Synthesis of 5,7-diacetamido-3,5,7,9-tetradeoxy-L-*glycero*-D-*galacto*- and -L-*glycero*-D-*talo*-nonulosonic acids, putative components of bacterial lipopolysaccharides

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The title acids were synthesised by condensation of 2,4-diacetamido-2,4,6-trideoxy-L-gulose with oxalacetic acid and characterised by ^{1}H and ^{13}C NMR spectroscopy.

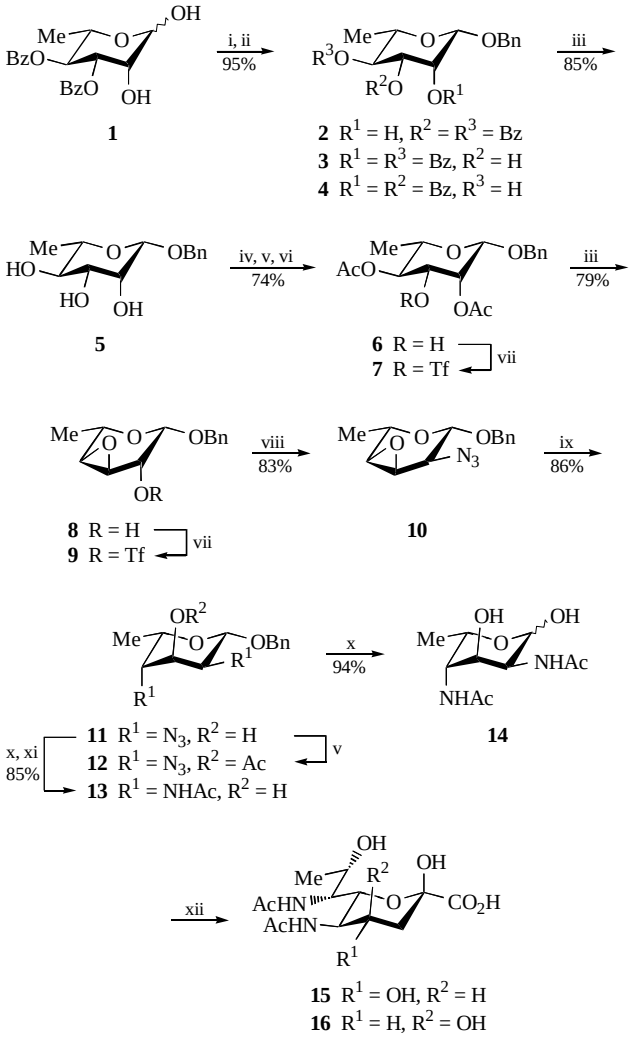
N-Acyl and *O*-acetyl derivatives of 5,7-diamino-3,5,7,9-tetradeoxynonulosonic acids are the components of lipopolysaccharides of Gram-negative bacteria, in which they play a role in serological specificity and endow the bacterial surface with peculiar physico-chemical properties. Pseudaminic and legionaminic acids were discovered in the mid-1980s, and the L-*glycero*-L-*manno* and D-*glycero*-L-*galacto* configurations, respectively, were ascribed to them.[1] More recently, the configuration of legionaminic acid was revised to L-*glycero*-D-*galacto*,[2,3] and its C4-epimer was isolated from a lipopolysaccharide from *Legionella pneumophila*.[4] Reliable identification of these sugars, including the determination of absolute configurations, requires authentic samples. Here, we report on the first synthesis of compounds of this class.

By analogy with the preparation of *N*-acetylneuraminic acid,[5] the condensation of 2,4-diacetamido-2,4,6-trideoxy-L-gulose **14**, in which the functionalisation and configuration of C2–C6 correspond to those of C5–C9 in the target nonulosonic acids, with oxalacetic acid was used at the key step of the synthesis. L-Rhamnose, a readily available 6-deoxyhexose, served as the progenitor of **14**. Since the introduction of an azido group (as a precursor of the acetamido function) is accompanied by inversion of configuration, azidation at the 2- and 4-positions in L-rhamnose would lead directly to the desirable configurations of C2 and C4. Hence, an additional inversion at C3 has to be performed to achieve the target L-*gulo* configuration.

The prerequisite for successful nucleophilic substitution of axial O2 sulfonates is that the substituent at C1 is equatorial.[6] Therefore, benzyl β-L-rhamnopyranoside **5** was thought to be the precursor of choice. It was prepared from 1,2-diol **1** by Bu_2SnO-mediated benzylation.[7] As expected,[8] the reaction occurred in a regio- and stereoselective manner, though it was accompanied by migration of benzoyl protecting groups (Scheme 1). As a result, a mixture of benzyl β-L-rhamnopyranoside dibenzoates **2**–**4** was obtained in a total yield of 90–95% with the **2**:**3**:**4** ratio approximately equal to 4:3:1. Debenzoylation of the mixture of **2**–**4** with NaOMe in methanol gave **5**.

The treatment of **5** with trimethyl orthoacetate in the presence of TsOH followed by acetylation of OH4 and hydrolytic opening of the orthoester ring in the resultant 2,3-orthoester yielded 2,4-diacetate **6**. The reaction of **6** with triflic anhydride in the presence of pyridine led to triflate **7**, which readily gave 3,4-anhydro-6-deoxyaltroside **8**† on treatment with NaOMe in methanol. Compound **8** was the key intermediate having (i) a necessary configuration of C3, (ii) a free OH2 group required for the subsequent introduction of an azido group and (iii) a 3,4-epoxy function suitable for the introduction of the second azido group at the 4-position.

As anticipated, the conversion of **8** to triflate **9** and subsequent reaction with NaN_3 in DMF resulted in azide **10** in a high yield. A large $J_{1,2}$ coupling constant of 7.6 Hz in the ^{1}H NMR spectrum of **10** showed that the azido group was *pseudo*-equatorial. The opening of the epoxide ring in **10** with NaN_3 in the presence of NH_4Cl in boiling aqueous ethanol[9] afforded diazide **11**. Large $J_{1,2}$ and small $J_{2,3}$, $J_{3,4}$ and $J_{4,5}$ coupling constants in the ^{1}H NMR spectrum of derived acetate **12**‡ proved unambiguously that **11** had the β-*gulo* configuration. The chemical shifts



Scheme 1 *Reagents and conditions*: i, Bu_2SnO, PhH, reflux; ii, BnBr, Bu_4NBr, PhH, reflux; iii, MeONa, MeOH; iv, $MeC(OMe)_3$, TsOH, MeCN; v, Ac_2O, pyridine; vi, aqueous 80% AcOH; vii, Tf_2O, pyridine, CH_2Cl_2, 0 °C; viii, NaN_3, DMF, 20 °C; ix, NaN_3, NH_4Cl, EtOH–H_2O, reflux; x, H_2, $Pd(OH)_2$/C, MeOH; xi, Ac_2O, MeOH; xii, oxalacetic acid, $Na_2B_4O_7$, pH 10.5.

† Compound **8**: $[\alpha]_D$ +87° (*c* 2.6, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 1.47 (d, 3H, H6, $J_{5,6}$ 7.0 Hz), 3.04 (d, H4, $J_{3,4}$ 3.9 Hz), 3.44 (dd, H3, $J_{2,3}$ 1.7 Hz), 4.02 (t, H2, $J_{1,2}$ 1.6 Hz), 4.07 (q, H5), 4.54 (d, H1), 4.58, 4.89 (2d, 2H, CH_2Ph, J_{gem} 11.9 Hz), 7.27–7.42 (m, 5H, Ph).

‡ Compound **12**: ^{1}H NMR ($CDCl_3$) δ: 1.38 (d, 3H, H6, $J_{6,5}$ 6.5 Hz), 2.05 (s, 3H, AcO), 3.48 (dd, H4, $J_{4,5}$ 1.7 Hz), 3.69 (dd, H2, $J_{2,3}$ 3.4 Hz), 4.03 (dq, H5), 4.69, 4.96 (2d, 2H, CH_2Ph, J_{gem} 11.8 Hz), 4.79 (d, H1, $J_{1,2}$ 8.1 Hz), 5.33 (t, H3, $J_{3,4}$ 3.5 Hz), 7.30–7.42 (m, 5H, Ph).

of H2–H4 in the ^{1}H NMR spectrum of **12** demonstrated the location of the azido groups at C2 and C4. The consecutive reduction of the azido groups in **11** by hydrogenation over $Pd(OH)_2$, N-acetylation, and removal of the protective benzyl group from **13** led to target sugar **14**§ in a high yield.

The condensation of **14** with oxalacetic acid in the presence of $Na_2B_4O_7$ at pH 10.5[5] resulted in a mixture of nonulosonic acids **15** and **16**. This mixture was isolated by anion-exchange chromatography (Dowex 1×8, 0.3 M formic acid) and separated by reversed-phase HPLC (C_{18}, 0.05% TFA) to afford **15**, $[\alpha]_D^{22}$ +15.4° (*c* 1.6, H_2O), and **16**, $[\alpha]_D^{22}$ –19.2° (*c* 1.7, H_2O), in 18 and 20% yields, respectively.

According to the ^{1}H and ^{13}C NMR spectral data,¶ both of the compounds were isolated as mixtures of anomers (α:β ≈ 1:19 and 1:8 for **15** and **16**, respectively). However, the concentration of **15**α was too small for the reliable assignment of the NMR spectra. The $J_{3a,4}$, $J_{4,5}$ and $J_{5,6}$ coupling constant values in the ^{1}H NMR spectra of **15** and **16** clearly indicated that the substituents at C4, C5, and C6 in **15** and at C5 and C6 in **16** were equatorial, whereas the hydroxy group at C4 in **16** was axial. The ^{13}C NMR spectrum showed upfield shifts by 1.6–4.5 ppm for the C3–C6 signals in **16**β, as compared to the corresponding signals in **15**β (*cf*. similar data for *N*-acetylneuraminic acid and its C4-epimer[10]). The most marked difference between **16**α and **16**β was observed for the chemical shifts of C6 (δ 72.7 and 68.6, respectively). This provides a basis for the determination of the anomeric configuration of nonulosonic acids from this class. A comparison of the synthetic and natural 5,7-diacetamido-3,5,7,9-tetradeoxynonulosonic acids will be published elsewhere.

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§ Compound **14**: mp 144–146 °C (MeOH–diethyl ether), $[\alpha]_D$ +6.3 → +76° (*c* 1.6, MeOH). ^{1}H NMR (D_2O) δ: **14**α, 1.16 (d, 3H, H6, $J_{5,6}$ 6.6 Hz), 2.06, 2.07 (2s, 6H, 2MeCON), 3.91 (t, 1H, H3, $J_{3,4}$ 3.8 Hz), 3.95 (dd, 1H, H4, $J_{4,5}$ 1.7 Hz), 4.11 (t, 1H, H2, $J_{2,3}$ 3.5 Hz), 4.62 (dq, 1H, H5), 5.15 (d, 1H, H1, $J_{1,2}$ 4.0 Hz); **14**β, 1.18 (d, 3H, H6, $J_{5,6}$ 6.5 Hz), 2.04, 2.08 (2s, 6H, 2MeCON), 3.85 (dd, 1H, H2, $J_{2,3}$ 3.2 Hz), 3.87 (dd, 1H, H4, $J_{4,5}$ 1.6 Hz), 3.94 (t, 1H, H3, $J_{3,4}$ 3.4 Hz), 4.29 (dq, 1H, H5), 4.94 (d, 1H, H1, $J_{1,2}$ 8.9 Hz). The ratio **14**α:**14**β ≈ 1:5.

¶ Spectral data (D_2O, 303 K, pD 1.7, acetone as an internal standard: δ_H 2.225, δ_C 31.45).

15β: ^{1}H NMR, δ: 1.16 (d, 3H, H9, $J_{8,9}$ 6.2 Hz), 1.85 (dd, H3a, $J_{3a,4}$ 12.2 Hz, $J_{3a,3e}$ 13.1 Hz), 1.96, 2.00 (2s, 6H, 2MeCON), 2.30 (dd, H3e, $J_{3e,4}$ 4.8 Hz), 3.70 (t, H5, $J_{4,5}$ 10.2 Hz), 3.89 (quintet, H8, $J_{7,8}$ 6.4 Hz), 3.92 (ddd, H4), 3.93 (dd, H7, $J_{6,7}$ 2.0 Hz), 4.14 (dd, H6, $J_{5,6}$ 10.3 Hz). ^{13}C NMR, δ: 20.0 (C9), 23.3, 23.6 (2*Me*CON), 40.6 (C3), 54.3 (C5), 54.7 (C7), 68.5 (C4), 69.5 (C8), 73.1 (C6), 96.7 (C2), 174.3 (C1), 175.4, 175.5 (2Me*C*ON).

16β: ^{1}H NMR, δ: 1.21 (d, 3H, H9, $J_{8,9}$ 5.7 Hz), 1.97, 2.01 (2s, 6H, 2MeCON), 2.13 (dd, H3a, $J_{3a,4}$ 3.3 Hz, $J_{3a,3e}$ 14.9 Hz), 2.18 (dd, H3e, $J_{3e,4}$ 2.9 Hz), 3.89 (dd, H5, $J_{4,5}$ 2.8 Hz, $J_{5,6}$ 10.6 Hz), 3.95 (m, H7), 3.96 (m, H8), 4.11 (m, H4), 4.48 (dd, H6, $J_{6,7}$ 1.3 Hz). ^{13}C NMR, δ: 19.9 (C9), 23.0, 23.1 (2*Me*CON), 37.6 (C3), 49.9 (C5), 54.8 (C7), 66.9 (C4), 68.6 (C6), 69.3 (C8), 96.2 (C2), 174.4 (C1), 174.6, 175.2 (2Me*C*ON).

16α: ^{1}H NMR, δ: 1.28 (d, 3H, H9, $J_{8,9}$ 6.3 Hz), 1.94 (dd, H3a, $J_{3a,4}$ 2.7 Hz, $J_{3a,3e}$ 14.4 Hz), 1.96, 2.04 (2s, 6H, 2MeCON), 2.65 (dd, H3e, $J_{3e,4}$ 3.6 Hz), 3.84 (dd, H5, $J_{4,5}$ 2.7 Hz, $J_{5,6}$ 10.5 Hz), 3.89 (H7), 4.08 (m, 2H, H4, H8), 4.47 (dd, H6). ^{13}C NMR, δ: 19.9 (C9), 40.0 (C3), 50.1 (C5), 54.8 (C7), 66.6 (C4), 69.8 (C8), 72.7 (C6).

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