Alkylation of Galactosyl, Mannosyl and Lactosyl Barbiturate

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Abstract
The reactions of galactosyl, mannosyl and lactosyl barbiturates with 4-benzyloxybenzyl chloride and α-halo amide with galactose barbiturate were investigated. The products (benzyloxybenzyl-galactosyl barbiturate, benzyloxybenzyl-mannosyl barbiturate, benzyloxybenzyl-lactosyl barbiturate and galactose amide) were characterised by high field nuclear magnetic resonance spectroscopy, mass spectrometry, carbon, hydrogen and nitrogen combustion analyses and further characterised as per-acetate. The alkylation or the barbituric salts proceeds well with the electrophiles and are routes to novel C-glycosyl lectin binders.
Keywords: Alkylation, galactosyl, mannosyl and lactosyl barbiturates.

Introduction

Carbohydrates play central roles in post translational modifications of proteins, cell-cell communication and immune response to pathogens. The importance of cell-surface carbohydrates (glycolipids and glycoproteins) in initiating a wide variety of biological and pathological processes (to determine cell-cell interactions with invading bacteria, viruses, and cancer cells) is now well recognized (Witczak, 1997). Carbohydrate-protein interactions are responsible for initiating the early stages of several microbial infections, such as bacterial and viral infections, inflammations, cancer metastasis, cardiovascular disorders, immune dsyfunctions or developmental disorders and biological functions such as cellular growth, recognition and adhesion (Kahn *et al*., 1996, Lis *et al*., 1998, Palmacci *et al*., 2001 de Andrade.*et al.,* 2012).

Glycodendrimers are neoglycoconjugates that can be considered as bioisosters of glycoproteins, as they can mimic the multivalent interactions of carbohydrate-proteins in several adhesion processes. The ability of glycodendrimers to present multivalent interactions with proteins (lectins) as compared to a monovalent ligand is referred to as "cluster effect". Increasing multivalency, however, does not always result in higher affinity. Several low molecular weight and low valence glycodendrimers (dimers, trimers and tetramers) are very potent and sometimes, even more potent than higher valency analogs in their interaction with lectins. High molecular weight glyco-based derivatives are not rapidly transported into various tissues and possess poor pharmacokinetic properties. This is very important from the medicinal chemistry point of view, since low molecular weight dendrimers are expected to have favourable

physicochemical properties compared to the high molecular weight, multivalent ones (de Andrade *et al*., 2012).

The complex structure of carbohydrates and additional modifications such as sulfation or sialation, was suggested recently to serve the evolutionary purpose of "herd immunity" (Franz *et al*. 2008). However, a distinct disadvantage of naturally occurring carbohydrates, e. g. O-glycosidically-linked oligosaccharides, is their metabolic instability in biological systems (Hanessian, 1983). Therefore, much effort has been expended on the development of feasible pathways towards carbohydrate mimics, including C-glycosidic sugars, which may compete with their O-glycosidic counterparts in cell surface adhesion, inhibit carbohydrate processing enzymes, and interfere in the biosynthesis of specific cell surface carbohydrates (Yang *et al*., 1992 and Hanessian, 1983). Carbohydrate mimics are potential therapeutic agents against HIV, cancer, diabetes and other metabolic diseases (Franz *et al*., 2008).

Mimics of cell-surface carbohydrates capable of competing with natural carbohydrate-protein interactions would be of interest in potentially developing anti-microbial agents. As an alternative to the use of antibiotics for the treatment of bacterial infections, significant efforts have been made in designing soluble multivalent carbohydrate ligands as anti-adhesive agents. Some of these approaches include carbohydrate based polymers, dendrimers, as well as anchoring of carbohydrate ligands on various solid supports. The need to block microbial lectins has driven carbohydrate chemists towards the production of original molecules specifically designed for their anti-adhesion potency. For example, L-fucoside and D-galactose-coated glycodendrimers were effective in blocking *P. aeruginosa* lectin, while D-mannose-coated ones were able to inhibit the binding of *E. coli* to epithelial cells (Arya *et al*., 1999). Recently, anti-inflammatory activity was reported for D-glucose-based glycodendrimers. Recent investigations of barbituric acid and its derivatives have provided scientists with information that they may have applications in antibacterial, anti-chlamydial, anti-viral, as well as anti-cancer treatments and may also act as immune modulators. Neumann (2004) has synthesised novel barbituric acid derivatives with potential applications in cancer treatment.

Due to the importance of neoglycoconjugates for these biological studies, there is a constant need for straight forward methods for the alkylation and purification of these compounds. In this work, we present reactions of galactose, mannose and lactose barbiturates with 4-benzyloxybenzyl chloride and galactose barbiturates with acetamide (as electrophiles) as a route towards synthesis of novel glycodendrimers.

Materials and Methods

Materials

^{1}H and ^{13}C NMR data were recorded on a Bruker DPX-400 (400MHz) spectrometer. Chemical shift, δ are in ppm. Carbon, hydrogen and nitrogen (CHN) analyses and mass spectrometry were performed by Warwick Analytical Services, UK. The chemicals used were purchased from Aldrich and used without further purification. Thin Layer Chromatography (TLC) was performed on alumina backed silica gel plates and visualized under UV and then stained with sulphuric acid and ethanol mixture with heating. Melting points (Perkin-Elmer) are uncorrected.

Methods

4-Benzyloxybenzyl chloride 2.

To a stirred solution of 4-benzyloxybenzyl alcohol 1, (5.0 g, 23.3 mmol, 1.0 eq) in DCM (60 ml) was added $SOCl_2$ (2.2 ml, 3.6 h, 30 mmol, 1.3 eq) and a catalytic amount of DMF. The reaction mixture was stirred at room temperature for 12 hours at which time thin layer chromatography (chloroform) showed that all the starting material had been consumed. The solvent was evaporated and the compound recrystallized from petrol as colourless plates. The compound was filtered under suction and washed with petrol; Yield 3.05 g, 56.2%. Mp 79^{0}C, (Lit 77-79^{0}C).

Benzyloxybenzyl-galactosyl barbiturate 4.

To a stirred solution of galactose barbituric salt 3, (1.0 g, 2.94 mmol, 1.3 eq) in DMSO (10.0 ml) was added 4-benzyloxybenzyl chloride (0.68 g, 2.9 mmol, 1.0 eq). The reaction was stirred at room temperature for 4 hours by which time TLC (EtOAc, MeOH, H_2O, 40:5:1) showed complete conversion. The solvent was then distilled off at 20-30^{0}C under high vacuum to give a brown solid and was extracted with portions of hot ethyl acetate (5 x 20 ml). A white powder was precipitated on the addition of petrol. This was purified by column chromatography using EtOAc containing an increasing gradient of MeOH/H_2O (5:1). The first product 4a came off at 2% MeOH/H_2O, (5:1) in EtOAc (10 mg, 20% yield), while the second product 4 came off at 3% MeOH/H_2O, (5:1) in EtOAc. Mp 122-123^{0}C, M^+ 514, ^{1}H NMR (400MHz: d_6- DMSO): δ:2.95 (3H, s, NCH_3), 3.00 (3H, s, NCH_3), 3.40 (4H, m, C_3H, C_5H, CH_2Ar), 3.44 (2H, m, $C_6HH`$), 3.61 (1H, dt, $J_{1,2}$- $J_{2,3}$ - $J_{2,OH}$= 94Hz, C_2H), 3.67 (1H, br t, $J_{3,4}$ - J_{4OH} = 3.3Hz, C_4H) 3.75 (1H, d, $J_{1,2}$ = 9.6Hz, C_1H), 4.29 (1H, d, $J_{4,OH}$ = 2.7Hz, C_4OH), 4.56 (1H, t, $J_{6,OH}$ = 5.6Hz, C_6OH), 4.72 (1H, d, $J_{3,OH}$ = 6.0Hz, C_3OH), 5.03 (2H, s OCH_2Ph), 5.07 (1H, d, $J_{2,OH}$ = 5.9Hz, C_2OH), 6.85 (2H, d, J_{AB} = 9.1Hz, ArH), 7.38 (5H, m, Ph). ^{13}C NMR (400MHz; d_6-DMSO), δ: 28.2, 28.3, 60.9, 68.6, 68.7, 69.4, 75.4, 80.4, 83.7, 114.9, 127.6, 128.0, 128.1, 128.7, 130.6, 137.3, 150.8, 157.7, 169.8, 170.1. $C_{26}H_{30}N_2O_9$ requires C; 60.69, H; 5.88, N; 5.44%. Found; C; 57.82, H; 6.07, N; 5.03%.

Benzyloxybenzyl mannosyl barbiturate 6

Mannose barbituric salt (1.0g, 3.1 mmol, 1.3 eq) was dissolved in DMSO (15.0 ml), then 4-benzyloxybenzyl chloride (0.74 g, 3.15 mmol, 1.3 eq) and $NaHCHO_3$ (0.25 g) were added. The reaction was stirred overnight by which time TLC (EtOAc, MeOH, H_2O, 40:5:1) showed complete conversion. The solvent was removed under high vacuum and the solid was extracted with EtOAc (5x20 ml). The combined EtOAc extracts were evaporated and the product was separated by column chromatography using EtOAc. The product was recrystallized from water as white plates. M^+514. ^{1}H NMR (400MH$_z$: d_6-DMSO); δ:2.98 (3H, s, NCH_3), 3.05 (3H, s, NCH_3), 3.10 (1H, m, C_5H), 3.14 (1H, d, J_{AB} =13.0Hz, CHH`ArH), 3.26 (2H, m, $C_3H$, $C_4H$), 3.47 (1H,dt, $J_{6,6`OH}$ - $J_{5,6`}$ = 6.0Hz, $J_{6,6`}$ = 12.0Hz, $C_6H`$ ), 3.54 (1H, br d, $J_{2,0H}$ = 4.5Hz, $C_2H$), 3.64 (1H, d, $J_{AB}$ = 13.0Hz, CHH` ArH), 3.74 (1H, ddd, $J_{5,6}$ = 1.9, $J_{6,OH}$ = 5.8, $J_{6,6`}$ = 12.0Hz, $C_6H$), 3.87 (1H, br s, $C_1H$), 4.37 (1H, t, $J_{6`,\ OH}$ = 5.8Hz, C_6OH), 4.78 (1H, d, $J_{3,OH}$ = 5.0Hz, C_3OH), 4.82 (1H, d, $J_{4,OH}$ = 4.9Hz, C_4OH), 4.89 (1H, d, J = 4.5Hz, C_2OH), 5.03 (2H, s, OCH_2Ph), 6.88 (2H, d, J= 8.9Hz, ArH), 6.91 (2H, d, J= 8.9Hz, ArH), 7.39 (5H, m, Ph). ^{13}C NMR (400MHz: d_6-DMSO); δ: 28.4, 28.5, 39.8, 59.4, 61.9, 67.3, 68.9, 69.4, 74.7, 82.7, 83.5, 114.9, 127.6, 128.0, 128.7, 130.8, 137.3, 150.3, 157.7, 167.8, 170.2. $C_{26}H_{30}N_2O_9$ requires C; 60.69, H; 5.88, N; 5.14%. Found; C: 59.00, H: 5.66, N: 5.14%.

Benzyloxybenzyl-lactosyl barbiturate 8

Lactose barbituric salt (1.0 g, 1.9 mmol, 1.3 eq) was dissolved in DMSO (20 ml) then 4-benzyloxybenzyl chloride (0.44 g, 1.9 mmol, 1.0 eq) and $NaHCO_3$ (0.25 g, 2.9 mmol, 2.0 eq) were added. The reaction mixture was stirred at room temperature overnight by which time TLC (EtOAc, MeOH, H_2O:20:5:1) showed all the starting material has been consumed. The solvent was removed under high vacuum to give a solid, which was extracted with hot EtOAc (4x20ml). The combined extracts were extracted further with water (2x40 ml). Combined aqueous extracts were saturated with sodium chloride and extracted further with chloroform (3x50ml). The organic extract was dried over $MgSO_4$, filtered and evaporated under reduced pressure leaving behind the white powdery solid. This was recrystallized from water as tiny plates. M^+754, Mp 98-99^{0}C, ^{1}H NMR (400MHz: d_6-DMSO); δ:2.94 (3H, s, NCH_3), 3.00 (3H, s, NCH_3), 3.22-3.80 (14H, m, C-2,3,4,5,6,6`gal & glu and CH_2Ar), 3.89 (1H, d, J=9.3Hz, C_1H glu), 34.21 (1H, d, $J_{1,2}$ = 7.1Hz,

C_1Hgal), 4.54 (2H, m, 2x OH), 4.64 (1H, br t, J= 5.0Hz, OH), 4.72 (1H, br s, OH), 4.79 (1H, br d, J= 3.7 Hz OH), 6.83 (2H, s, OCH_2Ph), 5.13 (1H, d, J=4.0Hz, OH), 5.50 (1H, d, J=5.5Hz,OH), 6.83 (2H, d, J_{AB} = 8.8Hz, ArH), 6.88 (2H, d, J_{AB} = 8.8Hz, Ar), 7.39 (5H, m, Ph). ^{13}C NMR (400MHz: d_6-DMSO); δ:28.2, 28.3, 40.7, 60.7, 61.1, 68.5, 69.5, 70.8, 71.6, 73.6, 75.8, 76.7, 80.3, 81.0, 82.4, 104.1, 115.0, 127.2, 128.0, 128.2, 128.7, 130.4, 137.3, 150.6, 157.8, 169.8, 169.9, 170.11. $C_{32}H_{40}N_2O_{14}$ requires C; 56.80, H; 59.6, N; 4.14%. Found; C: 55.39, H: 6.06, N: 2.02%.

Per-O-acetyl benzyloxybenzyl-galactosyl barbiturate 9

Acetic anhydride (5.0ml) and a catalytic amount of 4-DMAP were added to benzyloxybenzyl-galactosyl barbiturate **4** (about 0.5g). The reaction mixture was stirred overnight at room temperature. The reaction was hydrolysed by the addition of water (40ml), stirred for 10 minutes then extracted twice with DCM (2 x 50ml). The organic layer was further extracted with 1M HCl (2 x 50ml) and a saturated solution of $NaHCO_3$ (2 x 50ml) then dried over $MgSO_4$, and filtered. The solvent was removed under vacuum to give a white powder. M^+684. ^{1}H NMR (400MHz: $CDCl_3$); δ:1.89 (3H, s, OAc), 1.90 (3H, s, OAc), 1.93 (3H, s, OAc), 2.09 (3H, s, OAc), 2.99 (3H, s, NCH_3), 3.05 (3H, s, NCH_3), 3.09 (1H, d, J=12.8Hz, CHH`Ar), 3.27 (1H, d, J=12.8Hz, CHH`Ar), 3.81 (1H, br t, $J_{5,6}$ - $J_{5,6`}$ = 6.4Hz, $C_5$H) 3.97 (2H, m, $C_6$HH`) 4.29 (1H, d, $J_{1,2}$ = 9.8Hz, C_1H), 4.92 (2H, s, OCH_2Ph), 4.97 (1H, dd, $J_{2,3}$ = 9.7Hz, $J_{3,4}$ = 3.3Hz, C_3H), 5.32 (1H, d, $H_{3,4}$= 3.2Hz, C_4H), 5.65 (1H,t, $J_{1,2}$ - $J_{2,3}$= 9.7Hz, C_2H), 6.70 (2H, d, J_{AB}= 8.6Hz, ArH), 6.80 (2H, d, J_{AB} = 8.6Hz, ArH) 7.28 (5H, m, Ph). ^{13}C NMR (400MHz: $CDCl_3$); δ:20.9(7), 20.9(8), 21.0, 21.1, 28,6, 28.7, 40.7, 60.8, 61.9, 67.4, 67.7, 70.3, 73.3, 75.3, 81.2, 115.3, 126.1, 127.8, 128.4, 128.9, 130.8, 137.0, 150.5, 158.7, 169.4, 169.8, 170.0, 170.3, 170.5, 170.6.

Per-O-acetyl benzyloxybenzyl-mannosyl barbiturate 10

Acetic anhydride (1.0ml), pyridine (1.0ml) and a catalytic amount of 4-DMAP were added to benzyloxybenzyl-mannosyl barbiturate, **6** (0.11g). The reaction was stirred at room temperature overnight. Water (40ml) was added to the reaction mixture and stirred for 10 minutes then extracted with DCM. The DCM layer was further extracted with 1M HCl (2 x 40ml) and saturated $NaHCO_3$ solution (2 x 40ml), filtered and dried over $MgSO_4$. The solvent was removed under vacuum to give the per-O-acetyl benzyloxybenzyl-mannosyl barbiturate. M^+683, ^{1}H NMR (400MHz: $CDCl_3$); δ:1.91 (3H, s, OAc), 1.96 (3H, s, NCH_3), 3.19 (1H, d, J_{AB} =12.8Hz, CHH`ArG), 3.23 (1H, s, $J_{AB}$=12.8Hz, CHH`,ArH), 3.55 (1H, ddd, $J_{5,6}$=2.3, $J_{5,6`}$ = 6.7, $J_{4,5}$ = 9.9Hz, $C_5$H), 3.98 (1H, dd, $J_{5,6`}$ = 6.7, $J_{6,6`}$ 12.2Hz, $C_6$H), 4.03 (1H, s, $C_1$H), 4.08 (1H, dd, $J_{5,6`}$ = 6.7, $J_{6,6`}$ = 12.2Hz, C_6H), 4.90 (2H, s, OCH_2Ph), 4.97 (1H, dd, $J_{2,3}$=3.0, $J_{3,4}$= 9.9Hz, C_3H), 5.09 (1H, t, $J_{3,4}$ = 9.9Hz, C_4H), 6.68 (2H, d, J_{AB}=8.6Hz ArH), 6.90 (2H, d, J_{AB}= 8.6Hz ArH), 7.30 (5H, m, Ph). ^{13}C NMR (400MHz: $CDCl_3$); δ: 20.8, 20.9, 21.0(x2), 28.9, 29,1, 38.5, 60.4, 63.0, 66.3, 66.5, 70.2, 72.8, 77.6, 81.7, 115.1, 127.8, 128.3, 128.9, 131.6, 137.2, 150.8, 158.4, 167.9, 169.5, 170.0, 170.2, 170.5, 170.6. $C_{34}H_{38}N_2O_{13}$ requires C; 59.82, H; 5.61, N; 4.10%. Found; C: 59.08, H: 5.58, N: 3.98%.

Per-O-acetyl benzyloxybenzyl-lactosyl barbiturate 11

Acetic anhydride (2.0ml), pyridine (2.0ml) and a catalytic amount of 4-DMAP were added to benzyloxybenzyl-lactosyl barbiturate **7** (0.2g). The reaction was stirred at room temperature overnight. Water (40ml) was added to the reaction mixture and stirred for 10 minutes then extracted with DCM. The DCM layer was further extracted with 1 M HCl (2 x 40ml) and saturated $NaHCO_3$ solution (2 x 40ml), filtered and dried over $MgSO_4$. The solvent was removed under vacuum to give the per-O-acetyl benzyloxybenzyl-lactosyl barbiturate.

M^+971, ^{1}H NMR (400MHz: $CDCl_3$); δ:1.94 (3H, s, OAc), 1.97 (3H, s, OAc), 2.01 (6H, s, OAc), 2.02 (3H, s, OAc), 2.06 (3H, s, OAc) 3.04 (3H, s, NCH_3), 3.08 (1H, d, J_{AB} = 12.7Hz, CHH`ArH), 3.10 (3H, s, OAc), 3.04 (3H, s, $NCH_3$), 3.24 (1H, d, $J_{AB}$ = 12.7$H_z$, CHH`ArH), 3.51 (1H, m, C_5H), 3.70 (1H, t, $J_{3,4}$ - $J_{4,5}$ = 9.5Hz, C_4H glu), 3.85 (1H, br t, $J_{5,6}$ - $J_{5,6`}$ - $J_{5,6`}$ = 6.7Hz, C_5H gal), 3.93 (1H, dd, $J_{6,6`}$ = 12.1Hz, $J_{5,6}$ = 5.2Hz, $C_6$H`glu), 4.09 (2H, m, C_6HH gal), 4.30 (1H, d, $J_{1,2}$ = 10.0Hz, C_1H glu), 4.47 (1H, d, $J_{1,2}$ 7.9Hz, C_1H gal), 4.48 (1H, br d, $J_{6,6`}$ = 12.1Hz, C_6H glu), 4.93 (1H, dd, $J_{2,3}$ = 10.3Hz, C_3H gal), 4.97 (2H, s, OCH_2Ph), 5.09 (1H, dd, $J_{1,2}$=7.9, $J_{2,3}$=10.3Hz, C_2H gal), 5.15 (1H,t, $J_{2,3}$ - $J_{3,4}$= 9.0Hz, C_3H glu) 5.33 (1H, d, $J_{3,4}$ = 3.4Hz, C_4H gal), 5.52 (1H, t, $J_{1,2}$ - $J_{2,3}$ = 9.5Hz, C_2H glu) 6.76 (2H, d, J_{AB} = 8.7Hz,ArH), 7.78 (2H, d, J_{AB} = 8.7Hz, ArH), 7.25 (5H, m, Ph). ^{13}C NMR (400MHz: $CDCl_3$); δ : 20.8, 20.9, 21.1, 21.2 (x3), 28.6, 28.7, 40.3, 61.0, 61.2, 61.6, 67.0, 69.5, 70.2, 70.3, 71.1, 71.3, 75.2, 76.2, 77.2, 80.6, 101.4, 115.3, 126.1, 127.8, 128.3, 128.9, 130.7, 137.0, 150.4, 158.7, 170.0, 170.3, 170.4, 170.5, 170.6, 170.7.

Galactose amide 12

To a stirred solution of galactose barbituric salt (1.0g, 2.9mmol, 1.3eq) in DMSO (20ml) was added *N*-bromoacetamide (0.67g, 2.9mmol, 1.0eq). The reaction was stirred overnight at room temperature by which time TLC (EtOAc, MeOH, H_2O: 20:5:1) showed complete conversion. The solvent was removed under high vacuum and the solid extracted with ethyl acetate (3 x 40ml). The combined EtOAc extracts were evaporated at reduced pressure and the product was separated by column chromatography using EtOAc with an increasing concentration of methanol: water (5:1). The pure product eluted at 3% methanol: water (5:1) in EtOAc. The solvent was removed under vacuum to give the product. M^+467, ^{1}H NMR (400MHz; d6-DMSO), δ: 3.11 (3H, s, NCH_3), 3.13 (3H, s, NCH_3), 3.17 (1H, d, J_{AB} =12.9Hz, CHH`CONH), 3.29 (3H, m, $C_3$H, $C_5$H, $C_6$H`), 3.44 (2H, m, C_1H, C_6H), 3.53 (1H, dt, $J_{2,OH}$ = 5.7, $J_{1,2}$ - $J_{2,3}$ = 9.2Hz),3.60 (1H, d, J_{AB}=12.9Hz, CHH`CONH), 3.65 (1H, br t, $J_{4,3}$-$J_{4OH}$ = 3.3Hz, $C_4$H), 4.18 (1H, dd, $J_{HH`NH}$ = 6.0, $J_{HH`}$ = 15.5Hz, CHH`NHCO), 4.24 (1H, dd, $J_{HH`NH}$ = 6.0, JHH` =15.5Hz, CHH`NHCO), 4.34 (1H, d, $J_{4,OH}$ = 3.8Hz, $C_4$OH), 4.48 (1H, t, $J_{6H`OH}$ =5.4Hz, C_6OH), 4.70 (1H, d, $J_{3,OH}$= 6.0Hz, C_3OH), 4.92 (1H, d, $J_{2,OH}$=5.7Hz, C_2OH), 7.20 (2H, d, J=7.1Hz ArH), 7.27 (1H, t, J= 7.2Hz, ArH), 7.25 (2H, t, J= 7.2Hz, ArH), 8.76 (1H, t, J=6.0Hz, CONH). ^{13}C NMR (400MHz: d_6-DMSO); δ: 28.43, 28.56, 39.9, 56.65, 60.62, 68.09, 68.23, 75.36, 79.18, 83.54, 127.10, 127.33, 128.59, 139.51, 152.15, 170.14, 170.35, 171.68.

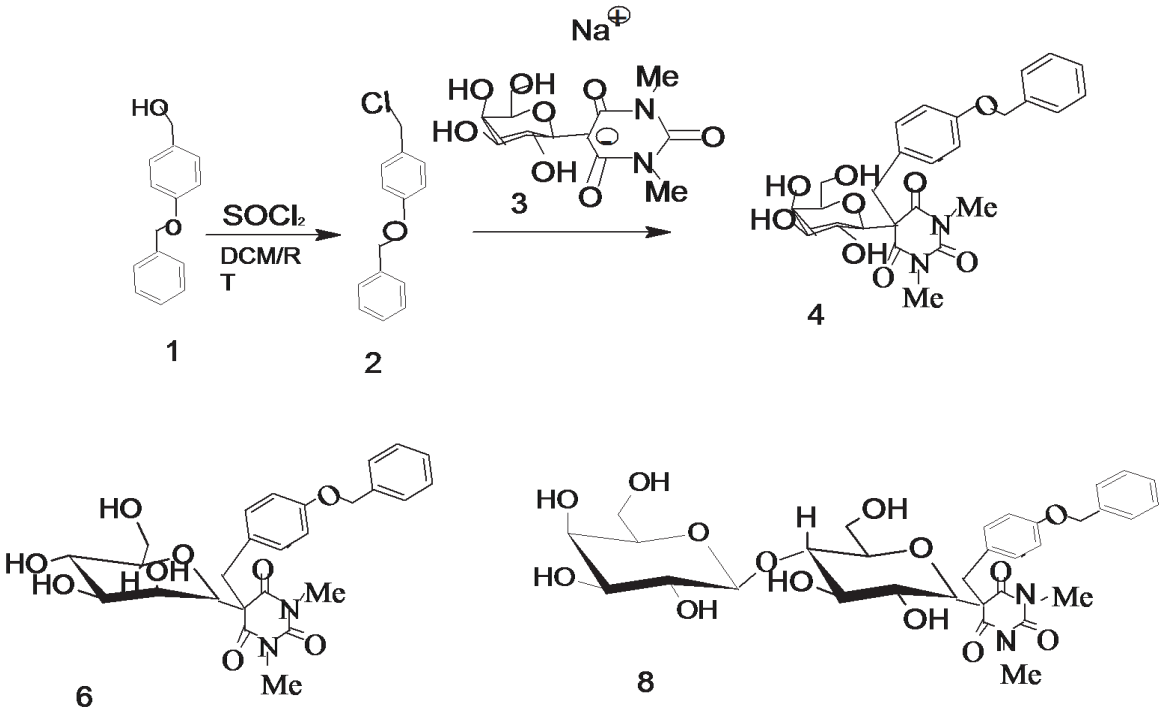
Per-O-acetyl-galactose amide 13

Acetic anhydride (2.0 ml), pyridine (2.0 ml) and a catalytic amount of 4-DMAP were added to galactose amide (20 mg). The reaction was stirred overnight at room temperature. Water (50ml) was added to hydrolyse unreacted acetic anhydride then, the mixture was extracted with DCM. The organic layer was further extracted with 1M HCl (3 x 50 ml) and a saturated solution of $NaHCO_3$ (3 x 50 ml), then dried over $MgSO_4$ and filtered. The solvent was removed under vacuum to give a white plate. M^+634, ^{1}H NMR (400MHz: $CDCl_3$); δ:1.94 (3H, s, OAc), 2.02 (3H, s, OAc), 2.11 (3H, s, OAc), 3.13 (2H, br s, CHH`CONH), 3.30 (3H, s, $NCH_3$), 3.34 (3H, s, $NCH_3$), 3.78 (1H, br t, $J_{5,6`}$ = 6.7Hz, C_5H), 3.87 (1H, dd, $J_{6,6`}$ = 7.0, $J_{6,6`}$=11,4Hz, C_6H`), 3.96 (1H, d, $J_{1,2}$= 9.6$H_z$, $C_1$H), 4.02 (1H, dd, $J_{5,6}$ = 6.6, $J_{6,6`}$ = 11.4Hz, C_6H), 4.28 (1H, dd $J_{H,NH}$ = 5.4, J_{AB}=14.6H_z, CHH`NHCO), 4.35 (1H, dd $J_{H`NH}$ =5.4, J_{AB} = 14.6Hz, CHH`NHCO), 4.95 (1H, dd, $J_{3,4}$=3.3, $J_{2,3}$=9.6Hz, C_3H), 5.29 (1H, d, $J_{3,4}$=3.3Hz, C_4H), 5.33 (1H, t, $J_{1,2}$ 9.6Hz, C_2H), 5.70 (1H, t, J=5.4Hz, CONH), 7.18 (2H, d, J = 6.7Hz, ArH), 7.26 (5H, m, ArH). ^{13}C NMR (400MHz: $CDCl_3$); δ:20.9, 21.0, 21.4(x2), 39.4, 44.3, 56.4, 62.1, 67.2, 67.6, 73.1, 74.9, 81.3, 128.2 (x2), 129.2, 137.3, 151.7, 168.8, 169.1, 170.2, 170.5, 170.6, 170.9. $C_{29}H_{35}N_3O_{13}$ requires C;

54.97, H; 5.57, N; 6.63%. Found; C: 54.92, H: 5.58, N: 6.32%.

Results And Discussion

A simple approach was employed where the C-glycoside could have a versatile reactive handle to attach to a lipid or even functionalised dendrimer core to make sugar balls (Dam *et al*., 2000). This was designed to use electrophiles, which had been shown to react cleanly with the glycol barbituric salts and simple deprotection chemistry to reveal the reactive handle. Additionally, any isomers formed during the alkylation could be easily separated and characterized due to the robust nature of the electrophile used. The idea is shown in Scheme 1 for the galactose, mannose and lactose barbituric salts. The chloride is available by substitution of commercially available 4-benzyloxybenzyl alcohol with $SOCl_2$.

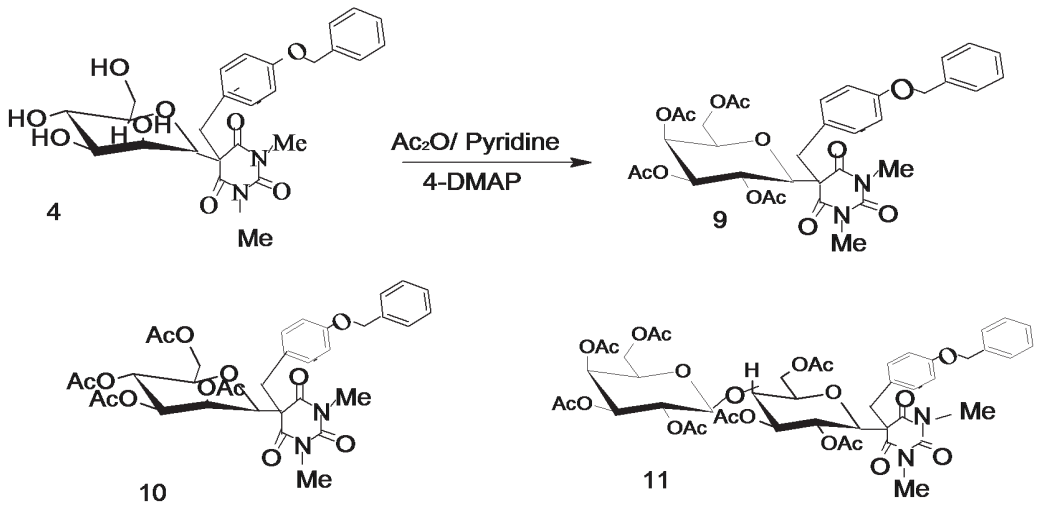


Scheme 1

The reaction of the sodium salts 3,5 and 7 with 4-benzyloxybenzyl chloride at 50-60^{0}C gave a complex mixture believed to be composed of isomers of the sugar and breakdown products. It was far superior to conduct the reaction at room temperature and in the presence of $NaHCO_3$ in DMSO which gave yields of between 50-60% of the expected β-C-glycosidic product 4, 6 and 8.

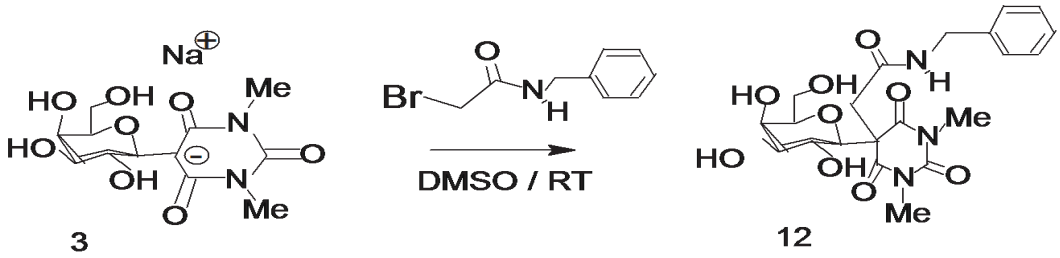
Characterization of the C-glycosides.

The 400 MHz spectra of the products were informative as they revealed the AB coupling system of the benzyl ring and multiplet for the phenyl ring. The sugar barbiturate moiety showed the usual $J_{1,2}$ 10Hz coupling indicative of the β conformation. In addition, the signal for the benzylic CH_2 near the barbituric moiety showed that they are not magnetically equivalent. This suggests that the above structures correspond with the expected product. The compounds were further characterized as the acetates (9,10 & 11) to verify their structures as shown in Scheme 2 using acetic anhydride and pyridine containing a catalytic amount of 4-dimethylaminopyridine in near quantitative yield.

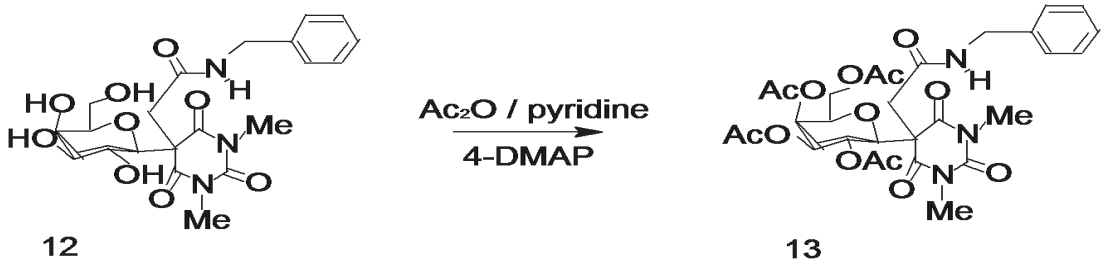


Scheme 2

Finally, bromo acetamide was investigated as electrophile in the alkylation of galactose barbiturate. The amide bond is one of the most useful connections to attach a carbohydrate moiety to a core or dendrimer (de Andrade *et al.,* 2012). As a simple test, the *N*-benzyl bromoacetamide was used as the electrophile. This worked well under our conditions to furnish the amide 12 which was fully characterized as the β- alkylation product as its per-acetate 13. No other isomers were detected during the alkylation of galactose barbituric salt with this electrophile. This is exemplified in scheme 3 and 4, respectively.



Scheme 3



Scheme 4

Conclusion

The simple reactions of the galactosyl, mannosyl and lactosyl barbiturates with 4-benzyloxybenzyl chloride and the more robust α-halo amide with galactose barbiturate as electrophiles were accomplished and characterised by high field nuclear magnetic resonance spectroscopy, mass spectrometry and elemental analyses. The alkylation of these salts proceeds well with the electrophiles and are routes to novel C-glycosyl lectin binders.

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