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Synthesis and identification in bacterial lipopolysaccharides of 5,7-diacetamido-3,5,7,9-tetradeoxy-D-glycero-D-galacto- and -D-glycero-D-talo-non-2-ulosonic acids

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Abstract

5,7-Diacetamido-3,5,7,9-tetradeoxy-D-glycero-D-galacto- and -D-glycero-D-talo-non-2-ulosonic acids were synthesized by condensation of 2,4-diacetamido-2,4,6-trideoxy-D-mannose with oxalacetic acid. Comparison of the ^{1}H and ^{13}C NMR data and the specific optical rotation values of these monosaccharides and the corresponding L-glycero-D-galacto and L-glycero-D-talo isomers synthesized earlier [Tsvetkov, Y. E.; Shashkov, A. S.; Knirel, Y. A.; Backinowsky, L. V.; Zähringer, U. *Mendeleev Commun.* **2000**, 90–92] with data of the natural compounds enabled the identification in bacterial lipopolysaccharides of derivatives of 5,7-diamino-3,5,7,9-tetradeoxy-D-glycero-D-galacto-non-2-ulosonic (legionaminic) acid and epimers of legionaminic acid at C-4 and C-8.

Keywords: 5,7-Diamino-3,5,7,9-tetradeoxynon-2-ulosonic acid; Synthesis; Legionaminic acid, identification of; Lipopolysaccharide components; *Legionella pneumophila*

N-Acyl and *O*-acetyl derivatives of 5,7-diamino-3,5,7,9-tetradeoxynon-2-ulosonic acids have been reported as components of the lipopolysaccharides (LPS) of gram-negative bacteria, which play a role in serological specificity and endow the bacterial surface with peculiar physicochemical properties.[1–3] The first monosaccharide of this class discovered in the mid-1980s in the LPS of *Pseudomonas aeruginosa* and *Shigella boydii* was identified as the L-glycero-L-manno isomer (pseudaminic acid).[4,5] The D-glycero-L-galacto configuration that was ascribed to the second isomer found first in *P. aeruginosa*,[6] was later revised to the L-glycero-D-galacto configuration.[7,8] A homopolymer of a derivative of a 5,7-diamino-3,5,7,9-tetradeoxynon-2-ulosonic acid, called legionaminic acid, was described as the O-chain of the LPS in *Legionella pneumophila* serogroup 1[3,9] and *Pseudomonas fluorescens* ATCC 49271,[10,11] and it was suggested that legionaminic acid has the same L-glycero-D-galacto configuration.[7] While the configurations of the higher sugars in these and some

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other LPS[12–14] (Table 1) were ascribed by NMR spectroscopy without their isolation as monosaccharides, two 5,7-diacetamido-3,5,7,9-tetradeoxynon-2-ulosonic acids were released by mild acid hydrolysis from the LPS of *Vibrio alginolyticus*[15] and *L. pneumophila*,[16] and their configurations were tentatively assigned to be L-glycero-D-galacto and L-glycero-D-talo, respectively.

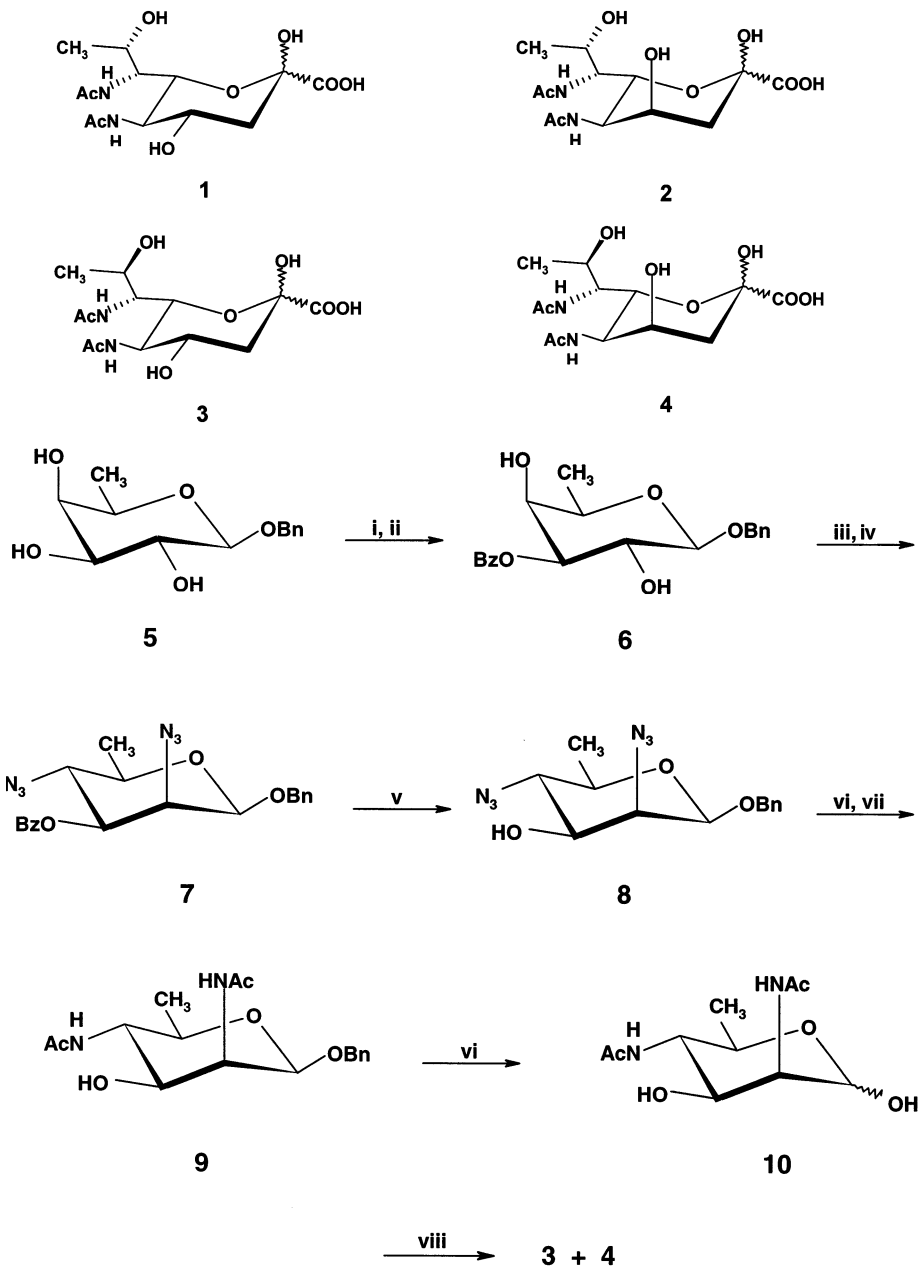
Aiming at unambiguous identification of these higher sugars, including determination of their absolute configuration, we have synthesized, for the first time, 5,7-diacetamido-3,5,7,9-tetradeoxy-L-glycero-D-galacto- (**1**) and -L-glycero-D-talo-non-2-ulosonic acids (**2**) from 2,4-diacetamido-2,4,6-trideoxy-L-gulose.[17] In this paper, we report the first synthesis of the corresponding D-glycero-D-galacto (**3**) and D-glycero-D-talo (**4**) isomers, which enabled determination of the configurations of the natural monosaccharides, legionaminic acid (Leg) and two of its epimers, 4-epi- and 8-epi-legionaminic acid (4eLeg and 8eLeg, respectively).

Synthesis was performed starting from benzyl β-D-fucopyranoside **5**,[18] which afforded 3-benzoate **6** on Bu_2SnO-mediated selective benzoylation (Scheme 1). Conversion of **6** into the corresponding 2,4-ditriflate, followed by bis-azidation with Bu_4NN_3, resulted in diazide **7** having the manno configuration. After debenzoylation (**7**→**8**), reduction of the azido groups was performed by hydrogenation over $Pd(OH)_2$–C without affecting the benzyl group[19] and followed by N-acetylation (**8**→**9**). Removal of the benzyl aglycon by hydrogenolysis gave 2,4-diacetamido-2,4,6-trideoxy-D-mannose (**10**) in 38% overall yield from **5**. All new compounds were fully characterized by NMR spectroscopy and elemental analysis.

Condensation of **10** with oxalacetic acid in the presence of sodium tetraborate at pH 10.5[20] gave a mixture of **3** and **4**. The individual compounds **3**, $[\alpha]_D$ +27.2° (*c* 1, water), and **4**, $[\alpha]_D$ −12.5° (*c* 1, water), were isolated in 7 and 10% yield, respectively, by anion-exchange chromatography on Dowex 1 × 8 (aq 0.3 M formic acid) followed by reversed-phase C_{18} HPLC (aq 0.05% CF_3CO_2H). The ^1H and ^{13}C NMR spectroscopic data (Table 2) showed that **3** and **4** are mixtures of α and β anomers in the ratio 1:18 and 1:5.4, respectively, and proved their configurations. Particularly, the $J_{3a,4}$, $J_{4,5}$, and $J_{5,6}$ coupling constant values clearly indicated that the substituents at C-4,5,6 in **3** and C-5,6 in **4** are equatorial, whereas the hydroxy group at C-4 in **4** is axial.

Table 1
Some naturally occurring isomers of 5,7-diamino-3,5,7,9-tetradeoxynon-2-ulosonic acid

Source (lipopolysaccharide of)	Configuration		
	Originally ascribed	Previously revised to[7,8]	Revised to/confirmed in this work
Legionaminic acid (Leg)			
Legionella pneumophila	D-glycero-L-galacto[9]	L-glycero-D-galacto	D-glycero-D-galacto
Pseudomonas fluorescens	D-glycero-L-galacto[10,11]	L-glycero-D-galacto	D-glycero-D-galacto
Vibrio salmonicida	L-glycero-D-galacto[8]		D-glycero-D-galacto
Acinetobacter baumannii	L-glycero-D-galacto[12]		D-glycero-D-galacto
Vibrio alginolyticus	D-glycero-L-galacto[15]	L-glycero-D-galacto	D-glycero-D-galacto
8-Epilegionaminic acid (8eLeg)			
Pseudomonas aeruginosa	D-glycero-L-galacto[6]	L-glycero-D-galacto	L-glycero-D-galacto
Salmonella arizonae	D-glycero-L-galacto[13]	L-glycero-D-galacto	L-glycero-D-galacto
Yersinia ruckerii	D-glycero-L-galacto[14]	L-glycero-D-galacto	L-glycero-D-galacto
4-Epilegionaminic acid (4eLeg)			
Legionella pneumophila	L-glycero-D-talo[16]		D-glycero-D-talo



Scheme 1. **i**, Bu_2SnO, benzene, reflux; **ii**, BzCl, rt; **iii**, Tf_2O, pyridine, CH_2Cl_2, 0 °C; **iv**, Bu_4NN_3, toluene, 100 °C; **v**, MeONa, MeOH, rt; **vi**, H_2, $Pd(OH)_2$-C, MeOH, rt; **vii**, Ac_2O, MeOH, rt; **viii**, oxalacetic acid, $Na_2B_4O_7$, pH 10.5, rt.

Comparison of the ^1H and ^{13}C NMR data of synthetic and natural compounds (Table 2) suggested that 5,7-diacetamido-3,5,7,9-tetradeoxynon-2-ulosonic acids from LPS of *V. alginolyticus* (Leg5Ac7Ac)[15] and *L. pneumophila* (4eLeg5Ac7Ac)[16] have the same relative configurations as **3** and **4** and differ from those of **1** and **2**, respectively. In addition to the $J_{H,H}$ coupling constant values, this conclusion was based on the ^{13}C NMR chemical shifts for C-6 and C-8, which are indicative of the configuration at the side chain. The specific optical rotation values of the natural compounds, $[\alpha]_D$ +25.2° (*c* 0.5, water) for Leg5Ac7Ac[15] and $[\alpha]_D$ −3.9° (*c* 0.2, water) for 4eLeg5Ac7Ac (this work), compared with the data for **3** and **4**, respectively (see above), indicated that they have the same absolute configurations as well. The identity of the monosaccharide from *L. pneumophila* was confirmed by GLC–MS of the acetylated (+)-2-butyl ester (+)-2-butyl glycosides.

Table 2
500 MHz ^{1}H and 125 MHz ^{13}C NMR data of synthetic compounds **1–4** and natural compounds Leg5Ac7Ac and 4eLeg5Ac7Ac for solutions in D_2O at 30 °C (δ, ppm, related to internal acetone, δ_H 2.225, δ_C 31.45; $J_{H,H}$, Hz)

Compound	H-3eq ($J_{3eq,3ax}$)	H-3ax ($J_{3ax,4}$)	H-4 ($J_{3eq,4}$)	H-5 ($J_{4,5}$)	H-6 ($J_{5,6}$)	H-7 ($J_{6,7}$)	H-8 ($J_{7,8}$)	H-9 ($J_{8,9}$)	CH_3CON	
^{1}H NMR										
1α	2.67 (12.9)	1.69 (12)	3.81 (4.6)	3.65 (10.2)	4.82 (10)	3.90	3.97	1.17 (6.3)		
1β [17]	2.30 (13.1)	1.85 (12.2)	3.92 (4.8)	3.70 (10.2)	4.14 (10.3)	3.93 (2.0)	3.89 (6.4)	1.16 (6.2)	1.96, 2.00	
2α [17]	2.65 (14.4)	1.94 (2.7)	4.08 (3.6)	3.84 (2.7)	4.47 (10.5)	3.89	4.08	1.28 (6.3)	1.96, 2.04	
2β [17]	2.18 (14.9)	2.13 (3.3)	4.11 (2.9)	3.89 (2.8)	4.48 (10.6)	3.95 (1.3)	3.96	1.21 (5.7)	1.97, 2.01	
3α	2.73 (12.9)	1.71 (11.9)	3.82 (4.7)	3.68	3.93 (10.3)	3.84	3.94	1.16	1.97, 2.00	
3β	2.31 (13.1)	1.87 (11.7)	3.98 (4.8)	3.72 (10.3)	4.31 (10.5)	3.91 (1.9)	3.85 (8.9)	1.16 (6.2)	1.99, 2.00	
4α	2.69 (14.4)	1.95 (3.5)	4.10 (3.0)	3.86 (2.9)	4.55 (10.8)	3.88 (2.3)	4.00 (8.6)	1.20 (6.4)	1.96, 2.00	
4β	2.19 (14.9)	2.14 (3.4)	4.13 (3.0)	3.90 (2.9)	4.63 (10.8)	3.92	3.92	1.18 (5.5)	1.98, 1.99	
Leg5Ac7Acβ [a]	2.20 (13)	1.80 (11.5)	3.93 (5)	3.70 (10.3)	4.22 (10.4)	3.84 (1.8)	3.84	1.14 (6.3)		
4eLeg5Ac7Acα [b]	2.67 (14.5)	1.95 (3.1)	4.09 (2.6)	3.86 (2.6)	4.54 (11)	3.86 (2.2)	4.00 (8.5)	1.20 (6.6)	1.96, 2.01	
4eLeg5Ac7Acβ [b]	2.17 (14.9)	2.13 (3.1)	4.12 (2.6)	3.90 (2.6)	4.62 (11)	3.91 (<2)	3.91	1.18 (5.3)	1.99, 2.00	

^{13}C NMR	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	CH_3CON	CH_3CON
1α			41.2	68.9	53.7	75.2	54.4	69.4	19.8		
1β [17]	174.3	96.5	40.4	68.3	54.1	72.9	54.4	69.3	19.8	23.1; 23.4	175.1; 175.2
2α [17]			40.0	66.6	50.1	72.6	54.9	69.7	19.9		
2β [17]	174.5 [c]	96.1	37.7	66.9	50.0	68.5	54.9	69.2	19.9	23.0; 23.1	174.6 [c]; 175.2
3α			41.4	69.2	53.4	73.2	54.4	68.0	20.4		
3β	174.4 [c]	96.6	40.3	68.4	53.9	70.9	54.4	67.5	20.4	23.0; 23.4	175.0 [c]; 175.2
4α			40.2	66.9	49.7	70.3	55.0	68.2	20.4		
4β	174.7	96.3	37.6	67.1	49.7	66.5	54.7	67.2	20.4	23.0; 23.1	174.7; 175.0
Leg5Ac7Acβ [a]	175.1	97.7	40.9	67.9	54.1	70.8	54.5	68.3	20.4	23.0; 23.4	175.1
4eLeg5Ac7Acα [b]			40.0	66.9	49.8	70.2	54.9	68.1	20.4		
4eLeg5Ac7Acβ [b]	174.9 [c]	96.5	37.6	67.2	49.7	66.5	54.6	67.4	20.4	22.9; 23.1	174.6 [c]; 174.9 [c]

[a] Obtained by mild acid hydrolysis of LPS of *V. alginolyticus* strain 945-80.[15]
[b] Obtained by mild acid hydrolysis of O-deacylated LPS of *L. pneumophila* serogroup 1, strain Philadelphia 1 (this work).
[c] Assignment within one row could be interchanged.

The data obtained enabled also determination of the configuration of the higher sugars that could not be isolated as monosaccharides. Based on the $J_{H,H}$ coupling constant and ^{13}C NMR chemical shift data, it was concluded that legionaminic acid in the homopolymer O-chain of LPS of *L. pneumophila*[9] has the same relative configuration as **3** (e.g., compare $\delta_{C\text{-}6}$ 73.5 and $\delta_{C\text{-}8}$ 68.2 in Leg5Ac7Acα[9] with the values $\delta_{C\text{-}6}$ 73.2 and $\delta_{C\text{-}8}$ 68.0 in **3**α but $\delta_{C\text{-}6}$ 75.2 and $\delta_{C\text{-}8}$ 69.4 in **1**α). It has also the same absolute configuration, as shown by GLC–MS of the acetylated (+)-2-butyl ester (+)-2-butyl glycosides derived from LPS. Therefore, the configuration of Leg in the *L. pneumophila* polysaccharide should be revised from L-glycero-D-galacto[7–9] to D-glycero-D-galacto (Table 1). In contrast, the L-glycero-D-galacto configuration that had been ascribed to the isomer found in LPS of *P. aeruginosa*[6–8], could be confirmed. Indeed, this sugar is characterized by the values $\delta_{C\text{-}6}$ 74.8 and $\delta_{C\text{-}8}$ 70.1[6] and thus represents 8eLeg5Ac7Ac. Similarly, it was concluded that derivatives of Leg are present in the O-chain polysaccharides of *Vibrio salmonicida* LPS,[8] *Pseudomonas fluorescens*[10,11] and *Acinetobacter baumannii*,[12] whereas derivatives of 8eLeg are components of LPS of *Salmonella arizonae*[13] and *Yersinia ruckerii*[14] (Table 1).

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