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Synthesis of a Sialic Acid Analog with the Acetamido Group at C-4

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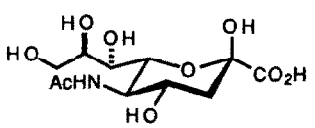
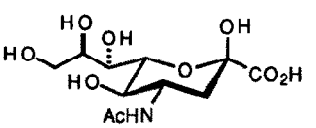
Key Words: aminoulosonic acid, *N*-acetylneuraminic acid, conjugate addition, trimethylsilyl azide.

Abstract: *An isomer of* N-*acetylneuraminic acid (Neu5Ac) with the acetamido group at C-4 (iso-Neu4Ac) has been synthesized through stereoselective 1,4-conjugate addition of trimethylsilyl azide to a 2-thiazolyl α,β-enone bearing a protected* D-*mannose moiety at C-β. The same approach employing benzylamine as aminating reagent was less viable. In both routes the thiazole ring serves as a precursor to the formyl group which is oxidized to carboxylic acid in the final step of the synthesis.*

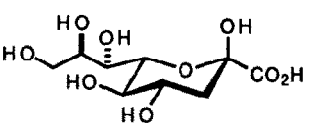
The participation of sialic acids,[1] especially *N*-acetylneuraminic acid (Neu5Ac, **1**), and their conjugates such as gangliosides and glycoproteins, in molecular recognition and cell adhesion and differentiation phenomena[2] has stimulated in recent years chemical and enzymatic synthesis[3] of **1** and various analogs[4] with potential inhibitory activity against sialidases and sialyltransferases. Based on a recent exploratory study of a route to 4-amino-3,4-dideoxyulosonic acids through a Wittig olefination and Michael-type amination sequence,[5] we would like to report here two synthetic approaches to an isomer of **1**, namely 4-acetamido-3,4-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid **2** that we call iso-Neu4Ac.



Neu5Ac, **1**



iso-Neu4Ac, **2**

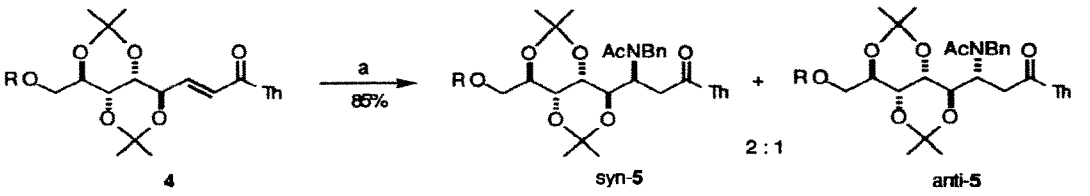


KDN, **3**

Given the satisfactory synthesis[6] of the deaminated analog of **1**, i. e. KDN **3**, by the 1,4-conjugate addition of benzyloxide anion to the α,β-enone **4**, this activated olefin appeared worth application in a route to iso-Neu4Ac **2** through reaction with nitrogen nucleophiles. The thiazole ring of **4** whose high stability ensured tolerance to various synthetic manipulations, served as a precursor to carboxylic acid through aldehyde.[7] Indeed, compound **4** prepared from a suitably protected D-mannose-derived aldehyde and a thiazole-armed carbonyl ylid as described,[6] reacted with benzylamine in CH_2Cl_2 at -50 °C to give a mixture of β-aminoketone diastereomers in ca. 2:1 ratio by NMR (Scheme 1). On standing at room temperature, the ratio changed to a constant 1:1 value, thus indicating a rapid equilibration of the adducts via retroaddition. Hence the crude reaction mixture was quenched at -50 °C with acetic anhydride and the stable acetamides syn-**5** and anti-**5** were isolated[8] in 2:1 ratio and 85% overall yield by HPLC (silica, 6 μm, 60 Å, 85:15 cyclohexane-AcOEt, λ = 254 nm). The configuration of the major isomer syn-**5** was established[9] through the peracetylated methyl pyranoside[8] **7** (Scheme 2) obtained by acid-catalyzed methanolysis of the protecting groups of syn-**5** and

ketalization to the tetrol **6** followed by acetylation of the latter. Syn-selectivity, at a much higher level however (9:1), had been observed[5] in the addition of benzylamine to the 2-thiazolyl α,β-enone derived from D-glyceraldehyde acetonide.

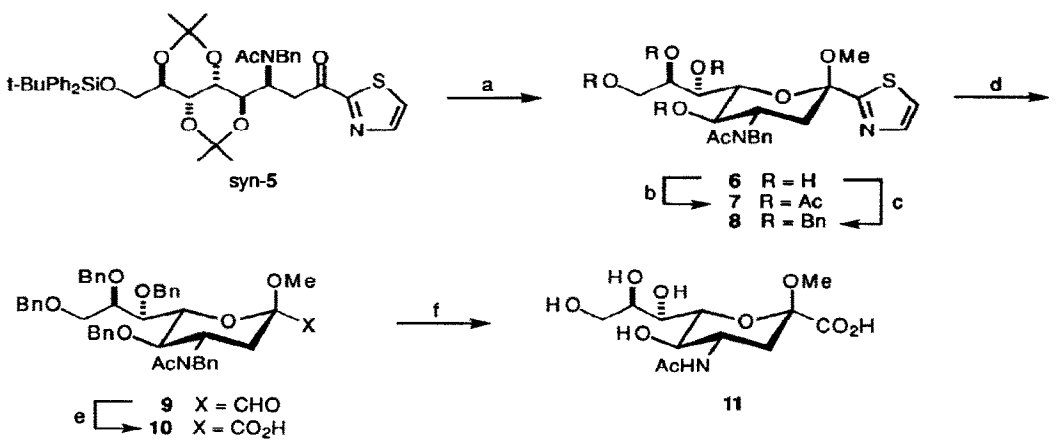
Scheme 1



Th = 2-thiazolyl; R = t-BuPh$_2$Si. Reagents: (a) BnNH$_2$ (3.5 eq.), CH$_2$Cl$_2$, -50 °C, 3.5 h; then Ac$_2$O, -50 °C, 30 min.

For a convenient synthesis, the crude tetrol **6** was transformed into the *O*-benzyl derivative[8] **8** in 56% yield from syn-**5** (Scheme 2). This compound subjected to the standard thiazolyl to formyl unmasking procedure[10] gave the aldehyde **9** which as a crude product was readily oxidized by wet Ag$_2$O to the carboxylic acid[8] **10** in 72% yield. While *O*-debenzylation of **10** was easily carried out by catalytic hydrogenation, the removal of the *N*-benzyl group was problematical. This reaction was carried out by the use of lithium in liquid ammonia to give methyl iso-Neu4Ac **11** (90%) contaminated by numerous side products. Since efforts to remove these impurities by chromatography were unsuccessful, further synthesis was not continued.

Scheme 2

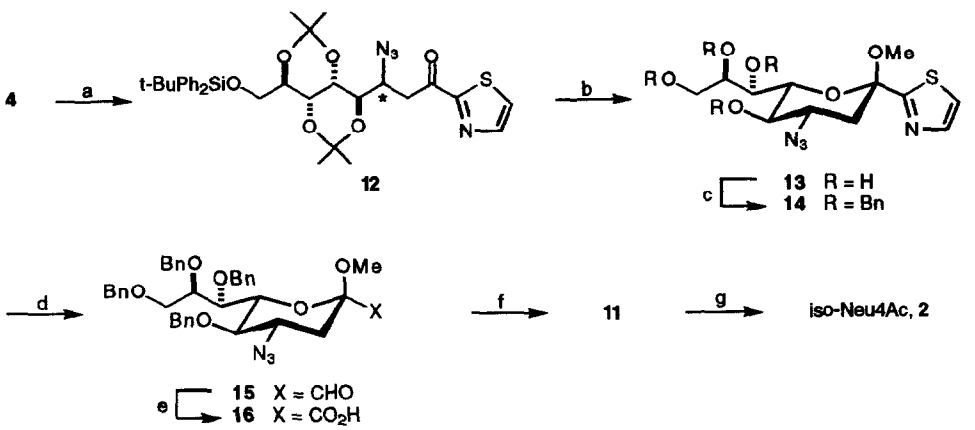


Reagents: (a) 2% w/w HCl-MeOH, r. t., 14 h; (b) Ac$_2$O, pyridine, r. t., 4 h; (c) BnBr, NaH, DMF, 0 °C, 1 h; (d) TfOMe, CH$_3$CN, r. t., 15 min; then NaBH$_4$, MeOH, 0 °C, 15 min; then CuCl$_2$, CuO, CH$_3$CN-H$_2$O, r. t., 15 min; (e) Ag$_2$O, THF-H$_2$O, r. t., 14 h; (f) Li, liquid NH$_3$, -33 °C, 15 min.

As the above synthetic route with benzylamine proved to be quite inefficient mainly because of the modest stereoselectivity and difficult *N*-debenzylation, we decided to employ the azido group as a nitrogen nucleophile precursor to the amino group. Hence, treatment of **4** with trimethylsilyl azide in the presence of 10% Bu$_4$NF in CH$_2$Cl$_2$ at -20 °C (Scheme 3) afforded after extraction with water the β-azido ketone[11] **12** (94%) as a 3:1 mixture of diastereomers by ^{1}H NMR. While the equilibration between these products did not occur on rising

the temperature of the reaction mixture up to room temperature, their separation by column chromatography was unsuccessful due to considerable decomposition. Thus, the crude reaction mixture was treated with HCl-MeOH at room temperature and the fraction containing the major product β-methyl glycoside[11] **13** was isolated by HPLC (silica C18, 6 μm, 60 Å, 80:20 H_2O-MeOH, λ = 254 nm). The benzylation of this material and further purification by HPLC (silica, 6 μm, 60 Å, 94:6 cyclohexane-AcOEt, λ = 254 nm)) afforded the tetra-*O*-benzyl derivative[11] **14** in 40% total yield from **4**. The unequivocally established stereochemistry of the azido group in this compound confirmed that the syn-isomer was the major product of the 1,4-conjugate addition of Me_3SiN_3 to the α,β-enone **4** as well. Therefore, this reaction had stereoselectivity in the same sense observed with benzylamine but at higher level. The synthesis was continued from **14** by standard operations, i. e. the unmasking of the aldehyde **15** and the oxidation to carboxylic acid[11] **16** (70% overall yield), then the concomitant reductive *O*-debenzylation and conversion of the azido to the amino group followed by *N*-acetylation to **11** (92%).[11] Finally, this compound was transformed into iso-Neu4Ac[11] **2** by hydrolysis with 4:1 AcOH-H_2O at 100 °C in 85% yield.[12]

Scheme 3



Reagents: (a) Me_3SiN_3 (1.2 eq), Bu_4NF (0.1 eq.), -20 °C, 4 days; (b) 2% w/w HCl-MeOH, r. t., 14 h; (c) BnBr, NaH, DMF, 0 °C, 1 h; (d) TfOMe, CH_3CN, r. t , 15 min; then $NaBH_4$, MeOH, 0 °C, 15 min; then $CuCl_2$, CuO, CH_3CN-H_2O, r. t., 15 min; (e) Ag_2O, THF-H_2O, r. t., 14 h; (f) Li, liquid NH_3, -33 °C, 15 min; then Ac_2O, MeOH, r. t., 15 min; (g) 4:1 AcOH-H_2O, 100 °C, 1 h.

In conclusion, besides the synthesis of an interesting sialic acid analog whose biological activity is under investigation, the above results point to trimethylsilyl azide as a convenient nitrogen nucleophile, hitherto unexploited to the best of our knowledge, in 1,4-conjugate addition reactions. The scope of this route for the synthesis of 4-aminoulosonic acids now becomes of interest.

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8. syn-**5**: $[\alpha]_D^{20}$ -60 (*c* 0.7, $CHCl_3$); ^1H NMR (DMSO d-6, 160 °C): δ 4.84 (app. q, 1 H, J 6.5 Hz, H-3), 4.71 and 4.47 (2 d, 2 H, J 16.8 Hz, PhCH_2), 3.62-3.45 (m, 2 H, 2 H-2), 2.12 (s, 3 H, Ac). anti-**5**: $[\alpha]_D^{20}$ +46 (*c* 0.6, $CHCl_3$); ^1H NMR (DMSO d-6, 140 °C): δ 4.94 (m, 1 H, H-3), 4.77 and 4.43 (2 d, 2 H, J 16.6 Hz, PhCH_2), 3.52 (dd, 1 H, $J_{2,2'}$ 17.6, $J_{2,3}$ 5.5 Hz, H-2), 3.31 (dd, 1 H, $J_{2',3}$ 7.4 Hz, H-2'), 2.20 (s, 3 H, Ac). **7**: $[\alpha]_D^{20}$ +24 (*c* 0.3, $CHCl_3$); ^1H NMR (DMSO d-6, 160 °C): δ 7.76 and 7.63 (2 d, 2 H, J 3.0 Hz, Th), 7.32-7.18 (m, 5 H, Ph), 5.35-5.28 (m, 2 H, H-6, H-7), 5.21 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.9 Hz, H-4), 4.66 (ddd, 1 H, $J_{2ax,3}$ 13.2, $J_{2eq,3}$ 4.4 Hz, H-3), 4.55 and 4.36 (2d , 2 H, J 17.3 Hz, PhCH_2), 4.47 (dd, 1 H, $J_{8,8'}$ 12.4, $J_{7,8}$ 3.3 Hz, H-8), 4.24 (dd, 1 H, $J_{5,6}$ 2.5 Hz, H-5), 4.19 (dd, 1 H, $J_{7,8'}$ 5.8 Hz, H-8'), 3.10 (s, 3 H, OMe), 2.40 (dd, 1 H, $J_{2eq,2ax}$ 13.2 Hz, H-2eq), 2.25 (dd, 1 H, H-2ax), 2.10, 2.04, and 1.98 (3 s, 15 H, 5 Ac). **8**: $[\alpha]_D^{20}$ -7 (*c* 0.9, $CHCl_3$); ^1H NMR (DMSO d-6, 140 °C): δ 2.97 (s, 3 H, OMe), 2.36 (dd, 1 H, $J_{2ax,2eq}$ 12.9, $J_{2eq,3}$ 4.1 Hz, H-2eq), 2.20-2.05 (m, 1 H, H-2ax), 2.03 (s, 3 H, Ac). **10**: $[\alpha]_D^{20}$ -26 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$, 25 °C) of one rotamer: δ 2.10 (dd, 1 H, $J_{3ax,3eq}$ 12.5, $J_{3eq,4}$ 2.8 Hz, H-3eq), 1.74 (dd, 1 H, $J_{3ax,4}$ 13.8 Hz, H-3ax).
9. The ^1H NMR spectrum of **7** showed large coupling constant values for the pyranoside ring protons, thus indicating a trans-diequatorial arrangement of the acetoxy and *N*-benzylacetamido groups at C-3 and C-4 in a $^1C_4$ conformation. The β-anomeric configuration of the methyl pyranosides **6-10** and **13-16** was proved by a n.O.e. between the methoxy group and the axial proton at C-6 in **11** (ulosonic acid numbering).
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11. **14**: $[\alpha]_D^{20}$ -36 (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$, 25 °C): δ 7.85 (d, 1 H, J 3.0 Hz, Th), 7.40-7.20 (m, 21 H, 4 Ph, Th), 4.28 (dd, 1 H, $J_{5,6}$ 1.5, $J_{6,7}$ 5.0 Hz, H-6), 4.16 (ddd, 1 H, $J_{2eq,3}$ 4.8, $J_{2ax,3}$ 12.2, $J_{3,4}$ 9.0 Hz, H-3), 4.14 (dd, 1 H, $J_{7,8}$ 2.0, $J_{8,8'}$ 10.5 Hz, H-8), 4.08 (ddd, 1 H, $J_{7,8'}$ 5.5 Hz, H-7), 4.05 (dd, 1 H, $J_{4,5}$ 10.0 Hz, H-5), 3.81 (dd, 1 H, H-8'), 3.61 (dd, 1 H, H-4), 2.95 (s, 3 H, OMe), 2.80 (dd, 1 H, $J_{2eq,2ax}$ 13.3 Hz, H-2eq), 1.80 (dd, 1 H, H-2ax). **16**: $[\alpha]_D^{20}$ -52 (*c* 1.3, $CHCl_3$); ^1H NMR ($CDCl_3$, 25 °C): δ 2.48 (dd, 1 H, $J_{3eq,3ax}$ 13.0, $J_{3eq,4}$ 4.2 Hz, H-3eq), 1.75 (dd, 1 H, $J_{3ax,4}$ 12.0 Hz, H-3ax). **11**: $[\alpha]_D^{20}$ -34 (*c* 0.4, MeOH); ^1H NMR (D_2O, 25 °C): δ 3.02 (s, 3 H, OMe), 1.96 (dd, 1 H, $J_{3eq,3ax}$ 13.6, $J_{3eq,4}$ 3.9 Hz, H-3eq), 1.40 (dd, 1 H, $J_{3ax,4}$ 11.7 Hz, H-3ax). **2**: ^1H NMR (D_2O, 25 °C): δ 1.87 (dd, 1 H, $J_{3eq,3ax}$ 13.5, $J_{3eq,4}$ 4.5 Hz, H-3eq), 1.60 (dd, 1 H, $J_{3ax,4}$ 12.4 Hz, H-3ax).
12. Compound **2** was contaminated by ca. 10% of unreacted **11** which could not be removed by chromatography. Prolonged hydrolysis led to a complex mixture of products. We are currently investigating the use of alcohols different from methanol for the chetalization reactions.

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