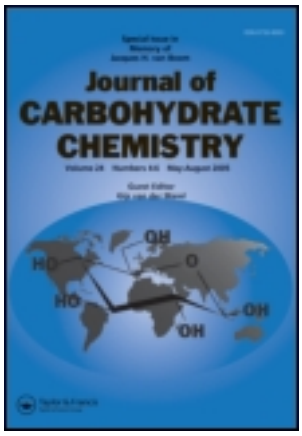

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An Efficient Synthesis of Derivatives of 2-Acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose

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An Efficient Synthesis of Derivatives of 2-Acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose[#]

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ABSTRACT

Methyl 2-acetamido-4-amino-2,4,6-trideoxy-α-D-galactopyranoside (**10**) was synthesized from D-glucosamine hydrochloride in eight steps in an overall yield of 31%. Key steps include the selective benzoylation at O-3 of methyl 2-acetamido-2,6-dideoxy-α-D-glucopyranoside in 89% yield and the subsequent Mitsunobu reaction using diphenylphosphoryl azide as the azide source which proceeded in 92% yield. Di- and mono-benzyloxycarbonyl derivatives of **10** were also prepared.

Key Words: Mitsunobu reaction; 2-Acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose; Selective benzoylation; C-polysaccharide.

[#]Dedicated to Professor Gérard Descotes on the occasion of his 70th birthday.

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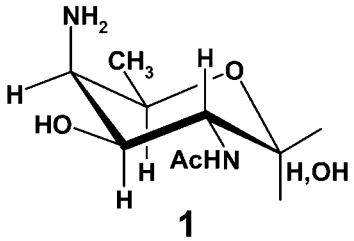
INTRODUCTION

2-Acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose (**1**), commonly abbreviated



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as AAT, is present at the cell surface of a number of bacterial polysaccharides. The *O*-specific side chains of the phase I lipopolysaccharide of the Gram-negative bacteria *Shigella sonnei*[1] consist of repeating units of AAT β-linked to O-4 of 2-amino-L-altruronic acid [→3)-β-AAT*p*-(1→4)-α-L-Alt*p*A-(1→]. α-Linked AAT occurs in capsules of the Gram-positive bacteriae *Streptococcus pneumoniae*,[2,3] *Streptococcus mitis*,[4] and *Bacteroides fragilis*.[5,6] In *S. pneumoniae*, it is one of three sugars constituting the repeating unit [→4)-α-Gal*p*A-(1→3)-α-Gal*p*A-(1→3)-α-AAT*p*-(1→] of the capsular polysaccharide of serotype 1[2,7] and it is also one of five sugars in the repeating unit of the C-polysaccharide, which, with slight structural variations, is common to all 90 serotypes of *S. pneumoniae*.[3,8,9] The C-polysaccharide is also present in some biovars of the closely related bacterium, *S. mitis*.[4] The capsule of *B. fragilis* contains two polysaccharides, of which the one containing AAT has the following repeating unit: [→3)-α-AAT*p*-(1→4)[β-D-Gal*f*-(1→3)]-α-D-Gal*p*NAc-(1→3)-β-D-Gal*p*-(1→].[5]

It was recently discovered that zwitterionic polysaccharides containing ammonium ions are potent T-cell activators.[10–13] All of the AAT-containing polysaccharides also have acidic groups in their structures: carboxylates, phosphonates, or sulfates.[13] The free amino group present in AAT will be present as an ammonium ion at physiological pHs. Thus, these AAT-containing polysaccharides have potential promise as immunostimulants and an efficient synthesis of AAT derivatives is highly desirable.

Previous syntheses of analogs of AAT are described as follows. The first report, the synthesis of the 4-acetamido derivative, in 1984 by Liav et al.,[14,15] proceeded from *N*-acetyl-D-glucosamine via benzyl 2-acetamido-3-*O*-benzyl-4-*O*-methanesulfonyl-2,6-dideoxy-α-D-glucopyranoside in 12 steps and 4% overall yield. Methyl 2-acetamido-4-azido-2,4,6-trideoxy-α-D-galactopyranoside (**2**) was synthesized in six steps with an overall yield of 18% starting from methyl 2-acetamido-4,6-benzylidene-α-D-glucopyranoside by Lönn and Lönngren.[16] Medgyes et al. used 11 steps to obtain 2-acetamido-4-azido-2,4,6-trideoxy-β-D-glucopyranoside in 11% yield starting from ethyl 3-*O*-acetyl-2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-1-thio-β-D-glucopyranoside.[17] This latter compound can be prepared in five steps from D-glucosamine hydrochloride.[18] Van Boom's group[19] prepared benzyl 2,4-diacetamido-2,4,6-trideoxy-α/β-D-galactopyranoside from 1,6-anhydro-2,3-*O*-(4-methoxybenzylidene)-β-D-mannopyranose in 12 steps with an overall yield of 4%.

We needed AAT for the synthesis of the pentasaccharide repeating unit of the C-polysaccharide of *S. pneumoniae*. None of the literature syntheses appeared to be scalable to produce the large amounts required to prepare the pentasaccharide. It was therefore

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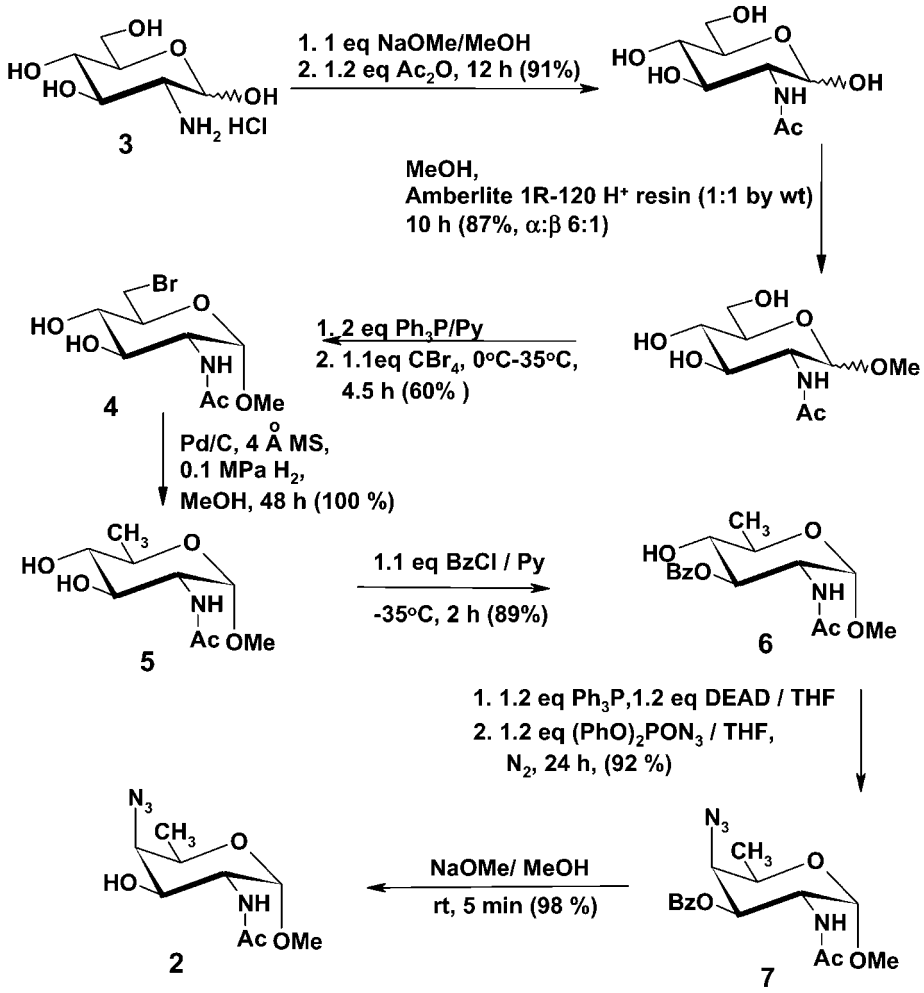
necessary to devise a short and efficient method from a readily available, cheap starting material. This paper describes such a route to methyl 2-acetamido-4-amino-2,4,6-trideoxy-α-D-galactopyranoside (**10**) and derivatives. Compound **10** was obtained here in eight steps from D-glucosamine hydrochloride in an overall yield of 31%, considerably better than that obtained with the previously available methods.

RESULTS AND DISCUSSION

D-Glucosamine hydrochloride (**3**) was converted into methyl 2-acetamido-6-bromo-2,6-dideoxy-α-D-glucopyranoside (**4**) largely using the method of Galemno et al. (Sch. 1).[20] However, it was found that the yield in the replacement of HO-6 by bromine



Scheme 1. Synthesis of methyl 2-acetamido-4-azido-2,4,6-trideoxy-α-D-galactopyranoside (**2**).

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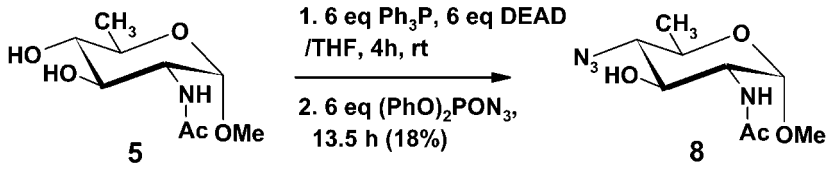


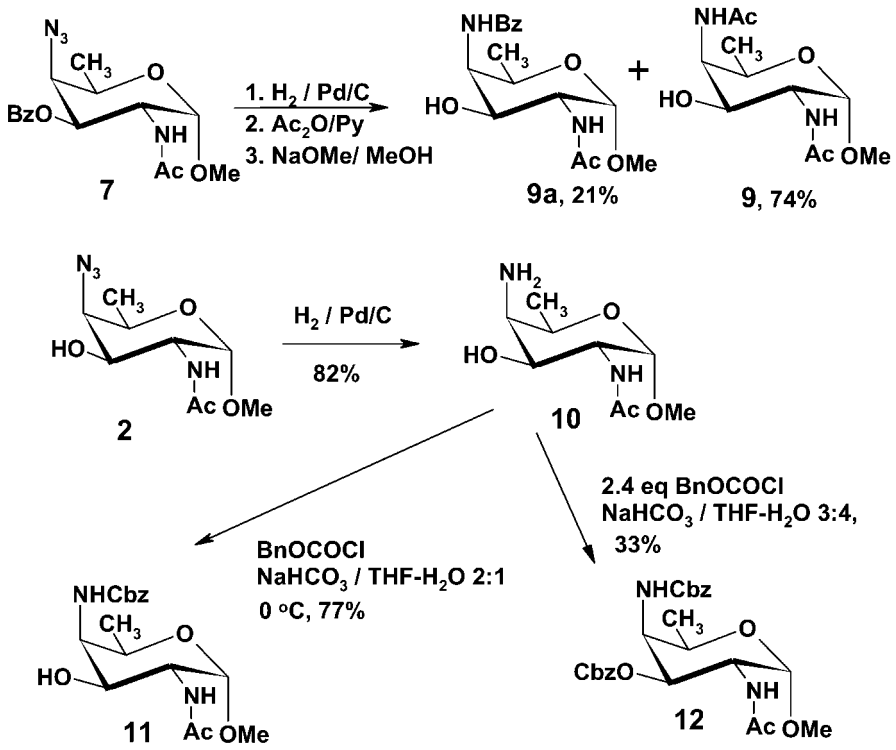
Scheme 2. Mitsunobu reaction on compound **5**.

using two equivalents of triphenylphosphine and one of carbon tetrabromide was somewhat higher (60% vs. 46%) if the reaction was performed at 30–35°C for 5 hr rather than 60–65°C for 30 min. It was also essential to recrystallize the carbon tetrabromide to obtain good yields. An alternative method used triphenylphosphine and *N*-bromosuccinimide for the same transformation of **4**.[21] Hydrogenolysis of the bromide to give the 6-deoxy compound **5** was conveniently performed in quantitative yield using 10% Pd/C and atmospheric pressure hydrogen. Either wet (Degussa type) or dry 10% Pd/C was found to be equally effective. The key step in the synthesis of **2** was the discovery that this product (**5**) could be benzoylated regioselectively at O-3 at −35°C in pyridine in excellent yield (89%). Wang and Lee had noted earlier that the main product of the reaction of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside with benzoyl chloride in pyridine at −60°C was 3,6-di-*O*-benzoate,[22] similar to the excellent regioselectivity obtained here for substitution at O-3 in preference to O-4. Introduction of the azide group at O-4 with inversion of configuration to give the 4-azidogalactose derivative **7** in 92% yield was conveniently performed using a Mitsunobu reaction with diphenylphosphoryl azide[23] as the azide donor. The zinc azide–pyridine complex[24] was ineffective as an azide donor here.

An initial attempt to perform the Mitsunobu reaction on **5** directly, that relied on the axial glycosidic group to inhibit displacement at C-3,[25] gave one azide-containing product, tentatively identified as methyl 2-acetamido-4-azido-2,4,6-trideoxy-α-D-glucopyranoside (**8**), in low yield (Sch. 2). Since Brandstetter and Zbiral had obtained methyl 4-azido-2,6-di-*t*-butyldimethylsilyl-4-deoxy-α-D-galactopyranoside by reaction of methyl 2,6-di-*t*-butyldimethylsilyl-α-D-glucopyranoside with triphenylphosphine, diethylazodicarboxylate, and hydrogen azide in 72% yield,[26] the result obtained here in the reaction on **5** was surprising. Compound **8** could have come from a double inversion process, perhaps via the 3,4-epoxide having the *galacto* configuration. Epoxides have been observed as intermediates and products in Mitsunobu reactions on pyranoside diols and polyols.[27,28] However, in this case, diequatorial opening of the azide is required to give the regiochemistry observed. Alternatively, it could have arisen by nucleophilic displacement at C-4 of a 3,4-oxyphosphonium intermediate with retention, displacement from the β-face being hindered by the large 3-oxyphosphonium group.[a]

Reduction of compound **7** with hydrogen over 10% Pd/C directly followed by *N*-acetylation and *O*-deacylation gave a mixture of the diacetamido-(**9**) and the 2-acetamido-4-benzamido-(**9a**) derivatives (Sch. 3). The latter product results from *O*- to *N*-migration of the benzoyl group after reduction but before *N*-acetylation. Deacylation

[a]We thank a referee for discussion of this point.



Scheme 3. Synthesis of **10** and derivatives thereof.

of **7** gave compound **2** that could be reduced to methyl 2-acetamido-4-amino-2,4,6-trideoxy-α-D-galactopyranoside (**10**). Compound **10** could be differentially protected as the 4-*N*-benzyloxycarbonyl derivative (**11**) or fully protected as the 3-*O*-,4-*N*-*bis*benzyloxycarbonyl derivative (**12**).

To our knowledge, this eight-step synthesis of **10** from commercially available D-glucosamine hydrochloride is the shortest and highest yielding to date. In addition, a number of derivatives, such as the differentially protected **11**, were prepared.

EXPERIMENTAL

General Methods

Melting points were determined with a Fisher–Johns melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at 300 K in 5 mm NMR tubes on Bruker AC-250 MHz or AMX-400 NMR spectrometers operating at 250.13 or 400.13 MHz and 62.9 or 100.08 MHz, respectively, on solutions in chloroform-*d*, unless otherwise indicated. Chemical shifts are given in parts per million (ppm) ($\pm$ 0.01 ppm) relative to that of tetramethylsilane (TMS) (0.00 ppm) in the case of ^{1}H NMR spectra, and to the central line of chloroform-*d* (δ 77.16) for the ^{13}C NMR spectra. All assignments were con-

firmed by COSY, HETCOR, HMQC, or HMBC experiments. Exact masses measured using electron ionization (EI) (70 eV) were done on a CEC 21-110B mass spectrometer. Electrospray mass spectra were recorded on a Fisons Quattro mass spectrometer with a Quattro source (cone voltage: 55 V, flow rate: 5 μL/min, complexing agent: potassium acetate, solvent: 75 : 25 v/v acetonitrile/water). Pyridine was dried by refluxing over calcium hydride for 12 hr, followed by distillation. Dichloromethane was refluxed over calcium hydride for 1 hr, then distilled. Chloroform was dried with magnesium sulfate, then distilled and stored over 4 Å molecular sieves. TLC was performed on aluminum-backed plates bearing 200 μm silica gel 60 F_{254} (Merck) or 250 μm ultra pure silica gel (Silicycle Inc., Quebec City, Canada). Compounds were visualized by UV where applicable and/or were located by spraying with a solution of 2% ceric sulfate in 1 M sulfuric acid followed by heating on a hot plate until color developed. Compounds were purified on 230–240 mesh ultra pure silica gel (Silicycle Inc., Quebec City, Canada) by flash chromatography using specified eluents. Elemental analyses were performed by the Canadian Microanalytical Service, Delta, BC.

Methyl 2-acetamido-6-bromo-2,6-dideoxy-α-D-glucopyranoside (4). A suspension of 2-acetamido-2-deoxy-α-D-glucopyranose[29] (20.52 g, 92.8 mmol) and dry Amberlite IR-120 (H^+ form) resin (20.5 g) in dry methanol (600 mL) was refluxed for 14 hr. The resin was removed, and washed with methanol (3 × 60 mL). The combined solutions were concentrated to give a solid residue of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (19.00 g, 87%). From ^{13}C NMR spectral peak heights, this product was an approximately 6 : 1 ratio of α to β products. ^{13}C NMR (D_2O) δ: 100.9 (C_β-1), 100.3 (C_α-1), 74.4 (C_α-5), 73.9 (C_α-3), 72.8 (C_α-4), 63.4 (C_α-6), 57.9 (OCH_3), 56.4 (C_α-2), 24.7 ($COCH_3$).

Part of the anomeric mixture from above (11.75 g, 50 mmol) was added to a solution of triphenylphosphine (26.0 g, 100 mmol) in pyridine (250 mL). Carbon tetrabromide (17.5 g, 55 mmol, recrystallized from ethanol) was added at 0°C, and the solution was stirred at 30–35°C for 5 hr, then concentrated. Azeotropic removal of pyridine with toluene gave a yellow solid that was purified by column chromatography using 30 : 1 (v/v) ethyl acetate–methanol as the eluent. Compound **4** was obtained as a colorless powder that was recrystallized from methanol–chloroform–hexane to give colorless needles: yield 6.65 g. Chromatographic separation of the mother liquor gave an additional 2.30 g, total yield 8.95 g (60%); m.p. 173–174°C, lit.[20] 175–176°C; ^1H NMR δ: 5.95 (d, 1H, $J_{NH,2} = 7.3$ Hz, NH), 4.72 (d, 1H, $J_{1,2} = 3.7$ Hz, H-1), 4.01 (m, 1H, H-2), 3.80–3.50 (complex m, 5H, H-3, H-4, H-5, H-6, H-6′), 3.45 (s, 3H, OCH_3), 2.08 (s, 3H, $COCH_3$); ^1H NMR (DMSO-d_6) δ: 7.80 (d, 1H, $J_{NH,2} = 9.6$ Hz, NH), 5.35 (s, 1H, OH-4), 4.88 (s, 1H, OH-3), 4.56 (d, 1H, $J_{1,2} = 3.1$ Hz, H-1), 3.79–3.42 (complex m, 5H, H-2, H-3, H-5, H-6, H-6′), 3.28 (s, 3H, OCH_3), 3.13 (m, 1H, H-4), 1.84 (s, 3H, $COCH_3$); ^{13}C NMR δ: 177.6 (C═O), 98.1 (C-1), 74.9, 73.7, 70.4 (C-3, C-4, C-5), 55.3 (OCH_3), 54.0 (C-2), 32.8 (C-6), 23.2 ($COCH_3$); ^{13}C NMR (DMSO-d_6) δ: 169.5 (C═O), 98.1 (C-1), 72.9 (C-4), 71.0 (C-3), 70.4 (C-5), 54.5 (OCH_3), 53.6 (C-2), 35.1 (C-6), 22.7 ($COCH_3$).

Methyl 2-acetamido-2,6-dideoxy-α-D-glucopyranoside (5). Compound **4** (3.09 g, 10 mmol) was dissolved in dry methanol (150 mL) and 10% palladium-on-charcoal (3.09 g) and finely ground 4 Å molecular sieves (12 g) were added in portions. The flask was evacuated and hydrogen was admitted at 1 atm. The mixture was stirred at r.t. and monitored by TLC (chloroform : methanol, 3 : 1). After 48 hr, the reaction was complete.

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The reaction mixture was filtered through a bed of Celite under suction and the filtrate was concentrated to a residue that was purified by column chromatography using 13 : 1 (v/v) ethyl acetate–methanol as the eluent to give a colorless amorphous solid: yield 2.30 g (100%); m.p. 173–175°C, lit.[20] 168–170°C, lit.[21] 172–173°C; ^{1}H NMR δ: 6.00 (d, 1H, $J_{NH,2} = 7.9$ Hz, NH), 4.62 (d, 1H, $J_{1,2} = 3.7$ Hz, H-1), 4.07 (m, 1H, H-2), 3.65 (m, 1H, H-5), 3.60 (dd, 1H, $J_{2,3} = 10.4$ Hz, $J_{3,4} = 9.2$ Hz, H-3), 3.38 (s, 3H, OCH_3), 3.24 (dd, 1H, $J_{4,5} = 9.1$ Hz, H-4), 2.06 (s, 3H, $COCH_3$), 1.31 (d, 3H, $J_{5,6} = 6.7$ Hz, CH_3-6); ^{13}C NMR δ: 172.3 (C═O), 98.2 (C-1), 76.9 (C-4), 74.7 (C-3), 67.1 (C-5), 55.0 (OCH_3), 54.2 (C-2), 23.3 ($COCH_3$), 17.4 (C-6).

Methyl 2-acetamido-4-azido-2,4,6-trideoxy-α-D-glucopyranoside (8). Compound **5** (93.2 mg, 0.43 mmol) and triphenylphosphine (674.2 mg, 2.55 mmol, 6 equiv.) were dissolved in dry THF (6 mL). Diethyl azodicarboxylate (400 μL, 2.55 mmol, 6 equiv.) was added dropwise to the mixture and the solution was stirred for 4 hr at r.t. Diphenylphosphoryl azide (560 μL, 2.55 mmol, 6 equiv.) was added to the clear pink reaction mixture, which was stirred for 13.5 hr, then concentrated to an orange-red syrup. Chromatography on silica gel (ethyl acetate : hexane, 1 : 1) gave a colorless syrup, yield 18 mg (18%); ^{1}H NMR δ: 6.61 (d, 1H, $J_{NH,2} = 9.1$ Hz, NH), 4.79 (d, 1H, $J_{1,2} = 3.7$ Hz, H-1), 4.62 (dq, 1H, $J_{2,3} = 11.3$ Hz, H-2), 4.34 (dd, 1H, $J_{3,4} = 10.2$ Hz, H-3), 3.99 (m, 1H, $J_{4,5} = 9.8$ Hz, H-5), 3.83 (dd, 1H, H-4), 3.39 (s, 3H, OCH_3), 2.04 (s, 3H, $COCH_3$), 1.37 (d, 3H, $J_{5,6} = 6.1$ Hz, CH_3-6); ^{13}C NMR δ: 170.2 (C═O), 99.1 (C-1), 81.7 (C-4), 79.4 (C-3), 66.7 (C-5), 55.8 (OCH_3), 51.9 (C-2), 23.1 ($COCH_3$), 17.5 (C-6).

Methyl 2-acetamido-3-*O*-benzoyl-2,6-dideoxy-α-D-glucopyranoside (6). Benzoyl chloride (70 mL, 0.60 mmol, 1.1 equiv.) was added dropwise to a solution of compound **5** (0.12 g, 0.55 mmol) in dry pyridine (4 mL) at −35°C. The resultant suspension was stirred at that temperature for 2 hr, then was allowed to warm gradually to r.t. over 3 hr. Methanol (1.5 mL) was added and the reaction mixture was stirred for 10 min, then concentrated. The white residue was dissolved in chloroform (15 mL), and the solution was washed successively with 3% hydrochloric acid (2 mL), cold saturated aqueous sodium bicarbonate (2 mL), and water (2 mL), then dried (anhydrous sodium sulfate) and filtered. The filtrate was concentrated to give a solid residue, that on crystallization from ethanol afforded colorless needles; yield 165 mg (89%); m.p. 158–160°C; $[\alpha]_D + 45.0^\circ$ (c 0.9, $CHCl_3$); ^{1}H NMR δ: 8.00 (d, 2H, $J = 7.3$ Hz, o-Ph), 7.56 (t, 1H, $J = 7.3$ Hz, p-Ph), 7.42 (t, 2H, $J = 7.3$ Hz, m-Ph), 6.10 (d, 1H, $J_{NH,2} = 9.5$ Hz, NH), 5.09 (t, 1H, $J_{2,3} = 10.4$ Hz, $J_{3,4} = 9.5$ Hz, H-3), 4.44 (d, 1H, $J_{1,2} = 3.2$ Hz, H-1), 4.15 (dt, 1H, H-2), 3.64 (s, 1H, OH-4), 3.56 (m, 1H, $J_{4,5} = 10.3$ Hz, H-5), 3.32 (t, 1H, H-4), 3.21 (s, 3H, OCH_3), 1.61 (s, 3H, $COCH_3$), 1.12 (d, 3H, $J_{5,6} = 5.5$ Hz, CH_3-6); ^{13}C NMR δ: 170.1, 168.3 (2 × C═O), 133.6, 130.0, 129.3, 128.6 (Ph), 98.2 (C-1), 74.7 (C-3), 73.9 (C-4), 67.8 (C-5), 55.0 (OCH_3), 52.1 (C-2), 22.9 ($COCH_3$), 17.7 (C-6).

HRMS (EI, m/z) Calcd for $[C_{16}H_{21}NO_6 - CH_3O]^+$: 292.1185. Found: 292.1188.

Methyl 2-acetamido-4-azido-3-*O*-benzoyl-2,4,6-trideoxy-α-D-galactopyranoside (7). Compound **6** (100 mg, 0.31 mmol) and triphenylphosphine (81 mg, 0.31 mmol) were dissolved in dry THF (5 mL). Diethyl azodicarboxylate (48 μL, 0.31 mmol) was added to the mixture, followed by diphenylphosphoryl azide (68 μL, 0.31 mmol) in THF (0.7 μL). The mixture was stirred at r.t. for 24 hr. The reaction mixture was diluted with chloroform (10 mL), and the resulting solution was washed with distilled water (2 mL), dried (anhydrous sodium sulfate), and filtered. Concentration of the filtrate gave a yellow residue. Chromatography on silica gel (ethyl acetate : hexane, 3 : 1) afforded a

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colorless solid, yield 99 mg (92%); m.p. 177–178°C; $[\alpha]_D$ − 8.3° (*c* 0.3, $CHCl_3$); ^{1}H NMR δ: 8.07 (d, 2H, J = 7.5 Hz, *o*-Ph), 7.59 (t, 1H, J = 7.5 Hz, *p*-Ph), 7.45 (t, 2H, J = 7.5 Hz, *m*-Ph), 5.79 (d, 1H, $J_{NH,2}$ = 9.3 Hz, NH), 5.46 (dd, 1H, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 3.4 Hz, H-3), 4.78 (dt, 1H, $J_{1,2}$ = 3.7 Hz, H-2), 4.71 (d, 1H, H-1), 4.11 (m, 1H, H-5), 3.93 (dd, 1H, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 2.1 Hz, H-4), 3.38 (s, 3H, OCH_3), 1.87 (s, 3H, $COCH_3$), 1.33 (d, 3H, $J_{5,6}$ = 7.6 Hz, CH_3-6); ^{13}C NMR δ: 169.9, 166.6 (2 × C═O), 133.6, 128.61, 128.57 (Ph), 98.8 (C-1), 71.9 (C-3), 64.8 (C-4), 64.2 (C-5), 55.3 (OCH_3), 47.7 (C-2), 23.3 ($COCH_3$), 17.3 (C-6).

Anal. Calcd for $C_{16}H_{20}N_4O_5$ (348.3616): C, 55.17; H, 5.79; N, 16.08. Found: C, 55.26; H, 5.71; N, 15.74.

Methyl 2,4-diacetamido-2,4,6-trideoxy-α-D-galactopyranoside (9). Compound **7** (49.9 mg, 0.13 mmol) in dry methanol (5 mL) containing 10% Pd/C (74.8 mg, 50% wet) was stirred under hydrogen at atmospheric pressure. After the disappearance of **7** (19 hr) on TLC (ethyl acetate : hexane, 3 : 1), the catalyst was removed by filtration. Concentration yielded a colorless solid, that was taken up in pyridine (2 mL) and acetic anhydride (2.0 μL, 0.2 mmol, 4 equiv.). The reaction mixture was stirred at r.t. until the amino compound had disappeared on TLC (chloroform : methanol, 8 : 1) (0.5 hr). The reaction mixture was quenched with methanol (5 mL), and evaporated to give the diacetamide as a syrup. This syrup was dissolved in methanol (4 mL) and sodium methoxide was added (0.8 mL, 30 mg sodium in 6 mL methanol). After being stirred at r.t. for 1 hr, the reaction mixture was neutralized with Amberlite IR-120(H^+), filtered, and concentrated to a yellow syrup. The syrup was separated on a column of silica gel (ethyl acetate : methanol, 6 : 1) into two components. The first component, compound **9** was a colorless solid, yield 33.8 mg (74%); m.p. 213–215°C; $[\alpha]_D$ + 125.9° (*c* 0.5, ethanol); ^{1}H NMR δ: 6.10 (d, 1H, $J_{NH,4}$ = 7.8 Hz, NH-4), 5.82 (d, 1H, $J_{NH,2}$ = 9.4 Hz, NH-2), 4.69 (d, 1H, $J_{1,2}$ = 4.1 Hz, H-1), 4.31 (m, 1H, H-4), 4.13–4.07 (m, 2H, H-2, H-5), 3.92 (dd, 1H, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 4.0 Hz, H-3), 3.35 (s, 3H, OCH_3), 2.12, 2.04 (2s, 2 × 3H, 2 × $COCH_3$), 1.17 (d, 3H, $J_{5,6}$ = 6.6 Hz, CH_3-6); ^{13}C NMR (D_2O) δ: 178.4, 177.3 (2 × C═O), 101.0 (C-1), 69.7 (C-3), 68.0 (C-5), 58.0 (OCH_3), 56.2 (C-4), 52.9 (C-2), 24.7 (2 × $COCH_3$), 18.3 (C-6).

HRMS (EI, m/z) Calcd for $[C_{11}H_{20}N_2O_5 - CH_3OH]^+$: 228.1110. Found: 228.1110.

The second component, methyl 2-acetamido-4-benzamido-2,4,6-trideoxy-α-D-galactopyranoside (**9a**) was isolated as a colorless syrup, yield: 9 mg (21%); $[\alpha]_D$ + 140.5° (*c* 0.9, ethanol); ^{1}H NMR δ: 7.85–7.42 (m, 5H, Ph), 6.69 (d, $J_{NH,4}$ = 7.4 Hz, NH-4), 6.01 (bs, 1H, NH-2), 4.75 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.51 (m, 1H, H-4), 4.16–3.98 (m, 3H, H-2, H-3, H-5), 3.36 (s, 3H, OCH_3), 1.96 (s, 3H, $COCH_3$), 1.22 (d, 3H, $J_{5,6}$ = 5.5 Hz, CH_3-6); ^{13}C NMR δ: 171.4, 169.9 (2 × C═O), 133.9, 131.9, 128.6, 127.3 (Ph), 98.5 (C-1), 69.4(C-3), 64.8 (C-5), 55.5 (OCH_3), 54.4 (C-4), 51.0 (C-2), 23.3 ($COCH3$), 16.9 (C-6).

Methyl 2-acetamido-4-azido-2,4,6-trideoxy-α-D-galactopyranoside (2). Sodium (0.02 g, 0.80 mmol) was dissolved in dry methanol (6 mL) and compound **7** (0.25 g, 0.71 mmol) was added. After 5 min, the solution was neutralized with Amberlite IR-120 (H^+), filtered, washed with methanol (2 × 5 mL) and evaporated to a colorless solid. Recrystallization from ethyl acetate–hexane gave colorless needles, yield: 0.15 g (98%); m.p. 195.5–197.0°C; $[\alpha]_D$ + 152.4° (*c* 0.6, ethanol); ^{1}H NMR δ: 5.92 (d, 1H, $J_{NH,2}$ = 9.5 Hz, NH), 5.09 (dd, 1H, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 4.64 (d, 1H, $J_{1,2}$ = 4.1 Hz, H-1), 4.28 (ddd, 1H, H-2), 3.64 (s, 1H, OH-4), 3.56 (m, 1H, H-5), 3.32 (dd, $J_{4,5}$ = 10.3 Hz, H-4), 3.21 (s, 3H, OCH_3), 1.61 (s, 3H, $COCH_3$), 1.12 (d, 3H,

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$J_{5,6} = 5.5$ Hz, C-6); ^{13}C NMR δ: 170.4, 168.4, 167.4 (C═O), 98.2 (C-1), 74.7 (C-3), 73.9 (C-4), 67.8 (C-5), 55.0 (OCH_3), 52.1 (C-2), 22.9 ($CO\mathit{C}H_3$), 17.7 (C-6).

Anal. Calcd for $C_9H_{16}N_4O_4$ (244.2506): C, 44.26; H, 6.60. Found: C, 44.00; H, 6.62.

Methyl 2-acetamido-4-amino-2,4,6-trideoxy-α-D-galactopyranoside (10). A solution of compound **2** (90.0 mg, 0.37 mmol) in dry ethanol (1.5 mL) was stirred with 10% palladium-on-charcoal (0.24 g) under hydrogen at r.t. for 16 hr. The reaction mixture was filtered through a bed of Celite under suction and the filtrate was concentrated to a colorless residue. The residue was purified by flash chromatography using 4 : 1 (v/v) dichloromethane–methanol to give the title compound (**10**) as a colorless solid: 65.0 mg (82%); m.p. 194.5–196.0°C; $[\alpha]_D + 159.4^\circ$ (*c* 1.2, ethanol); ^{1}H NMR δ: 6.99 (d, 1H, $J_{2,NH} = 8.5$ Hz, NH), 4.65 (d, 1H, $J_{1,2} = 3.8$ Hz, H-1), 4.08 (ddd, 1H, $J_{2,3} = 10.8$ Hz, H-2), 3.97 (qd, 1H, $J_{5,6} = 6.6$ Hz, $J_{4,5} = 1.8$ Hz, H-5), 3.73 (dd, 1H, $J_{3,4} = 4.2$ Hz, H-3), 3.35 (s, 3H, OCH_3), 2.96 (dd, 1H, H-4), 2.05 (s, 3H, $COCH_3$), 1.25 (d, 3H, H-6); ^{13}C NMR δ: 171.7 (C═O), 98.5 (C-1), 69.7 (C-3), 65.4 (C-5), 55.2 (OCH_3), 54.8 (C-4), 50.5 (C-2), 23.5 ($CO\mathit{C}H_3$), 16.8 (C-6).

HRMS (EI, m/z). Calcd for $[C_9H_{18}N_2O_4 - NH_2CH_3]^+$: 187.0845. Found: 187.0878. ESI MS (m/z). Calcd for $[C_9H_{18}N_2O_4 + K]^+$: 257.2. Found: 257.5.

Methyl 2-acetamido-4-(benzyloxylcarbonyl)amino-2,4,6-trideoxy-α-D-galactopyranoside (11). Benzyl chloroformate (64 μL, 0.45 mmol, 1.5 equiv.) was added to a solution of compound **10** (65.2 mg, 0.30 mmol) and sodium bicarbonate (75.6 mg, 0.90 mmol, 3 equiv.) in 2 : 1 (v/v) THF–water (1.5 mL) dropwise at 0°C. The mixture was stirred at 0°C for 2 hr. The result two-phase mixture was diluted with ethyl acetate (3 mL) and extracted with ethyl acetate (3 × 3 mL). The combined organic layers were concentrated in vacuo and purified by silica gel chromatography using 20 : 1 (v/v) dichloromethane–methanol ($R_f = 0.2$) as eluent to give the title compound (**11**) as a colorless solid: 81.1 mg (77%); m.p. 62.5–64.5°C; $[\alpha]_D + 87.0^\circ$ (*c* 0.7, $CHCl_3$); ^{1}H NMR δ: 7.36 (bs, 5H, Ph), 5.79 (d, 1H, $J_{2,NH} = 8.7$ Hz, NHAc), 5.30 (d, 1H, $J_{4,NH} = 9.8$ Hz, NHCbz), 5.17, 5.09 (2d, 2H, $J = 12.2$ Hz, $C\mathit{H}_2Ph$), 4.63 (d, 1H, $J_{1,2} = 3.7$ Hz, H-1), 4.12–4.02 (m, 3H, H-2, H-4, H-5), 3.86 (m, 1H, H-3), 3.34 (s, 3H, OCH_3), 3.22 (d, 1H, $J_{4,OH} = 6.1$ Hz, OH), 2.01 (s, 3H, $COCH_3$), 1.18 (d, 3H, $J_{5,6} = 6.4$ Hz, CH_3-6); ^{13}C NMR δ: 170.1 (C═O, NHAc), 154.8 (C═O, NHCbz), 128.6, 128.2 (Ph), 98.4 (C-1), 69.8 (C-3), 67.3 ($\mathit{C}H_2Ph$), 64.9 (C-5), 65.6 (C-4), 55.3 ($O\mathit{C}H_3$), 50.9 (C-2), 23.4 ($CO\mathit{C}H_3$), 16.6 (C-6); MS (EI, m/z) 321 ($[C_{17}H_{24}N_2O_6 - CH_3O]^+$, 16%), 320 ($[C_{17}H_{24}N_2O_6 - CH_3OH]^+$, 20%), 303 (321 − H_2O, 5%), 91 ($C_7H_7^+$, 100%).

HRMS (EI, m/z). Calcd for $[C_{17}H_{24}N_2O_6 - CH_3OH]^+$: 320.1372. Found: 320.1364.

Methyl 2-acetamido-4-(benzyloxylcarbonyl)amino-3-*O*-benzyloxylcarbonyl-2,4,6-trideoxy-α-D-galactopyranoside (12). Compound **10** (106.5 mg, 0.49 mmol) was dried in vacuo and then dissolved in 3 : 4 (v/v) THF–water (7 mL). Addition of sodium bicarbonate (205.8 mg, 2.44 mmol, 5 equiv.) to the clear solution gave a milky mixture. Benzyl chloroformate (180 μL, 1.18 mmol, 2.4 equiv.) was added to the mixture dropwise at 0°C. The mixture was stirred at 0°C for 0.5 hr and then at r.t. for 1 hr. The resulting two-phase mixture was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were concentrated in vacuo and the residue was purified by the column chromatography using 2 : 1 (v/v) ethyl acetate–hexane as eluent to give the title compound (**12**) as a colorless solid: 0.18 g (33%); m.p. 120.0–123.0°C; $[\alpha]_D + 98.8^\circ$ (*c* 0.5, $CHCl_3$); ^{1}H NMR (acetone-d_6) δ: 7.42–7.26 (m, 10H, 2 × Ph), 7.00 (d, 1H, $J_{2,NH} = 8.5$ Hz, NHAc), 6.77 (d, 1H, $J_{4,NH} = 10.0$ Hz, NHCbz), 5.17, 5.10 (2d, 2H, $J = 12.4$ Hz, 4-$C\mathit{H}_2Ph$), 5.08

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(s, 2H, 3-CH_2Ph), 4.89 (dd, 1H, $J_{2,3} = 11.8$ Hz, $J_{3,4} = 4.20$ Hz, H-3), 4.64 (d, 1H, $J_{1,2} = 3.7$ Hz, H-1), 4.41 (ddd, 1H, H-2), 4.30 (ddd, 1H, $J_{4,5} = 1.8$ Hz, H-4), 4.08 (qd, 1H, $J_{5,6} = 6.48$ Hz, H-5), 3.34 (s, 3H, OCH_3), 1.83 (s, 3H, $COCH_3$), 1.18 (d, 3H, H-6); ^{13}C NMR δ: 170.2 (C═O, NHAc), 157.0 (C═O, OCbz), 154.9 (C═O, NHCbz), 136.4, 135.2 (q Ph), 128.41, 128.39, 128.37, 128.2, 127.9, 127.4 (Ph), 98.3 (C-1), 73.46 (C-3), 69.8, 66.8 (2 × CH$_2$Ph), 64.5 (C-5), 55.3 (OCH_3), 52.7 (C-4), 48.0 (C-2), 23.0 (COCH$_3$), 16.4 (C-6).

MS (ESI, m/z). Calcd for $[C_{25}H_{30}N_2O_8 + K]^+$: 525.0. Found: 525.0.

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