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Formation and stability of oxocarbenium ions from glycosides

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Structural, protecting group and leaving group effects in the formation of oxocarbenium intermediates were studied in the gas phase. It is found that significant stabilization of oxocarbenium cations is achieved by protecting groups that interact with the cationic center via neighboring group participation despite the electron-withdrawing character of these moieties. On the other hand, ethereal protecting groups do not facilitate the formation of oxocarbenium intermediates. The experimental findings are supported by DFT calculations that show the following order of stabilization by the group adjacent to the cationic center: RCO > SiR_3 > R, where R is an alkyl group. This indicates that the SN1-like mechanism that is commonly proposed for this reaction is not always valid. Moderate leaving group effect is also detected in a series of thioaryl glucopyranosides. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: glycosylation; oxocarbenium; mass spectrometry; electrospray ionization

INTRODUCTION

The study of carbohydrate biology has seen enormous growth in the past decade, and as a result there is an increasing need for synthetic methods for their preparation.[1–4] The key reaction in the synthesis of oligosaccharides is the glycosylation step, in which the various monosaccharide units are bound according to a well designed sequence. The acid-catalyzed synthetic strategies for glycosylation are based on the idea that the reactions are stepwise SN1-like, mediated by the formation of oxocabenium species.[3] Several mechanisms have been considered either dissociative or partially dissociative through the participation of ion pairs.[5–12] Regarding the stereoselectivity of the glycosylation (α vs β), a useful synthetic strategy is based on anchimeric assistance of 2-*O*-acyl groups of the so-called donors, supporting the notion that oxocarbenium intermediates are indeed formed in these reactions.[13,14] It has also been proposed that the involvement of solvent-separated ion pair between the anomeric carbon and the leaving group result in better reactivity in polar solvents that stabilize charge-separated species.[2] For reactions performed under basic conditions, concerted SN2-like mechanisms were also considered. Specifically for glucopyranosides, a stepwise transacetalization mechanism that involves ring opening and closure was proposed.[3] There are several factors that may affect the course of reaction: solvent, leaving group at the anomeric position, the Lewis acid (promoter) that is being used to activate the glycosyl donor, and, as carbohydrates possess several hydroxy groups, the stereochemistry of the donor and the nature of the protecting groups. Much effort has been invested in the design of suitable leaving groups at the anomeric position, which allow selective activation of the donor, while the functions of the counterpart sugar (acceptor) are all protected except for the site that is to be linked.[15–20] Several reports show that a large enhancement in the rate of glycosylation is observed when the hydroxy group at position 4 of the sugar ring of the donor is axial (e.g. galactose).[21–24] Other kinetic effects that were observed are attributed to the nature of the protecting groups.[21–23,25,26] Thus, electron-withdrawing protecting groups are thought to destabilize the oxocarbenium intermediate while electron-donating moieties stabilize the reaction intermediate and thus enhance the reaction.[21–23] An ultimate goal in this field is to establish a reactivity scale that will allow the design of the oligosaccharides and prepare them in a one pot synthesis of a mixture of donors with different reactivities.[21,27,28]

Mass spectrometry allows the isolation of reactive intermediates in the gas phase for the study of their relative stability. Electrospray ionization (ESI) enables the formation of cationized sugar moieties from solution in order to follow their decomposition in the gas phase. Collision induced dissociation (CID) spectra of protonated or ionized isomeric pyranosides have been measured by others using chemical ionization (CI), electron impact (EI) or fast atom bombardment (FAB) as ionization methods.[29–34] The purpose of these studies was to develop methodologies for isomeric distinction using mass spectrometry or the determination of linkage position.[35] The experimental conditions chosen for this investigation are less energetic than previous studies as the ions are produced with electrospray ionization and the CID carried out using low energy on $[M + NH_4]^+$ ion. We report here a systematic study that was conducted in order to elucidate the structural, protecting group and leaving group

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effects on the formation of oxocarbenium intermediates from protected glucosides and galactosides using ESI FT-ICR (Fourier Transform Ion Cyclotron Resonance) mass spectrometry.

EXPERIMENTAL

Mass spectrometry

All ESI FT-ICR experiments were carried out using Bruker BioAPEX III 47e FT-ICR spectrometer (Bruker Analytical Systems, Inc., Billerica, MA) equipped with a 4.7 T superconducting magnet, an external source (Apollo ESI Source), and an infinity analyzer cell. The samples were dissolved in an ammonium acetate solution (10.0 mg ammonium acetate in 100 ml CH_3OH) and introduced into the ESI source at a flow rate of 0.3 ml h^{-1}. Ions were detected using the broadband detection mode covering a mass range from 50 to 3000 amu. Typically, 8 individual transients were accumulated to improve the signal-to-noise ratio. For CID experiments, precursor ions were isolated using swept frequency ejection pulses of 250–500 μs duration to eject all other ions. A pulsed valve introduced the argon collision gas prior to ion activation. With the pulsed valve open for 30 ms, a peak pressure of ~8 exp-7 mbar was obtained. The precursor ions were excited using a variable amplitude off-resonance excitation pulse.

Materials

p-methylphenyl 2, 3, 4, 6-tetra-O-acetyl-1-thio-β-D-galactopyranoside (1)

Galactose pentaacetate (33.1 g, 0.084 mol) and *p*-thiocresol (5.8 g, 0.13 mol) were dissolved in CH_2Cl_2, under nitrogen, at 0 °C. Boron trifluoride diethyl etherate (14 ml, 0.11 mol) was added and the reaction mixture was stirred for 18 h at room temperature. After dilution with CH_2Cl_2, the organic layer was washed with saturated $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, the solvent was removed and the residue was purified by column chromatography on silica gel (hexane/ethylacetate 3 : 1) to afford (**8**) in 57% yield (20.2 g). ^{1}H NMR ($CDCl_3$) δ 7.37 (dd, J = 8.1 Hz, 2H), 7.08 (dd, J = 7.8 Hz, 2H), 5.37 (d, J = 5.7 Hz, 1H), 5.16 (t, J = 5.4 Hz, 1H), 4.98 (dd, J = 9.9, 3.3 Hz, 1H), 4.6 (d, J = 9.9 Hz, 1H), 4.05 (m, 2H), 3.86 (t, J = 6.9 Hz, 1H), 2.3 (s,3H), 2.09 (s,3H), 2.07 (s,3H), 2.07 (s,3H), 1.94 (s,3H). ^{13}C NMR ($CDCl_3$) δ 170.7, 170.5, 170.4, 169.8, 133.4, 133.2, 130.1, 129.9, 21.5, 21.2, 21.0, 20.93, 20.89. HRMS: $C_{21}O_9NSH_{30}$ $[M + NH_4]^+$ calcd 472.1636, found 472.1653.

p-methylphenyl 2, 3, 4, 6-tetra-O-benzyl-1-thio-β-D-galactopyranoside (2)

Galactopyranoside (**1**) (10.6 g, 0.02409 mol) was dissolved in a 64-ml mixture of MeOH/ethylacetate (1 : 1). After cooling to 0 °C, a solution of sodium methoxide in methanol (4.8 ml, 0.024 mol) was added and the reaction was allowed to warm to room temperature. The reaction was quenched by addition of Amberlite HR-120 (H^+) in methanol, then filtrated and concentrated. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 5 : 1) to afford *p*-methylphenyl-1-thio-β-D-galactopyranoside (5.5 g, 80%). *p*-Methylphenyl 1-thio-β-D-galactopyranoside (2.0 g, 7.0 mmol) was dissolved in 40 ml of freshly distilled DMF and cooled to −10 °C under nitrogen. NaH (1.7 g, 70 mmol) was added, the mixture was stirred for 10 minutes at room temperature followed by dropwise addition of benzyl bromide (7.6 ml, 70 mmol). The reaction mixture was stirred at room temperature for 5 h, quenched by water at 0 °C and extracted with ethylacetate. The organic layer was dried over $MgSO_4$ and concentrated. Purification with flash chromatography (ethylacetate/hexane, 5 : 1) yielded a white solid that was recrystallized from an ethylacetate/hexane mixture to afford pure (**2**) (3.1 g, 70%). ^{1}H NMR ($CDCl_3$) δ 7.42 (d, J = 9 Hz, 3H), 7.23 (m, aromatic protons), 6.95 (d, J = 8.1 Hz, 3H), 4.91 (d, J = 11.4 Hz, 1H), 4.72 (m,2H), 4.69 (d, J = 3.6 Hz, 1H), 4.58 (d, J = 1.5 Hz, 2H), 4.55 (d, J = 1.8 Hz, 1H), 4.36 (q, J = 13.8 Hz, 2H), 3.94 (d, J = 2.4 Hz, 1H), 3.84 (t, J = 9.3 Hz, 1H), 3.53 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 127–132 (aromatic carbons), 88.47, 84.62, 77.75, 77.65, 76.06, 74.84, 73.99, 73.96, 73.14, 69.18, 21.5. HRMS: $C_{41}O_5NSH_{46}$ $[M + NH_4]^+$ calcd 664.3091, found 664.3120.

p-methylphenyl 2, 3, 4, 6-tetra-O-methyl-1-thio-β-D-galactopyranoside (3)

p-Methylphenyl-1-thio-β-D-galactopyranoside (0.3 g, 1.057 mmol, see preparation of (**1**)) was dissolved in 40 ml of freshly distilled DMF and cooled to −10 °C under nitrogen atmosphere. NaH (0.31 g, 12.5 mmol) was then added and the mixture was allowed to warm up to room temperature and was further stirred for 10 min. Methyl iodide (1.6 ml, 25 mmol) was added dropwise and the reaction mixture was further stirred for 5 h, cooled to 0 °C, quenched with water and extracted with ethylacetate. The organic layer was dried over $MgSO_4$ and concentrated. Flash chromatography (ethylacetate/hexane, 3 : 1) yielded a white solid that was recrystallized from an ethylacetate/hexane mixture to afford pure (**3**) (0.29 g, 60%).^{1}H NMR ($CDCl_3$) δ 7.40 (d, J = 8.1 Hz, 2H), 7.04 (d, J = 7.8 Hz, 2H), 4.44 (d, J = 7.8 Hz, 1H), 3.72 (d, J = 3 Hz, 1H), 3.6 (m, 4H), 3.58 (s, 3H) 3.56 (s, 3H), 3.52 (s, 3H), 3.34 (s, 3H), 3.14 (dd, J = 9, 3 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 132.7, 129.8, 88.5, 86.1, 79.5, 77.75, 75.16, 70.85, 61.59, 61.47, 59.47, 58.49, 21.4. HRMS: $C_{17}O_5NSH_{30}$ $[M + NH_4]^+$ calcd 360.1839, found 360.1847.

p-methylphenyl 2, 3, 4, 6-tetra-O-trimethyl silyl-1-thio-β-D-glucopyranoside (4)

To a solution of *p*-methylphenyl-1-thio-β-D-glucopyranoside (0.13 g, 0.045 mmol) (synthesized according to the described above procedure for the preparation of *p*-methylphenyl-1-thio-β-D-galactopyranoside) in dry pyridine at 0 °C, TMSCl (0.25 ml, 1.9 mmol) and catalytic amount of 4-DMAP were added. The reaction was carried out at room temperature while monitored by TLC (ethylacetate/hexane, 6 : 4) and was quenched by addition of ethylacetate. The mixture was washed with saturated $NaHCO_3$ and brine, after which the organic layer was dried over $MgSO_4$ to allow (**5**) (0.17 g, 63%). ^{1}H NMR ($CDCl_3$) δ 7.48 (d, J = 4.8, 2H), 7.22 (d, J = 4.8, 2H), 4.75 (m,1H), 4.33 (m,1H), 4.00 (m,1H), 3.75 (m,1H), 3.60 (m, 2H), 3.35 (m, 1H), 0.37 (s, 3H), 0.30 (s, 3H), 0.28 (s, 3H), 0.193 (s, 3H). HRMS: $C_{25}O_5NSSiH_{54}$ $[M + NH_4]^+$ calcd 592.2794, found 592.2829.

1, 2, 3, 4, 6-O-benzoyl-β-D-glucopyranoside (5)

Benzoyl chloride (3.9 ml, 33.5 mmol) was added to a solution of D-glucose (1.1 g, 6.1 mmol) in dry pyridine, at 0 °C followed by catalytic amount of 4-DMAP. The reaction was stirred overnight at room temperature, then quenched by addition of ethylacetate and washed with aqueous H_2SO_4, saturated $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and concentrated. The product was crystallized from ethylacetate/hexane to allow (**5**) (2.8 g, 68%). ^{1}H NMR ($CDCl_3$) δ 7.26–8.1 (m, aromatic protons), 6.82 (d, J = 3.6 Hz, 1H), 6.25 (t, J = 9.6 Hz, 1H), 5.80 (t, J = 9.9 Hz, 1H), 5.63 (dd, J = 14.1, 3.6 Hz, 1H), 4.56 (m, 2H), 4.42 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 166.7, 166.4, 165.7, 165.4, 164.9, 128.7–130.5 (aromatic carbons), 90.3, 70.76, 70.75, 70.71, 69.1, 62.7. HRMS: $C_{41}H_{36}O_{11}N$ $[M + NH_4]^+$ calcd 718.2283, found 718.2324.

p-nitrophenyl 2, 3, 4, 6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (6)

To the solution of 2, 3, 4, 6-Tetra-*O*-acetyl-1-bromo-α-glucopyranoside (5 g, 12.17 mmol) and TBAHS (6.15 g, 18.3 mmol) in 50 ml of ethyl acetate, Na_2CO_3 1 M (50 ml) and *p*-nitrothiophenol (3.2 g, 24.35 mmol) was added and the reaction mixture was stirred overnight at room temperature. After dilution with ethylacetate, the organic layer was washed with saturated $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, the solvent was removed and the residue was purified by crystallization from hexane/ethylacetate to afford (**6**) in 83% yield. ^{1}H NMR ($CDCl_3$) δ 8.14 (d J = 8.64 Hz, 2H), 7.57 (d, J = 8.67 Hz, 2H), 5.26 (dd, J = 9.19 Hz, 1H), 5.08 (t, J = 4.83 Hz, 1H), 5.03 (t, J = 4.59 Hz, 1H), 4.84 (d, J = 9.98 Hz, 1H), 4.21 (d, J = 5 Hz, 2H), 3.8 (m, 1H), 2.09 (s, 3H), 2.06 (s, 3H), 2.03 (s, 3H), 1.99 (s, 3H). CI-MS (MH^+) 485.0.

4-acetamidophenyl 2, 3, 4, 6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (7)

A solution of (**6**) (1.95 g, 5.18 mmol) in ethyl alcohol (60 ml) and $SnCl_2$*H_2O (6 g, 27 mmol) was refluxed for 3 h, then it was cooled and $NaHCO_3$ was added to adjust pH 7. After dilution with ethylacetate, the organic layer was washed with saturated $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, the solvent was removed and the residue was purified by flash chromatography to allow pure 4-aminophenyl 2, 3, 4, 6-Tetra-*O*-acetyl-1-thio-β-D-glucopyranoside, that was acetylated by acetic anhydride in pyridine. Flash chromatography yielded pure (**7**). ^{1}H NMR ($CDCl_3$) δ 7.3–7.44 (m, 4H), 5.18 (t, J = 9.15 Hz, 1H), 4.99 (t, J = 9.66 Hz, 1H), 4.89 (t, J = 9.31 Hz, 1H), 4.17 (m, 2H), 3.66 (m, 1H), 2.17 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.96 (s, 3H), 1.64 (s, 1H). CI-MS (MH^+) 496.1.

Tiophenyl 2, 3, 4, 6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (8)

Glucose pentaacetate (5 g, 12.9 mmol) and thiophenol (1.4 ml, 13.5 mmol) were dissolved in CH_2Cl_2, under argon, at 0 °C. TMS triflate (0.2 ml) was added and the reaction mixture was stirred for 18 h at room temperature. After dilution with CH_2Cl_2, the organic layer was washed with saturated $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, the solvent was removed and the residue was purified by column chromatography on silica gel (hexane/ethylacetate 1 : 1) to allow (**8**) as an anomeric mixture. ^{1}H NMR ($CDCl_3$) δ 7.45 (m, 2H, aromatic protons), 7.40 (d, J = 7.83 Hz, 4H), 7.27 (d, J = 7.83, 4H), 5.88(d, J = 4.7 Hz, 1H), 5.4 (t, J = 10 Hz, 1H), 5.18 (t, J = 9.39 Hz, 1H), 5.07 (t, J = 6.27, 1H), 5.02 (m, 1H), 4.99 (dd, 5.73 Hz, 1H), 4.93 (t, J = 9.39, 1H), 4.67 (d, J = 9.39 Hz, 1H), 4.53 (dd, J = 4.7, 1H), 4.17 (d, J = 3.77, 2H), 3.99 (d, J = 3.77, 2H), 3.69 (dd, J = 4.52 Hz, 1H), 2.09 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H), 1.9 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H). CI-MS ($[M-H]^-$) 439.0.

p-methylphenyl 2, 3, 4, 6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (9)

(**9**) was prepared from glucose pentaacetate in the same manner as described for the preparation of (**1**). ^{1}H NMR ($CDCl_3$) δ 7.37 (dd, J = 4.31 Hz, 2H), 7.1 (dd, J = 4.66 Hz, 2H), 5.17 (dd, J = 9.14 Hz, 1H), 5.0 (t, J = 9.78 Hz, 1H), 4.91 (t, J = 9.25, 1H), 4.61 (d, J = 9.9 Hz, 1H), 4.17 (d, 2H), 3.68 (m, 1H), 2.3 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 1.99 (s, 3H), 1.96 (s, 3H). CI-MS ($[M-H]^-$) 453.1.

p-methoxyphenyl 2, 3, 4, 6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (10)

β Glucose pentaacetate (5 g, 12.8 mmol) and *p*-metoxybenzenethiol (2.4 ml, 19.32 mmol) were dissolved in CH_2Cl_2, under argon, at 0 °C. TMS triflate was added and the reaction mixture was stirred overnight at room temperature. After dilution with CH_2Cl_2, the organic layer was washed with saturated $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, the solvent was removed and the residue was purified by column chromatography on silica gel (hexane/ethylacetate) to afford (**10**) in 37% yield (20.2 g). ^{1}H NMR ($CDCl_3$) δ 7.42 (d, J = 8.85 Hz, 2H), 6.83 (d, J = 8.63 Hz, 2H), 5.2 (dd, J = 9.42 Hz, 1H), 4.97 (t, J = 9.87 Hz, 1H), 4.87 (t, J = 9.43, 3.3 Hz, 1H), 4.53 (d, J = 9.95 Hz, 1H), 4.17 (d, J = 3.58 Hz, 2H), 3.79 (s, 1H), 3.65 (m, 1H), 2.08 (s,3H), 2.06 (s,3H), 1.99 (s,3H), 1.96 (s,3H). CI-MS ($[M-H]^-$) 469.0.

p-methylphenylsulfenyl 2, 3, 4, 6-tetra-O-benzyl-β-D-galactopyranoside (13)

Into a 25-ml round-bottomed flask was weighted 2.5 g of Merck silica gel 60 that has been equilibrated with atmosphere at 110 °C for 72 h. The flask was stoppered and the content was allowed to cool to room temperature after which 0.5 ml of water was added and the absorbent was tumbled on rotary evaporator at atmospheric pressure until uniformly free-flowing. A solution of (**2**) (0.646 g, 1.0 mmol) in 5 ml CH_2Cl_2 was added with stirring followed by addition of 462 mg of OXONE. The mixture was stirred at room temperature for 18 h. The silica was then removed by filtration and washed with ethylacetate. Then the organic fraction was washed with 30 ml of saturated, aqueous solution of $FeSO_4$, dried over Na_2SO_4 and concentrated. Further purification was achieved by column chromatography on silica gel to allow a mixture of the diastereomeric sulphoxides (**13**). ^{1}H NMR ($CDCl_3$) δ 7.32(d, j = 8 Hz, 2H), 7.23 (m, 20H, aromatic protons), 7.23(m, 2H), 4.97 (d, J = 3.9 Hz, 1H), 4.92 (d, J = 10 Hz, 1H), 4.7

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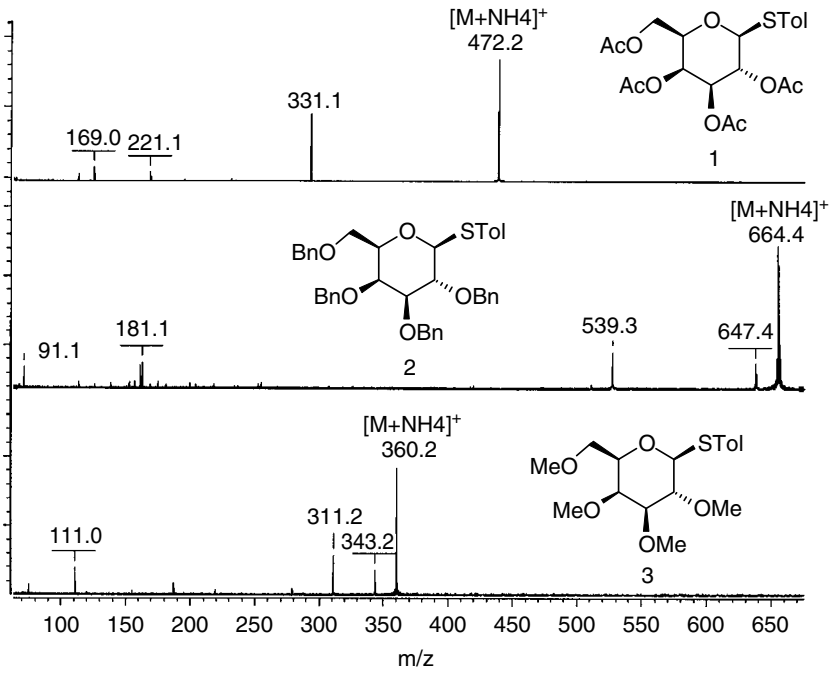

Figure 1. CID spectra that were recorded for ESI-generated $[M + NH_4]^+$ ions of galactose based sugars (**1**), (**2**) and (**3**) that contain an S-Tol group at the anomeric position and acetyl (Ac), benzyl (Bn) and methyl (Me) protecting groups, respectively.

(d, 3.6 Hz, 2H), 4.57 (d, $J = 11.7$ Hz, 1H), 4.39 (t, $J = 9.6$ Hz, 1H), 4.11 (s,2H), 3.8 (m, 2H)3.59(m, 2H), 3.38 (m, 2H), 2.26 (s, 3H).

^{13}C NMR ($CDCl_3$) δ 140.8, 137.8, 137.5, 137.4, 137.4, 129–124.9 (aromatic carbons), 93.59, 8.79, 78.75, 75.47, 73.92, 73.4, 72.99, 72.76, 72.18, 68.72. HRMS: C_{41} $H_{42}O_6Na$ $[M + Na]^+$ calcd 685.2594, found 685.2594.

p-methylphenylsulfenyl 2, 3, 4, 6-tetra-O-acetyl-β-D-galactopyranoside (14)

A diastereomeric mixture of (**14**) was prepared in the same manner as (**13**) from *p*-methylphenyl 2, 3, 4, 6-tetra-*O*-acetyl-1-thio-*β*-D-galopyranoside. Further purification was achieved by column chromatography on silica gel to allow a mixture of the diastereomeric sulfoxides. ^1H NMR ($CDCl_3$) δ 7.51 (m, 2H), 7.28 (d, $J = 7.8$, 2H), 5.42 (m, 1H), 5.31(m, 1H), 5.01 (m,1H), 4.27 (d, $J = 9.9$ Hz, 0.5H), 4.19 (m, 0.5H), 4.01 (d, $J = 7.2$ Hz, 0.5H), 3.98 (m, 1H), 3.95 (d, $J = 6.3$ Hz, 0.5H), 3.8 (m, 1H), 2.39 (s,3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.94 (s.3H), 1.91 (s,3H). ESI-MS (MH^+) 471.2.

Theoretical methods

Calculations were carried out using Gaussian 98 package of programs.[36] The clusters and molecules under study were optimized at B3LYP/6-31G* hybride density functional level of theory and the optimized structures were analyzed using analytical frequencies calculations. Energies are presented with zero point energy correction.

RESULTS

Protecting group effect

Galactose-based (**1**)–(**3**) (Fig. 1) possess a thiotolyl leaving group at the anomeric position while all other positions are acetylated (**1**), benzylated (**2**) or methylated (**3**). The thiotolyl group is frequently used as a leaving group in glycosylation[21] since it is readily activated with commonly used promoters such as NIS-T_fOH or DMTST, and it was therefore chosen as a leaving group for this study.

Figure 1 presents CID spectra of $[M + NH_4]^+$ ions that were measured for (**1**)–(**3**). The $[M + NH_4]^+$ ion (m/z 472) of the acetylated thiotolyl galactose (**1**) gives rise to an abundant oxocarbenium ion of m/z 331 (relative abundance (RA) = 52%). This ion further dissociates to afford consecutive products at lower masses. However, the CID of the $[M + NH_4]^+$ ion of methoxy derivatized (**3**) gives rise to very low abundance of the oxocarbenium ion of m/z 219 (RA = 0.05%), while the analogous oxocarbenium ion of m/z 523 is totally absent from the CID spectrum of benzyloxy protected (**2**). Instead, fragmentation reactions that occur involve the losses of ammonia and benzyl alcohol or methanol with retention of the thiotolyl group in (**2**) and (**3**), respectively.

We further examined the fragmentation behavior of the $[M + NH_4]^+$ ion of per-silylated glucose-based (**4**) (Fig. 2). The RA of the oxocarbenium ion of m/z 451 in the CID spectrum of (**4**) is 11%, much higher than the abundance of oxocarbenium ions in the CID spectra of ethereal galactose-based (**2**) and (**3**) but significantly lower than what is seen in the CID spectrum of (**1**), in which the RA of the oxocarbenium ion of m/z 331 is higher than 60% under the same energetic conditions. Apparently, silyl ethers enhance the formation of the oxocarbenium cation and stabilize it more than ethers but far less than acetyl groups. On the other hand, prominent loss of ammonia and benzoic acid is observed in the CID spectrum of per-benzyloxy (**5**) (Fig. 2). The resulting ion of m/z 579 (an oxocarbenium ion, RA = 45%) is relatively stable and an

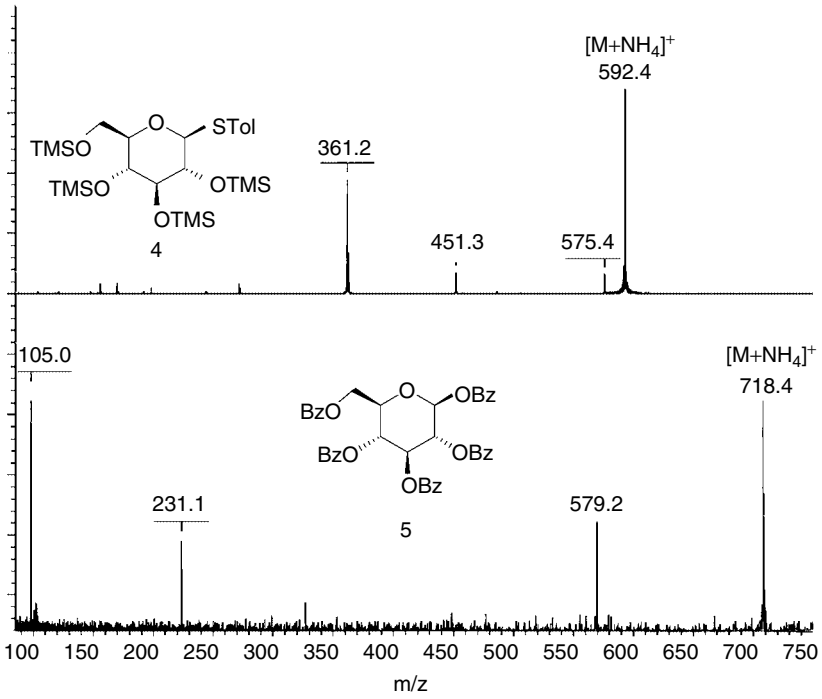

Figure 2. CID spectra that were recorded for ESI-generated $[M + NH_4]^+$ ions of glucose based (**4**) and (**5**) that contain trimethylsilyl (TMS) and benzoyl (Bz) protecting groups, respectively.

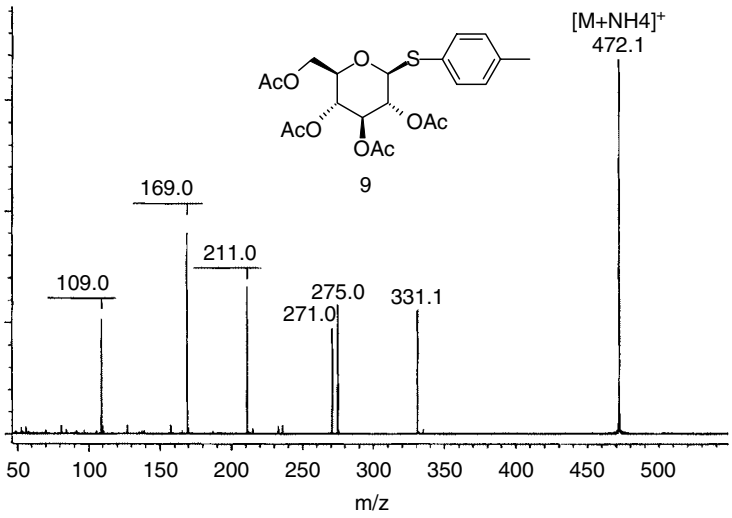

Figure 3. CID spectrum measured for the $[M + NH_4]^+$ ion of glucose-based **9**.

MS^3 measurement shows that it does not readily dissociate (not shown).

Leaving group effect

CID spectra were also measured for a series of glucopyranosides that possess a variety of thioaryl leaving groups. Ammonium cationized (**6**–**10**) undergo fragmentation in two different ways, upon collisions. The major fragmentation pathway involves the loss of ammonia and substituted thiophenol to form an oxocarbenium ion of m/z 331. This ion further dissociates to afford product ions at m/z 271, 211, 169 and 109 by consecutive losses of acetic acid or ketene. Another, less favored route of reaction is the loss of ammonia and three acetic acid moieties in one step, giving rise to an ion that retains the thioaryl group. For example, Fig. 3 presents the CID spectrum of ammonium cationized glucose-based (**9**) that contains a thiotolyl group at the anomeric carbon. Upon collisions, this $[M + NH_4]^+$ ion forms an $[M + NH_4–NH_3–3HOAc]^+$ ion of m/z 275 with a relative abundance of 34%.

CID spectra of ammonium cationized (**6**–**10**) show similar oxocarbenium to $[M + NH_4]^+$ ion ratios indicating no apparent effect of the nature of the leaving group on the cleavage at the anomeric position (Scheme 1). However, the small intensities of $[M + NH_4–NH_3–180]^+$ ions that were measured depend on the substituent at the thioaryl group, as summarized in Table 1. It is worth adding that an analogous ion is absent from the CID spectrum of the isomeric galactose per-acetate (**1**).

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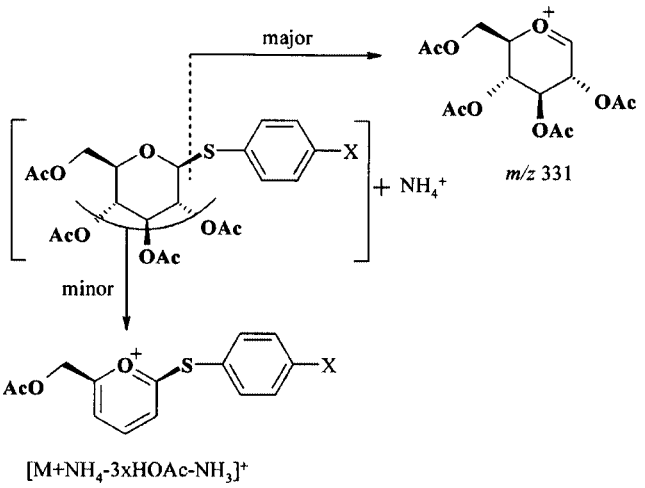


6-X=NO_2; **7**-X=NHAc; **8**-X=H; **9**-X=Me; **10**-X=OMe

Scheme 1. Competitive routes of fragmentation: formation of oxocarbenium ions versus combined loss of three acetic acid moieties with retention of the thioaryl group.

Table 1. Relative abundances of $[M + NH_4 - 3HOAc - NH_3]^+$ ions in the CID spectra of (**6**)–(**10**) and relative intensities of these ions corrected according to the total ion intensity

	Substituent (*X*)	$[M + NH_4 - 3HOAc - NH_3]^+$ (%)	RA (%)
(**6**)	NO_2	2.4	5.6
(**7**)	NHAc	8.9	30
(**8**)	H	9.7	32
(**9**)	Me	10	34
(**10**)	Ome	13	54

DISCUSSION

A stepwise glycosylation process involves the formation of an oxocarbenium intermediate, and the formation of this intermediate may be the rate-determining step (Scheme 2). If so, the stability of this intermediate as well as the rate of its formation may determine the activity of the so-called donor counterpart in this reaction.

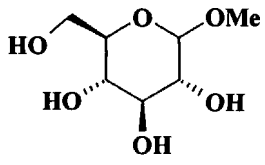
The rate of formation of isomeric oxocarbenium ions depends on stereochemical effects, dominated by the configuration of a specific isomer, the nature of the protecting groups and the leaving group at the anomeric position. The systems that were chosen for this study were designed in order to gain insight into each of these effects.

The relative reactivity of stereoisomeric acetylated α- and β- glycosides towards the formation of oxocarbenium ions at m/z 331 was examined by us previously.[37] Comparison of CID spectra of their $[M + NH_4]^+$ ions shows that *trans* diacetoxy groups at C_1-C_2 are more reactive, towards the loss of acetic acid, as the group at C_2 enhances the reaction by neighboring group participation. It was also found that the presence of an axial substituent at C_4 (i.e. galactose) results in favored formation of oxocarbenium ions. Moreover, β-xylose per-acetate and α-fucose per-acetate that lack an acetyl group at C_6 exhibit reactivities that are comparable with β-glucose per-acetate with *trans* diacetoxy groups at C_1-C_2.

Protecting groups

According to the presented experimental results, the protecting groups at the different positions show the strongest influence on the stability of the oxocarbenium intermediate. Moreover, in the case of (**2**) and (**3**) that contain methyl and benzyl groups, an oxocarbenium cation is not generated in the gas phase at all. This could indicate that glycosylation reactions with donors that contain ethereal protecting groups proceed via a mechanism that does not involve the formation of an oxocarbenium intermediate.

Alternatively, it can be argued that the ammonium group in $[M + NH_4]^+$ ions of (**2**) and (**3**) is not attached at the anomeric position, therefore oxocarbenium cations are absent from the CID spectra of (**2**) and (**3**) even if their formation is favorable. Nevertheless, the basicity of acetyloxy groups is higher than that of alkoxy groups and therefore the behavior of (**1**) indicates that even if initial cationization is not at the anomeric position, the ammonium cation (or proton) can travel within the $[M + NH_4]^+$ attachment ion. In order to reassure this assumption a CID spectrum was also measured for ammonium cationized unprotected methyl glucopyranoside (**11**), in which the ammonium ion is likely to interact with the methoxy group at the anomeric position and the heterocyclic oxygen atom.



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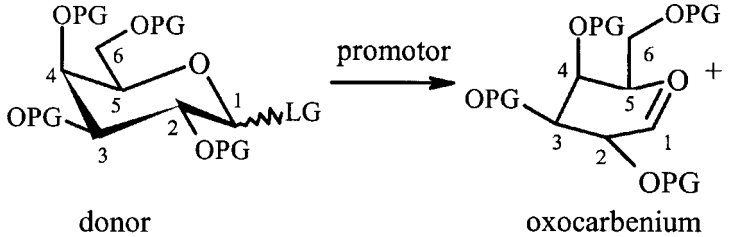
Nevertheless, the CID of the $[M + NH_4]^+$ ion of (**11**) (not shown) does not reveal the formation of an oxocarbenium ion of m/z 163. Instead, consecutive losses of

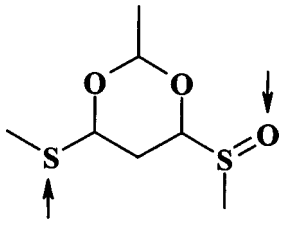


Scheme 2. Oxocarbenium intermediates formed in glycosylation reactions of galactose based glycosides. Glucose based systems differ by the configuration at C_4.

water molecules occur. This indicates that despite the higher topical basicity at the anomeric position, proton migration and water loss from other sites takes place.

An oxocarbenium ion is present in the CID spectra of the $[M + NH_4]^+$ ions of per-silylated glucose (**4**) (11%) and per-benzyloxy (**5**) (45%) as seen in Fig. 2. Although the RA of the analogous ion in the CID spectrum of per-acetylated (**1**) is higher (~60%, Fig. 1), MS^3 experiments reveal that the per-benzylated oxonium ion is more stable than the per-acetylated analog upon collisional activation. It is therefore concluded that in the case of ammonium cationized glycosides, elimination of the group at the anomeric position occurs when the resulting oxocarbenium cation is stabilized by the substituents, with the following order of stability: Bz > Ac > $(CH_3)_3Si$ > R, where R is an alkyl group. When the formation of an oxocarbenium is disfavored competing reactions, like alcohol elimination, occur.

Mobility of the cation (proton ammonium etc.) between different basic sites can be avoided if the anomeric position is significantly more basic. Thioaryls are used in solution because they are selectively activated by specific reagents. Since in the gas phase the promoter is an acid, it is desirable to have a basic group at the anomeric position. For example, sulphoxides are better candidates for the formation of MH^+ ions than sulphides because of their higher proton affinity. This is supported by the calculated topical proton affinity at the two basic sites of the model system (**12**). DFT calculations (B3LYP/6-31G* with ZPE correction) show that protonation of the sulphoxide oxygen atom is favored by 29.0 kcal mol^{-1} when compared with protonation of the sulphide sulfur atom. Indeed, we find that (**13**), that contains a sulphoxide group at the anomeric position, gives rise to MH^+ ions upon addition of ammonium acetate. This MH^+ ion is isolated in the gas phase and dissociates to afford the corresponding oxocarbenium ion of m/z 523 upon collisions, despite the benzyl protecting groups. Nevertheless, a comparison of the CID spectra that were measured for MH^+ ions of (**13**) and (**14**) (Scheme 3) shows that the amount of oxocarbenium ion (relative to the sum of fragment ions) in the CID of the MH^+ ion of (**14**), that contains acetyloxy protecting groups, is much higher than the amount of oxocarbenium ion in the CID of the MH^+ ion of (**13**) (90% versus 26%, respectively).



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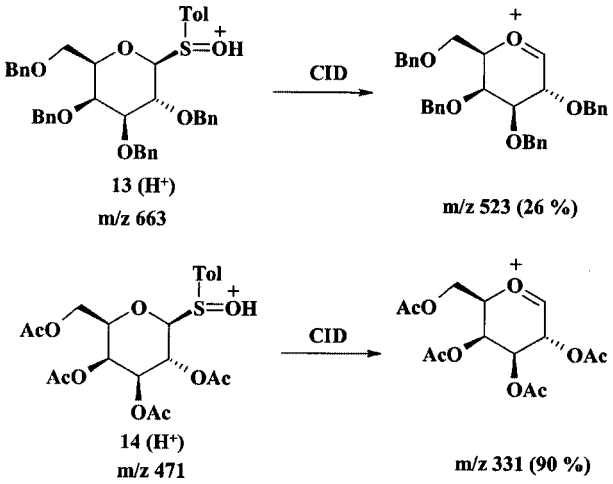
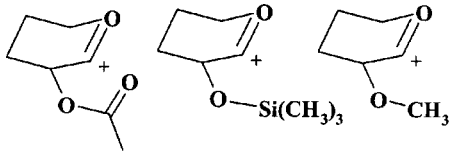
Theoretical support for the effect of protecting groups

In previous work, we calculated the stabilization of the developing cation at the anomeric position by neighboring



Scheme 3. Fraction of oxocarbenium ions in the sum intensity of fragment ions present in the CID spectra of MH^+ ions of (**13**) and **14**, as a measure for stabilization by acetyl versus benzyl groups.

group interaction with acetyl groups at C_2 and C_4 of glycosides. We could show that the strongest effect is caused by the group at C_2. Other experimental evidence indicate that an acetyl group at C_6 does not interact with the cation. Here, in order to support the order of stabilization by protecting groups at C_2, as deduced from the relative reactivity towards the formation of an oxocarbenium ion upon CID, relative hydride affinities (HA) of model cations (**15**)–(**17**) that contain an acetyl, methyl or trimethylsilyl group at C_2 were calculated. It is assumed that a cation that shows a higher hydride affinity value is less stable. DFT calculations, at the B3LYP/6–31G* level of theory (with ZPE correction), indicate that acetoxy substituted (**15**) has the smallest HA value while the relative HAs of model cations (**16**) and (**17**) are 13.2 and 16.5 kcal mol^{-1} higher, respectively. It is therefore shown that despite the electron-withdrawing character of acyl groups they stabilize the oxocarbenium ion efficiently through π overlap and the favored formation of a 5-membered ring.



	15	**16**	**17**
Relative HA (kcal mol^{-1})	**0**	**13.2**	**16.5**

Examination of the HOMO orbital of cation (**15**) (Fig. 4) also leads to the conclusion that there is an overlap between the nonbonding p-orbital of the acetyl oxygen atom and a sp^2 orbital of the anomeric carbon. On the other hand, no analogous π interaction is identified in the HOMO orbitals of model cations (**16**) and (**17**).

Considering the electronegativity of a methyl versus trimethylsilyl groups, it is reasonable that model cation (**17**) is less stable than (**16**). Indeed, the sum of Mulliken charges

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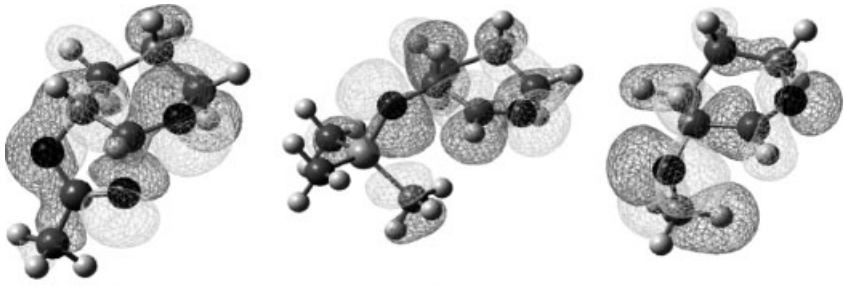

Figure 4. Calculated HOMO orbitals (B3LYP/6-31G*) of model oxocarbenium cations (**15**), (**16**) and (**17**). A clear overlap between the nonbonding p-orbital of the acetyl oxygen atom and a sp^2 orbital of the anomeric carbon of (**15**) is observed. The electron density around the methyl group in (**17**) is higher than the electron density on the trimethylsilyl group in (**16**).

on the trimethylsilyl group in (**16**) is 0.78 in comparison with 0.19 on the methyl group in (**17**). Also the HOMO orbitals that are shown in Fig. 4 illustrate higher electron density around the methyl group in (**17**) in comparison with the electron density around the trimethylsilyl group in (**16**).

Our findings indicate that per-benzoylated protected glucose (**5**) (Fig. 2) affords a particularly stable oxocarbenium ion of m/z 579, more stable than the acetate analog. It is known that benzoyl thio-donors are less reactive towards glycosylation reactions than their acetate thio-analogs[21]. It might be explained by the formation of a more stable oxocarbenium ion in the presence of benzoyl protecting groups, an intermediate that is too stable to react, possibly an ion pair that collapses back to the starting material. In this case, the rate-determining step is no longer the formation of a cationic intermediate but the successive reaction with the acceptor.

We also find that the effect of the leaving group on the rate of formation of an oxocarbenium ion is small. This does not mean that there is no leaving group effect in glycosylation. It only suggests that if glycosylation proceeds via the formation of an oxocarbenium intermediate it should be less sensitive to the leaving group.

CONCLUSIONS

Our experiments show that protecting groups play a significant role in the generation and stabilization of oxocarbenium intermediates. It is clear that oxocarbeniums are not generated from $[M + NH_4]^+$ ions in the presence of alkyl protecting groups instead of carboxy groups at proper positions. Adequate stabilization is achieved in the present of trimethylsilyl groups. It is concluded that the order of stabilization by protecting groups at C_2 and/or C_4 are Bz > Ac > $(CH_3)_3Si$ > R, where R is an alkyl group. These results point out that efficient glycosylation reactions that occur with benzylated or methylated donors might take place without the formation of an oxocarbenium intermediate. Other mechanisms that can be considered are SN2- like or ring opening (mutarotation). Moreover, the formation of some stabilized oxocarbenium ions (e.g. **5**) is not necessarily a rate-determining step in this reaction. Thus, protecting groups should be chosen with care in order to allow the formation of a reactive intermediate. We find only a mild leaving group effect when comparing a series of thioaryl leaving groups.

Acknowledgement

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