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Crystal structures of *O*-acetylated 2-acylamino-2-deoxy-D-galactose derivatives

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Abstract

The X-ray structures of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-galactopyranoside derivatives with four different 2-(acylamino) substituents have been determined with Mo K_α radiation at 123 K. The structure of the 2-acetylamino derivative and of its acyl-homologs with a 2-(propanoylamino)-, 2-(butanoylamino)-, and 2-(2-methyl-propanoylamino)-group crystallized in the monoclinic space group *C*2. The pyranose unit of all compounds has the usual $^4C_1$ shape. The different orientations of the 6-*O*-acetyl-groups are discussed. Conformations of the acylamino-group are compared to those found in the crystal structure of *N*-acetyl-α-D-galactosamine.

Keywords: Amino sugars; 2-(Acylamino)-2-deoxy-D-galactose; X-ray structures

1. Introduction

N-Acetyl-D-galactosamine is a naturally occurring building block of glycoproteins, glycolipids, and glycosaminoglycans implicated in cell recognition events.[1] Cell surfaces can be modified by the exogenous addition of carbohydrate-[2,3] and lipid[4] analogs to cells, followed by their metabolic incorporation into components of the plasma membrane. Such cell surface engineering can lead to functional alteration of cellular properties, like virus binding, or membrane receptor function.[2–4] The metabolic transformation of amino sugar analogs into non-natural sialic acids and their subsequent incorporation into glycoconjugates has been demonstrated in 1992[5] using *N*-acyl-modified D-mannosamine derivatives like *N*-propanoyl- and *N*-butanoyl-D-mannosamine.

We have been especially interested in analogs of *N*-acetyl-D-galactosamine (GalNAc). This amino sugar is biosynthetically incorporated into glycosphingolipids of the ganglio-series[6,7] by a β-*N*-acetyl-D-galactosaminyl-transferase.[8,9] Here, we report on the preparation and structural characterization of a series of *N*-acyl D-*galacto* configured sugar analogs. Per-*O*-acetylated *N*-acetyl-α-D-galactosamine (**9**) and the acyl-homologs target molecules **10**–**12** are easily accessible, as indicated in Fig. 1. They have been prepared as potential cell permeable prodrugs, which might intracellularly generate the free sugar analogs after enzymatic ester cleavage.[10–12] The structures of the 2-acetylamino derivative **9** and of its acyl-homologs with a 2-(propanoylamino)- (**10**), 2-(butanoylamino)- (**11**), and 2-(2-methyl-propanoylamino)-group (**12**) are discussed.

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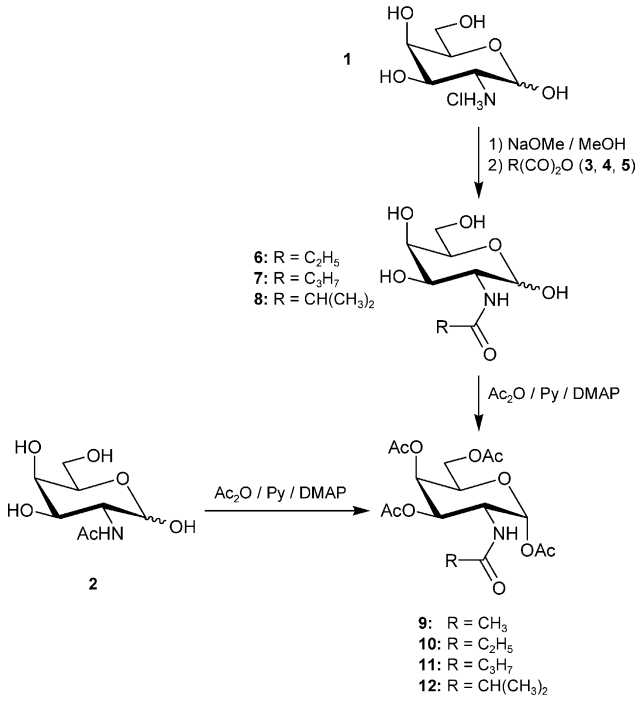

Fig. 1. Synthesis scheme for compounds **9**–**12**. DMAP, 4-dimethylaminopyridine.

2. Results and discussion

2.1. Synthesis

The following target compounds were prepared according to modified procedures described in the literature[13,14] (Fig. 1): 1,3,4,6-tetra-*O*-acetyl-2-(acetylamino)-2-deoxy-α-D-galactopyranoside (**9**), 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(propanoyl-amino)-α-D-galactopyranoside (**10**), 1,3,4,6-tetra-*O*-acetyl-2-(butanoylamino)-2-deoxy-α-D-galactopyranoside (**11**), and 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(2-methyl-propanoylamino)-α-D-galactopyranoside (**12**). The starting material for compounds **10**–**12** was D-galactosamine-hydrochloride (**1**), and *N*-acetyl-D-galactosamine (**2**) for compound **9**. The amino function of **1** was converted to an acylamino group with the appropriate acid anhydrides **3**–**5**, respectively. The free hydroxyl groups were protected with acetyl groups resulting in an anomeric mixture of the title compounds. Recrystallization from ethyl acetate resulted in the pure α-configured compounds **9**–**12**.

2.2. Crystal structure determination

The structures of **9**–**12** with the atom numbering[15] are shown in Figs. 2–5. Selected geometrical parameters are listed in Table 1. Like in *N*-acetyl-α-D-galactosamine,[16,17] the pyranose ring of the title compounds is in the typical $^4C_1$ conformation. This conformation can be described by the endocyclic torsion angles (Table 2). The range of the ring torsion angles, 52.8(2)–58.9(2)°, show only a slight deviation from 60° of an ideal chair conformation as in cyclohexane, and of 55.8–61.7° as in an ideal pyranose ring.[18] These values are in agreement with the torsion angles of 54.9(3)–59.0(3)°[16] and 55.8(5)–60.2(5)°[17] found in *N*-acetyl-α-D-galactosamine.

The acetylamino-group in *N*-acetyl-α-D-galactosamine is in a Z-*anti* conformation. This Z-*anti* conformation is also found in the acylamino-groups of the title compounds as indicated by the torsion angles C2–N21–C21–C22 with values in the range of 164.5(2)–179.5(4)° (Table 2; ideal: ±180° for *anti* conformation). In *N*-acetyl-α-D-galactosamine, this torsion angle is 179.0(5)°.[17] The torsion angle C2–N21–C21–O21(2) ranging from 1.8(8) to −11.8(7)° show in part considerable deviation from planarity of the acylamino-group (Table 2; ideal: ±0° for planar arrangement). In contrast to these values in the title compounds, the acetylamino-group in *N*-acetyl-α-D-galactosamine shows nearly ideal planarity as indicated by the torsion angle of −0.4(8)°.[17] Further bond lengths and angles of the acylamino-groups are given in Table 2.

The *O*-acetyl-groups in the target compounds are in a *cisoid*-planar conformation. This is in agreement with those found in other *O*-acetylated pyranoses.[19] The *cisoid*-planar conformation is characterized by the torsion angles C*i*–O*i*1–C*i*1–C*i*2 (*i* = 3, 4, 6) and C*i*–O*i*1–C*i*1–Oi2 (*i* = 3, 4, 6) (Table 2). The first torsion angles are in the range from −173.6(4) to 180.0(2)° (ideal: ±180° for *cisoid* conformation), and the second torsion angles from −0.2(3) to 8.2(8)° (ideal: ±0° for *cisoid* conformation). Only the 3-*O*-acetyl-group and the 4-*O*-acetyl-group in **9** show a small deviation from planarity. This deviation has no influence on the *cisoid*-planar arrangement of the carbon-atom of the ring and the oxygen-atom of the carbonyl group. The preferred *cis* over the *trans* conformation is apparently a result of electronic effects.[20]

The orientations of the 6-*O*-acetyl-groups relative to the pyranose ring are different in the four title compounds. For compound **9**, this group is found in a *trans–gauche* (*tg*) arrangement, whereas in **10**–**12**, it is in a *gauche–trans* (*gt*) conformation. The respective conformation is characterized by the torsion angles O1–C5–C6–O6 and C4–C5–C6–O6, respectively (Table 2).[21] A graphical presentation is given in Fig. 6. In the ideal case, a torsion angle for a *gauche* conformation is ±60°, and ±180° for the *trans* conformation. In the title compounds, the torsion angles for the *gauche* conformation are in the range from 53.1(5) to 77.5(5)°, and for the *trans* conformation in a range from 158.3(4) to 174.4(4)° (Table 2). In contrast to this observation, in the crystal structure of *N*-acetyl-α-D-galactosamine, a

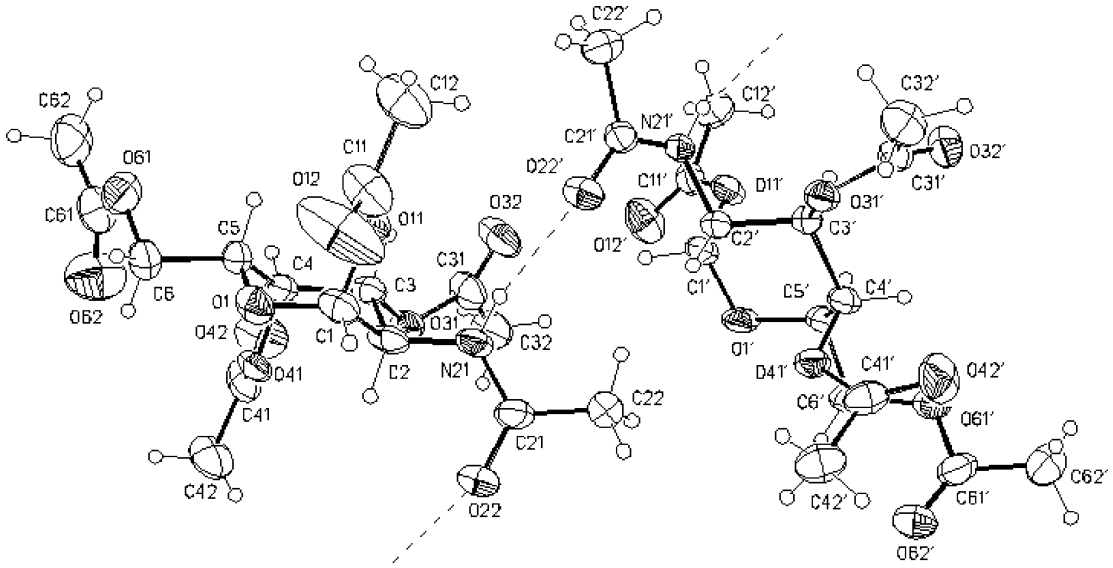

Fig. 2. The structure of **9**, showing the atom-numbering scheme and displacement ellipsoids at the 50% probability level for non-H atoms.[15a] The asymmetric unit contains two molecules.

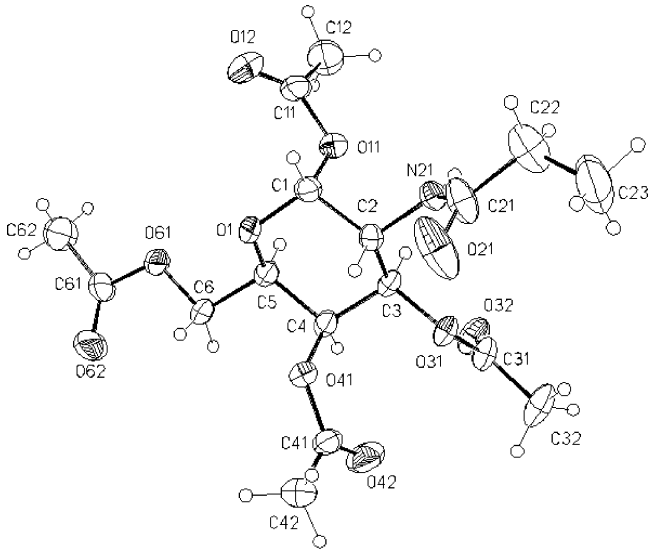

Fig. 3. The structure of **10**, showing the atom-numbering scheme and displacement ellipsoids at the 50% probability level for non-H atoms.[15a]

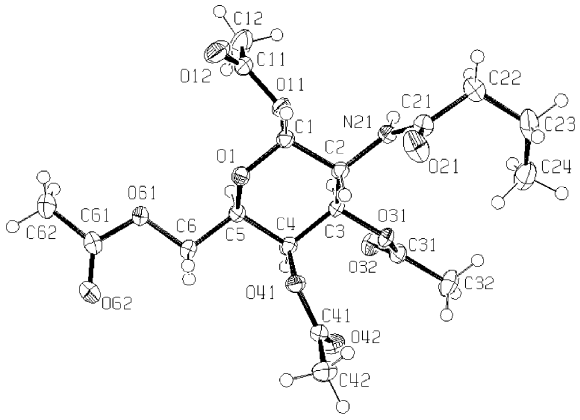

Fig. 4. The structure of **11**, showing the atom-numbering scheme and displacement ellipsoids at the 50% probability level for non-H atoms.[15b]

gauche–gauche (*gg*) conformation of the torsion angles O1–C5–C6–O6 and C4–C5–C6–O6 is found.[16]

The bond lengths and angles around the anomeric center (Table 3) are of special interest, because they are strongly correlated to theories and models of the anomeric effect.[22,23] The (*endo*-) anomeric effect describes the preferred axial orientation of an electronegative substituent at the anomeric center compared to the equatorial position relative to cyclohexane.

In the title compounds, the 1-*O*-acetyl-groups are in an axial position. The anomeric torsion angle C5–O1–C1–O11 between the carbon-atoms C-5 and the exocyclic oxygen-atoms O-11 are in a *gauche* (+*g*) orientation. The values for this torsion angle are in the range from 59.6(2) to 63.1(4)° (Table 3). The endocyclic C1–O1 bond lengths are in the range from 1.39(1) to 1.42(1) Å (Table 3) and are slightly shorter then the averaged C–O bond lengths of 1.43(1) Å.[24]

The *exo*-anomeric effect[25] describes the preferred *gauche* orientation of the carbon-atom of the aglycon (C-11) with respect to a rotation around the exocyclic bond (C1–O11) to the endocyclic oxygen-atom O-1. This arrangement is defined by the *exo*-anomeric torsion angle O1–C1–O11–C11 (Table 3).

In the title compounds, the 1-*O*-acetyl groups show a *gauche* (+*g*) arrangement. Therefore, the aglycon is oriented towards the direction of the endocyclic oxygen-atom O-1. The conformation around the anomeric

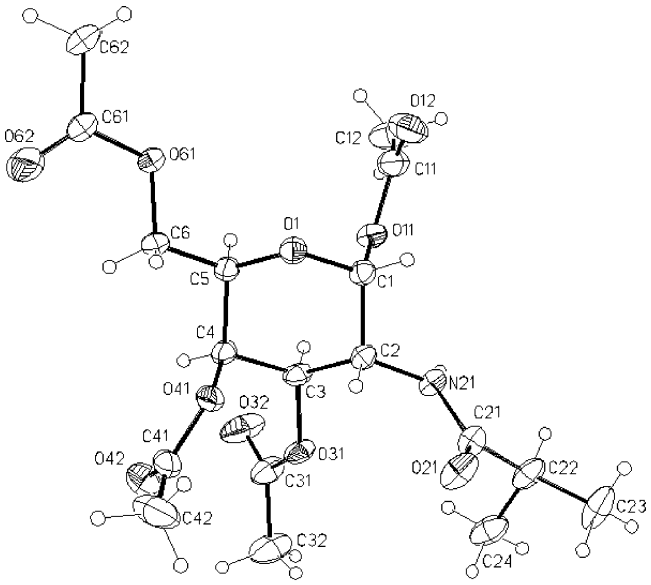

Fig. 5. The structure of **12**, showing the atom-numbering scheme and displacements ellipsoids at the 50% probability level for non-H atoms.[15a]

carbon-atom can be described as a $(+g, +g)$-conformation, or as an *ax-exo-syn* conformation, respectively.[26] The exocyclic C1–O11 bond lengths (Table 3) are in the range of the averaged C–O bond lengths of 1.43(1) Å.[24]

Tables 4–7 show geometric details and the symmetry codes of the hydrogen-bonding geometry in the crystals of the title compounds. The compounds **10**–**12** show a similar network of their hydrogen bonds. One intermolecular N–H···O hydrogen bond of normal strength[27] is present. The proton attached to the nitrogen atom of the acylamino group of a given molecule forms a hydrogen bond to the carbonyl group oxygen of the acylamino group of another molecule. Only compound **11** with a bond angle N21–H21–O21 of 143° shows a slight deviation from the nearly linear arrangement (ideal: 180° for linear arrangement) in the crystal (Table 6). Within such hydrogen bonds, the value of this bond angle is most often found to be in the range from 160 to 170°. Furthermore, this arrangement spreads like a chain inside the crystal as illustrated for compound **10** (Figs. 7 and 8). Additional five weak[27]

Table 1
Crystal data and structure refinement

	9	**10**	**11**	**12**
Empirical formula	$C_{16}H_{23}NO_{10}$	$C_{17}H_{25}NO_{10}$	$C_{18}H_{27}NO_{10}$	$C_{18}H_{27}NO_{10}$
Formula weight	389.35	403.38	417.41	417.41
Unit cell dimensions				
a (Å)	35.2322(6)	16.8743(3)	17.1624(2)	16.8087(3)
b (Å)	9.2859(2)	9.2640(3)	9.3055(1)	9.4611(2)
c (Å)	12.4737(2)	14.8649(4)	15.1385(2)	15.1526(3)
β (°)	107.062(2)	117.347(2)	119.3363(6)	118.772(1)
V (Å^3)	3901.32(12)	2064.03(9)	2107.64(4)	2112.20(7)
Formula units Z	8	4	4	4
D_{calcd} (mg m^{-3})	1.326	1.298	1.315	1.313
Absorption coefficient (mm^{-1})	0.111	0.108	0.108	0.108
$F(000)$	1648	856	888	888
Crystal size (mm)	0.60 × 0.40 × 0.20	0.55 × 0.35 × 0.15	0.79 × 0.28 × 0.14	0.55 × 0.50 × 0.40
θ range (°)	1.71–25.00	2.72–25.00	2.95–24.99	2.76–27.48
Limiting indices	$-41 \le h \le 41$, $-11 \le k \le 11$, $-14 \le l \le 14$	$-20 \le h \le 19$, $-10 \le k \le 10$, $-17 \le l \le 17$	$-20 \le h \le 20$, $-11 \le k \le 11$, $-18 \le l \le 18$	$-21 \le h \le 21$, $-12 \le k \le 12$, $-19 \le l \le 19$
Reflections collected/unique	36,586/6876 [R_{int} = 0.0529]	9429/3641 [R_{int} = 0.0384]	30,215/1984 [R_{int} = 0.0914]	10,044/4640 [R_{int} = 0.0438]
Data/restraints/parameters	6876/3/503	3641/2/261	1984/1/267	4640/1/269
Goodness-of-fit on F^2	1.073	1.026	1.062	0.973
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0718$, wR_2 = 0.1970	$R_1 = 0.0406$, wR_2 = 0.0967	$R_1 = 0.0288$, wR_2 = 0.0752	$R_1 = 0.0367$, wR_2 = 0.0788
R indices (all data)	$R_1 = 0.0768$, wR_2 = 0.2012	$R_1 = 0.0501$, wR_2 = 0.1003	$R_1 = 0.0305$, wR_2 = 0.0768	$R_1 = 0.0491$, wR_2 = 0.0837
Flack-parameter	−0.3(15)	0.0(10)	−0.1(9)	−0.1(7)
Largest difference peak/hole (e Å^{-3})	0.571/−0.442	0.359/−0.264	0.191/−0.212	0.227/−0.181

All compounds crystallized in the monoclinic space group $C2$ ($\alpha = \gamma = 90°$).

Table 2
Selected geometric parameters (Å, °, estimated standard deviations in parentheses)

	9	10	11	12
Endocyclic torsion angles				
C1–C2–C3–C4	−54.5(5); −56.0(4)	−56.3(2)	−57.1(2)	−57.6(2)
C2–C3–C4–C5	53.4(4); 57.7(4)	55.0(2)	54.9(2)	55.4(2)
C3–C4–C5–O1	−54.6(5); −57.0(4)	−54.1(2)	−53.3(2)	−52.8(2)
C4–C5–O1–C1	58.5(5); 56.9(5)	56.9(2)	56.8(2)	55.6(2)
C5–O1–C1–C2	−57.6(5); −55.1(5)	−58.5(2)	−58.9(2)	−58.0(2)
O1–C1–C2–C3	55.1(5); 53.7(5)	57.3(2)	58.3(2)	58.2(2)
Acylamino-group				
N21–C21	1.350(6); 1.329(6)	1.334(3)	1.342(3)	1.347(2)
C21–O21(2)	1.228(6); 1.224(6)	1.244(3)	1.233(3)	1.238(2)
C21–C22	1.498(7); 1.531(7)	1.503(4)	1.507(3)	1.511(2)
C22–C23		1.442(4)	1.530(3)	1.518(2)
C23–C24			1.522(4)	1.532(3)
C2–N21–C21–C22	179.5(4); 166.3(4)	−177.4(2)	175.1(2)	164.5(2)
C2–N21–C21–O21(2)	1.8(8); −11.8(7)	3.5(4)	−4.6(3)	−11.7(2)
N21–C21–C22–C23		−138.2(3)	−114.3(2)	148.7(2)
C21–C22–C23–C24			55.3(2)	
N21–C21–C22–C24				−88.4(2)
Planarity of the O*-acetyl-groups*				
C3–O31–C31–C32	−174.9(4); 174.3(4)	−177.2(2)	180.0(2)	−177.8(2)
C3–O31–C31–O32	8.2(8); −5.1(6)	0.3(3)	−0.3(3)	1.4(3)
C4–O41–C41–C42	−175.2(4); 177.7(4)	−179.2(2)	−179.4(2)	179.2(2)
C4–O41–C41–O42	5.8(7); −1.8(6)	−0.2(3)	−0.2(3)	−0.7(2)
C6–O61–C61–C62	175.6(5); −173.6(4)	178.7(3)	−179.0(3)	−178.1(2)
C6–O61–C61–O62	−3.6(8); 1.9(8)	−1.9(4)	−0.4(4)	0.7(3)
*Conformation of the 6-*O*-acetyl-group*				
O1–C5–C6–O61	158.3(4); −174.4(4)	70.4(2)	72.1(2)	73.7(2)
C4–C5–C6–O61	−77.5(5); −53.1(5)	−167.5(2)	−165.2(2)	−164.1(1)

Compound **9** contains two molecules in the asymmetric unit, whereas the second value is correlated with the (′) marked molecule (Fig. 2).

intermolecular hydrogen bonds are found in the crystals (Tables 5–7).

The reason for the nearly isomorphous crystal structures and the similar hydrogen bond pattern of compounds **10**–**12** may be a result of the intermolecular N–H···O hydrogen bond formation. The development of this pattern during the crystallization process seems to occur regardless of the steric demands of the acylamino-group.

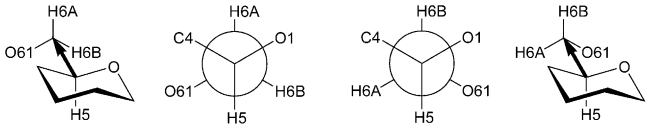

Fig. 6. Newman projection along the C5–C6 bond (arrow) in the *trans–gauche* (*tg*) and the *gauche–trans* (*gt*) conformation. The (*tg*) conformation is found in **9**, and the (*gt*) conformation in **10**–**12**.

The intermolecular hydrogen bond of normal strength described above is also found in **9**. However, there are two molecules in the asymmetric unit, and the values found for this bonding are slightly different (Table 4). Nevertheless, they also show a nearly linear arrangement as indicated by their bond angles of 164(5) and 169(5)°, respectively. These intermolecular hydrogen bonds can also be described like a chain and are shown in Figs. 9 and 10. Eight additional weak intermolecular hydrogen bonds are found in the crystal (Table 4).

The structural data of the ring system and the acylamino-groups constitute an improved basis for molecular modeling approaches towards neoglycoconjugates containing these structures.

Table 3
Selected geometric parameters (Å, °) around the anomeric center

	9	10	11	12
C1–O1	1.387(6); 1.421(5)	1.404(3)	1.402(2)	1.399(2)
O1–C5	1.440(6); 1.434(5)	1.434(3)	1.442(2)	1.448(2)
C1–O11	1.432(6); 1.427(5)	1.443(3)	1.444(2)	1.440(2)
C1–O1–C5	113.8(3); 114.7(3)	114.0(2)	114.0(2)	114.6(1)
O1–C1–C2	111.2(4); 111.8(3)	111.3(2)	110.9(2)	110.9(1)
C5–O1–C1–O11	59.8(5): 63.1(4)	59.9(2)	59.6(2)	61.9(2)
O1–C1–O11–C11	89.1(5); 92.5(4)	76.6(2)	75.5(2)	75.8(2)
C1–O11–C11–C12	179.0(5); 171.3(4)	– 175.0(2)	– 174.3(2)	– 176.0(2)
C1–O11–C11–O12	1.1(1); – 10.4(7)	4.3(3)	5.2(3)	3.7(3)

Table 4
Hydrogen-bonding geometry (Å, °) for **9**

D–H···*A*	d(*D*–H)	d(H···*A*)	d(*D*···*A*)	< (DHA)
N21–H21···O22′	0.87(3)	2.19(3)	3.047(5)	169(5)
N21′–H21′···O22 #1	0.87(2)	1.98(2)	2.827(5)	164(5)
C1′–H1′···O32	1.00	2.57	3.517(7)	159
C4–H4···O62′ #2	1.00	2.52	3.081(7)	115
C5–H5···O62′ #2	1.00	2.56	3.234(7)	124
C32–H32A···O62 #3	0.98	2.49	3.436(8)	163
C32′–H32E···O42 #4	0.98	2.43	3.326(7)	152
C42′–H42E···O61 #5	0.98	2.54	3.484(7)	162
C62–H62A···O12′ #6	0.98	2.52	3.291(8)	136
C62′–H62F···O32 #5	0.98	2.50	3.422(8)	157

Symmetry codes: #1, x, $y-1$, z; #2, x, y, $1+z$; #3, $1/2-x$, $y-1/2$, $2-z$; #4 $-x$, y, $-z$; #5, x, y, $z-1$; #6, $1/2-x$, $1/2+y$, $2-z$.

3. Experimental

3.1. General methods

Melting points (mp) were determined on a Büchi SMP 20 capillary melting-point apparatus and are uncorrected. Optical rotations were measured at 25 °C on a

Table 5
Hydrogen-bonding geometry (Å, °) for **10**

D–H···*A*	d(*D*–H)	d(H···*A*)	d(*D*···*A*)	< (DHA)
N21–H21···O21 #1	0.83(2)	2.03(2)	2.829(3)	165(3)
C5–H5···O62 #2	1.00	2.41	3.334(4)	153
C6–H6A···O42 #3	0.99	2.35	3.291(3)	158
C32–H32A···O1 #4	0.98	2.43	3.280(4)	144
C62–H62A···O32 #5	0.98	2.53	3.403(3)	148
C62–H62C···O32 #6	0.98	2.56	3.461(4)	153

Symmetry codes: #1, $3/2-x$, $y-1/2$, $1-z$; #2, $3/2-x$, $y-1/2$, $2-z$; #3, $2-x$, y, $2-z$; #4, $1/2+x$, $y-1/2$, z; #5, $3/2-x$, $1/2+y$, $2-z$; #6, $x-1/2$, $1/2+y$, z.

Table 6
Hydrogen-bonding geometry (Å, °) for **11**

D–H···*A*	d(*D*–H)	d(H···*A*)	d(*D*···*A*)	< (DHA)
N21–H21···O21 #1	0.88	2.08	2.831(2)	143
C5–H5···O62 #2	1.00	2.43	3.370(3)	156
C6–H6A···O42 #3	0.99	2.35	3.293(3)	159
C32–H32A···O1 #4	0.98	2.59	3.349(3)	134
C62–H62A···O32 #5	0.98	2.48	3.334(4)	145
C62–H62C···O32 #6	0.98	2.57	3.526(3)	166

Symmetry codes: #1, $1/2-x$, $y-1/2$, $2-z$; #2, $1/2-x$, $y-1/2$, $1-z$; #3, $-x$, y, $1-z$; #4, $x-1/2$, $y-1/2$, z; #5, $1/2-x$, $1/2+y$, $1-z$; #6, $1/2+x$, $1/2+y$, z.

Table 7
Hydrogen-bonding geometry (Å, °) for **12**

D–H···*A*	d(*D*–H)	d(H···*A*)	d(*D*···*A*)	< (DHA)
N21–H21···O21 #1	0.86(2)	2.05(2)	2.912(2)	174(2)
C5–H5···O62 #2	1.00	2.42	3.371(3)	158
C6–H6A···O42 #3	0.99	2.31	3.276(2)	164
C32–H32B···O1 #4	0.98	2.49	3.370(2)	149
C62–H62A···O32 #5	0.98	2.55	3.382(3)	143
C62–H62C···O32 #6	0.98	2.40	3.351(3)	165

Symmetry codes: #1, $1/2-x$, $y-1/2$, $1-z$; #2, $1/2-x$, $1/2-y$, $-z$; #3, $-x$, y, $-z$; #4, $x-1/2$, $y-1/2$, z; #5, $1/2-x$, $1/2+y$, $-z$; #6, $1/2+x$, $1/2+y$, z.

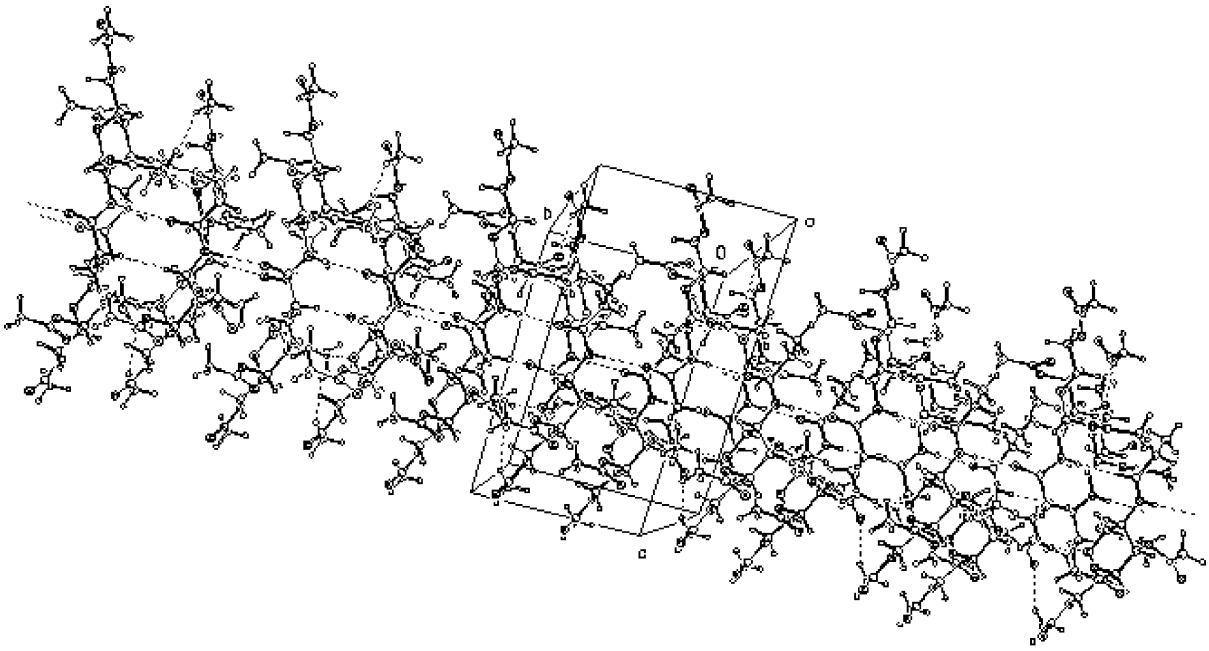

Fig. 7. The chain like arrangement of the normal intermolecular hydrogen bonds (dashed lines) in the crystal, here shown for compound **10**.

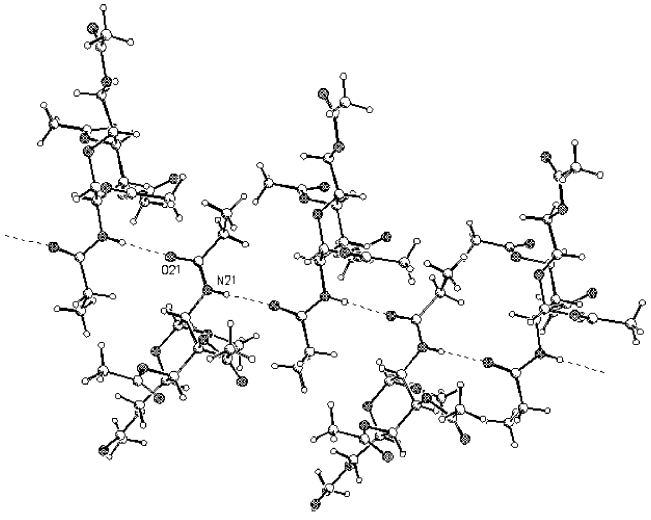

Fig. 8. Enlarged excerpt of the graphic given above for **10**.

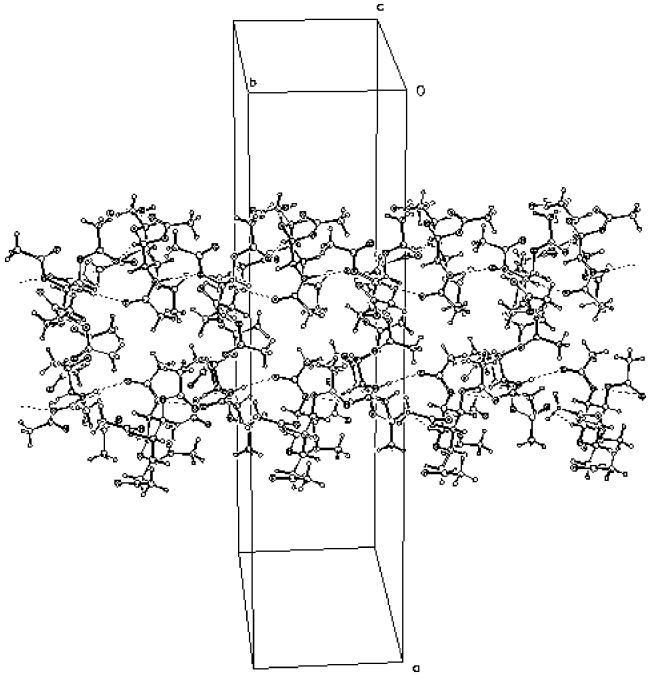

Fig. 9. The chain like arrangement of the normal intermolecular hydrogen bonds (dashed lines) in the crystal in the case of compound **9**.

Perkin–Elmer P341 polarimeter and are given in units of 10^{-1} deg cm^2 g^{-1}. Infrared spectra were recorded on a Perkin–Elmer 1600 Series FT-IR instrument. ^{1}H NMR spectra were recorded on a Bruker DPX 400 (400 MHz) spectrometer. The chemical shifts δ (ppm) are recorded relative to Me_4Si and the multiplicity of a signal is designated by one of the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; sep, septet; and m, multiplet. All coupling constants values J are in Hertz. ^{13}C NMR spectra were recorded on a Bruker DPX 400 (100 MHz) spectrometer. The spectra were recorded with a broadband decoupled mode, and the multiplicities obtained using a DEPT sequence. The carbon atoms of the pyranose ring were assigned by additional HMQC experiments using a Bruker DPX 400 spectrometer. The high-resolution mass spectra (HRMS) were obtained on an AEI MS 50 spectrometer. The spectrometer was operated at a source temperature

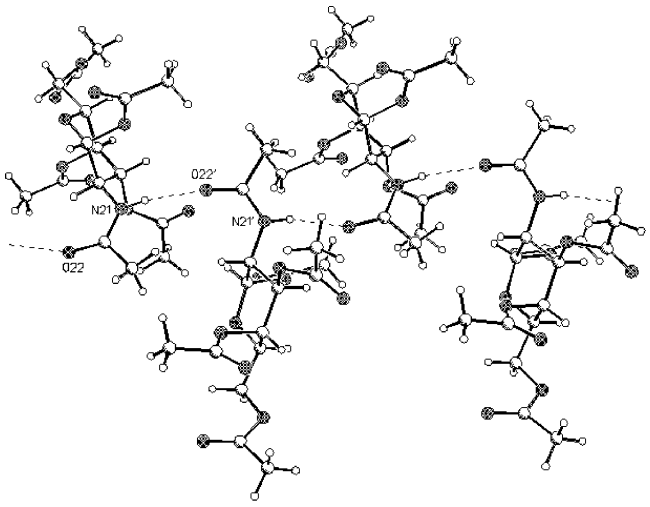

Fig. 10. Enlarged excerpt of the graphic given above for **9**.

of 180 °C, and an ionizing potential of 70 eV. Perfluorokerosene was used as an internal calibration-standard.

Flash chromatography was carried out using Merck silica gel 60 (230–400 mesh), $CHCl_3$ from Fisher, and MeOH from Acros. Thin-layer chromatography (TLC) was carried out on commercially available pre-coated aluminum plates (Merck silica gel 60 silica). Spots were visualized with Kägi–Miescher solution.[28]

3.2. X-ray measurements, determination and refinement

Crystallographic measurements were carried out on a Bruker Nonius Kappa-CCD diffractometer using graphite monochromatized Mo K_α radiation ($\lambda = 0.71073$ Å) at 123 K. The structures were solved by direct methods using SHELXS[29] and refined on F^2, by full matrix least-squares procedures using SHELXL,[30] with no absorption correction. The Friedel pairs of **11** were merged prior to the final refinement. The absolute configuration[31] of the title compounds could not be determined by the X-ray data but is fixed by the absolute stereochemistry of the starting material *N*-acetyl-D-galactosamine and D-galactosamine-hydrochloride, respectively. The position of the amide H atom of **9**, **10** and **12** were determined from a difference Fourier map and the coordinates were refined freely, with isotropic displacement parameter constrained to $U_{iso}(H) = 1.2U_{eq}(N)$ or free (**10**). All remaining H atoms were treated as riding, with C–H = 0.98–1.00 and N–H = 0.88 Å (**11**). $U_{iso}(H) = 1.2U_{eq}(CH, CH_2)$, $1.5U_{eq}(CH_3)$ and $1.2U_{eq}(NH)$ (**11**). The hydrogen-bonding geometry and the symmetry codes were determined using PLATON.[32]

3.3. General procedure for N-acylation (6–8)

Glassware was flame-dried under an Ar atmosphere and allowed to cool. D-Galactosamine hydrochloride (**1**), (1.0 equiv) in a methanolic $NaOCH_3$ solution (0.5 M, 0.99 equiv) was stirred for 10 min at room temperature (rt). The insoluble material was filtered off and the filter was washed with dry MeOH (molecular sieve). To the filtrate was added the appropriate acid anhydride **3** (propionic anhydride), **4** (butyric anhydride) or **5** (isobutyric anhydride), respectively (1.2 equiv), and the mixture was stirred for 12 h under an Ar atmosphere. The solution was evaporated under reduced pressure at 40 °C to leave a solid, which was stirred in dry EtOAc (molecular sieve) for 1 h at rt. The solid was filtered off, washed with dry EtOAc (molecular sieve) and dried in vacuo. The obtained solid **6**, **7**, respectively **8** was used without further purification.

3.4. General procedure for O-acetylation (9–12)

Glassware was flame-dried under an Ar atmosphere and allowed to cool. To the flask are added **2**, **6**, **7**, respectively **8** (1.0 equiv), anhyd Py (67.0 equiv), Ac_2O (33.5 equiv) and a catalytic amount of 4-dimethylaminopyridine (DMAP). The mixture was stirred for 24 h at rt, diluted with $CHCl_3$ and extracted 2 times with aq 1.0 M HCl. The organic phase was separated and the combined aqueous phases extracted 2 times with $CHCl_3$. The combined organic phases were washed 2 times with cold, satd aq $NaHCO_3$, dried (Na_2SO_4), concentrated, and dried in vacuo. The crude product was purified by flash chromatography on silica, eluted with $CHCl_3$–MeOH (30:1 for **9**, 40:1 for **10**–**12**) to give an anomeric mixture of the title compound as a solid.

Recrystallization from EtOAc afforded colourless crystals of **9**–**12** suitable for X-ray analysis.

3.5. 1,3,4,6-Tetra-*O*-acetyl-2-(acetylamino)-2-deoxy-α-D-galactopyranoside (9)

Mp 183–184 °C (EtOAc), Lit. 33 182–183 °C ($CHCl_3$–Et_2O), Lit. 34 178 °C (EtOH–petroleum ether), 169–171 °C (EtOH); $[\alpha]_D$ +96° (*c* 0.25, $CHCl_3$), Lit. 33 +102° (*c* 3.2, $CHCl_3$), Lit. 34 +102° (*c* 1.6, $CHCl_3$), Lit. 35 +97° (*c* 1.0, $CHCl_3$); IR (KBr): 3300, 2925, 1749 (*v* C=O, ester), 1658 (amide I), 1563 (amide II), 1436, 1375, 1256, 1221, 1127, 1043, 1011, 940 and 682 cm^{-1}; ^1H NMR (400 MHz, d_5-Py): δ 9.14 (d, 1 H, $J_{NH,2}$ 8.5, NH), 6.86 (d, 1 H, $J_{1,2}$ 3.5, H-1), 5.89 (dd, 1 H, $J_{4,3}$ 3.3, $J_{4,5}$ 1.1, H-4), 5.74 (dd, 1 H, $J_{3,2}$ 11.9, H-3), 5.33 (ddd, 1 H, H-2), 4.64 (ddd, 1 H, $J_{6',5}$ 6.6, $J_{6,5}$ 6.5, H-5), 4.40 (dd, 1 H, $J_{6',6}$ 11.2, H-6′), 4.31 (dd, 1 H, H-6), 2.06 (s, 3 H, CH_3), 2.03 (s, 3 H, CH_3), 2.02 (s, 3 H, CH_3), 1.89 (s, 3 H, CH_3), 1.86 (s, 3 H, CH_3), Lit. 35 coupling constants are in a good agreement (400 MHz, $CDCl_3$); ^{13}C NMR (100

MHz, d_5-Py): δ 170.8 (CO), 170.6 (CO), 170.6 (CO), 170.2 (CO), 169.4 (CO), 92.0 (C-1), 69.2 (C-5), 68.5 (C-3), 67.7 (C-4), 61.9 (C-6), 47.5 (C-2), 22.7 (CH_3), 20.6 (CH_3), 20.5 (CH_3), 20.4 (CH_3), 20.3 (CH_3); EI-HRMS *m/z* Calcd for $[C_{16}H_{23}NO_{10}]H^+$: 390.1400. Found: 390.1376; Anal. Calcd for $C_{16}H_{23}NO_{10}$: C, 49.36; H, 5.95; N, 3.60. Found: C, 48.91; H, 5.97; N, 3.56.

3.6. 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(propanoylamino)-α-D-galactopyranoside (10)

Mp 202–203 °C (EtOAc); $[\alpha]_D$ +89° (*c* 0.25, $CHCl_3$); IR (KBr): 3310, 2987, 1750 (*v* C=O, ester), 1650 (amide I), 1549 (amide II), 1434, 1374, 1242, 1217, 1133, 1047, 824, 761 and 711 cm^{-1}; ^1H NMR (400 MHz, d_5-Py): δ 9.02 (d, 1 H, $J_{NH,2}$ 8.5, NH), 6.86 (d, 1 H, $J_{1,2}$ 3.7, H-1), 5.90 (dd, 1 H, $J_{4,3}$ 3.2, $J_{4,5}$ 0.9, H-4), 5.75 (dd, 1 H, $J_{3,2}$ 11.8, H-3), 5.34 (ddd, 1 H, H-2), 4.64 (ddd, 1 H, $J_{6',5}$ 6.6, $J_{6,5}$ 6.6, H-5), 4.40 (dd, 1 H, $J_{6',6}$ 11.2, H-6′), 4.31 (dd, 1 H, H-6), 2.35 (q, 2 H, J_{HH} 7.5, CH_2CH_3), 2.03 (s, 3 H, CH_3), 2.02 (s, 3 H, CH_3), 1.89 (s, 3 H, CH_3), 1.87 (s, 3 H, CH_3), 1.16 (t, 3 H, CH_2CH_3); ^{13}C NMR (100 MHz, d_5-Py): δ 174.8 (CO), 170.6 (CO), 170.6 (CO), 170.2 (CO), 169.4 (CO), 92.0 (C-1), 69.2 (C-5), 68.5 (C-3), 67.7 (C-4), 61.9 (C-6), 47.4 (C-2), 29.3 (CH_2, CH_2CH_3), 20.6 (CH_3), 20.5 (CH_3), 20.4 (CH_3), 20.3 (CH_3), 10.3 (CH_3, CH_2CH_3); EI-HRMS *m/z* Calcd for $[C_{17}H_{25}NO_{10}]^+$: 403.1478. Found: 403.1476; Anal. Calcd for $C_{17}H_{23}NO_{10}$: C, 50.62; H, 6.25; N, 3.47. Found: C, 50.02; H, 6.21; N, 3.16.

3.7. 1,3,4,6-Tetra-*O*-acetyl-2-(butanoylamino)-2-deoxy-α-D-galactopyranoside (11)

Mp 185–186 °C (EtOAc); $[\alpha]_D$ +89° (*c* 0.25, $CHCl_3$); IR (KBr): 3309, 2974, 1749 (*v* C=O, ester), 1645 (amide I), 1548 (amide II), 1435, 1374, 1244, 1216, 1133, 1085, 1037, 1009, 927, 824, 760 and 711 cm^{-1}; ^1H NMR (400 MHz, d_5-Py): δ 9.02 (d, 1 H, $J_{NH,2}$ 8.6, NH), 6.86 (d, 1 H, $J_{1,2}$ 3.5, H-1), 5.90 (dd, 1 H, $J_{4,3}$ 3.2, $J_{4,5}$ 1.1, H-4), 5.75 (dd, 1 H, $J_{3,2}$ 11.8, H-3), 5.35 (ddd, 1 H, H-2), 4.64 (ddd, 1 H, $J_{6',5}$ 6.7, $J_{6,5}$ 6.6, H-5), 4.40 (dd, 1 H, $J_{6',6}$ 11.2, H-6′), 4.31 (dd, 1 H, H-6), 2.33 (t, 2 H, J_{HH} 7.2, (CO)CH_2), 2.05 (s, 3 H, CH_3), 2.01 (s, 3 H, CH_3), 1.89 (s, 3 H, CH_3), 1.88 (s, 3 H, CH_3), 1.72 (m, 2 H, CH_2CH_3), 0.85 (t, 3 H, J_{HH} 7.4, CH_2CH_3); ^{13}C NMR (100 MHz, d_5-Py): δ 173.9 (CO), 170.6 (CO), 170.6 (CO), 170.2 (CO), 169.4 (CO), 92.0 (C-1), 69.2 (C-5), 68.5 (C-3), 67.7 (C-4), 61.9 (C-6), 47.3 (C-2), 38.1 (CH_2, (CO)CH_2), 20.6 (CH_3), 20.5 (CH_3), 20.4 (CH_3), 20.3 (CH_3), 19.6 (CH_2, CH_2CH_3), 13.7 (CH_3, CH_2CH_3); EI-HRMS *m/z* Calcd for $[C_{18}H_{27}NO_{10}]^+$: 417.1635. Found: 417.1649; Anal. Calcd for $C_{18}H_{27}NO_{10}$: C, 51.79; H, 6.52; N, 3.36. Found: C, 51.63; H, 6.47; N, 3.27.

3.8. 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(2-methylpropanoylamino)-α-D-galactopyranoside (12)

Mp 180–181 °C (EtOAc); $[\alpha]_D$ +88° (*c* 0.25, $CHCl_3$); IR (KBr): 3313, 2975, 1752 (*v* C=O, ester), 1653 (amide I), 1540 (amide II), 1434, 1374, 1243, 1215, 1133, 1092, 1046, 1010, 934, 821, 759 and 713 cm^{-1}; ^1H NMR (400 MHz, d_5-Py): δ 8.92 (d, 1 H, $J_{NH,2}$ 8.6, NH), 6.86 (d, 1 H, $J_{1,2}$ 3.7, H-1), 5.90 (dd, 1 H, $J_{4,3}$ 3.2, $J_{4,5}$ 1.1, H-4), 5.76 (dd, 1 H, $J_{3,2}$ 11.9, H-3), 5.33 (ddd, 1 H, H-2), 4.64 (ddd, 1 H, $J_{6',5}$ 6.6, $J_{6,5}$ 6.6, H-5), 4.39 (dd, 1 H, $J_{6',6}$ 11.2, H-6′), 4.31 (dd, 1 H, H-6), 2.67 (sep, 1 H, J_{HH} 6.8, $CH(CH_3)_2$), 2.03 (s, 3 H, CH_3), 2.00 (s, 3 H, CH_3), 1.89 (s, 3 H, CH_3), 1.88 (s, 3 H, CH_3), 1.19 (d, 3 H, $CH(CH_3)_2$), 1.18 (d, 3 H, $CH(CH_3)_2$); ^{13}C NMR (100 MHz, d_5-Py): δ 178.1 (CO), 170.6 (CO), 170.6 (CO), 170.2 (CO), 169.4 (CO), 91.9 (C-1), 69.3 (C-5), 68.6 (C-3), 67.7 (C-4), 61.9 (C-6), 47.3 (C-2), 35.1 (CH, $CH(CH_3)_2$), 20.6 (CH_3), 20.5 (CH_3), 20.4 (CH_3), 20.3 (CH_3), 20.0 (CH_3), 19.8 (CH_3); EI-HRMS *m/z* Calcd for $[C_{18}H_{27}NO_{10}]^+$: 417.1635. Found: 417.1649; Anal. Calcd for $C_{18}H_{27}NO_{10}$: C, 51.79; H, 6.52; N, 3.36. Found: C, 51.48; H, 6.42; N, 3.23.

4. Supplementary material

Supplementary material is available at the Cambridge Crystallographic Data Center (CCDC) for the title compounds **9** (CCDC 189829), **10** (CCDC 189830), **11** (CCDC 189831), and **12** (CCDC 208437). These crystallographic data may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (Fax: +44-1223-336-033; e-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk/conts/retrieving.html).

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