Novel Synthesis of Disaccharides Containing the 2-Amino-2-deoxy-β-D-glucopyranosyl Unit and L-*Glycero*-D-*Manno*- and 7-Deoxy-L-*Glycero*-D-*Galacto*-heptopyranoses

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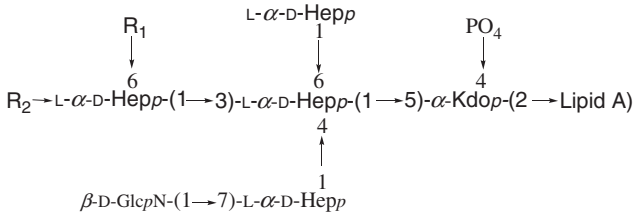
Stereocontrolled syntheses of immunologically relevant heptopyranose disaccharides were achieved in excellent yields by the trimethylsilyl triflate promoted glycosylation of the donor 1,3,4,6-tetra-*O*-acetyl-2-*N*-allyloxycarbonyl-2-amino-2-deoxy-β-D-glucopyranose with the adequate acceptors.

Lipopolysaccharides of Gram-negative bacteria (LPS) have a profound effect on the mammalian immune system and have great importance in various human diseases. The LPS structure is composed of three distinct regions: the lipid A, the core oligosaccharides (composed of an inner core and outer core) and the *O*-polysaccharide chain. L-*Glycero*-D-*manno*-heptose[1,2] (LD-Hep) is considered as a ubiquitous constituent of the inner core-region of the LPS.[3,4]

In the course of structural investigations on the precise molecular structure of lipopolysaccharides of Gram-negative bacteria *Vibrio ordalii* and *Aeromonas salmonicida*[5] we have isolated the disaccharide, 7-*O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-L-*glycero*-D-*manno*-heptose (**1**), by hydrolysis of the core oligosaccharides with 2 M hydrochloric acid for 1 h at 100 °C. This aminoglucosylheptose disaccharide **1** appears to be one of the antigenic determinants of the rough lipopolysaccharide. The core oligosaccharides have the following structure:

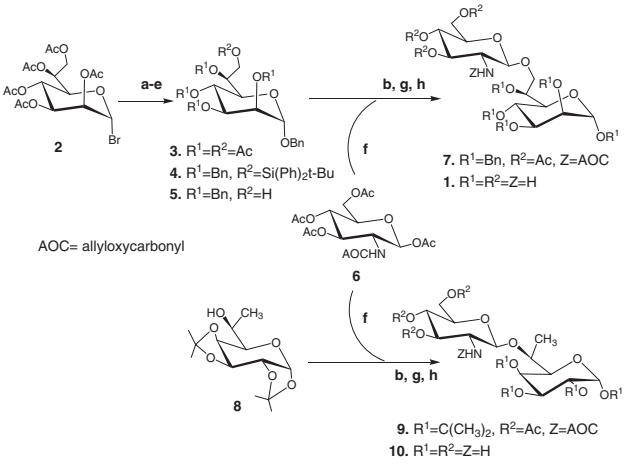


R_1= R_2 =α-D-Glc*p*, lipopolysaccharide core of *V. ordali*
R_1= H, R_2 =α-D-Gal*p*-(1→4)-α-D-Gal*p*NAc-(1→4)-α-D-Hep*p*-(1→4)-, lipopolysaccharide core of *A. salmonicida*

Similarly, during the studies of the precipitin reaction antigens, possessing a β-D-Glc*p*NAc-(1 → 4)-α-D-galacto-pyranosyl haptenic structure and their specific antibodies, it was found that the immunologically relevant disaccharide, β-D-Glc*p*NAc-(1 → 6)-7-deoxy-L-glycero-α-D-galactoheptopyranose (**10**), was a potent inhibitor.[6] Owing to their close resemblance, the syntheses of both aminoglucosylheptose disaccharides **1** and **10** were investigated simultaneously.

We have published the uses of 1,3,4,6-tetra-*O*-acetyl-2-*N*-allyloxycarbonyl-2-amino-2-deoxy-β-D-glycopyranose (**6**) as a potent 1,2-*trans*-glycosylating agent using Lewis acid as condensation promotor.[7,8] Using this *N*-allyloxycarbonyl approach, a variety of disaccharides were stereospecificly and regioselectively synthesised. The *N*-allyloxycarbonyl protective group was easily removed with Pd(0) complexes, thus affording the free amino group containing glycoside. We report in this communication the successful extension of this method to the synthesis of the immunogically relevant aminoglucosylheptose disaccharides **1** and **10**.

The glycosyl acceptor benzyl-2,3,4,6-tetra-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranoside (**5**) was synthesized in 70% overall yield starting from 2,3,4,6,7-penta-*O*-acetyl-L-*glycero*-α-D-*manno*-heptopyranosyl bromide (**2**).[9] The transformation of glycosyl bromide **2** into the corresponding glycosyl acceptor **5** was realized via the intermediates **3** and **4** according to the Hanessian and Banoub procedure (Scheme 1).[10]



a. AgOTf, tetramethylurea, BnOH, 10 °C; **b.** MeONa, MeOH; **c.** ClSi(Ph)2t-Bu, imidazole; **d.** BrBn, NaH, DMF; **e.** 3 % HCl, MeOH; **f.** TMSOTf, CH_2Cl_2,-30 °C; **g**. $Pd(PPh_3)_4$, $CH_2(COOMe)_2$; **h.** 10% Pd/C, H_2.

Scheme 1.

The disaccharide benzyl-7-*O*-(3,4,6-tri-*O*-acetyl-2-*N*-allyloxycarbonyl-2-amino-2-deoxy-β-D-glucopyranosyl)-2,3,4,6-tetra-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranoside was synthetisized by reaction between the glycosyl acceptor **5** and the donor **6**[7,8] in the presence of TMSOTf in stoichiometric amounts in dry CH_2Cl_2 at −30 °C for 18 h, to afford, after conventional work up, a chromatographically pure, white solid foam disaccharide (**7**) in 89% yield.[11]

Electrospray using a QqTOF MS/MS hybrid instrument was performed on disaccharide **7**. The conventional ES showed a diagnostic protonated molecule $[M + H]^+$ at m/z 1033.4460 and the sodiated molecule $[M + Na]^+$ at m/z 1055.7670. CID

MS/MS of these last selected two molecular ions afforded series of expected diagnostic product ions, thus confirming the proposed structure.

The 2-*N*-allyloxycarbonyl-2-deoxy-β-D-disaccharide **7** was then subjected to the Zemplen deacetylation, and the *N*-allyloxycarbonyl group was deprotected to the free amino-group using $Pd(PPh_3)_4$ in the presence of an allyl acceptor as previously reported.[7,8] This was followed by a hydrogenolysis in the presence of 10% Pd/C in methanol, leading to the desired aminoglycosylheptose disaccharide β-D-Glc*p*N-(1-7)-L-*glycero*-D-*manno*-heptopyranose **1**, in 82% overall yield.[12]

It may be noted that another approach for the synthesis of the aminoglucosylheptose disaccharide hydrochloride **1** was achieved using 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl bromide (3.7 equiv.) and benzyl-2,3,5,6-tetra-*O*-benzyl-L-*glycero*-D-*manno*-heptofuranoside (1 equiv.) in the presence of silver silicate at $-50\,^{\circ}C$ and led to an anomeric mixture of the expected disaccharides.[13]

The synthesis of disaccharide 6-*O*-(3,4,6-tri-*O*-acetyl-2-*N*-allyloxycarbonyl-2-amino-2-deoxy-β-D-glucopyranosyl)-7-deoxy-1,2:3,4-di-*O*-isopropylidene-L-*glycero*-α-D-*galacto*-heptopyranose (**9**) was performed by reacting stoichiometric amounts of the glycosyl donor **6** with 1,2:3,4-di-*O*-isopropylidene-7-deoxy-L-*glycero*-α-D-*galacto*-heptopyranose (**8**)[14] as the glycosyl acceptor and TMSOTf in dry CH_2Cl_2 at $-30\,^{\circ}C$ for 18 h to afford, after conventional work up, a chromatographically pure white solid foam in 79% yield which was crystallized from ether/EtOAc (72% yield).[15]

Finally, the **9** was recovered by the deprotection pathway used in case of disaccharide **7**, leading to the expected 6-*O*-(-2-*N*-acetamido-2-deoxy-β-D-glucopyranosyl)-7-deoxy-L-*glycero*-D-*galacto*-heptopyranose disaccharide (**10**) in 80% overall yield.[16]

To sum up, this work provided two stereocontrolled syntheses of immunogically relevant disaccharides which were achieved in excellent yields using the *N*-allyloxycarbonyl approach.

The preparation of antigenic branched glycoconjugates of the poly-*N*-acetyllactosamine series [β-D-Gal*p*-(1 $\rightarrow$ 4)-Glc*p*NAc-(1-]$_n$, using the *N*-allyloxycarbonyl derivatives of lactosamine is under studies in our laboratories and will be reported in due course.

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11 mp 120–121 °C; $[\alpha]_D^{23}$ +1.6° (*c* 0.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.89 (m, 1H, *CH*=CH_2), 5.33–5.15 (m, 3H, CH=*CH*$_2$, H-3′), 5.07 (t, 1H, $J_{3',4'} = 9.5$ Hz, H-4′), 4.92 (m, 1H *NH*′), 4.81(m, 1H, H-2′); 4.72–4.68 (m, 2H, 2*CH*-Ph), 4.62–4.48 (m, 4H, *CH*$_2$-CH=CH$_2$, H-1′, H-1, α-Hep), 4.60 (d, 1H, $J = 12.0$ Hz, *CH*-Ph), 4.56 (d, 1H, $J = 12.0$ Hz, *CH*-Ph), 4.53 (d, 1H, $J = 11.8$ Hz, *CH*-Ph), 4.50 (dd, 1H, H-4), 4.44 (d, 1H, $J = 11.9$ Hz, *CH*-Ph), 4.21 (m, 2H, $J_{6'a,6'b} = 12.3$ Hz, $J_{5,6} = 4.7$ Hz, $J_{5,6'} = 4.2$ Hz, H-6, H-6′ non reducing end unit), 4.25 (dd, 1H, $J_{1,2} = 4.0$ Hz, $J_{2,3} = 4.1$ Hz, H-2), 4.27 (dd, 1H, $J_{3,4} = 2.7$ Hz, H-3), 4.31 (dd, 1H, $J_{4,5} = 7.1$ Hz, H-5), 3.70 (m, 1H, H-5′), 2.09, 2.04, 2.03 (3s, 9H, 3CO-*CH*$_3$); ESI-QqTOF-MS-MS: Calcd $[M + H]^+$ *m*/*z* 1033.4460, Found $[M + H]^+$ *m*/*z* 1033.6680; Anal. Calcd for $C_{58}H_{66}O_{16}N$ (1033.15): C, 67.42, H, 6.43, N, 1.35. Found: C, 67.72, H, 6.59, N, 1.20%.
12 $[\alpha]_D^{23}$ +7.0° (*c* 1.0, H_2O); ^{1}H NMR (300 MHz, D_2O): δ 4.73 (d, 1H, H-1′), 4.09 (d, 1H, H-1). 3.92 (dd, 1H, $J_{1,2} = 1.8$ Hz, $J_{2,3} = 4.6$ Hz, H-2), 3.19 (dd, 1H, H-5), 4.13 (m, 1H, $J_{5,6} = 1.8$ Hz, $J_{6,7} = 6.4$ Hz, H-6), 3.69 (dd, 1H, H-3), 3.64–3.52 (m, 3H, $J_{1,2} = 1.2$ Hz, $J_{2,3} = 9.4$ Hz, $J_{7a,7b} = 11.7$ Hz, H-2′, H-7a, H-7b), 3.52 (dd, 1H, $J_{3',4'} = 9.0$ Hz, H-3′), 3.48 (d, 2H, H-6′), 3.28 (dd, 1H, H-4′), 3.17 (dd, 1H, H-5′). ^{13}C NMR (125 MHz, $CDCl_3$): δ 102.4 (C-1′), 94.9 (C-1α), 94.7 (C-1β), 77.3 (C-5), 76.0 (C-5′), 73.0 (C-3′), 72.5 (C-3α), 71.9 (C-3β), 71.6 (C-2β), 71.5 (C-2α), 68.1 (C-6), 67.1 (C-4α), 65.6 (C-4′), 66.5 (C-4β), 63.8 (C-7α), 63.4 (C-7β), 61.3 (C-6′), 55.3 (C-2′). ESI-QqTOF-MS-MS: Calcd $[M + H]^+$ *m*/*z* 372.1427, Found $[M + H]^+$ *m*/*z* 372.1627; Anal. Calcd for $C_{58}H_{66}O_{16}N$ (371.30): C, 42.05, H, 6.78, N, 3.77. Found: C, 42.51, H, 6.61, N, 3.92%.
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15 mp 165–166 °C; $[\alpha]_D^{23}$ +4.1° (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.99 (m, 1H, *CH*=CH_2), 5.49 (d, 1H, $J_{1,2} = 5.0$ Hz, H-1), 5.28, 5.15 (2m, 2H, CH=*CH*$_2$), 5.25 (dd, 1H, $J_{2',3'} = 10.1$ Hz, $J_{3',4'} = 9.5$ Hz, H-3′, β-GlcNAOC), 5.06 (d, 1H, $J_{1',2'} = 8.5$ Hz, H-1′), 4.96 (dd, 1H, $J_{4',5'} = 10.0$ Hz, H-4′), 4.60 (dd, 1H, $J_{2,3} = 2.4$ Hz, $J_{3,4} = 8.0$ Hz, H-3, α-Hep), 4.59, 4.43 (2m, 2H, *CH*$_2$–CH=CH$_2$), 4.33 (dd, 1H, H-2), 4.29 (dd, 1H, $J_{4,5} = 1.5$ Hz, H-4), 4.28 (dd, 1H, $J_{5',6'a} = 5.1$ Hz, $J_{6'a,6'b} = 12.2$ Hz, H-6′a), 4.10 (m, 2H, $J_{5',6'b} = 2.5$, $J_{5',6a'} = 2.5$ Hz, H-6′b), 3.93 (m, 2H, $J_{5,6} = 8.0$ Hz, $J_{6,CH3} = 6.6$ Hz, H-6), 3.80 (m, 1H, H-5′), 3.70 (m, 1H, H-5), 3.62 (m, 1H, $J_{2',NH} = 9.2$ Hz, H-2′), 2.02, 1.99, 1.93 (3s, 9H, CO-*CH*$_3$), 1.46, 1.40, 1.31, 1.30 (4s, 12H, *CH*$_{3iso}$), 1.27 (d, 3H, H-7); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.7, 170.4, 170.1 (*CO*–CH_3), 156.2 (*CO*, allyloxycarbonyl), 134.7 (CH=*CH*$_2$), 116.8 (*CH*=CH$_2$), 109.6 (C$_{iso}$), 102.4 (C-1′, β-GlcNAOC), 97.0 (C-1, α-Hep), 76.2 (C-6), 74.4 (C-5), 72.2 (C-5′), 72.0 (C-3′) 71.7 (C-2), 71.6 (C-3), 71.2 (C-4), 70.0 (C-4′), 65.5 (*CH*$_2$–CH=CH$_2$), 63.0 (C-6′), 51.2 (C-2′), 26.4, 26.3, 25.3, 24.5 (*CH*$_{3iso}$), 18.1 (C-7, Hep), 21.2, 21.0, 20.9 (3s, 9H, CO-*CH*$_3$).
16 $[\alpha]_D^{23}$ +10.7° (*c* 1.0, H_2O); ^{1}H NMR (300 MHz, D_2O): δ 5.12 (d, 1H, $J_{1,2} = 4.0$ Hz, H-1α), 5.09 (d, 1H, $J_{1,2} = 9.0$ Hz, H-1β), 4.69 (d, 1H, $J_{1',2'} = 8.0$ Hz, H-1′), 4.68 (m, 1H, H-6a′), 4.18 (m, 1H, H-6b′), 3.98 (dd, 1H, $J_{2,3} = 5.1$ Hz, H-2), 3.76 (m, 1H, H-4), 3.70 (dd, 1H, $J_{2,3} = 3.9$ Hz, $J_{3,4} = 10.1$ Hz, H-3), 3.61 (m, 1H, H2′), 3.54 (m, 1H, H-5), 3.50 (dd, 1H, $J_{3',4'} = 9.0$ Hz, H-3′), 3.28 (dd, 1H, H-4′), 3.17 (dd, 1H, H-5′), 1.30 (d, 3H, H-7);. ESI-QqTOF-MS-MS: Calcd $[M + H]^+$ *m*/*z* 356.1481, Found $[M + H]^+$ *m*/*z* 356.1691; Anal. Calcd for $C_{13}H_{25}O_{10}N$ (355.31): C, 42.97, H, 6.94, N, 3.86. Found: C, 42.61, H, 6.82, N, 3.80%.

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