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Synthesis and characterization of *N*-acyl-tetra-*O*-acyl glucosamine derivatives†

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Novel 1,3,4,6-tetra-*O*-acyl-*N*-acyl-D-glucosamine derivatives were synthesized from glucosamine hydrochloride (GlcN·HCl) by the acylation with pyridine as a catalyst. A derivative of tetra-*O*-acetyl glucosamine contained ketoprofen, a non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic effects, was first synthesized. In analysis of the NMR spectra, the ratio of α:β-anomer showed that penta-acyl-D-glucosamine derivatives and *N*-acetylated glucosamines containing *O*-acyl groups have been only the α-anomer. Meanwhile, both the intermediates and the glucoconjugate compound of ketoprofen have only the β-anomer.

Introduction

Rheumatoid arthritis[1–4] is a common disease in the elderly. Glucosamine is a precursor for glucosaminoglycans, which are major components of joint cartilage. The glucosamine supplement may influence the cartilage structure. Therefore, the glucosamine may be proposed to treat the arthritis and alleviate symptoms.[5–8] In recent years, the drugs containing glucosamine derivatives[9–11] have been widely used to treat and restore the damaged cartilage. Advantages of the drug containing glucosamine strictly treated the arthritis without causing side effects. The research of Poustie[12] showed that the acyl derivatives of glucosamine on the amino group ($-NH_2$) possessed stronger activity than glucosamine itself in the treatment of arthritis. In particular, *N*-butyryl-D-glucosamine was also the most active component. Moreover, Some tetra-*O*-acyl-*N*-acyl-D-glucosamine[13,14] were important intermediates in the oligosaccharide synthesis. The acetate and the *n*-butyrate esters of glucosamine have demonstrated activity against cancer cells.[15,16]

NSAIDs[17–19] have also been used in the treatment of rheumatoid arthritis. In this purpose, Numerous NSAIDs ester derivatives have been synthesized to reduce gastro intestinal side-effect. It was also known that glucoconjugates could be transported by cellular glucose transporters. Therefore, the glucoconjugates of NSAIDs were recently prepared with the objective of increasing the bioavailability of antioxidant and anti-inflammatory drugs such as, ibuprofen,[20] aspirin, diclofenac and indomethacin.[21,22] These findings indicated that the glucoconjugates of NSAIDs might lead to reduce ulcerogenic potency, increase bioavailability and possess coordinative effects on osteoarthritis.

Some acyl derivatives of this amino sugar with fatty acids have been previously reported in the literature.[14,15,23] However, their NMR spectra data has not revealed whether or not influence of *N*- and *O*-substituted groups to the α:β-isomer ratio of these derivatives. Our initial research[24] revealed that the short fatty acid groups have been giving the isomer ratio by the rules. In this paper, we describe the synthesis and NMR spectral data analysis of a series penta-acyl-D-glucosamine, 1,3,4,6-tetra-*O*-acyl-*N*-acyl-D-glucosamine derivatives and a novel glucoconjugate of ketoprofen, which is prepared from GlcN·HCl. In synthesis of the glucoconjugate compound, a series of the compounds containing *O*-acetyl groups was also prepared.

Results and discussion

Synthesis

The glucosamine derivatives containing both *N*-acyl groups and *O*-acyl groups were prepared *via* two ways. First, 1,3,4,6-tetra-*O*-acyl-*N*-acylglucosamine, which was generated in two steps from commercially available GlcN·HCl, was synthesized by *N*-acetylation reaction of GlcN·HCl carried out with the various acid anhydrides in sodium methoxide solution to give the compounds **1–4**. Esterification of these compounds was then performed by treatment with acid anhydrides in the presence of pyridine as the method previously reported.[24] In the second approach, the penta-*O*-acyl glucosamine derivatives **13–15** were directly prepared from GlcN·HCl and acetic anhydride, propionic anhydride or butyric anhydride by using pyridine as a catalyst (Scheme 1).

The synthesis of glucoconjugate of NSAIDs has been described by different methods using various acetylated glucosamine analogues as glycosyl donors.[12,21] A synthesis of **18**

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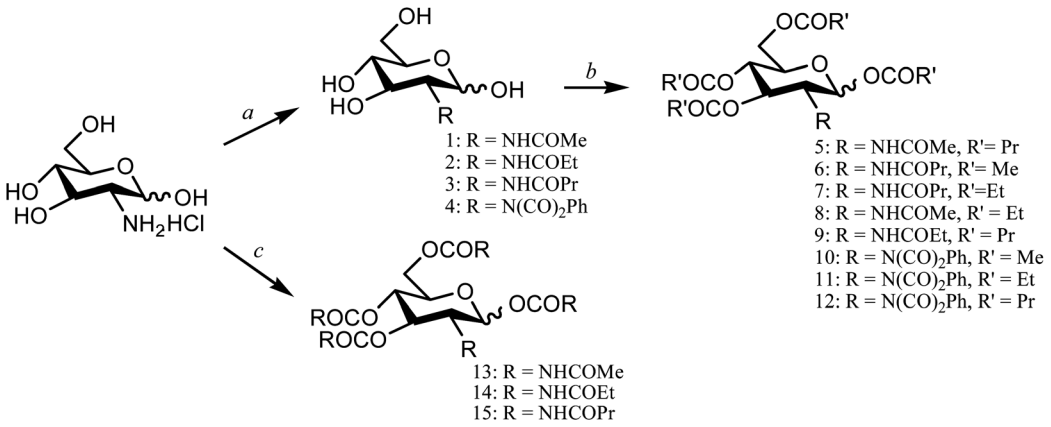
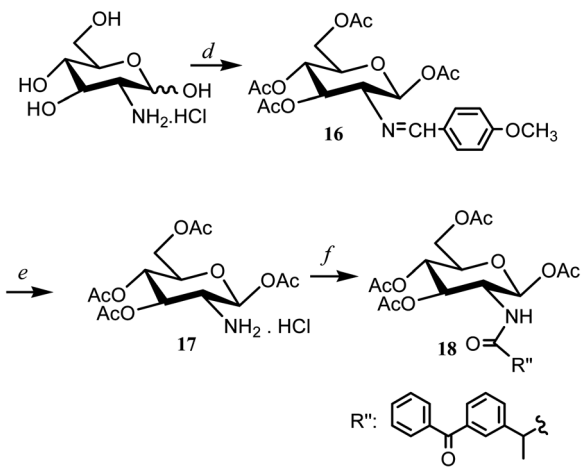


Reagents. (a) 1. Na/MeOH; 2. $(RCO)_2O$, Py; (b) $(R'CO)_2O$, Py; (c) $(RCO)_2O$, Py.

Scheme 1 Synthesis of tetra-*O*-acyl-*N*-acyl-D-glucosamine derivatives from GlcN·HCl.

from GlcN·HCl using esterification of NH_2 of glucosamine by ketoprofen chloride[12] and followed the acetylation of all four OH groups of sugar ring by acetic anhydride was attempted. However, in our experiment, ketoprofen chloride as a key intermediate obtained from ketoprofen and thionyl chloride were synthesized in a low yield. Alternatively, the use of 1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine hydrochloride (**17**) as a glycosyl donor[12,25] gave good yield as described in Scheme 2.

Compound **16** was obtained from commercially available GlcN·HCl in a procedure that used *p*-anisaldehyde for protection of $-NH_2$ group in 67.82% yield. Acetylation and removal of the *p*-methoxybenzylidene group with HCl in acetone were to give **17** in 91.3% yield. The ester **18** was obtained by esterifying ketoprofen with amine glycosyl compound **17** in the presence of DCC/DMF–Py–Et_3N in 51.5% yield after purification by silica gel column chromatography.



Reagents. (d) 1. *p*-anisaldehyde/NaOH 1M; 2. Ac_2O/Py; (e) HCl 5M/acetone; (f) 1. Na_2CO_3 1M; 2. Ketoprofen/DMF, DCC, Py, Et_3N

Scheme 2 Synthesis of D-glucosamine derivatives from GlcN·HCl and ketoprofen.

NMR spectra

The structure of the synthesized *N*-acyl, tetra-*O*-acyl-*N*-acyl-D-glucosamine and glucoconjugate derivatives was confirmed by ^1H and ^{13}C NMR spectroscopy (see Table 1 and 3, respectively). Analysis of the NMR spectra of **7** and **9–12** showed that structure and size of *N*-acyl group affected the chemical shifts of α and β-anomer. Simultaneously, the α:β-anomer ratios of the acylated glucosamines, that were determined by comparison of proton coupling constants of the H-1 peaks in the ^1H NMR spectra, depended on the length of the carbon chain of the acyl groups at C-1 and C-2 of glucosamine. However, the chemical shifts of the sugar ring protons or carbons of **16–18** were not affected by the substituted groups which had the source from *p*-anisaldehyde or ketoprofen.

Physical characteristics and ratio of α- and β-isomer of the glucosamine derivatives were showed in Table 2. The α-anomer was preferred for *N*-acetylated glucosamine with different *O*-acyl groups such as acetyl, *n*-propionyl, *n*-butyryl or even indomethacinyl-substituent.[22] With *N*-phthalated glucosamine, a mixture of the isomers was generated from which derivatives were substituted by shortly fatty groups on the O atom. In exception of the above cases, the β-anomer was preferred for the derivatives containing *O*-acetyl. The interesting results from Table 2 also showed that penta-acyl-D-glucosamine derivatives (**13–15**) gave more stable α-stereoisomer. In addition, *N*-acyl-D-glucosamine derivatives were obtained as a mixture of isomers in which the α-stereoisomer predominated.

Analysis of the NMR spectra of compounds **7** and **9** showed that the H-1 signal of α-anomer appeared at higher δ values than the H-1 signal of β-anomer, owing to their different equatorial and axial orientations ($\Delta\delta \sim 0.5$ ppm). Meanwhile, the C-1 carbon atom of the α-anomer resonated at higher field than of the β-anomer ($\Delta\delta \sim 2.1$ ppm). Especially, the H-1 chemical shifts of the α-anomer of *N*,*N*-phthaloyl glucosamine derivatives were lower than chemical shifts of the β-anomer. This showed that influence of two carbonyl groups (C=O) and benzene ring in *N*,*N*-phthaloyl glucosamine structure caused

Table 1 Chemical shifts (ppm) of the protons in the ^{1}H NMR spectra of tetra-*O*-acyl-*N*-acyl-D-glucosamine derivatives[a]

		Sugar moiety								Acyl groups	
							H-6				
		H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	N–H	*N*-acyl	*O*-acyl
5		6.19 (d, 3.5 Hz)	3.96 (m)	5.26–5.22 (m)		4.44 (ddd, 19.5, 10, 4 Hz)	4.07 (dd, 12.5, 2.5 Hz)	4.17 (dd, 12.5, 4.5 Hz)	5.56 (d, 9 Hz)	N–COMe: 1.91 (3H, s)	OCOPr: 2.20–2.42 (8H, m); 1.56–1.75 (8H, m); 0.80–1.00 (12H, m)
6		5.69 (d, 9 Hz)	4.31 (m)	5.1 (t, 9.5 Hz)	5.17 (t, 9.5 Hz)	3.82 (m)	4.12 (dd, 12.5, 2.5 Hz)	4.25 (dd, 12.5, 4.5 Hz)	5.84 (d, 9.5 Hz)	N–COPr: 2.01 (2H, m); 1.57 (2H, dd, 15.75 Hz); 0.88 (3H, t, 7.5 Hz);	O–COMe: 2.01–2.11 (12H, m)
7	α	6.21 (d, 3.5 Hz)	4.48 (ddd, 12.5, 9, 1.5 Hz)	5.21–5.5.29 (m)		4.01 (m)	4.07 (d, 11.5 Hz)	4.23 (dd, 12.5, 4.5 Hz)	5.72 (d, 8.5 Hz)	N–COPr: 2.05 (2H, m); 1.54 (2H, m). 0.88 (3H, t, 2.5 Hz)	O–COEt: 2.27–2.49 (8H, m); 1.07–1.22 (16H, m)
	β	5.73 (d, 9 Hz)	4.33 (q, 10 Hz)			3.85 (m)			6.13 (d, 9.5 Hz)		
8		6.19 (d, 3.5 Hz)	4.47 (ddd, 13, 9, 1.25 Hz)	5.24 (quint, 9 Hz)		3.98–4.01 (m)	4.07 (dd, 12.5, 2.5 Hz)	4.22 (dd, 12.5, 4.5 Hz)	5.53 (d, 8.5 Hz)	N–COMe: 1.92 (3H, s)	O–COEt: 2.25–2.48 (8H, m); 1.09–1.23 (12H, m)
9	α	6.18 (d, 3.5 Hz)	4.43 (ddd, 12.5, 9, 1.5 Hz)	5.22–5.18 (quint, 10 Hz)		3.95 (m)	4.06 (dd, 12.5, 2 Hz)	4.15 (dd, 12.5, 4.5 Hz)	5.52 (d, 9 Hz)	NHCOEt: 2.06 (2H, m); 1.04–1.08 (3H, m)	O–COPr: 2.15–2.25 (8H, m); 1.55–1.73 (8H, m); 0.89–0.99 (12H, m)
	β	5.67 (d, 8.5 Hz)	4.3 (m)	5.13 (quint, 10 Hz)		3.78 (m)	4.13 (dd, 12.5, 2.5 Hz)	4.19 (dd, 12.5, 4.5 Hz)	5.57 (d, 9 Hz)		
10	α	6.29 (d, 3.5 Hz)	4.70 (dd, 11.5, 3.5 Hz)	4.35 (dd, 12.5.4 Hz)	6.54 (dd, 11, 2.5 Hz)	5.15 (dd, 10.5, 1 Hz)	4.13 (dd, 12, 2 Hz)	4.35 (dd, 12.5, 4 Hz)	—	$N(CO)_2C_6H_4$: 7.83 (2H, m); 7.74 (2H, m)	O–COMe: 1.87–2.12 (9H, s)
	β	6.51 (d, 9 Hz)	4.45 (dd, 11.5, 9 Hz)	4.02 (m)	5.19 (dd, 10, 1 Hz)	5.87 (dd, 11.5, 1.5 Hz)	4.14 (dd, 12, 2.5 Hz)	4.3 (dd, 12.5; 4 Hz)	—		
11	α	6.31 (d, 3.5 Hz)	4.72 (dd, 11.5, 3.5 Hz)	4.31 (m)	6.54 (dd, 11.5; 2.5 Hz)	5.17 (t, 9.5 Hz)	4.12 (m)	4.31 (m)	—	$N(CO)_2C_6H_4$: 7.83 (2H, m); 7.75 (2H, m)	O–COEt: 2.04–2.41 (8H, m); 0.87–1.27 (12H, m)
	β	6.52 (d, 9 Hz)	4.46 (dd, 10.5, 1.5 Hz)	4.06 (m)	5.24 (t, 9.75 Hz)	5.89 (dd, 10.5, 1.5 Hz)			—		
12	α	6.30 (d, 3.5 Hz)	4.72 (dd, 11.5, 3.5 Hz)	4.29 (m)	6.54 (dd, 11.5, 2.5 Hz)	5.17 (t, 9.5 Hz)	4.08 (m)	4.29 (m)	—	$N(CO)_2C_6H_4$: 7.82 (2H, m); 7.71 (2H, m)	O–COPr: 2.07–2.35 (8H, m); 1.24–1.62 (8H, m); 0.65–0.98 (12H, m)
	β	6.52 (d, 9 Hz)	4.44 (dd, 12, 2 Hz)	3.8 (m)	5.22 (t, 9.5 Hz)	5.9 (dd, 10.5, 1.5 Hz)	4.08 (m)	4.29 (m)	—		
13		6.17 (d, 2.5 Hz)	4.48 (m)	5.14–5.27 (m)		4.0 (d, 9.5 Hz)	4.05 (d, 12.5 Hz)	4.23 (dd, 12, 3.5 Hz)	5.77 (m)	N–COMe: 2.19 (3H, s)	O–COMe: 1.93–2.11 (12H, s)
14		6.19 (d, 3.5 Hz)	4.47 (m)	5.24 (quint, 9.25 Hz)		3.99 (m)	4.07 (d, 7.5 Hz)	4.22 (dd, 12.5, 4.5 Hz)	5.55 (m)	N–COEt: 2.44 (2H, q, 7 Hz); 1.21 (3H, t, 7.5 Hz);	OCOEt: 2.09–2.4 (8H, m); 1.07–1.16 (12H, m)
15		6.21 (d, 3.5 Hz)	3.97 (m)	5.20–5.24 (m)		4.45 (ddd, 19, 10, 3.5 Hz)	4.07 (dd, 12.5, 2 Hz)	4.17 (dd, 2.5, 4.5 Hz)	5.54 (d, 9 Hz)	N–COPr: 2.04 (2H, m); 1.54 (2H, m); 0.87 (3H, m);	OCOPr: 2.2–2.41 (8H, m); 1.54–1.78 (8H, m); 1.02–0.87 (12H, m)
18		5.64 (d, 8.5 Hz)	3.74 (m)	5.07–5.18 (m)		4.26 (m)	4.07 (dd, 12.5, 2 Hz)	4.22 (dd, 12.5, 4 Hz)	6.08 (d, 9.5 Hz)	1.47 (3H, d, 7 Hz); 3.52 (1H, q, 7 Hz); 7, 4 (1H, dt, 7.5; 3 Hz); 7.48 (3H, m); 7.59 (2H, m); 7.69 (1H, s); 7.78 (2H, dd, 7; 3.5 Hz)	4 OAc: 1.82–2.06 (12H, s)

[a] NMR experimental conditions: 500 MHz, $CDCl_3$.

Table 2 Ratio of α- and β-isomer and physical characteristics of compounds **1–18**

N-Acyl groups (or *N*-substituent)	*O*-Acyl groups (or *O*-substituent)	Ratio of isomers		Substances	Mp (°C)	$[\alpha]_D^{20}$ (solvent)	R_f (EtOAc : PE[a])
		α	β				
CH_3CO	—	Priority	Lower	**1**	200–201	+65 (H_2O)	—
	CH_3CO	100%	0%	**13**	113–114	+86.5 (EtOH)	0.3 (2 : 1)
	CH_3CH_2CO	100%	0%	**8**	155–156	+95.5 (EtOH)	0.5 (1 : 1)
	$CH_3CH_2CH_2CO$	100%	0%	**5**	151–152	+80 (EtOH)	0.6 (3 : 2)
	Indomethacin[22]	100%	0%	—	—	—	—
CH_3CH_2CO	—	Priority	Lower	**2**	184–185	+36 (H_2O)	—
	CH_3CO[23]	0%	100%	—	—	—	—
	CH_3CH_2CO	100%	0%	**14**	105–106	+81 (EtOH)	0.4 (1 : 1)
	$CH_3CH_2CH_2CO$	83%	27%	**9**	108–110	+94 (EtOH)	0.5 (2 : 1)
$CH_3CH_2CH_2CO$	—	Priority	Lower	**3**	208–209	+33 (H_2O)	—
	CH_3CO	0%	100%	**6**	141–142	+33 (EtOH)	0.4 (2 : 1)
	CH_3CH_2CO	59%	41%	**7**	Liquid	+64 (EtOH)	0.4 (1 : 1)
	$CH_3CH_2CH_2CO$	100%	0%	**15**	94–95	+65 (EtOH)	0.5 (1 : 1)
$C_6H_4(CO)_2$	—	54%	46%	**4**	67–69	+25 (H_2O)	—
	CH_3CO	66%	34%	**10**	89–90	+66 (EtOH)	0.3 (1 : 1)
	CH_3CH_2CO	66%	34%	**11**	50–52	+117 (EtOH)	0.5 (1 : 1)
	$CH_3CH_2CH_2CO$	55%	45%	**12**	48–49	+110 (EtOH)	0.4 (2 : 1)
Anisaldehyde	CH_3CO	0%	100%	**16**	175–176	+98 ($CHCl_3$)	0.4 (1 : 1)
$NH_2 \cdot HCl$	CH_3CO	0%	100%	**17**	220–222	+27 (H_2O)	-
Ketoprofen	CH_3CO	0%	100%	**18**	134–135	+7 ($CHCl_3$).	0.5 (1 : 1)

[a] PE: petroleum ether; EtOAc: ethyl acetate.

deshielding of H-1 in the β-anomer ($\Delta\delta \sim 0.22$ ppm) in comparison with the corresponding H-1 signal of the α-anomer. In this case, the C-1 carbon atom of the α-anomer resonated at lower fields than C-1 carbon atom of the β-anomer ($\Delta\delta \sim 0.7$ ppm). Interestingly, the various *N*-acyl substituted groups had a significant influence on the resonance frequency of the sugar ring protons and carbons.

Analysis of the NMR spectra of **1–4** confirmed the empirical rules in the NMR spectroscopy of carbohydrates. Firstly, the axial orientation of the anomeric OH group in the α-anomer caused absorption at higher δ values of equatorially oriented H-1 protons in comparison with the corresponding β-anomer ($\Delta\delta \sim 0.5$ ppm). The C-1 carbon atom of the β-anomer resonated at lower fields than the C-1 carbon atom of the α-anomer ($\Delta\delta \sim 4$ ppm). These were compatible with the previous report.[26] Moreover, the C-2 carbon atom of the β-anomer was deshielded in comparison with the corresponding C-2 carbon atom of the α-anomer ($\Delta\delta \sim 2.5$ ppm).

Experimental

General methods

Melting points were measured with a Electrothermal Model 9200 (England). The ^1H and ^{13}C NMR spectra ($CDCl_3$, d_6-DMSO or D_2O, internal TMS) were measured with a Bruker Avance 500 NMR Spectrometer. Optical rotations were measured on a Polarimeter Model ADP 220 (Bellingham – Stanley Ltd, England) in a 1 cm^3 cell at 20 °C. Thin layer chromatography (TLC) was performed on the E. Merck Kieselgel 60 F-254 plates using the following eluent system (v/v) 1 : 1 petroleum ether – AcOEt and visualized by treatment with an acidic solution of 10% H_2SO_4 and EtOH. Flash chromatography was performed with Silica Gel 60 (230–400 mesh, E Merck, Darmstadt, Germany).

Procedure for the synthesis of 1,3,4,6-tetra-*O*-acyl-*N*-acyl-D-glucosamine derivatives and penta-acyl-D-glucosamine derivatives

General procedure for synthesis of *N*-acyl-D-glucosamine derivatives (1–4). Sodium (8.0 g, 34.8 mmol) was added slowly to 50 mL of anhydrous methanol followed by 5.0 g (23.2 mmol) of Glu·HCl under nitrogen atmosphere. The solution was stirred for 15 min and the precipitate was filtered off. To this filtrate, anhydride acid (34.8 mmol) was added under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 1 h and then cooled to 0 °C overnight. The *N*-acylated compounds were collected by filtration, washed with cold methanol (25 mL), diethyl ether (50 mL) and dried at 60 °C.

*N-Acetyl-D-glucosamine (**1**).* ^1H NMR (500 MHz, D_2O) δ 5.17 (1H, $J = 3.5$ Hz), 4.68 (1H, d, $J = 8.5$ Hz), 3.8 (2H, m), 3.74 (2H, dd, $J = 7.0, 2.5$ Hz), 3.69 (2H, ddd, $J = 15.5, 5.5, 1.5$ Hz), 3.62 (2H, dd, $J = 10.0, 8.5$ Hz), 3.42 (2H, m), 2.02 (6H, s). ^{13}C NMR (125 MHz, D_2O) δ 174.80, 174.54, 94.98, 90.89, 76.00, 73.59, 71.62, 70.74, 70.14, 69.91, 60.81, 60.66, 56.76, 54.13, 22.23, 21.96.

*N-Propyl-D-glucosamine (**2**).* ^1H NMR (500 MHz, D_2O) δ 5.17 (1H, $J = 3.5$ Hz), 4.66 (1H, d, $J = 12.0$ Hz), 3.90–3.71 (8H, m), 3.65 (2H, m), 3.5 (1H, m), 3.43 (1H, m), 2.32–2.26 (4H, q, 7.5 Hz), 1.12–1.08 (6H, t, 7.5 Hz). ^{13}C NMR (125 MHz, D_2O) δ 178.77, 178.55, 95.04, 90.95, 76.02, 73.93, 71.65, 70.72, 70.20, 69.98, 60.84, 60.69, 56.67, 54.03, 29.43, 29.12, 9.55, 9.50.

*N-Butyl-D-glucosamine (**3**).* ^1H NMR (500 MHz, D_2O) δ 5.16 (1H, d, $J = 3.5$ Hz), 4.67 (1H, d, $J = 10.5$ Hz), 3.80 (6H, m), 3.69 (2H, ddd, $J = 30.5, 11.5, 5.0$ Hz), 2.22 (4H, m), 1.55 (4H, m), 0.86

Table 3 Chemical shifts (ppm) of the carbon atoms in the ^{13}C NMR spectra of tetra-*O*-acyl-*N*-acyl-D-glucosamine derivatives[a]

		Sugar moiety						Acyl groups	
		C-1	C-2	C-3	C-4	C-5	C-6	*N*-acyl	*O*-acyl
5		90.44	51.30	70.40	67.20	69.96	61.43	169.76 (C=O) 22.99 (CH_3)	172.44; 173.23; 171.24; 171.66 (C=O); 36.02; 36.00; 35.86; 35.85 (–$COCH_2$–); 18.40; 18.36; 18.30; 18.24 (CH_2CH_2–); 13.60; 13.56; 13.50; 13.50 (–CH_3)
6		92.64	52.62	72.88	67.98	72.62	61.75	172.98 (C=O) 38.56 (–$COCH_2$) 19.00 (–CH_2CH_2) 13.49 (–CH_3)	171.17; 170.64; 169.48; 169.27 (C=O); 20.81; 20.67; 20.62; 20.53 (–CH_3)
7	α	90.41	50.94	70.43	67.30	69.74	61.39	172.03 (C=O) 38.19 ($COCH_2$) 18.79 (–CH_2CH_2) 13.36 (–CH_3)	175.05; 173.97; 172.83; 172.49 (C=O); 27.33; 27.22; 27.18 ($COCH_2$); 8.91; 8.82; 8.78 (CH_3)
	β	92.51	52.48	72.42	67.68	72.85	61.50	172.91 (C=O) 38.41 ($COCH_2$) 18.90 (–CH_2CH_2) 18.38 (–CH_3)	174.48; 172.87; 172.63 (C=O); 27.41; 27.31; 27.28 ($COCH_2$); 8.87; 8.75; 8.54 (CH_3)
8		90.60	51.21	70.62	67.34	69.88	61.45	169.80 (C=O) 22.99 ($COCH_3$)	172.09; 172.55; 174.06; 175.25 (C=O); 27.57; 27.49; 27.36; 27.31 (CH_2CO); 9.14; 9.05; 8.95; 8.91 (CH_3CH_2)
9	α	90.46	51.21	70.44	67.21	69.98	61.45	171.21 (C=O) 29.47 ($COCH_2$) 9.49 (CH_3)	174.44; 173.48; 173.22; 171.66 (C=O); 36.00; 35.99; 35.86; 35.85 ($COCH_2$); 18.40; 18.32; 18.29; 18.23 (CH_2); 13.58; 13.55; 13.49 (CH_3)
	β	92.66	52.87	73.14	67.44	72.35	61.46	171.22 (C=O) 29.69 ($COCH_2$) 9.63 (CH_3)	171.66; 173.23; 173.49; 173.77 (C=O); 35.85; 35.86; 35.99; 36.01 ($COCH_2$); 18, 24; 18.29; 18.33; 18.41 (CH_2); 13.42; 13.5; 13.55; 13.58 (CH_3)
10	α	90.52	52.84	70.19	67.04	69.42	61.53	167.39 (C=O) 134.44 (CH) 131.17 (C)	170.63; 169.99; 169.75; 169.49; 169.44; 169.28; 168.60 (C=O); 20.94; 20.73; 20.69; 20.61; 20.57; 20.36 (CH_3)
	β	89.80	53.53	70.53	68.36	72.66	61.56	123.71 (CH)	
11	α	90.43	52.90	70.29	66.84	69.21	61.39	167.31 (C=O) 134.37 (CH) 132.12 (C)	174.00; 173.37; 173.08; 172.83172.74; 172.03 (C=O); 27.48; 27.29; 27.27; 27.20 ($COCH_2$); 20.93; 15.18; 14.12; 8.91; 8.85; 8.78; 8.54; 8.45 (CH_3)
	β	89.72	53.60	68.16	65.75	72.83	60.29	123.58 (CH)	
12	α	90.33	52.97	70.30	66.82	69.10	60.38	167.33 (C=O) 134.39 (CH) 131.26 (C)	173.21; 172.50; 172.20; 171.95; 171.93; 171.82; 171.19; 171.08 (C=O); 35.95; 35.88; 35.85; 35.74; 35.66; 29.67 ($COCH_2$);
	β	89.67	53.71	70.20	68.13	72.80	61.43	123.59 (CH)	18.28; 18.21; 18.19; 18.11; 18.07; 17.96; 17.90 (CH_2); 14.17; 13.61; 13.59; 13.57; 13.52; 13.42; 13.21; 13.12 (CH_3)
13		90.61	51.00	70.61	67.56	69.66	61.53	171.55 (C=O) 20.44 (CH_3)	170.57; 169.99; 169.02; 168.57 (C=O); 22.87; 20.79; 20.58; 20.56 (CH_3)
14		90.60	51.09	70.61	67.32	69.88	61.47	172.08 (C=O) 29.49 ($COCH_2$) 9.57 (CH_3)	175.18; 174.05; 173.55; 172.55 (C=O); 27.52; 27.47; 27.34; 27.30 ($COCH_2$); 9.08; 9.04; 8.97; 8.90 (CH_3)
15		90.44	51.25	70.40	67.26	70.00	61.48	174.48 (C=O) 18.28 (–$COCH_2$) 35.89 (–CH_2CH_2) 13.55 (–CH_3)	173.27; 172.73; 171.71; 171.24 (C=O); 38.38; 36.06; 36.02; 35.91 ($COCH_2$); 18.89; 18.44; 18.33; 18.28 (–CH_2CH_2); 13.63; 13.60; 13.58; 13.56 (–CH_3)
18		92.31	52.92	67.86	72.43	72.84	61.66	18.12 (CH_3); 46.95 (CH); 128.36 (2C; CH); 128.59 (CH); 129.0 (CH); 129.18 (CH); 130.05 (2C, CH); 131.15 (CH); 132.63 (CH); 137.32 (C); 138.03 (C); 141.34 (C); 173.67 (C=O); 196.43 (C=O).	169.25; 169.42; 170.61; 170.94 (C=O); 20.44; 20.52; 20.65; 20.71 (CH_3)

[a] NMR experimental conditions: 125 MHz, $CDCl_3$.

(6H, m). ^{13}C NMR (125 MHz, D_2O) δ 177.82, 177.59, 95.04, 90.93, 75.98, 73.87, 71.61, 70.61, 70.22, 70.00, 60.82, 60.67, 56.62, 54.03, 38.03, 37.63, 19.01, 12.69.

*N,N-Phthaloyl-D-glucosamine (**4**).* ^{1}H NMR (500 MHz, D_2O) δ 7.67 (4H, m), 7.52 (4H, d, 2.5 Hz), 5.39 (1H, d, 3 Hz), 4.87 (1H, d, 8.5 Hz), 3.87–3.62 (8H, m), 3.43 (2H, m), 3.22 (2H, dd, J = 10, 9 Hz). ^{13}C NMR (125 MHz, D_2O) δ 174.18, 133.92, 130.83, 128.8, 92.79, 89.19, 76.23, 72.05, 71.66, 69.77, 69.66, 60.55, 60.42, 56.82, 54.38.

General procedure for synthesis of *N*-acyl-1,3,4,6-tetra-*O*-acyl-D-glucosamine derivatives (5–12). A round-bottomed flask equipped with a three-way stopcock was heated under reduced pressure and then cooled room temperature under a nitrogen atmosphere. *N*-acyl glucosamine (4.52 mmol), pyridine (10 mL) and anhydride acid (27.12 mmol) were replaced in the flask. The mixture was stirred at room temperature for 10 h. The cold water was then added and the resulting solution was extracted with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ aqueous solution, brine, dried over $MgSO_4$ and concentrated *in vacuo* to provide the desired products.

General procedure for synthesis of penta-acyl-D-glucosamine derivatives (13–15). Using the same procedure as described for compounds **5–12**, with replacement of *N*-acyl glucosamine by Glu·HCl.

Procedure for the synthesis of glucosamine conjugated with ketoprofen

Synthesis of *N*-((*p*-methoxyphenyl)methyliden)-1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-amino-β-D-glucopyranose (16). Into a round-bottomed flask were introduced 20.0 g of GlcN·HCl (92.76 mmol) and 100 mL of NaOH solution (1 M). After cooling down to 0 °C, 13.5 mL of *p*-anisaldehyde was added *via* dropwise under stirring. The resulting mixture was maintained at 0 °C for 1 h. The residue was collected by suction filtration, washed with the cold water, the cold ethanol, diethyl ether and dried *in vacuo* to give *N*-((*p*-methoxyphenyl)methyliden)-2-deoxy-2-amino-D-glucopyranose as a white solid (22.3 g, 80.87%).

A round-bottomed flask equipped with a three-way stopcock was heated under reduced pressure and then cooled room temperature under a nitrogen atmosphere. The above intermediate (22.3 g, 75.03 mmol) and pyridine (120 mL) were placed in the flask. After cooling down to 0 °C, acetic anhydride (12.5 mL) was added under stirring *via* a syringe. The mixture was stirred at 0 °C during 2 h and stirring was continued at room temperature overnight. The reaction was ended by adding 500 mL the water at 0 °C. The residue was collected by suction filtration, washed with the cold water and dried *in vacuo* to give **16** as a white solid (28.7 g, 83.87%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (2H, dd, J = 7.0, 2.0 Hz), 6.92 (2H, dd, J = 9.0, 2.0 Hz), 5.93 (1H, d, J = 8 Hz), 5.43 (1H, t, 10 Hz), 5.14 (1H, t, J = 10 Hz), 4.36 (1H, dd, J = 12.5, 4.5 Hz), 4.12 (1H, dd, J = 12.5, 2.5 Hz), 3.96 (1H, m), 3.84 (3H, s), 3.43 (1H, dd, J = 10.0, 8.0 Hz), 2.13–1.88 (12H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.68, 169.89, 169.54, 168.76, 164.27, 130.27, 128.35, 114.09, 93.20, 73.30, 72.98, 72.81, 68.11, 61.87, 55.42, 20.81, 20.76, 20.68, 20.51.

Synthesis of 1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine hydrochloride (17). A 28.0 g (60.0 mmol) sample of **16** was dissolved in acetone (200 mL). Then 12.5 mL of aq. HCl (5 M) was added. After 30 min, diethyl ether was added to the reaction mixture and stirring for 1 hour. The residue was collected by suction filtration, washed with the cold diethyl ether and dried *in vacuo* to give the product **17** (20.2 g, 91.3%). ^{1}H NMR (500 MHz, d_6-DMSO) δ 8.85 (3H, s), 5.91 (1H, d, J = 8.5 Hz), 5.37 (1H, t, J = 9.5 Hz), 4.92 (1H, t, J = 9.5 Hz), 4.17 (1H, dd, J = 12.0, 4.0 Hz), 3.99 (2H, m), 3.54 (1H, t, J = 9.5 Hz), 2.17–1.97 (12H, s). ^{13}C NMR (125 MHz, d_6-DMSO) δ 169.89, 169.70, 169.25, 168.56, 90.07, 71.58, 70.32, 67.82, 61.23, 52.14, 20.88, 20.79, 20.41, 20.27.

Synthesis of *N*-(2-(3-benzoylphenyl)propanoyl)-1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-amino- β-D-glucopyranose (18). A 6.0 g (16.2 mmol) sample of **17** was dissolved in CH_2Cl_2 (80 mL). Then 80 mL of Na_2CO_3 solution (1 M) was added. After 30 min, the mixture was extracted with CH_2Cl_2. The organic layer was washed with water, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give 1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine as a white solid (5.1 g, 92.8%). Mp (138 °C).

A round-bottomed flask equipped with a three-way stopcock was heated under reduced pressure and then cooled room temperature under a nitrogen atmosphere. Ketoprofen (1.0 g, 3.9 mmol) in dry DMF (15 mL) and DCC (1.22 g, 5.8 mmol) were placed in the flask and the mixture was stirred at 0 °C. Into the flask was added 1,3,4,6-tetra-*O*-acetylglucosamine (0.5 g, 1.5 mmol) in dry DMF *via* a syringe. The whole was stirred at 0 °C for 4 h and stirring was continued at room temperature overnight. The mixture was diluted by ethyl acetate and then the solid was filtered off. Ethyl acetate layer was washed with water, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The pure product **18** was recrystallized in diethyl ether as a white solid (0.44 g, 51.5%).

Conclusions

We synthesized successfully five novel tetra-*O*-acyl-*N*-acyl-D-glucosamine derivatives **7**, **9**, **11**, **12**, **18** with a good yield. The most important influence of α- and β-anomer ratio was related to acyl group at the amine ($-NH_2$) position and at *O*-acyl group of C-1 carbon position. Synthesis of substituent acyl groups containing longer carbon chain and the testing of biological activities of compounds **5–12** and **18** were planned.

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