5 (C-1), 170.2, 170.3, 170.7 (CH_3CO_2, CH_3CONH); FABMS: 457 $(M+1)^+$, 414 $(M^+ - N_3)$.

Alternatively, **6** was prepared by treatment of **5** (35 g, 84.7 mmole) in dry *tert*-butanol (280 mL) with Me_3Si-azide (47 mL, 0.35 mol) with stirring at 80°C for a period of 4 h. The mixture was worked up in the manner described above affording **6** (82.5%).

*Methyl 5-acetamido-7,8,9-tri-*O*-acetyl-4-*S*-acetyl-2,6-anhydro-3,4,5-trideoxy-*D*-glycero-*D*-galacto-non-2-enonate* (*7, 4-deoxy-4-thio-Neu4,5,7,8,9Ac$_3$2en1Me*).—Under Ar a mixture of 4,5-oxazolino-Neu7,8,9Ac$_3$2en1Me (**5**) (560 mg, 1.36 mmol) and thiolacetic acid (532 mg, 6.99 mmol) in anhyd DMF (10 mL) was stirred for 20 h at ~83°C. The resulting mixture was vacuum evaporated to remove DMF, and the residue triturated with ether (2 × 50 mL). The crude product was then purified by flash chromatography on silica gel (100 g) (solvent *A*, 10:1 CH_2Cl_2—acetone; solvent *B*, EtOAc), to yield 4-deoxy-4-thio-Neu4,5,7,8,9Ac$_3$2en1Me (**7**) (429 mg, 64.5%); $[\alpha]^{25}_D$ + 69.5° (*c* 1, MeOH); ν_{max} ($CHCl_3$) cm^{-1} 1740 (OAc, SAc, CO_2Me), 1688 (Amide); ^{1}H NMR data (300 MHz, $CDCl_3$) δ 1.84 (s, 3 H, CH_3CONH), 2.04, 2.05, 2.10 (s, 9 H, 3 AcO), 2.36 (s, 3 H, CH_3COS), 3.79 (s, 3 H, CO_2CH_3), 4.16 (dd, 1 H, $J_{9',8}$ 7.2, $J_{9',9}$ 12.5 Hz, H-9′), 4.24–4.32 (m, 3 H, H-4,5,6), 4.69 (dd, 1 H, $J_{9,8}$ 2.6, $J_{9,9'}$ 12.5 Hz, H-9), 5.30 (ddd, 1 H, $J_{8,7}$ 4.9, $J_{8,9'}$ 7.2, $J_{8,9}$ 2.6 Hz, H-8), 5.49 (dd, 1 H, $J_{7,6}$ 1.1, $J_{7,8}$ 4.9 Hz, H-7), 5.72 (d, 1 H, $J_{NH,5}$ 8.2 Hz, CONH), 5.94 (d, 1 H, $J_{3,4}$ 2.4 Hz, H-3); ^{13}C NMR data (300 MHz, $CDCl_3$) δ 20.8 ($3 \times CH_3CO_2$), 23.0 (CH_3CONH), 30.5 (CH_3COS); 42.8 (C-4), 46.9 (C-5), 52.4 (CO_2CH_3), 62.1 (C-9), 67.8 (C-7), 71.4 (C-8), 75.6 (C-6), 110.5 (C-3), 144.9 (C-2), 161.6, 170.2, 170.5 ($3 \times CH_3CO_2$, CH_3CONH, C-1), 195.5 (CH_3COS), FABMS: 490 $(M+1)^+$. Anal. Calcd for $C_{20}H_{27}NO_{11}S$ (489.49): C, 49.1; H, 5.6; N, 2.9; S, 6.5. Found C, 49.1; H, 5.8; N, 2.9; S, 6.5.

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REFERENCES

1 M.P. DeNinno, *Synthesis* (1991) 583–593.
2 A.P. Corfield and R. Schauer, *Sialic Acids, Chemistry, Metabolism and Function*, in R. Schauer (Ed.), *Cell Biology Monographs*, Vol. 10, Springer Verlag, New York, 1982, pp 5–39.
3 A. Lubineau and J. Le Gallic, *J. Carbohydr. Chem.*, 10 (1991) 263–268.
4 G. Estenne, A. Saroli, and A. Doutheau, *J. Carbohydr. Chem.*, 10 (1991) 181–195.
5 J. Roth, C. Zuber, P. Wagner, I. Blaha, D. Bitter-Suermann, and P.U. Heitz, *Lab. Invest.*, 62 (1988) 55–60.
6 W. Reutter, E. Köttgen, C. Bauer, and W. Gerok, *Sialic Acids, Chemistry, Metabolism and Function*, in R. Schauer (Ed.), *Cell Biology Monographs*, Vol. 10, Springer Verlag, Wien, New York, 1982, pp 263–305.
7 E. Schreiner, E. Zbiral, R.G. Kleineidam, and R. Schauer, *Liebigs Ann.Chem.*, (1991) 129–134.
8 V. Kumar, S.W. Tanenbaum, and M. Flashner, *Carbohydr. Res.* 101 (1982) 155–159.