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Note

Improved synthesis and characterization of 1,3,4,6-tetra-*O*-acetyl-2-(*N*-acetylacetamido)-2-deoxy-β-D-glucopyranose

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Abstract

A method from the 1960s to synthesize the *N*,*N*-diacetyl derivative of peracetylated β-D-glucosamine was improved by assistance of molecular sieves. The melting point of the title compound was revised and the structure determined by means of X-ray diffraction.

Keywords: Glucosamine; Peracetylated *N*-acetylacetamido derivatives; Molecular sieves in acetylation; Crystal structure

1. Introduction

Glucosamine is a common constituent of natural glycoconjugates. Prior to synthetic glycosylation, the neighboring amino group and the leaving group must be adapted. For β linkages, the neighboring *N*-diacetyl group, introduced after placement of a methylthio group at C-1 as a leaving group has given encouraging results.[1] The present study introduces an intermediate for an alternative route toward this kind of donor.

The methods for acetylation of amines or amides are traditionally the same as for the hydroxyl groups, acetic anhydride being one of the most popular reagents. Nevertheless, the mutarotation of the sugar, the thermodynamic stability of the peracetylated α anomer,[2] and the anomeric effect[3] tend to complicate the stereocontrol for acetylated β-products. Hence, it is essential to acetylate the hydroxyl and amido groups in separate stages. For *N*,*N*-diacetylation, isopropenyl acetate (IPA), including a trace of *p*-toluenesulfonic acid (TsOH) monohydrate as a catalyst, was discovered in the 1960s to be a promising reagent, especially for synthesizing peracetylated 2-acetylacetamido derivatives of gluco-, manno-, and galactopyranose.[4–8] In the present study, this method was applied starting from 2-acetamido-,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucose (**1**) and compared to the acetic anhydride method starting from 2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-β-D-glucose (**2**).

Rauhut and Tseng have found that 3–5 Å molecular sieves are effective adsorbents of acidic byproducts in a series of amide preparations.[9] In the present N-acetylation study, the effect of 3 Å sieves, added to the isopropenyl acetate solvent, was tested. The *N*,*N*-diacetyl

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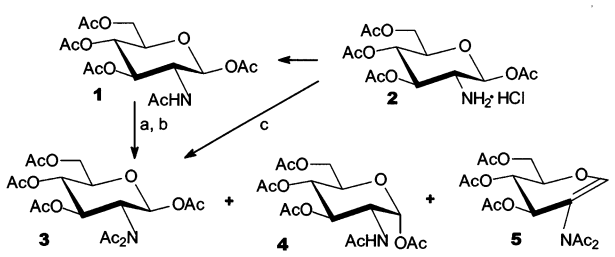

Scheme 1. Methods used for the acetylation of the acetamido- or amino group of peracetylated glucosamine. (a) Isopropenyl acetate, TsOH monohydrate, molecular sieves, 94 °C; (b) isopropenyl acetate, TsOH monohydrate, 94 °C; (c) Ac_2O, NaOAc, 140 °C.

Table 1
Influence of the molecular sieves on the yield of the title compound using isopropenyl acetate as an acetylating reagent and TsOH monohydrate as a catalyst

Entry	Molecular sieves (Å)	Column eluent	Yield (%) [a]	Mp (°C)
a	3	5:2 $CHCl_3$–Me_2CO	98	111–113 [b]
b		5:1 $CHCl_3$–Me_2CO	32	[c]
Ref. 4		1:1 C_6H_6–Et_2O	56	84–85 [d]

[a] From column chromatography, pure according to ^1H NMR.
[b] Recrystallized from diethyl ether.
[c] Not recrystallized.
[d] Recrystallized from diethyl ether–heptane.

derivative **3**, which was obtained in nearly quantitative yield by this method, was characterized by X-ray diffraction in addition to general spectroscopic methods and melting point measurement.

2. Results and discussion

Three procedures were used to synthesize the *N*-acetylacetamido derivative of peracetylated β-GlcNAc. The best result was obtained by starting from **1** (Scheme 1, entry a) following the method developed by Inch and Fletcher,[9] but with the addition of 3 Å molecular sieves. The reaction mixture referred to consists of the starting material and refluxing isopropenyl acetate as a solvolytic acetylating reagent, including a trace of the protonic catalyst, *p*-toluenesulfonic acid (TsOH) monohydrate. The addition of the sieves to the reaction mixture raised the yield of the title compound **3** from 13 to 32% (entry b) to 76–98% (Table 1).

The method of Bergmann and Zervas for *N*-monoacetylation of **2** with refluxing acetic anhydride and NaOAc during 2 min worked also for *N*,*N*-diacetylation prolonging the reaction time (Scheme 1, entry c).[10] Production of the monoacetylated compound **1** as one of the byproducts indicates that the ionic form of the amino group requires a refluxing period of more than 1–2 h in order to yield the purely diacetylated amino product. The reaction conditions and the results of the three procedures are summarized in Table 2.

When examining the influence of anomeric orientation on the melting point of peracetylated carbohydrates in the D-series that the melting point is usually observed to be higher for the β form.[6,11] In this respect, the melting points for the *N*-acetylacetamido compounds published by Inch and Fletcher: 111–112 °C for the α anomer and 84–85 °C for the β anomer,[4] seem contradictory. In the present study, a melting point of 111–113 °C was found for the β anomer, the structure of which was confirmed by X-ray crystallographic data.

1,3,4,6-Tetra-*O*-acetyl-2-(*N*-acetylacetamido)-2-deoxy-β-D-glucopyranose (**3**) crystallizes in the monoclinic space group $P2_1$. The asymmetric unit contains two independent molecules, in which the 6-membered sugar ring adopts the $^4C_1$ chair conformation common for D-aldohexopyranoses (Fig. 1). In both molecules, the corresponding bond

Table 2
Comparison of the methods used for synthesizing the title compound **3**

Entry	Starting compound	Reagents	Temperature (°C)/time (h)	Yield of **3** (%)	Other products
a	**1**	IPA, $TsOH·H_2O$, MS	94/12	98	**4**, **5** (traces)
b	**1**	IPA, $TsOH·H_2O$	94/12	32	**4**, **5**
c	**2**	Ac_2O, NaOAc	140/1	43	**1**, **4**, **5**

IPA, isopropenyl acetate; TsOH, *p*-toluenesulfonic acid; MS, molecular sieves.

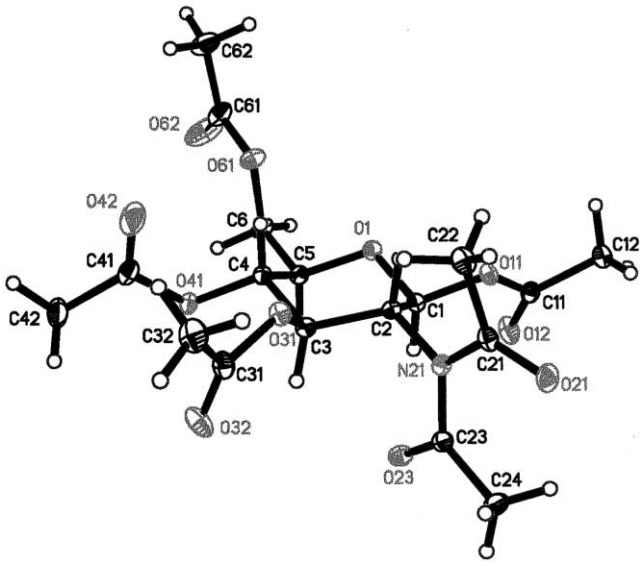

Fig. 1. Crystal structure of the compound **3**.

lengths are equal and quite comparable to those found in 1,3,4,6-tetra-*O*-acetyl-2-(*N*-acetylacetamido)-2-deoxy-β-D-galactopyranose.[12] The bonding of the additional *N*-acetyl group is evident in the ^{1}H NMR spectrum of **3** by the downfield shift of the H-1 doublet 6.55 ppm and by two singlets at 2.39 and 2.36 ppm. The large coupling constant of the H-1 doublet, 8.2 Hz, indicates retention of the β-anomeric orientation.

Observation of the elimination product **5** as a component of every product mixture supports the observation made by Fletcher's group, that 2-acetylamido-pyranoses with the trans arrangement at C-1 and C-2 have the tendency to undergo 1,2-elimination.[6,8] They found that prolonging the N-acetylation time of peracetylated β-GalNAc using isopropenyl acetate favored formation of the elimination product (lyxo derivative) at the cost of the β-anomeric *N*,*N*-diacetyl product, whose yield was best after 1 h of refluxing.[6] In the present study, ^{1}H NMR monitoring of the reaction of the peracetylated β-GlcNAc in isopropenyl acetate after 1 h of refluxing showed that half of the starting compound **1** was cleanly converted into the title compound **3**. This finding suggests that as little as a few hours of refluxing is sufficient for satisfactory results. Nevertheless, as evidenced by the yield of 98% after 12 h of refluxing, the prolonged reaction time did no harm in the presence of 3 Å molecular sieves.

The yield-enhancing effect of the sieves is presumably based on their ability to absorb the eliminating amino proton during the reaction. Absence of the sieves facilitates departure of the anomeric acetyl group, giving rise to the byproducts **4** and **5** (3,4,6-tri-*O*-acetyl-2-(*N*-acetylacetamido)-1,5-anhydro-2-deoxy-D-*arabino*-hex-1-enitol).

In conclusion, it can be stated that molecular sieves are useful IPA, preventing the formation of byproducts in N-acetylation of peracetylated GlcNAc. In the acetic anhydride method starting from the ionic amine hydrochloride, a prolonged reaction time is recommended. Keeping the temperature high is a requirement for stereocontrol of the β product in both of the methods.

3. Experimental

General methods.—All solvents were freshly distilled and the starting compounds were dried in vacuo prior to use. Silica gel 60 70–230 mesh was used for column chromatography. Melting points were measured on a GWB Gallenkamp melting-point apparatus and are uncorrected. TLC was performed on SiO_2 60 F_{254} and plates were sprayed with 10% H_2SO_4 in EtOH. Specific rotations were measured on a JASCO DIP-1000 digital polarimeter. Infrared spectra were obtained on a Nicolet Avatar 320 FT-IR spectrometer. NMR spectra were produced by a Bruker Avance 250 MHz spectrometer. Chemical shifts are reported in δ (ppm) with reference to internal $CHCl_3$ (^{1}H NMR: 7.27, ^{13}C NMR: 77.0 ppm) and coupling constants are in Hz. Mass spectra were obtained on a MALDI-TOF Proflex mass spectrometer (Bruker Daltonics, Billerica, MA, USA). Elemental analyses were obtained on an EA 1110 CHNS-O (CE Instruments). Crystal data were obtained by means of a Nonius Kappa-CCD diffractometer using Mo K_α radiation ($\lambda =$ 0.71073 Å). DENZO and SCALEPACK programs were used for cell refinement and data reduction.[13] The structure was solved by direct-methods with SHELXS97.[14] Structure refinement was carried out with SHELXL97.[15] All hydrogen atoms were placed in idealized positions.

*1,3,4,6-Tetra-*O*-acetyl-2-(*N*-acetylacetamido)-2-deoxy-β-*D*-glucopyranose* (**3**) *from 1-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-*D*-glucose* (**1**).—(a) A solution of **1**[11] (210 mg, 0.54 mmol) in isopropenyl acetate (8 mL, 71.91 mmol) containing TsOH monohydrate (0.8 mg, 0.004 mmol) and 3 Å molecular sieves (38 mg) was boiled under reflux for 12 h. The sieves were removed and the mixture was concentrated in vacuo and chromatographed (5:2 $CHCl_3$–Me_2CO) to remove the catalyst, giving foamy **3** (225 mg, 98%), which was pure according to ^{1}H NMR. Recrystallization (Et_2O) yielded colorless crystals; mp 111–113 °C (the structure confirmed by X-ray crystallographic data), lit. 84-85 °C for the β anomer, 111–112 °C for the α anomer;[4] $[\alpha]_D^{20}$ +8.90° (*c* 2.06, $CHCl_3$), lit. +9°;[4] R_f 0.62 (5:2 $CHCl_3$–Me_2CO); IR (KBr): *ν* 1756 (OAc), 1719 and 1691 cm^{-1} (NAc_2), lit. 1770, 1755 and 1720 cm^{-1} (Nujol);[4] ^{1}H NMR ($CDCl_3$): δ 6.55 (d, 1 H, $J_{1,2}$ 8.2, H-1), 5.91 (dd, 1 H, $J_{2,3}$ 9.5, $J_{3,4}$ 9.0, H-3), 5.14 (dd, 1 H, $J_{4,5}$ 9.5, H-4), 4.39 (dd, 1 H, $J_{5,6a}$ 4.4, $J_{6a,6b}$ 12.5, H-6a), 4.09 (dd, 1 H, $J_{5,6b}$ 1.8, H-6b), 3.97 (m, 1 H, H-5), 3.90 (dd, 1 H, H-2), 2.39, 2.36 (2s, 6 H, NAc_2), 2.11 (2s, 6 H, OAc_2), 2.05, 2.02 (s, 6 H, OAc_2), lit. similar peaks;[4,16] ^{13}C NMR ($CDCl_3$): δ 173.9, 173.1(2 × s N*C*OCH$_3$), 170.1, 169.3, 169.2, 167.8 (4 × s, O*C*OCH$_3$), 90.4 (d, *J* 176.5, C-1), 71.9 (d, *J* 144.2, C-3), 69.9 (d, *J* 156.4, C-4), 68.3 (d, *J* 151.6, C-5), 61.1 (t, *J* 149.1, C-6), 60.6 (d, *J* 138.2, C-2), 27.3, 24.6 (dq, *J* 129.9, NCO*C*H$_3$), 20.4, 20.4, 20.2, 20.1 (qq, *J* 130.3 OCO*C*H$_3$); MS: Calcd for $C_{18}H_{25}NO_{11}$ [M + Na] 454.13. Found *m/z* 454.14. Anal. Calcd C, 50.12; H, 5.84; N, 3.25. Found C, 50.13; H, 5.90; N, 3.22. ^{1}H NMR of the crude product showed traces of **4** and **5**.

(b) The synthesis was carried out from compound **1** (100 mg, 0.26 mmol) as in (a) but without the use of molecular sieves. The eluent for the chromatography was 5:1 $CHCl_3$–Me_2CO. The method gave **3** (35 mg, 32%) and compounds **4** and **5** as byproducts; for **5** R_f 0.55 (5:2 $CHCl_3$–Me_2CO); ^{1}H NMR ($CDCl_3$): δ 6.60 (s, 1 H, H-1), 5.57 (d, 1 H, $J_{3,4}$ 5.1, H-3), 5.26 (dd, 1 H, $J_{4,5}$ 6.3, H-4), 4.49 (m, 2 H, H-5, H-6a), 4.18 (d, 1 H, $J_{6a,6b}$ 9.0, H-6b), 2.36, 2.33 (2s, 6 H, NAc_2), 2.092 (s, 3 H, OAc), 2.089 (s, 3 H, OAc), 2.01 (s, 3 H, OAc); ^{13}C NMR ($CDCl_3$), characteristic peaks: δ 147.5 (d, *J* 189.9, C-1), 112.8 (s, C-2), 77.2 (d, *J* 210.8, C-3); MS: Calcd for $C_{16}H_{21}NO_9$ [M + H] 372.13. Found *m/z* 372.64.

*From 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-*D*-glucose hydrochloride* (**2**). (c) Compound **2**[10] (3.45 g, 8.99 mmol) was added to a refluxing suspension of Ac_2O (36 mL, 381 mmol) and NaOAc (1.12 g, 13.7 mmol) and boiled for 1 h. The mixture was concentrated and poured into ice–water to produce **3** (0.81 g, 23%) as a white precipitate. An additional fraction (0.70 g, 43% total yield) of the product was obtained by extracting the water filtrate with $CHCl_3$, water and brine, and chromatographed as in (a). Byproducts proved to be **5**, **4**, and **1** (in order of elution).

Crystallographic summary.—$C_{18}H_{25}NO_{11}$, $M_r = 431.39$, monoclinic, $P2_1$, $Z = 4$, $a = 8.2286(1)$, $b = 13.2314(2)$, $c = 19.4224(3)$ Å, $\beta = 94.159(1)°$, $V = 2109.06(5)$ A^3, $D_{calcd} = 1.359$ g/cm^3, Mo K_α radiation, $\lambda = 0.71073$ Å, $\mu = 0.11$ mm^{-1}, $F(000) = 912$, $T = 150$ K, conventional $R(F) = 0.0281$ for 8321 observed reflections [$F_o \geq 4\sigma(F_o)$], $wR(F^2) = 0.0661$ for all 8898 unique data, goodness-of-fit = 1.056, largest difference peak/hole = 0.201/−0.163 e/A^3.

4. Supplementary material

Full crystallographic details, excluding structure features, have been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 160409. Copies of this information can be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or www: http://www.rsc.org/suppdata/).

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