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Synthesis of methyl 2-acetamido-2,6-dideoxy-α- and β-D-*xylo*-hexopyranosid-4-ulose, a keto sugar which misled the analytical chemists

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Abstract—To understand the contradictory results on the structure of the lipopolysaccharide isolated from a *Yersinia enterocolitica* O:3, both anomers of methyl 2-acetamido-2,6-dideoxy-D-*xylo*-hexopyranosid-4-ulose were prepared. The key steps of the synthetic pathway were the selective acetylation of the methyl 2-acetamido-2,6-dideoxy-α,β-D-glucopyranosides, the oxidation of the 4-position to form the keto-sugars, and deacetylation to provide the target compound. Surprisingly, the last step was accompanied by a disproportionation to give methyl 2-acetamido-2,6-dideoxy-α- and β-D-glucopyranosides and *N*-(5-hydroxy-6-methyl-4-oxo-4*H*-pyran-3-yl)acetamide as side-products.

Keywords: D-Glucosamine; Reduction; Oxidation; Anomerization; Keto-sugar; Selective acetylation

1. Introduction

The lipopolysaccharides (LPS) of the wild type strain *Yersinia enterocolitica* (Ye) 75S consist of an O-specific polysaccharide, an outer and an inner core oligosaccharide, and lipid A, whereas the LPS of the rough (R) mutant YeO3-R1 bears only the core oligosaccharide and lipid A.[1] In contrast to earlier structural analyses in which an *N*-acetyl-D-fucosamine (Fuc*p*NAc)[2] or an *N*-acetyl-D-quinovosamine (Qui*p*NAc) unit was believed to be the link between outer and inner core oligosaccharides, improved NMR spectroscopic and mass spectrometric investigations [K. A. Duda et al. unpublished results] indicated that a 4-keto sugar takes on this role and Fuc*p*NAc and Qui*p*NAc are artefacts formed by reduction during various analytical protocols. The ratio of the 4-epimeric sugars depended on the reducing agents and the experimental conditions.[3] To study this phenomenon by using a model compound and, additionally, to obtain information on the influence of the structure at the anomeric centre on the ratio of the 4-epimers, both anomers of methyl 2-acetamido-2,6-dideoxy-D-*xylo*-hexopyranosid-4-ulose (**9α**,**β**) were synthesized.

2. Results and discussion

The α- and β-isomers of 4-ulose **9** were synthesized via a synthetic approach shown in Scheme 1 using methyl 2-acetamido-2-deoxy-α/β-D-glucopyranoside (**1α**,**β**) as starting material. Applying conventional Fischer glycosylation reaction conditions, *N*-acetyl-D-glucosamine in methanol was converted in the presence of IR120 (H^+)

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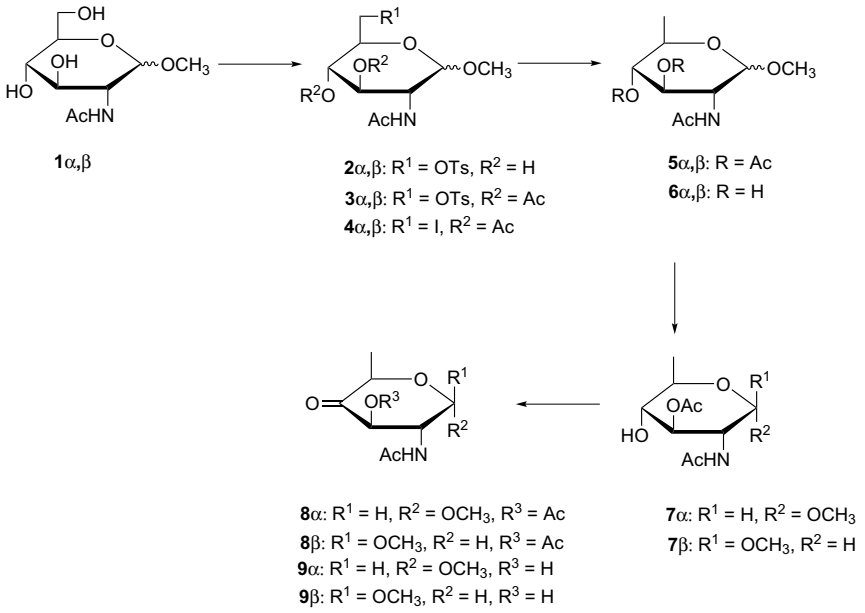


Scheme 1. Synthetic pathway for the preparation of both anomers of methyl 2-acetamido-2,6-dideoxy-D-*xylo*-hexopyranosid-4-ulose (**9α** and **9β**) starting from *N*-acetyl-D-glucosamine.

Amberlite resin into the corresponding methyl glycoside. In contrast to the work of Bornaghi and Poulsen,[4] we did not use anhydrous methanol and after 8 h at 70 °C we reproducibly observed α/β ratios from 2:1 through 5:1. The total yield of isolated methyl glycosides **1α,β** was 74%. Although it was possible by flash-chromatography to separate the α,β-isomers formed during each synthetic step, further reactions were carried out with the anomeric mixtures until the oxidation step, since (i) both isomers were needed for reduction experiments and (ii) each synthetic step with a pure β-isomer was always accompanied by anomerization. Consequently, it made sense under these conditions to work on a multi-step pathway with anomeric mixtures as long as possible.

The synthesis of the 6-deoxy-sugars **6α,β** was carried out by using a procedure developed by Kajihara et al.[5] for the β-anomer (Scheme 1). The sequence comprised selective tosylation (**2α,β**), acetylation (**3α,β**), exchange of tosyl function by iodine (**4α,β**), reduction (**5α,β**) and deacetylation (**6α,β**). It was of advantage to conduct the synthesis of **3α,β** from **1α,β** as an one-pot reaction. Otherwise, the total yield was significantly lower. As mentioned before, each anomer was separated for analytical characterization and since the Japanese authors had not published the ^{13}C NMR data for the β-anomers, the complete set of analytical data for both α- and β-isomers (**2**–**6**) are given in the experimental part.

It had to be proved that an acetyl group could be introduced regioselectively at O-3 position of compound **6**. Dropwise addition of acetyl chloride diluted by toluene to a solution of both compounds **6α** and **6β** in pyridine at −40 °C gave exclusively the desired derivatives **7α** and **7β** in nearly 40% yields. As side-products only the completely acetylated compounds **5α** and **5β** were isolated by flash chromatography, respectively, which could be used again in the previous reaction steps. The ^{1}H NMR data proved the acetylation of the O-3 position of **7α** and **7β**. The multiplets at δ 3.51–3.34 (2H, H-3, H-5) and δ 3.26–3.11 (2H, H-3, H-5) of **6α** and **6β**, respectively, were splitted up in the spectra of **7α** and **7β**. As expected, the acetyl function in the O-3 position caused a considerable downfield shift of about 1.5 ppm of the H-3 signals of both anomers to δ 4.99. Furthermore, the O-3 position of the acetyl group was also confirmed by the HMBC spectrum recorded for **7α**. In this spectrum, a correlation between the proton H-3 and the carbonyl carbon atom of the acetyl group was observed involving interaction through three bonds.

Additionally, X-ray diffraction studies were performed to establish the structures of **7α** and **7β** (Figs. 1

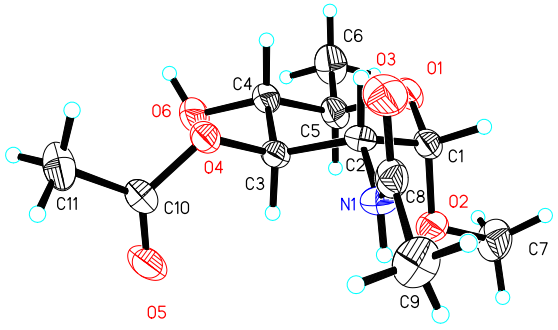


Figure 1. An ORTEP plot of **7α** with 50% probability for the thermic ellipsoids.

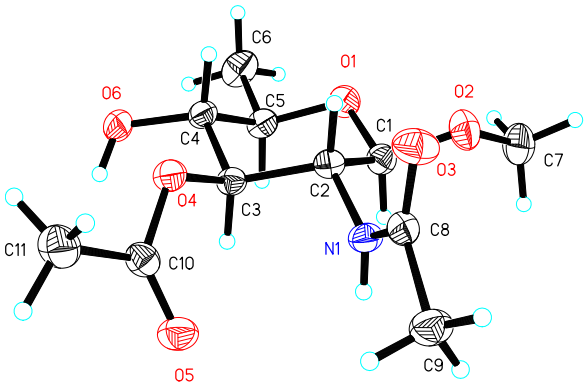


Figure 2. An ORTEP plot of **7β** with 50% probability for the thermic ellipsoids.

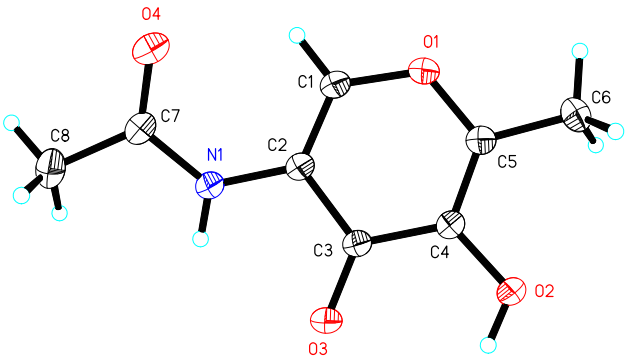
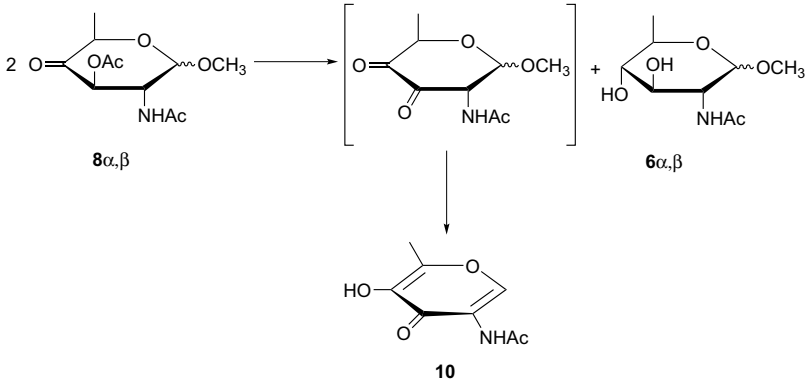


Figure 3. An ORTEP plot of **10** with 50% probability for the thermic ellipsoids. In compound **10** the molecules form hydrogen bonded dimers around a centre of inversion.

and 2). Both structures showed a number of hydrogen bond interactions. In the tetragonal crystal of **7α** (Hall space group: *P*4*abw* 2*nw*), there are two donating groups and two acceptor sites per molecule. The starting molecule is linked to its translational equivalent molecule parallel to *a*-axis via pairs of again translational equivalent molecules with symmetry codes $0.5 - x$, $0.5 + y$, $0.25 - z$ and $0.5 - x$, $1.5 + y$, $0.25 - z$, thus forming a planar network of hydrogen bonded molecules perpendicular to the *c*-axis. In the monoclinic **7β** (Hall space group: *P*2*yb*) there is also a planar network of hydrogen bonded molecules perpendicular to the *c*-axis. However, this plane consists of two symmetry related chains of molecules (symmetry code: $-x$, $0.5 + y$, $-z$). The molecules in each chain are connected to each other via hydrogen bonds between the OH group at C-4 and the carbonyl oxygen of the *N*-acetyl group in the next molecule. The symmetry related chains are then connected via a link between the NH-group in one chain to the oxygen at 4-position of the neighbouring chain.

The crucial point of the synthetic route was the oxidation step to gain 4-uloses **8α** and **8β**. The first experiments using pyridinium chlorochromate (PCC)[6] or the conditions of Jones oxidation[7] were not very auspicious. The yields (5–10%) such as the reaction rate were pretty low. Switching to pyridinium dichromate (PDC)[8] **8α** and **8β** were obtained in 75% and 50% yields, respectively, but the reaction time of 3 weeks was still unacceptable. A significant improvement of the oxidation with PDC was achieved by the addition of molecular sieves[9] and a catalytic amount of glacial acetic acid[10] to the reaction mixture. Now, 4-uloses **8α** and **8β** were obtained after 12 h in 91% and 60% yield, respectively. Parallely, the reaction conditions of Swern-oxidation[11] were applied to **7α** and **7β**. The observed yields for **8α** (93%) and **8β** (57%) were comparable with the improved PDC oxidation. However, Swern-oxidation necessitated purification by column chromatography while PDC oxidation provided analytically pure products. The structure of the 4-uloses **8α** and **8β** was confirmed by the analytical and by the NMR data. Thus, in the ^{13}C NMR spectra of the 4-uloses the oxidation of the 4-position of **7α** and **7β** resulted in a characteristic downfield shifts δ 74.2 and δ 72.0 to δ 197.9 and δ 198.3, respectively.

Surprisingly, the final step of the synthesis of the model compounds was quite cumbersome and accompanied by an unexpected side reaction. Deacetylation under Zemplén conditions furnished the target



Scheme 2. Suggested mechanism of the side-reaction occurring during deacetylation of compounds **8α** and **8β**.

compounds **9α** and **9β** in yields of 72% and 40%, respectively. The application of methanolic 0.28 M hydrochloric acid[12] gave only 44% and 12%, respectively.

Both reactions yielded an unknown side-product (**10**) together with the reduced derivative **6α** originating from **8α** or the anomeric mixture **6α,β** starting from **8β**. The structural determination of the side-product **10** was finally achieved by X-ray diffraction studies (Fig. 3). All other analytical data were in good agreement with the proposed structure of **10**. Thus, in the $^{1}H,^{13}C$ HMBC spectrum recorded for **10** the following correlations have been found: H-2 with C-3,4 and 6; methyl group with C-5 and C-6; CH_3CO with CH_3CO; and NH with C-2 and CH_3CO. The formation of **10** together with compounds **6α,β** suggested that a disproportionation took place during deacetylation either under base or acid catalysis. The diketo structure shown in Scheme 2 was not stable and, consequently, by the elimination of methanol the side-product **10** was formed.

An investigation of the reduction of the model compounds **9α** and **9β** under conditions which are typical for analytical procedures of bacterial polysaccharides is presently underway.

3. Experimental

3.1. General methods

Melting points were determined with a Boetius microheating plate BHMK 05 (Rapido, Dresden) and were not corrected. Optical rotation was measured for solutions in a 2-cm cell with an automatic polarimeter GYROMAT (Dr. Kernchen Co.). ^{1}H NMR spectra (250.13 MHz, 300.13 MHz, and 500.13 MHz) and ^{13}C NMR spectra (62.89 MHz; 75.47 MHz; and 125.76 MHz) were recorded on Bruker instruments AC 250, ARX 300, and AVANCE 500, respectively, with $CDCl_3$, CD_3OD or Me_2SO-d_6 as solvents. The calibration of spectra was carried out on solvent signals ($CDCl_3$: δ ^{1}H 7.25, δ ^{13}C 77.0; CD_3OD: δ ^{1}H 4.78, δ ^{13}C 49.0; Me_2SO-d_6: δ ^{1}H 2.50, δ ^{13}C 39.7). ^{1}H and ^{13}C NMR signals were assigned by DEPT and two-dimensional $^{1}H,^{1}H$ COSY, and $^{1}H,^{13}C$ correlation spectra (HMBC and HSQC). Mass spectra were recorded on an AMD 402/3 spectrometer (AMD Intectra GmbH). Elemental analysis was performed on a CHNS-Flash-EA-1112 instrument (Thermoquest). For the X-ray structural determination of compounds **7α**, **7β** and **10** an X8Apex system with CCD area detector was used ($\lambda = 0.71073$ Å, graphite monochromator). The structures were solved by direct methods (Bruker-SHELXTL). The refinement calculations were done by the full-matrix least-squares method of Bruker SHELXTL, Vers.5.10, Copyright 1997, Bruker Analytical X-ray Systems. All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were put into theoretical positions and refined using the riding model. Crystallographic data for the structural analysis has been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 669295–CCDC 669297 for compounds **7α**, **7β** and **10**. Copies of this information may be obtained free of charge from: The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ UK, Fax. (int code) +44(1223)336-033 or via Email: deposit@ccdc.cam.ac.uk or www:http://www.ccdc.cam.ac.uk. All washing solutions were cooled to ~5 °C. The $NaHCO_3$ soln was saturated. Reactions were monitored by thin-layer chromatography (TLC, Silica Gel 60 F_{254}, Merck KGaA). The followings solvents systems (v/v) were used: (A_1) 4:1, (A_2) 9:1 chloroform–ethanol; (B_1) 3:1, (B_2) 6:1, (B_3) 10:1 chloroform–methanol. The spots were made visible by dipping the TLC plates into a methanolic 10% H_2SO_4 soln and charring with a heat gun for 3–5 min. Preparative flash chromatography was performed by elution from columns of slurry-packed Silica Gel 60 (Merck, 63–200 μm). All solvents and reagents were purified and dried according to standard procedures.[13] After classical workup of the reaction mixtures, the organic layer was dried over $MgSO_4$, and then concentrated under reduced pressure (rotary evaporator).

3.2. Selective tosylation of methyl 2-acetamido-2-deoxy-α,β-D-glucopyranoside (1α,β)

A soln of *p*-tolysulfonyl chloride (930 mg, 4.90 mmol) in dry pyridine (3 mL) was added dropwise to a soln of anomeric mixture **1α,β** (1.0 g, 4.25 mmol)[4] in dry pyridine (10 mL) at −10 °C. The mixture was stirred at that temperature for 3 h, then at 5 °C for 12 h, and finally at ambient temperature for 24 h. Ice-water (5 mL) was added, and after 30 min the aqueous phase was extracted with chloroform (4 × 70 mL). The combined extracts were successively washed with ice-water (3 × 30 mL), aq 1 M H_2SO_4 (30 mL), ice-water (30 mL), aq $NaHCO_3$ (30 mL), ice-water (30 mL), dried and concentrated. Traces of pyridine were removed by evaporation with repeated addition of toluene. The residue was then subjected to column chromatography (solvent A_2) to provide **2α** and **2β**.

3.3. Methyl 2-acetamido-2-deoxy-6-*O*-*p*-tolysulfonyl-α-D-glucopyranoside (2α)

Colourless crystals (744 mg, 45%); mp 69–72 °C; lit.[14] 70–73 °C; $[\alpha]_D^{24}$ +55.6 (*c* 1.0, $CHCl_3$), lit.[14] $[\alpha]_D^{23}$ +68.0 (*c* 2.0, $CHCl_3$); R_f 0.40 (solvent A_1); ^{1}H NMR (300.13 MHz, $CDCl_3$): δ 7.78 (m, 2H, *o*-C_6H_4), 7.32 (m, 2H, *m*-C_6H_4), 6.22 (d, 1H, $^{3}J_{NH,2}$ 8.8 Hz, NH), 4.59 (d, 1H, $^{3}J_{1,2}$ 3.6 Hz, H-1), 4.34–4.21 (m, 2H, H-6), 3.98 (ddd, 1H, $^{3}J_{2,3}$ 10.2 Hz, H-2), 3.77–3.66 (m, 1H, H-5), 3.61 (dd, 1H, $^{3}J_{3,4}$ 9.2 Hz, H-3), 3.43 ('t',

1H, $^3J_{4,5}$ 9.2 Hz, H-4), 3.31 (s, 3H, OCH_3), 2.42 (s, 3H, $CH_3C_6H_4$), 2.00 (s, 3H, $COCH_3$); ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 172.08 ($COCH_3$), 144.90 (*p*-C_6H_4), 132.82 (*i*-C_6H_4), 129.88 (*m*-C_6H_4), 127.98 (*o*-C_6H_4), 98.25 (C-1), 73.61 (C-3), 70.71 (C-4), 69.41 (C-5), 69.19 (C-6), 55.23 (OCH_3), 53.45 (C-2), 23.21 ($COCH_3$), 21.62 ($CH_3C_6H_4$); CIMS (*m*/*z*, %) 390 (100) $[M+H]^+$. Anal. Calcd for $C_{16}H_{23}NO_8S$ (389.11): C, 49.35; H, 5.95; N, 3.60; S, 8.23. Found: C, 49.14; H, 5.97; N, 3.46; S, 8.18.

3.4. Methyl 2-acetamido-2-deoxy-6-*O*-*p*-tolysulfonyl-β-D-glucopyranoside (2β)

Colourless crystals (33 mg, 2%); mp 101–102 °C; $[\alpha]_D^{24}$ −30.7 (*c* 1.0, $CHCl_3$); R_f 0.27 (solvent A_1); ^1H NMR (500.13 MHz, Me_2SO-d_6): δ 7.67 (d, 1H, $^3J_{NH,2}$ 9.0 Hz, NH), 7.79 (m, 2H, *o*-C_6H_4), 7.48 (m, 2H, *m*-C_6H_4), 4.25 (dd, 1H, $^2J_{6a,6b}$ 10.7 Hz, $^3J_{5,6a}$ 1.8 Hz, H-6a), 4.17 (d, 1H, $^3J_{1,2}$ 8.3 Hz, H-1), 4.07 (dd, 1H, $^3J_{5,6b}$ 6.6 Hz, H-6b), 3.40–3.28 (m, 2H, H-2, H-5), 3.24 (dd, 1H, $^3J_{2,3}$ 10.1 Hz, $^3J_{3,4}$ 8.5 Hz, H-3), 3.24 (s, 3H, OCH_3), 3.01 (dd, 1H, $^3J_{4,5}$ 9.2 Hz, H-4), 2.42 (s, 3H, $CH_3C_6H_4$), 1.79 (s, 3H, $COCH_3$); ^{13}C NMR (125.76 MHz, Me_2SO-d_6): δ 169.24 ($COCH_3$), 145.10 (*p*-C_6H_4), 132.47 (*i*-C_6H_4), 130.31 (*m*-C_6H_4), 127.78 (*o*-C_6H_4), 101.75 (C-1), 74.05 (C-3), 73.47 (C-5), 70.27 (C-6), 70.15 (C-4), 55.68 (OCH_3), 55.12 (C-2), 23.25 ($COCH_3$), 21.27 ($CH_3C_6H_4$); CIMS (*m*/*z*, %) 390 (2) $[M+H]^+$. Anal. Calcd for $C_{16}H_{23}NO_8S$ (389.11): C, 49.35; H, 5.95; N, 3.60; S, 8.23. Found: C, 49.55, H, 6.25; N, 3.44; S, 8.36.

Starting from the anomeric mixture of **1α,β** (10.0 g, 43.0 mmol)[4] compounds **3α,β** (16,7 g, 83%) were obtained according to a procedure of Kajihara et al.[5] Both anomers were separated by flash-chromatography (ethyl acetate) to provide analytical samples.

3.5. Methyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-*p*-tolysulfonyl-α-D-glucopyranoside (3α)

Mp 67–70 °C; $[\alpha]_D^{21}$ +87.6 (*c* 1.0, $CHCl_3$); R_f 0.48 (ethyl acetate); ^1H NMR (250.13 MHz, $CDCl_3$): δ 7.77 (m, 2H, *o*-C_6H_4), 7.34 (m, 2H, *m*-C_6H_4), 5.65 (d, 1H, $^3J_{NH,2}$ 9.5 Hz, NH), 5.15 (dd, 1H, $^3J_{3,4}$ 9.5 Hz, H-3), 4.91 ('t', 1H, $^3J_{4,5}$ 10.0 Hz, H-4), 4.61 (d, 1H, $^3J_{1,2}$ 3.7 Hz, H-1), 4.21 (ddd, 1H, $^3J_{2,3}$ 10.7 Hz, H-2), 4.10–4.01 (m, 2H, H-6), 3.94 (ddd, 1H, $^3J_{5,6b}$ 3.4 Hz, $^3J_{5,6a}$ 5.3 Hz, H-5), 3.33 (s, 3H, OCH_3), 2.44 (s, 3H, $CH_3C_6H_4$), 2.02, 1.98 (2s, 2 × 3H, 2 × $COCH_3$), 1.92 (s, 3H, $NHCOCH_3$); ^{13}C NMR (62.89 MHz, $CDCl_3$): δ 171.25, 169.84, 169.34 (3 × CO), 145.06 (*p*-C_6H_4), 132.54 (*i*-C_6H_4), 129.81 (*m*-C_6H_4), 128.04 (*o*-C_6H_4), 97.97 (C-1), 71.00 (C-3), 68.37 (C-4), 67.93 (C-6), 67.52 (C-5), 55.45 (OCH_3), 51.66 (C-2), 23.16 ($NHCOCH_3$), 21.64 ($CH_3C_6H_4$), 20.65, 20.52 (2 × $COCH_3$); EIMS (*m*/*z*, %) 473 (5) $[M+H]^+$. Anal. Calcd for $C_{20}H_{27}NO_{10}S$ (473.14): C, 50.73; H, 5.75; N, 2.96; S, 6.77. Found: C, 50.54; H, 5.77; N, 2.77; S, 6.70.

3.6. Methyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-*p*-tolysulfonyl-β-D-glucopyranoside (3β)

Mp 179 °C (ethanol, dec), lit.[5] mp 192 °C (dec; this melting point was not reproducible even after several crystallisations); $[\alpha]_D^{22}$ +21.8 (*c* 1.0, $CHCl_3$), lit.[5] $[\alpha]_D^{23}$ +19.7 (*c* 0.6, CH_2Cl_2); R_f 0.34 (ethyl acetate); ^1H NMR (250.13 MHz, $CDCl_3$): δ 7.77 (m, 2H, *o*-C_6H_4), 7.32 (m, 2H, *m*-C_6H_4), 5.52 (d, 1H, $^3J_{NH,2}$ 9.0 Hz, NH), 5.22 (dd, 1H, $^3J_{2,3}$ 10.5 Hz, $^3J_{3,4}$ 9.3 Hz, H-3), 4.88 ('t', 1H, $^3J_{4,5}$ 10.0 Hz, H-4), 4.50 (d, 1H, $^3J_{1,2}$ 8.4 Hz, H-1), 4.14–4.02 (m, 2H, H-6), 3.85–3.70 (m, 2H, H-2, H-5), 3.41 (s, 3H, OCH_3), 2.44 (s, 3H, $CH_3C_6H_4$), 2.00, 1.99 (2s, 2 × 3H, 2 × $COCH_3$), 1.92 (s, 3H, $NHCOCH_3$); ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 170.86 ($NHCOCH_3$), 170.22, 169.47 (2 × $COCH_3$), 145.15, 132.41 (2 × *i*-C_6H_4), 129.88, 128.05 (2 × *o*-, 2 × *m*-C_6H_4), 101.44 (C-1), 72.10 (C-3), 71.58 (C-5), 68.88 (C-4), 68.01 (C-6), 56.76 (OCH_3), 54.41 (C-2), 23.32 ($NHCOCH_3$), 21.65 ($CH_3C_6H_4$), 20.62, 20.56 (2 × $COCH_3$); CIMS (*m*/*z*, %) 474 (76) $[M+H]^+$. Anal. Calcd for $C_{20}H_{27}NO_{10}S$ (473.14): C, 50.73; H, 5.75; N, 2.96; S, 6.77. Found: C, 51.04; H, 5.90; N, 2.85; S, 6.48.

For transformation of compounds **3α,β** (10.0 g, 21.14 mmol) into methyl 3,4-di-*O*-acetyl-2-acetamido-2,6-dideoxy-α,β-D-glucopyranosides (**5α,β**) via the corresponding iodine compound **4α,β** conditions of Kajihara et al.[5] were used again. Purification by column chromatography (eluent ethyl acetate gradient 80→100% in petrol ether) afforded **5α,β** (5.33 g, 83%). Analytical samples were obtained by column chromatography using ethyl acetate as eluent.

3.7. Methyl 2-acetamido-3,4-di-*O*-acetyl-2,6-dideoxy-α-D-glucopyranoside (5α)

Mp 104–109 °C; $[\alpha]_D^{23}$ +76.4 (*c* 1.0, $CHCl_3$); R_f 0.42 (ethyl acetate); ^1H NMR (300.13 MHz, $CDCl_3$): δ 5.69 (d, 1H, $^3J_{NH,2}$ 9.5 Hz, NH), 5.14 (dd, 1H, $^3J_{3,4}$ 9.5 Hz, H-3), 4.82 ('t', 1H, $^3J_{4,5}$ 9.9 Hz, H-4), 4.63 (d, 1H, $^3J_{1,2}$ 3.6 Hz, H-1), 4.28 (ddd, 1H, $^3J_{2,3}$ 10.7 Hz, H-2), 3.79 (dq, 1H, $^3J_{5,6}$ 6.3 Hz, H-5), 3.36 (s, 3H, OCH_3), 2.01, 1.99 (2s, 2 × 3H, 2 × $COCH_3$), 1.93 (s, 3H, $NHCOCH_3$), 1.17 (d, 3H, H-6); ^{13}C NMR (62.89 MHz, $CDCl_3$): δ 171.42, 169.83, 169.57 (3 × CO), 98.09 (C-1), 73.30 (C-3), 71.35 (C-4), 65.40 (C-5), 55.17 (OCH_3), 52.16 (C-2), 23.20 ($NHCOCH_3$), 20.72, 20.68 (2 × $COCH_3$), 17.30 (C-6); CIMS (*m*/*z*, %) 304 (87) $[M+H]^+$. Anal. Calcd for $C_{13}H_{21}NO_7$ (303.13): C, 51.48; H, 6.98; N, 4.62. Found: C, 51.42; H, 7.21; N, 4.45.

3.8. Methyl 2-acetamido-3,4-di-*O*-acetyl-2,6-dideoxy-β-D-glucopyranoside (5β)

Mp 169–171 °C (dec); $[\alpha]_D^{23}$ −2.2 (*c* 1.0, $CHCl_3$); lit.[5] $[\alpha]_D^{23}$ −38.0 (*c* 0.3, Me_2SO); R_f 0.28 (ethyl acetate); ^1H NMR (300.15 MHz, $CDCl_3$): δ 5.56 (d, 1H, $^3J_{NH,2}$ 9.0 Hz, NH), 5.17 (dd, 1H, $^3J_{3,4}$ 9.5 Hz, H-3), 4.80 ('t', 1H, $^3J_{4,5}$ 9.5 Hz, H-4), 4.49 (d, 1H, $^3J_{1,2}$ 8.4 Hz, H-1), 3.87 (ddd, 1H, $^3J_{2,3}$ 10.7 Hz, H-2), 3.55 (dq, 1H, $^3J_{5,6}$ 6.2 Hz, H-5), 3.47 (s, 3H, OCH_3), 2.02, 2.01 (2s, 2 × 3H, 2 × $COCH_3$), 1.93 (s, 3H, $NHCOCH_3$), 1.23 (d, 3H, H-6); ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 171.07, 170.21, 169.64 (3 × CO), 101.42 (C-1), 73.57 (C-3), 72.59 (C-5), 69.95 (C-4), 56.50 (OCH_3), 54.66 (C-2), 23.35 ($NHCOCH_3$), 20.69 (2 × $COCH_3$), 17.42 (C-6); CIMS (*m*/*z*, %) 304 (82) $[M+H]^+$. Anal. Calcd for $C_{13}H_{21}NO_7$ (303.13): C, 51.48; H, 6.98; N, 4.62. Found: C, 51.19; H, 6.68; N, 4.39.

3.9. Deacetylation of 5α,β

Methanolic sodium methoxide soln (0.5 M, 1.0 mL) was added to a soln of compounds **5α,β** (5.0 g, 16.45 mmol) in dry methanol (60 mL). After stirring at ambient temperature for 2 h (TLC, solvent B_1), the reaction mixture was neutralized with IR120 (H^+) Amberlite resin, filtered, dried and concentrated. Column chromatography (solvent B_3) of the residue provided the separated isomers **6α** (2.05 g, 57%) and **6β** (323 mg, 9%).

3.10. Methyl 2-acetamido-2,6-dideoxy-α-D-glucopyranoside (6α)

Mp 172–173 °C (ethanol); $[\alpha]_D^{24}$ +55.5 (*c* 0.9, $CHCl_3$); R_f 0.45 (solvent B_2); ^1H NMR (300.13 MHz, Me_2SO-d_6): δ 7.70 (d, 1H, $^3J_{NH,2}$ 8.5 Hz, NH), 5.01 (d, 1H, $^3J_{OH,4}$ 5.7 Hz, OH-4), 4.68 (d, 1H, $^3J_{OH,3}$ 5.7 Hz, OH-3), 4.47 (d, 1H, $^3J_{1,2}$ 3.6 Hz, H-1), 3.67 (ddd, 1H, $^3J_{2,3}$ 10.7 Hz, H-2), 3.51–3.34 (m, 2H, H-3, H-5), 3.22 (s, 3H, OCH_3), 2.85 (ddd, 1H, $^3J_{4,5}$ 9.3 Hz, $^3J_{3,4}$ 8.5 Hz, H-4), 1.82 (s, 3H, $NHCOCH_3$), 1.15 (d, 3H, $^3J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR (62.89 MHz, Me_2SO-d_6): δ 169.55 ($NHCOCH_3$), 98.23 (C-1), 76.63 (C-3), 70.64 (C-4), 67.36 (C-5), 54.55 (OCH_3), 54.10 (C-2), 22.79 ($NHCOCH_3$), 18.08 (C-6); CIMS (*m*/*z*, %) 220 (100) $[M+H]^+$. Anal. Calcd for $C_9H_{17}NO_5$ (219.11): C, 49.31; H, 7.82; N 6.39. Found: C, 49.25; H, 7.98; N, 6.21.

3.11. Methyl 2-acetamido-2,6-dideoxy-β-D-glucopyranoside (6β)

Mp 155–157 °C (ethanol); $[\alpha]_D^{26}$ −27.6 (*c* 1.0, CH_3OH); lit.[5] $[\alpha]_D^{23}$ −46.3 (*c* 2.0, H_2O); R_f 0.23 (solvent B_2); ^1H NMR (300.13 MHz, Me_2SO-d_6): δ 7.66 (d, 1H, $^3J_{NH,2}$ 9.0 Hz, NH), 5.02 (d, 1H, $^3J_{OH,4}$ 5.3 Hz, OH-4), 4.86 (d, 1H, $^3J_{OH,3}$ 5.2 Hz, OH-3), 4.17 (d, 1H, $^3J_{1,2}$ 8.5 Hz, H-1), 3.43 (ddd, 1H, $^3J_{2,3}$ 10.3 Hz, H-2), 3.28 (s, 3H, OCH_3), 3.26–3.11 (m, 2H, H-3, H-5), 2.82 (ddd, 1H, $^3J_{4,5}$ 9.3 Hz, $^3J_{3,4}$ 8.8 Hz, H-4), 1.79 (s, 3H, $COCH_3$), 1.17 (d, 3H, $^3J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR (62.89 MHz, Me_2SO-d_6): δ 169.20 ($COCH_3$), 101.84 (C-1), 76.06 (C-3), 74.26 (C-5), 71.78 (C-4), 55.62 (OCH_3), 55.51 (C-2), 23.28 ($COCH_3$), 18.09 (C-6); CIMS (*m*/*z*, %) 220 (100) $[M+H]^+$. Anal. Calcd for $C_9H_{17}NO_5$ (219.11): C, 49.31; H, 7.82; N, 6.39. Found: C, 49.45; H, 8.07; N, 6.18.

3.12. Selective acetylation of compounds 6α and 6β

A soln of acetyl chloride (0.4 mL) in dry toluene (2.0 mL) was added dropwise to a stirred soln of compound **6α** or **6β** (each 1.0 g, 4.56 mmol) in dry pyridine (20 mL) at −40 °C. The mixture was kept at that temperature for additional 30 min, then at −20 °C for 30 min, and finally stored at 5 °C for 48 h (TLC, solvent B_1), and concentrated. The only observed side-products were the diacetylated compounds **5α** and **5β** which were removed easily by column chromatography (ethyl acetate) to give crystalline **7α** (480 mg, 40%) and **7β** (456, 38%), respectively.

3.13. Methyl 2-acetamido-3-*O*-acetyl-2,6-dideoxy-α-D-glucopyranoside (7α)

Mp 160–163 °C; $[\alpha]_D^{24}$ +71.2 (*c* 1.0, $CHCl_3$); R_f 0.20 (ethyl acetate); ^1H NMR (250.13 MHz, $CDCl_3$): δ 5.85 (d, 1H, $^3J_{NH,2}$ 9.5 Hz, NH), 4.99 (dd, 1H, $^3J_{3,4}$ 9.2 Hz, H-3), 4.60 (d, 1H, $^3J_{1,2}$ 3.6 Hz, H-1), 4.22 (ddd, 1H, $^3J_{2,3}$ 10.7 Hz, H-2), 3.69 (dq, 1H, $^3J_{5,6}$ 6.2 Hz, H-5), 3.36 (s, 3H, OCH_3), 3.35 (t, 1H, $^3J_{4,5}$ 9.5 Hz, H-4), 2.06 (s, 3H, $COCH_3$), 1.93 (s, 3H, $NHCOCH_3$), 1.31 (d, 3H, H-6); ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 172.43, 170.1 (2 × CO), 98.26 (C-1), 74.54 (C-3), 74.18 (C-4), 67.48 (C-5), 55.04 (OCH_3), 52.03 (C-2), 23.18 ($NHCOCH_3$), 20.98 ($COCH_3$), 17.54 (C-6); CIMS (*m*/*z*, %) 262 (63) $[M+H]^+$. Anal. Calcd for $C_{11}H_{19}NO_6$ (261.12): C, 50.57; H, 7.33; N, 5.36. Found: C, 50.59; H, 7.65; N, 5.09.

3.14. Methyl 2-acetamido-3-*O*-acetyl-2,6-dideoxy-β-D-glucopyranoside (7β)

Mp 181–184 °C; $[\alpha]_D^{26}$ −90.6 (*c* 1.0, CH_3OH); R_f 0.10 (ethyl acetate); ^1H NMR (300.13 MHz, $CDCl_3$): δ 5.57 (d, 1H, $^3J_{NH,2}$ 9.2 Hz, NH), 4.99 (dd, 1H, $^3J_{3,4}$ 10.5 Hz, H-3), 4.41 (d, 1H, $^3J_{1,2}$ 8.4 Hz, H-1), 3.86 (ddd, 1H, $^3J_{2,3}$ 8.8 Hz, H-2), 3.47 (s, 3H, OCH_3), 3.45–3.30 (m, 2H, H-4, H-5), 2.10 (s, 3H, $COCH_3$), 1.94 (s, 3H, $NHCOCH_3$), 1.36 (d, 3H, $^3J_{5,6}$ 5.8 Hz, H-6); ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 172.21, 170.20 (2 × CO), 101.54 (C-1), 75.98 (C-3), 74.35 (C-5), 71.96

(C-4), 56.45 (OCH_3), 54.45 (C-2), 23.40 ($NHCOCH_3$), 20.94 ($COCH_3$), 17.60 (C-6); CIMS (*m*/*z*, %) 262 (100) $[M+H]^+$. Anal. Calcd for $C_{11}H_{19}NO_6$ (261.12): C, 50.57; H, 7.33; N, 5.36. Found: C, 50.83; H, 7.67; N, 5.06.

3.15. Methyl 2-acetamido-3-*O*-acetyl-2,6-dideoxy-α- and β-D-*xylo*-hexopyranosid-4-ulose (8α and 8β)

3.15.1. Via pyridinium dichromate (PDC) oxidation. A catalytic amount of glacial acetic acid (100 μL) and freshly activated and powdered 3-Å molecular sieves (800 mg) were added to a stirred suspension of compound **7α** or **7β** (261 mg, 1.0 mmol) and PDC (564 mg, 1.5 mmol) in dry dichloromethane (5 mL). After stirring for 18 h at ambient temperature (TLC, ethyl acetate), Celite was added (500 mg) and, 20 min later, the insoluble constituents were filtered off and washed with dichloromethane (2 × 3 mL). The combined filtrates were dried, concentrated and the residue was repeatedly evaporated with toluene to remove traces of acetic acid. The brown residue was then extracted with diethyl ether. The ethereal extracts were combined, dried and evaporated to give colourless, crystalline and analytically pure **8α** (235 mg, 91%) and **8β** (155 mg, 60%), respectively.

3.15.2. Via Swern-oxidation. Dimethyl sulfoxide (214 μL, 3.0 mmol) was added dropwise to a stirred soln of oxalyl chloride (128 μL, 1.5 mmol) in dry dichloromethane (3 mL) at −78 °C. After the mixture had been stirred for 15 min, a soln of compound **7α** or **7β** (261 mg, 1.0 mmol) in dry dichloromethane (9 mL) was added dropwise at −78 °C. Stirring was continued for another 1 h before triethylamine (690 μL, 5.0 mmol) was added dropwise. The mixture was warmed to room temperature and then concentrated. The slightly yellow coloured crude product was purified by column chromatography (ethyl acetate) to provide **8α** (241 mg, 93%) and **8β** (148 mg, 57%), respectively.

3.16. Methyl 2-acetamido-3-*O*-acetyl-2,6-dideoxy-α-D-*xylo*-hexopyranosid-4- ulose (8α)

Mp 136–139 °C; $[\alpha]_D^{25}$ +126.2 (*c* 1.0, $CHCl_3$); R_f 0.24 (ethyl acetate); ^1H NMR (250.13 MHz, $CDCl_3$): δ 5.85 (d, 1H, $^3J_{NH,2}$ 9.5 Hz, NH), 5.44 (d, 1H, $^3J_{2,3}$ 11.5 Hz, H-3), 4.82 (d, 1H, $^3J_{1,2}$ 3.5 Hz, H-1), 4.64 (ddd, 1H, H-2), 4.28 (q, 1H, $^3J_{5,6}$ 6.5 Hz, H-5), 3.48 (s, 3H, OCH_3), 2.14 (s, 3H, $COCH_3$), 1.98 (s, 3H, $NHCOCH_3$), 1.30 (d, 3H, H-6); ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 197.94 (C-4), 170.69, 169.62 (2 × CO), 98.28 (C-1), 74.35 (C-3), 69.42 (C-5), 55.99 (OCH_3), 53.74 (C-2), 23.20 ($NHCOCH_3$), 20.52 ($COCH_3$), 13.75 (C-6); CIMS (*m*/*z*, %) 260 (97) $[M+H]^+$. Anal. Calcd for $C_{11}H_{17}NO_6$ (259.11): C, 50.96; H, 6.61; N, 5.40. Found: C, 50.89; H, 6.92; N, 5.13.

3.17. Methyl 2-acetamido-3-*O*-acetyl-2,6-dideoxy-β-D-*xylo*-hexopyranosid-4-ulose (8β)

Mp 120–122 °C; $[\alpha]_D^{24}$ +11.2 (*c* 1.0, $CHCl_3$); R_f 0.16 (ethyl acetate); ^1H NMR (300.13 MHz, $CDCl_3$): δ 6.07 (d, 1H, $^3J_{NH,2}$ 8.0 Hz, NH), 5.78 (d, 1H, H-3), 5.10 (d, 1H, $^3J_{1,2}$ 7.2 Hz, H-1), 4.19 (q, 1H, $J_{5,6}$ 6.5 Hz, H-5), 3.86 (ddd, 1H, $^3J_{2,3}$ 10.8 Hz, H-2), 3.51 (s, 3H, OCH_3), 2.15 (s, 3H, $COCH_3$), 1.99 (s, 3H, $NHCOCH_3$), 1.36 (d, 3H, H-6); ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 198.28 (C-4), 170.47, 170.05 (2 × CO), 100.58 (C-1), 74.10, 73.93 (C-3, C-5), 57.99 (C-2), 56.84 (OCH_3), 23.42 ($NHCOCH_3$), 20.49 ($COCH_3$), 14.51 (C-6); CIMS (*m*/*z*, %) 260 (100) $[M+H]^+$. Anal. Calcd for $C_{11}H_{17}NO_6$ (259.11): C, 50.96; H, 6.61; N, 5.40. Found: C, 50.96; H, 6.53; N, 5.09.

3.18. Deacetylation of compounds 8α and 8β

3.18.1. Via Zemplén procedure. A methanolic sodium methoxide soln (0.5 M, 0.75 mL) was added to a soln of compound **8α** or **8β** (65 mg, 0.25 mmol) in dry methanol (2 mL). After stirring at ambient temperature for 2 h (TLC, solvent B_1), the soln was neutralized by the addition of IR120 (H^+) Amberlite resin, filtered, and concentrated. The purification of the crude material by column chromatography (solvent B_3) afforded **9α** (39 mg, 72 %) and **9β** (22 mg, 40%), respectively.

3.18.2. Via methanolic HCl soln. Compound **8α** or **8β** (130 mg, 0.5 mmol) was dissolved in methanolic HCl (0.28 M, 19 mL, prepared by adding of 0.4 mL acetyl chloride to 18.6 mL ice-cold dry methanol) and the soln was stirred at ambient temperature for 12 h (TLC, solvent B_1). The soln was then neutralized with $PbCO_3 \times Pb(OH)_2$ (1.6 g), filtered, and concentrated. The residue was purified by column chromatography (solvent B_3) to provide **9α** (48 mg, 44%) and **9β** (13 mg, 12%), respectively.

Under both deacetylation conditions compounds **6α**, **10** and **6α**, **6β** and **10** were determined as side-products starting from **8α** and **8β**, respectively. The overall yield of isolated side-products in each case was about 10%.

3.19. Methyl 2-acetamido-2,6-dideoxy-α-D-*xylo*-hexopyranosid-4-ulose (9α)

Mp 121–123 °C; $[\alpha]_D^{22}$ +141.7 (*c* 1.0, CH_3OH); R_f 0.52 (solvent B_1); ^1H NMR (250.13 MHz, $CDCl_3$): δ 6.04 (br s, 1H, NH), 4.82 (d, 1H, $^3J_{1,2}$ 3.5 Hz, H-1), 4.42–4.31 (m, 2H, H-2, H-3), 4.31 (q, 1H, $^3J_{5,6}$ 6.5 Hz, H-5), 3.48 (s, 3H, OCH_3), 2.05 (s, 3H, $COCH_3$), 1.33 (d, 3H, H-6); ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 203.91 (C-4), 170.46 ($COCH_3$), 98.37 (C-1), 74.21 (C-3), 68.53 (C-5), 57.36 (C-2), 56.12 (OCH_3), 23.30 ($COCH_3$), 13.60 (C-6); CIMS (*m*/*z*, %) 218 (100) $[M+H]^+$. Anal.

Calcd for $C_9H_{15}NO_5$ (217.10): C, 49.76; H, 6.96; N, 6.45. Found: C, 49.68; H, 7.15; N, 6.58.

3.20. Methyl 2-acetamido-2,6-dideoxy-β-D-*xylo*-hexopyranosid-4-ulose (9β)

Colourless syrup; $[\alpha]_D^{22}$ −45.9 (*c* 0.5, CH_3OH); R_f 0.47 (solvent B_1); ^1H NMR (300.13 MHz, CD_3OD): δ 4.35 (s, 1H, $^3J_{1,2}$ 10.7 Hz, H-1), 3.83–3.75 (m, 1H, H-2), 4.21–4.14, 3.72–3.62 (2m, 2 × 1H, H-3, H-5), 3.39 (s, 3H, OCH_3), 1.95 (s, 3H, $COCH_3$), 1.27 (d, 3H, $^3J_{5,6}$ 6.5 Hz, H-6); ^{13}C NMR (75.47 MHz, CD_3OD): δ 205.31 (C-4), 173.52 ($COCH_3$), 102.94 (C-1), 75.66 (C-3), 74.49 (C-5), 60.94 (C-2), 57.05 (OCH_3), 22.98 ($COCH_3$), 14.59 (C-6); CIMS (*m*/*z*, %) 218 (57) $[M+H]^+$. Anal. Calcd for $C_9H_{15}NO_5$ (217.10): C, 49.76; H, 6.96; N, 6.45. Found: C, 49.60; H, 7.21; N, 6.66.

3.21. *N*-(5-Hydroxy-6-methyl-4-oxo-4*H*-pyran-3-yl)acetamide (10)

Mp 172 °C (dec); R_f 0.67 (solvent B_1); ^1H NMR (500.13 MHz, $CDCl_3$): δ 9.13 (s, 1H, H-2), 8.01 (br s, 1H, NH), 6.99 (br s, 1H, OH), 2.38 (s, 3H, CH_3), 2.18 (s, 3H, $COCH_3$); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ 168.75 ($COCH_3$), 166.39 (C-4), 150.33 (C-6), 144.23 (C-2), 140.77 (C-5), 126.03 (C-3), 23.86 ($COCH_3$), 14.63 (CH_3); CIMS (*m*/*z*, %) 184 (100) $[M+H]^+$. Anal. Calcd for $C_8H_9NO_4$ (183.16): C, 52.46; H, 4.95; N, 7.65. Found: C, 52.39; H, 5.01; N, 7.55.

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