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Authentic standards for the reductive-cleavage method. The positional isomers of partially methylated and acetylated or benzoylated methyl 2-(acetylmethylamino)-2-deoxy-β-D-glucopyranoside

Larry E. Elvebak II [a], Sarah O. Smith [b], Gary R. Gray [b,*]

[a] *Primedica, Worcester, MA 01608, USA*

[b] *Department of Chemistry, University of Minnesota, 207 Pleasant Street S.E., Minneapolis, MN 55455-0431, USA*

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Abstract

Described herein is the synthesis of eight positional isomers of methylated and acetylated or benzoylated methyl 2-(acetylmethylamino)-2-deoxy-β-D-glucopyranoside. The compounds were generated simultaneously from methyl 2-(acetylmethylamino)-2-deoxy-β-D-glucopyranoside by sequential partial methylation and benzoylation and isolated in pure form by high-performance liquid chromatography (HPLC). The desired acetates were obtained by debenzoylation and acetylation of the pure isomers. Reported herein are the ^{1}H NMR spectra of the benzoates and the electron-ionization mass spectra of the acetates and the tri-*O*-methyl derivative. Also reported for the acetates and the tri-*O*-methyl derivative are their linear temperature-programmed gas–liquid chromatography (GLC) retention indices on three different capillary columns.

Keywords: Reductive-cleavage; Methyl 2-(acetylmethylamino)-2-deoxy-β-D-glucopyranoside; Partially methylated and acylated

1. Introduction

In contrast to aldose residues, in which glycosidic linkages are reduced to give the corresponding anhydroalditols [1–4], fully methylated 2-acetamido-2-deoxy-D-glucopyranosyl (GlcNAc) residues do not undergo reduction under reductive-cleavage conditions but, instead, undergo transglycosidation when the reactions are quenched with an alcohol [5]. Mechanistic studies have shown that transglycosidation proceeds via an oxazolinium ion intermediate, which then acts as a glycosyl donor to yield an anomerically pure (β) product [5]. At room temperature, only β-linked 2-acetamido-2-deoxy-D-glucopyranosyl residues give the intermediate oxazolinium ion [5,6], whereas, at elevated temperature (70 °C) α-linked 2-acetamido-2-deoxy-D-glucopyranosyl residues also give the oxazolinium ion via anomerization [7]. Since quenching of reductive-cleavage reactions with methanol is particularly convenient [5], fully methylated GlcNAc residues are thus stereospecifically converted to their corresponding β-methyl glycosides. Described herein is the synthesis of authentic standards derivable from GlcNAc residues, namely the eight positional isomers of partially methylated and acetylated or ben-

* Corresponding author. Tel.: +1-612-6251068; fax: +1-612-6267541.

E-mail address: gray@chem.umn.edu (G.R. Gray).

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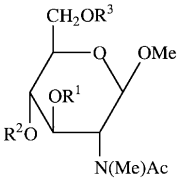
zoylated methyl 2-(acetylmethylamino)-2-deoxy-β-D-glucopyranosides (**1**–**8**). As an aid to those who wish to use the reductive-cleavage method, ^{1}H NMR spectra of the seven methylated and benzoylated positional isomers (**2b**–**8b**) are reported, as are the electron-ionization mass spectra of the corresponding acetates (**2a**–**8a**) and the tri-*O*-methyl derivative (**1**). Also reported for the acetates (**2a**–**8a**) and the tri-*O*-methyl derivative (**1**) are their linear temperature-programmed gas–liquid chromatography (GLC) retention indices (LTPGLCRI) [8] on three different capillary columns [9].



	R^1	R^2	R^3
1	Me	Me	Me
2a	Ac	Me	Me
2b	Bz	Me	Me
3a	Me	Ac	Me
3b	Me	Bz	Me
4a	Me	Me	Ac
4b	Me	Me	Bz
5a	Ac	Ac	Me
5b	Bz	Bz	Me
6a	Ac	Me	Ac
6b	Bz	Me	Bz
7a	Me	Ac	Ac
7b	Me	Bz	Bz
8a	Ac	Ac	Ac
8b	Bz	Bz	Bz

2. Results

Synthesis.—The tri-*O*-methyl- (**1**), tri-*O*-acetyl- (**8a**) and tri-*O*-benzoyl- (**8b**) derivatives of methyl 2-(acetylmethylamino)-2-deoxy-β-D-glucopyranoside were prepared from the latter [10] by total methylation [11], acetylation, and benzoylation, respectively. The remaining six partially methylated and benzoylated positional isomers **2b**–**7b** were prepared from the same starting material by partial methylation followed by benzoylation in situ [9]. The resultant mixture of benzoates was then separated by semipreparative reversed-phase high performance liquid chromatography (HPLC) using a Rainin C_{18} column (Table 1). Compounds **2b**–**4b**, which were poorly resolved under these conditions, were separated by normal-phase chromatography using a Regis silica column (Table 1). The individual components were isolated and, after removal of solvent, were identified by ^{1}H NMR spectroscopy. A portion of each benzoate was then debenzoylated (NaOMe in MeOH), and the product was acetylated, affording the partially methylated methyl 2-(acetylmethylamino)-2-deoxy-β-D-glucopyranoside acetates in chromatographically pure form.

Table 1
Reversed-phase and normal-phase HPLC capacity factors of compounds **2b**–**8b**

Compound (position of benzoyl)	Capacity factor (k') [a]	
	Reversed-phase [b]	Normal-phase [c]
2b (3-)	[d]	2.02
3b (4-)	[d]	4.48
4b (6-)	[d]	4.02
5b (3,4-)	3.5	
6b (3,6-)	4.2	
7b (4,6-)	4.0	
8b (3,4,6-)	4.6	

[a] Capacity factors (k') were calculated from the equation $k'(x) = (t_{r(x)} - t_m)/t_m$ where $k'(x)$ is the capacity factor of the compound of interest (x), $t_{r(x)}$ the absolute retention time of the compound of interest (x), and t_m is the dead time. Dead time was estimated from the equation $t_m = (0.5Ld_c^2)/F$ where 0.5 is a unitless constant, L is the length of the column in centimeters, d_c is the column diameter in centimeters, and F is the column flow rate in mL/min [12].

[b] Reversed-phase HPLC was conducted using a 5 μm particle-size Rainin Dynamax Microsorb semipreparative C_{18} column (1 × 25 cm) equilibrated in 40:60 MeCN–H_2O at 3.0 mL/min. After injection, the column was eluted for 10 min, then programmed with a linear gradient to 95:5 MeCN–H_2O over 20 min.

[c] Normal-phase HPLC was conducted using a 5 μm particle-size Regis Spherisorb S5W Hi-Chrom silica column (0.46 × 25 cm) equilibrated in 95:5 EtOAc–MeOH at 1.0 mL/min.

[d] Compounds coeluted.

^1H NMR spectra of partially methylated methyl 2-(acetylmethylamino)-2-deoxy-β-D-glucopyranoside benzoates (**2b**–**8b**).—Given in Table 2 are ^1H NMR spectral data for compounds **2b**–**8b**. The individual components of the mixture (see Table 2) were identified based upon an analysis of the chemical shifts and coupling constants of the ring hydrogen resonances. All resonances displayed the multiplicities and coupling constants expected for a GlcNAc derivative in the $^4C_1$ conformation and, in addition, the positions of substitution of benzoyl groups were readily discerned based upon the large downfield shift of the respective ring hydrogen resonances. It should be noted, however, that the spectra were complicated by the presence of rotomers about the amide bond [10], so multiple signals were observed for many resonances, especially, those of the *O*-methyl, *N*-methyl, and *N*-acetyl groups. It should also be noted that distinguishing between positional isomers bearing benzoates at the 3- and 4-positions was difficult based solely upon the magnitudes of coupling constants since both H-3 and H-4 have two *trans*-diaxial nearest neighbors. In order to confirm the structural assignments given in Table 2, the 3,6- and 4,6-di-*O*-benzoyl derivatives (**6b** and **7b**, respectively) were distinguished by ^1H–^1H COSY NMR spectroscopy and the 3- and 4-*O*-benzoyl derivatives (**2b** and **3b**, respectively) were distinguished by comparing the mass spectrum of the acetate **3a** derived from the latter with that of the major product derived by reductive-cleavage analysis of chitotriose, a β-(1→4)-linked trisaccharide of GlcNAc.

Mass spectra of the methylated methyl 2-(acetylmethylamino)-2-deoxy-β-D-glucopyranoside acetates (**1**, **2a**–**8a**).—Compounds **1** and **2a**–**8a** were analyzed by chemical-ionization (CI) mass spectrometry with ammonia as the reagent gas and by EI mass spectrometry. The CI (NH_3) mass spectra of all compounds displayed the expected $(M+H)^+$ and $(M+NH_4)^+$ ions, which, because of their unique molecular weights, readily identify them as derivatives of methyl 2-(acetylmethylamino)-2-deoxy-β-D-glucopyranoside. The EI mass spectra of the compounds are shown in Fig. 1. Inspection of the spectra reveals that they are diagnostically different. Furthermore, 3-*O*-methyl derivatives (**1**, **3a**, **4a** and **7a**) are easily identified based on the presence of fragment ions at *m*/*z* 87 and 129, whereas, 3-*O*-acetyl derivatives (**2a**, **5a**, **6a**, and **8a**) are easily identified by the presence of a fragment ion at *m*/*z* 115, in agreement with past work [13].

GLC retention indices of methylated methyl 2-(acetylmethylamino)-2-deoxy-β-D-glucopyranoside acetates (**1**, **2a**–**8a**).—Given in Table 3 are the linear temperature-programmed gas–liquid chromatography retention index [8] (LTPGLCRI) values for compounds **1** and **2a**–**8a** determined on three different capillary columns [9]: one (DB-5), a relatively nonpolar stationary phase (5% phenyl–95% methyl polysiloxane); one (DB-17), a more polar stationary phase (50% phenyl–50% methyl polysiloxane); and one (RT_x-200); a relatively polar stationary phase (50% trifluoropropyl–50% methyl polysiloxane). Analyses were performed in triplicate on each column using a mixture of all six compounds and a mixture of *n*-alkanes $C_{11}H_{24}$ to $C_{34}H_{70}$ as retention index standards [9]. With the exception of compounds **3a** and **4a**, which coeluted on the RT_x-200 column, the other positional isomers were readily separable on all three columns.

3. Discussion

This is one of a series of papers describing the synthesis and spectral characterization of authentic standards for the reductive-cleavage method. The goal of these studies is to provide such data for standards representing all possible combinations of position(s) of linkage and ring formation for the most frequently encountered sugars. The present report describes the synthesis of such standards for 2-acetamido-2-deoxy-D-glucopyranosyl residues, which are widely distributed in nature. The previously developed method for preparing such standards, namely, partial methylation of the anhydroalditol of the parent sugar [9], was not applicable in this case since partial methylation of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside would yield a mixture of isomers involving partial methylation of the acetamido group (at N) as well as the hydroxyl groups.

Table 2
^{1}H-NMR data (Δ in ppm, *J* in Hz in brackets) for partially methylated methyl 2-(acetylmethylamino)-2-deoxy-β-D-glucopyranoside benzoates **2b**–**8b** [a]

Compound	H-1	H-2	H-3 [b]	H-4 [b]	H-5	H-6	H-6′	*O*-Methyl	*N*-Methyl	Acetyl
2b	4.48 d (7.5)	3.38–3.82 complex	5.55 dd (9.0, 10.5)	3.38–3.82 complex	3.38–3.82 complex	3.38–3.82 complex	3.38–3.82 complex	3.40, 3.41 3.45, 3.46, 3.50, 3.51	2.86, 2.92	1.92 br 2.05
3b	4.47 d (8.0)	3.30–3.78 complex	3.30–3.78 complex	5.27 t (9.3)	3.30–3.78 complex	3.30–3.78 complex	3.30–3.78 complex	3.32, 3.33 3.36, 3.51 3.53	2.92 3.11 br	2.12, 2.17
4b	4.39 d (8.5)	3.30–3.74 complex	3.30–3.74 complex	3.30-3.74 complex	3.30–3.74 complex	4.67 dd, (2.0, 12.0) 4.62 dd (2.0, 12.0)	4.45–4.51 complex	3.45, 3.47 3.51, 3.53 3.54, 3.58	2.88, 2.92 3.08 br 3.12 br	2.12, 2.17
5b	4.63 d (8.0)	3.96 dd (8.0, 10.7)	5.79 dd (9.3, 10.7)	5.54 t (9.6)	3.82 ddd (2.6, 5.2, 10.0)	3.64 dd, (2.6, 10.8) 3.61 dd (2.7, 10.8)	3.53–3.56 complex	3.35, 3.36 3.55, 3.57	2.91, 2.97	1.98 br 2.10
6b	4.54–4.60 complex	3.82 dd (8.0, 10.8)	5.62 dd (8.8, 10.8)	3.59 br t, (9.2)	3.74 ddd (2.2, 5.3, 9.8)	4.70 dd (2.2, 11.9) 4.67 br d (12.0)	4.54–4.60 complex	3.40, 3.43 3.50, 3.51	2.88, 2.95	1.94 br 2.09
7b	4.52 d (8.1)	3.75 dd (8.1, 10.3)	3.68 dd (8.8, 10.3)	5.42 dd (8.8, 10.0) 5.32 complex	3.89 ddd (3.2, 5.8, 10.0) 3.97 m	4.58 dd (3.2, 12.1) 4.55 dd (3.0, 12.1)	4.41 dd (5.8, 12.1) 4.37 dd (5.5, 12.0)	3.38, 3.49 3.51	2.93 3.13 br	2.13, 2.19
8b	4.69 d (8.0)	4.00 dd (8.0, 10.7)	5.84 t (9.9)	5.63 t (9.8)	4.07 ddd (3.3, 5.5, 10.0)	4.57–4.65 complex	4.47 dd (5.0, 12.0) 4.51 dd (5.5, 12.0)	3.54 3.56	2.92, 2.99	2.12 2.00 br

[a] Additional resonances were observed for benzoyl hydrogens at Δ7.36–8.13; the spectra were acquired at 25 °C.
[b] The resonances assigned as a triplet (t) were actually doublets of doublets (dd) with a pair of coupling constants having nearly equal magnitude.

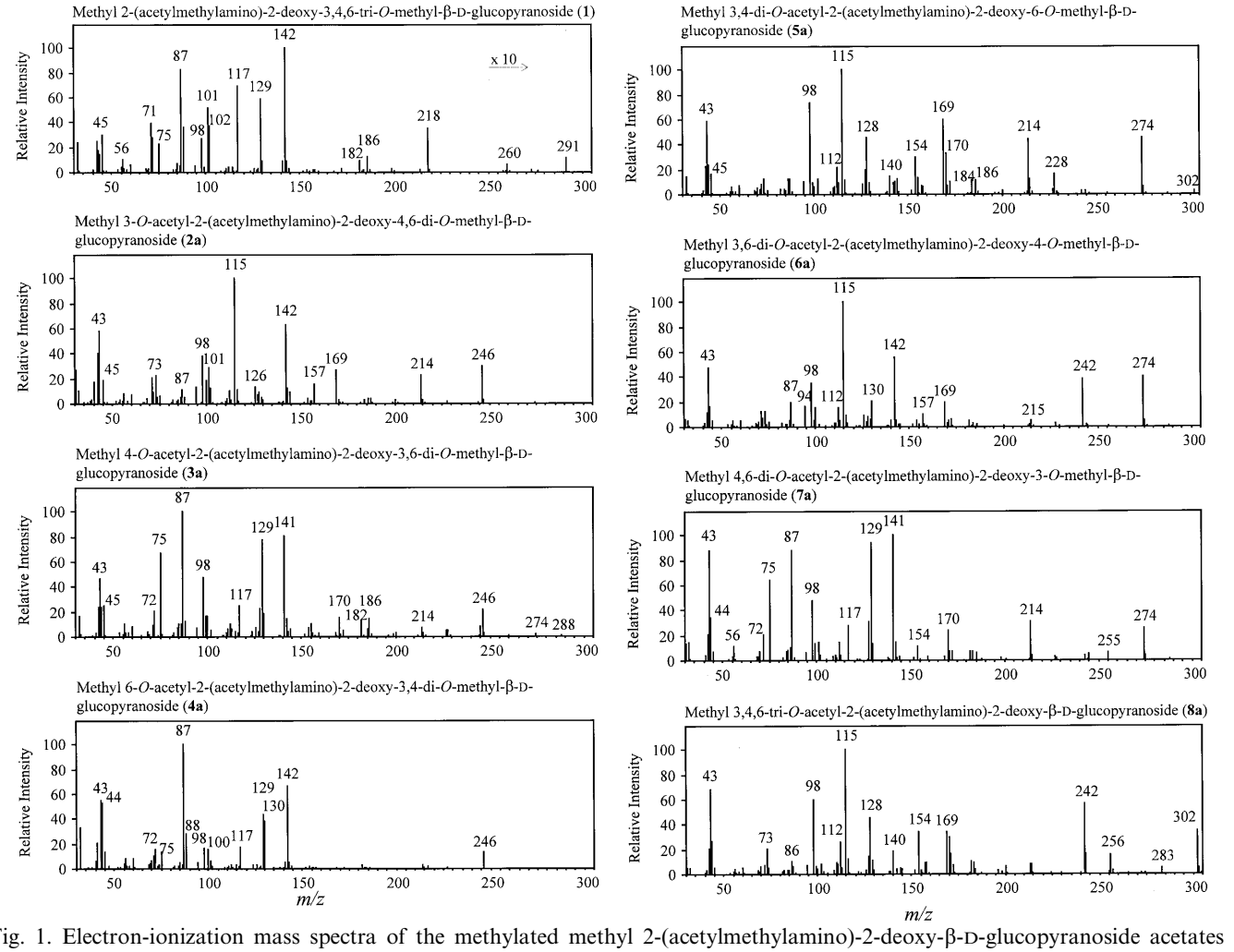


Fig. 1. Electron-ionization mass spectra of the methylated methyl 2-(acetylmethylamino)-2-deoxy-β-D-glucopyranoside acetates (compounds **1** and **2a**–**8a**).

The standards were prepared readily, however, by partial methylation of the known methyl 2-(acetylmethylamino)-2-deoxy-β-D-glucopyranoside [10]. Although separation of the benzoyl derivatives of the resultant mixture of positional isomers proved challenging due to the presence of rotamers about the acetylmethylamino group, they were nonetheless obtained in pure form by a combination of reversed-phase and normal-phase HPLC.

For the benefit of those who use the chromatographic GLC retention data for the acetyl derivatives as reported herein to identify such residues in a sample of unknown structure, it should be pointed out that their retention indices are much larger than those of most of the corresponding anhydrohexitol derivatives [14–16]. Their longer retention times combined with their unique molecular weights and EI mass spectra thus make their identification quite easy.

4. Experimental

General.—Reagents, solvents and materials were prepared as described previously [9]. Alkane standards were obtained from Aldrich Chemical Company. A stock solution of the homologous series of alkanes from $C_{11}H_{24}$ to $C_{34}H_{70}$ was prepared by combining 20–30 mg of each alkane and diluting to 10 mL with hexane.

Instrumentation.—HPLC was performed using a Beckman model 338 System Gold chromatograph. Reversed-phase chromatography was performed on a 5 μm particle-size Rainin Dynamax Microsorb semi-preparative

C_{18} reversed-phase column (1 × 25 cm) as described previously [9]. Analytical GLC was performed on a Hewlett–Packard 5890 gas–liquid chromatograph equipped with two flame ionization detectors, and HP 3365 Series II Chemstation recording software. The columns and conditions were the same as described previously [9]. GLC–MS analyses were performed using a Finnegan MAT 95 high-resolution double-focusing, reverse-geometry mass spectrometer equipped with a Hewlett–Packard 5890A Series II gas chromatograph and a DEC model 2100 workstation. Chemical ionization mass spectra and electron-ionization mass spectra were acquired as described previously [9]. ^{1}H NMR spectra were recorded on a Varian VXR-500S NMR spectrometer in $CDCl_3$ as the solvent.

Partially methylated methyl 2-(acetylmethylamino)-2-deoxy-β-D-glucopyranoside benzoates (**2b**–**7b**).—Methyl 2-(acetylmethylamino)-2-deoxy-β-D-glucopyranoside, prepared from methyl 2-acetamido-2-deoxy-β-D-glucopyranoside by sequential O-benzylation, methylation and debenzylation [10], was subjected to sequential partial methylation and benzoylation as described for 1,5-anhydro-D-fucitol [9]. Fractionation of the resultant mixture of six partially methylated benzoates (**2b**–**7b**) was accomplished by reversed-phase HPLC (Table 1) using a semipreparative C_{18} column to give **5b**–**7b** in pure form, but compounds **2b**–**4b** eluted together as a complicated mixture of rotamers. The latter were subsequently separated by normal-phase HPLC (Table 1) and isolated in pure form. After removal of the chromatographing solvents by evaporation under vacuum, the pure compounds were dissolved in $CDCl_3$ and identified by ^{1}H NMR spectroscopy.

Methylated methyl 2-(acetylmethylamino)-2-deoxy-β-D-glucopyranoside acetates (**1**, **2a**–**8a**).—Approximately one-third to one-half of each pure benzoate (**2b**–**7b**), obtained as described above, was debenzoylated (NaOMe, MeOH) and acetylated (Ac_2O, 1-methyl-imidazole) as described previously [9] to afford the partially methylated methyl 2-(acetylmethylamino)-2-deoxy-β-D-glucopyranoside acetate standards (**2a**–**7a**) in pure form. Compounds **2a**–**7a** as well as compounds **1** and **8a** were then chromatographed individually on the three GLC columns under the conditions described previously in order to establish the relative orders of elution of the standards on each column. In order to expedite acquisition of their mass spectra, further studies used mixtures of the standards prepared by acetylation of a portion of the partial methylation reaction that was saved.

Determination of LTPGLCRI values of methylated methyl 2-(acetylmethylamino)-2-deoxy-β-D-glucopyranoside acetates (**1**, **2a**–**8a**).—In order to ensure that the mixture of standards contained only the title compounds, aliquots of the individual pure standards were combined such that the integral of the area (flame ionization detection) of each component was at least 5% of the area of the most abundant component. An aliquot of the stock solution of *n*-alkanes from $C_{11}H_{24}$ to $C_{34}H_{70}$ was diluted 20-fold with hexane, then amounts of the alkane standard solution and the methylated methyl 2-(acetylmethylamino)-2-deoxy-β-

Table 3
Linear temperature-programmed gas–liquid chromatography retention indices (LTPGLCRI) of compounds **1** and **2a**–**8a** [a]

Compound (position of acetyl)	Stationary phase		
	DB-5	DB-17	RT_x-200
1 (none)	1824.63 [b]	2179.36	2238.13
3a (4-)	1971.01	2366.26	2533.30
4a (6-)	1983.04	2383.55	2533.30
2a (3-)	1991.85	2403.02	2593.38
5a (3,4-)	2065.95	2494.48	2723.34
7a (4,6-)	2109.46	2547.44	2778.59
6a (3,6-)	2143.65	2600.00	2885.36
8a (3,4,6-)	2197.34	2668.36	2958.48

[a] Indices were determined using a mixture of all compounds co-injected with the homologous series of *n*-alkanes from $C_{11}H_{24}$ to $C_{34}H_{70}$. Values were calculated from the equation $LTPGLCRI_{(x)} = 100n + [100\Delta n(t_{r(x)} - t_{r(n)})/(t_{r(n+\Delta n)} - t_{r(n)})]$, where $LTPGLCRI_{(x)}$ is the linear temperature-programmed gas–liquid chromatography retention index of the compound of interest (x), n is the carbon number of the *n*-alkane standard eluting just before the compound of interest (x), Δn is the difference in carbon number between the *n*-alkane standard eluting just before and just after the compound of interest (x), $t_{r(x)}$ is the absolute retention time of the compound of interest (x), and $t_{r(n)}$ and $t_{r(n+\Delta n)}$ are the absolute retention times of the *n*-alkanes eluting just before and just after the compound of interest (x).

[b] Values are listed according to increasing retention index on the DB-5 column.

D-glucopyranoside acetate standard solution were chosen for injection such that their area responses were comparable. The sample was injected manually and the individual components separated using a temperature program of 80–300 °C at 2 °C/min. LTPGLCRI values were determined in triplicate on each of the columns using the equation given in Table 3.

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