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SYNTHESIS OF MONO AND DI DIGOXIGENIN AND STROPHANTHIDIN CARDIAC GLYCOSIDES

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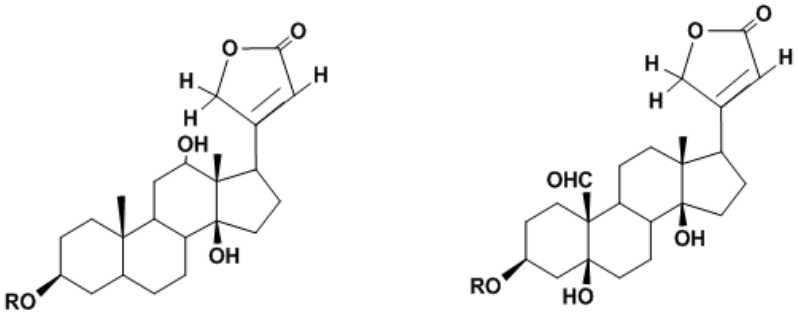
Abstract: Cardiac glycosides are the efficacious groups of naturally occurring compounds that influences the vital blood pumping mechanism. They are constituted of two parts i.e. steroidal aglycon part and the glycon. The glycon part may contain a monosaccharide or di to oligosaccharide. In this present paper we have made glycosyl donars of glucose, mannose, galactose and gentiobiose a disaccharide, glycosyl donars of these mono and disaccaharide were prepared, further they were glycosidically linked to two cardenolides i.e. digoxigenin and strophanthidin by TMS-OTF method. The structure of synthetically prepared six monoglycosides and two diglycosides of digoxigenin and strophanthidin were confirmed by ^{1}HNMR



Digoxigenin **Strophanthidin**

Keywords: Cardiac glycosides, cytotoxic, carcinoma, digoxigenin, strophanthidin.

1. Introduction

The significance of cardiac drugs is growing day by day[1], the major pharmacological effect of these compounds, which are still largely used in clinic derives from inhibition of the plasma membrane Na^{+}/K^{+} ATPase[2]. The cardiac glycosides are well known for improving the

myocardial contractility in the treatment of congestive heart failure[3] and arrhythmia[4]. Since human cancer cells tend to express particular isoforms of the subunits that build up the Na+/ K+ ATPase[5], they may be especially sensitive to the cytotoxic effect of cardiac glycosides[6] on carcinoma and leukemia cells[7]. For synthesis of Cardiac glycosides various avenues are being probed to develop expeditious methods for the stereo controlled construction of these compounds. Besides duration of action, there are other secondary, but important therapeutic parameters that have to be considered[8]. The compounds must be water soluble, orally acceptable, and must be unable to cross the blood brain barrier and thus be free of central effects[9]. Moreover certain other points that have to be kept in mind while carrying out any type of synthesis, is the availability of the raw materials (i.e. starting material) and reagents, the final output (i.e. the yield) and the easy addition and removal of the protecting groups. The synthesis of cardiac glycosides has been beset with difficulties because of the labile nature of C-14 hydroxyl genin which is easily removed by acids to give anhydrogenins[10] and the presence of methanolic alkali leads to the formation of isogenins[11] in these compounds. The synthesis of the glycon portion of these glycosides is also not easy due to high sensitivity of the sugar moieties, for masked within a given hexose are many steric, electronic, spatial, orientational, conformational and reactivity features that differ from one diastereomer to another. The union of a glycosyl donor and an glycosyl acceptor presents major challenges[12] such as (a) stereoselectivity for 1,2-cis or 1,2-trans glycosidic bonds: (b) site-selectivity traditionally achievable by selective O-protecting strategy in the acceptor: (c) protection and deprotection of hydroxy groups in donor and acceptor molecules (d) structure specificity (e) formation of a specific type of glycosidic bond: (f) sequential assembly of oligosaccharides. Amongst the various biologically active cardiac glycosides digoxigenin and strophanthidin constitute the aglycon part of the glycoside and here in the present paper we have synthesized mono and diglycosides of digoxigenin and strophanthidin as biologically cardiac glycosides

2. Materials and methods

All solvents used in the synthesis of cardiac glycosides were of analytical grade and were purified and dried according to standard procedures. Organic solvents were dried over anhydrous sodium sulphate and all the compounds were dried in a high vacuum over P_2O_5 before use. Pyridine and acetic anhydride were distilled over a direct flame before use. THF was dried over sodium and distilled over LAH. Chloroform was distilled over anhydrous $CaCl_2$. Dichloromethane was refluxed over P_2O_5 and stored over 4 Å molecular sieves. Acetonitrile was dried and distilled over CaH_2. K_2CO_3 was dried by heating to approximately 400°C in a muffle furnace and was then cooled to room temperature in a desiccators under vaccum over P_2O_5. The 4 Å molecular sieves were activated and cooled to room temperature as K_2CO_3. All the glycosidation reactions were carried out under an atmosphere of N_2. Sugars and the genins were used as supplied. All the evaporation were carried out in an electrically heated Boeitus micromelting point apparatus. Column chromatographies were carried over silica gel (60-120 mesh) using organic solvents like hexane, ethyl acetate, chloroform and methanol. ^{1}H NMR & ^{13}C NMR spectrum were recorded in $CDCl_3$ at Bruker AM 300 FT NMR. Optical rotations were determined on a Perkin-Elmer 241 Polarimeters, Polarimeter Autopol 3 Jasco DIP 180 digital polarimeters at ambient temperature. Chemicals and reagents purchased from sigma Aldrich.

1,2,3,4,6-Penta-*O*-acetyl-glucopyranoside (2) (scheme 1)

Conventional acetylation of glucose (200 mg,

1.10 mmol) was done by dissolving it a mixture of pyridine:aceticanhydride (30 ml:15 ml), which yielded **2.** Column chromatography of the residue, after removing the volatiles under reduced pressure, gave **2** (180 mg) as a crystalline compound.

Anal. Calcd. for $C_{16}H_{22}O_{11}$: C, 49.22 % , H, 5.69%
Found: C, 49.11% , H, 5.65%

2,3,4,6-Tetra-*O*-acetyl-glucopyranose (3) (scheme 1)

To a stirred solution of ethylenediamine (0.003 ml) in THF added glacial acetic acid (0.003 ml). Then the compound **2** (100 mgs, 0.253 mmol) dissolved in THF (3 ml) was added to this reaction mixture which was stirred for 24 hr at room temperature. Evaporation of the reaction mixture under reduced pressure yielded **3.** Column chromatography of the residue gave the tetra acetate **3** (75 mg) as a crystalline compound.

^{1}HNMR: δ4.74 (d, 1H, *J* = **8**.1 Hz, H-1), δ4.11 (m, 1H, H-5), δ5.53 (t, 1H, *J* = 9.6 Hz, H-2), δ5.24 (t, 1H, J=9.3 Hz, H-4), δ5.02 (m, 3H, H-6a & H-6b, H-3), δ5.43 (d, lH, J=3.7Hz, H-l), δ2.01, 2.03, 2.08, 2.09 (s, 12H, -4 OAc)
Anal. Calcd. for $C_{14}H_{20}O_{10}$: C, 48.26 % ; H, 5.79%
Found : C, 48.23% ; H, 5.81%

1,2,3,4,6-Penta-*O*-acetyl-mannopyranoside (6) (scheme 1)

Mannose (200 mg, 1.10 mmol) was acetylated by dissolving it in a mixture of pyridine: aceticanhydride (30 ml : 15 ml) which yielded **6**. Column chromatography of the residue gave **6** (180 mg) as a crystalline compound.
Anal. Calcd. for $C_{16}H_{20}O_{11}$. C, 49.22 % ; H, 5.69%
Found : C, 49.21% ; H, 5.68%

2,3,4,6-Tetra-*O*-acetyl-mannopyranose (7) (scheme 1)

To a stirred solution of ethylenediamine (0.003 ml) and glacial acetic acid (0.003 ml) was added compound **6** (100 mg, 0.253 mmol) dissolved in THF (3 ml). The reaction mixture was stirred for 24 hrs at room temperature. Evaporation of the reaction mixture under reduced pressure yielded **7**. Column chromatography of the residue gave the tetra acetate **7** (75 mg) as a crystalline compound.

^{1}HNMR: δ4.14 (lH, H5), δ4.21 (dd, lH, H3, *J* = 4.8, 9.3 Hz), δ4.28 (dd, lH, H4, *J* = 10.1, 9.2 Hz), δ4.97 (s, lH, H-l), δ5.30 (m, 2H, H-6 & H-6b), δ5.40 (d, lH, Hl, *J* = 3 Hz), δ5.43 (d, lH, H-2, J = 3.3Hz), δ1.99, 2.04, 2.09, 2.15 (4s, 12H, 4OAc).
Anal. Calcd. for $C_{14}H_{20}O_{10}$: C, 48.26 % ; H, 5.79%
Found . C, 48.19% ; H, 5.75%

1,2,3,4,6-Penta-*O*-acetyl-galactopyranoside (10) (scheme 1)

Conventional acetylation of galactose (200 mg, 1.10 mmol) was done by dissolving it in a sloution containing pyridine:aceticanhydride (30 ml : 15 ml) which yielded **10**. Column chromatography of the residue gave **10** (180 mg) as a crystalline compound.
Anal. Calcd. for $C_{16}H_{22}O_{11}$: C, 49.22 % ; H, 5.69%
Found : C, 49.19% ; H, 5.65%

2,3,4,6-Tetra-*O*-acetyl-galactopyranose (11) (scheme 1)

To a stirred solution of ethylenediamine (0.003 ml) in THF was added glacial acetic acid (0.003 m1). Then the compound **10** (100 mg, 0.253 mmol) dissolved in THF (3 ml) was added to this reaction mixture which was stirred for 24 hrs at room temperature. Evaporation of the reaction mixture under reduced pressure and column chromatography of the residue gave the hepta acetate **11** (75 mg) as a crystalline

compound.

[1]HNMR: δ4.07 (m, 1H, H-5), δ4.10 (dd, 1H, J = 3.1, 6.6 Hz, H-4), δ4.15 (d, lH, J = 8.1 Hz, H-l), δ4.35 (t, lH, J = 6.6 Hz, H-2), δ5.17 (dd, lH, J = 3.6, 7.2, H-3), δ5.42 (m, 2H, H-6a & H-6b), δ5.50 (d, lH, J = 3.3 Hz, H-l), δ1.98, 2.04, 2.09, 2.141 (s, 12H, 4 OAc)
Anal. Calcd. for $C_{14}H_{20}O_{10}$: C, 48.26 % ; H, 5.79%
Found : C, 48.11% ; H, 5.75%

Strophanthidin 3-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside) (13) (scheme 2)

To a solution of **3** (75 mg, 0.214 mmol) in dry CH_2Cl_2 was added anhydrous K_2CO_3 (31.20 mg) and trichloroacetonitrile (0.214 ml). The suspension was stirred at room temperature for 48hours under N_2. The reaction mixture was filtered through celite and washed with CH_2C1_2 (10 ml). The combined filtrate was concentrated under reduced pressure. The oily residue of trichloroacetimidate donor **4,** without further purification was used immediately for glycosidation. A solution of **strophanthidin** (40 mg) and **4** (35 mg, 0.877 mmol) were dissolved in dry CH_2C1_2 (16.33 ml). TMS-OTf (0.011 ml, 0.677 meq) was added and the reaction mixture was stirred at 0°C for 1 hour under an atmosphere of N_2. The reaction mixture was filtered through celite and the residue washed with CH_2C1_2. Combined filtrates and washings were concentrated and purified by column chromatography which gave **13** (20 mg, 57%) as a crystalline product: m.p. l65-166°C, $[\alpha]_D^{25}$ +113° (c, .02 $CHCl_3$), Lit.m.p.166- 167°C, $[\alpha]_D^{25}$ +l 16° (c, .02 $CHCl_3$).

[1]HNMR: δ4.11 (m, lH, H-5'), δ5.44 (t, lH, *J* = 9.9 Hz, H-2'), δ4.82 (d, lH, *J* = 18.1 Hz, H-21), δ4.96 (d, 1H, *J*= 18.1 Hz, H-21), δ5.11 (m, 3H, H-3, H-6a', H-6b'), δ5.73 (d, lH, *J* = 8.1 Hz, H-1'), δ5.89 (s, 1 H, H-22), δ0.88 (s, 3H,-$^{18}CH_3$), δ4.26 (m, 1H, H-3), δ9.99 (s, 1H, CHO), δ5.17 (t, lH, H-4, *J* = 9.3 Hz), δ2.15, 2.05, 2.11, 2.10 (s, 12H, -4 OAc)
Anal. Calcd. for $C_{37}H_{50}O_{15}$: C, 60.46 % , H, 6.87%
Found : C, 60.39% ; H, 6.83%

Digoxigenin 3-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside) (16) (scheme 3)

A solution of **digoxigenin** (40 mg) and **4** (35 mg, 0.87 mmol) were dissolved in dry CH_2C1_2 (16.33 ml). TMS-OTf (0.011 ml, 0.677 meq) was added and the reaction mixture was stirred at 0°C for 1 hr under an atmosphere of N_2. The reaction mixture was filtered through celite and the residue washed with CH_2C1_2. Combined filtrate and washing were concentrated and purified by column chromatography using Hex-EtOAc which gave **16** (16 mg, 48%) as a crystalline product. m.p, 195-198°C, $[\alpha]_D^{25}$ -33.3°(c, 0.76, EtOH), Lit-m.p l94-199°C, $[\alpha]_D^{25}$ -33° (c, 0.76, EtOH).

[1]HNMR: δ5.12 (m, 3H, H-3', H-6a', H-6b'), δ5.47 (t, lH, J = 9.9 Hz, H-2), δ4.89 (d,1H, J = 18.1 Hz, H-21), δ4.78 (d, 1H, J = 18.1 Hz, H-21), δ4.24 (m, 1 H, H-3), δ5.72 (d, 1H, J = 8 4,H-1'), 53.33 (m, 1 H, H-12), δ5.22 (t, 1H, H-4', J = 9.3 Hz), δ4.11 (m, 1H, H-5), δ5.93 (s, 1H, H-22), 0.96 (s, 3H,^{19}CH3), 0.81 (s, 3H, -$^{18}CH_3$), δ2.03, 2.081, 2.08 and 2.09 (s, 12H, -4 OAc)
Anal. Calcd. for $C_{37}H_{52}O_{14}$: C, 61.64 % ; H, 7.28%
Found : C, 61.61% ; H, 7.29%

Strophanthidin 3-*O* -(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranoside) (14) (scheme2)

To a solution of **7** (75mg, 0.214 mmol) in dry CH_2Cl_2 was added anhydrous K_2CO_3 (31.20 mg) and trichloroacetonitrile (0.214 ml). Workup of the reaction mixture as in **13** gave an oily residue of trichloroacetimidate donor **8** which was used immediately for glycosidation. **A** solution of **strophanthidin (40** mg) and **8** (35 mg, 0.877 mmol) in dry CH_2C1_2 (16.33 ml). TMS-OTf (0.011 ml, 0.677 meq) was added

and the reaction mixture was stirred at 0°C for 1 hr under an atmosphere of N_2. The reaction mixture was filtered through celite and the residue washed with CH_2C1_2. Combined filtrate and washings were concentrated and purified by column chromatography to give **14** (14mg, 40%) as a crystalline product. m.p.259-263°C, $[\alpha]_D^{25}$ +78.7° (c. 0.41 MeOH).

^{1}HNMR: δ4.08 (m, lH, H-5'), δ5.48 (d, lH, *J* = 2.7 Hz, H-2'), δ4.27 (dd, lH, *J* = 4.8, 9.3 Hz, H-3'), δ4.96 (d, lH, *J* = 18.l Hz, H-21), δ4.79 (d, lH, *J* = 8.l Hz, H-21), δ4.16 (dd, lH, *J* = 9.1, 10.2 Hz, H-4'), δ5.29 (m, 2H, H-6a' & H-6b'), δ4.08 (m, lH, H-3), δ5.86 (s, lH, H-22), δ5.89 (s, lH, H-r), δ9.98 (s, lH, CHO), δ0.86 (s, 3H, -$^{18}CH_3$), δ2.01, 2.06, 2.04, 2.16 (s,12H, -4 OAc)
Anal. Calcd for $C_{37}H_{50}O_{15}$: C, 60.46 % ; H, 6.87% Found : C, 60.34% , H, 6.75%

Digoxigenin 3-O-(2,3,4,6-Tetra-*O*-acetyl -α-D mannopyranoside) (17) (scheme 3)

A solution of **digoxigenin** (40 mg) and **8 (35** mg, 0.87 mmol) were dissolved in dry CH_2C1_2 (16.33 ml). TMS-OTf (0.011ml, 0.677meq) was added and the reaction mixture was stirred at 0°C for 1 hr under an atmosphere of N_2. The reaction mixture was filtered through celite and the residue washed with CH_2Cl_2. Combined filtrate and washings were concentrated and purified by column chromatography which gave **17** (12 mg, 35%) as a crystalline product, m.p. 277-279°C, $[\alpha]_D^{25}$ -48.7° (c, 0.40 MeOH).

^{1}HNMR: δ4.07 (m, lH, H-5'), δ5.49 (d, lH, *J* = 2.4 Hz, H-2'), δ4.31 (dd, 1H, *J* = 4.8, 9.3 Hz, H-3'), δ5.99 (s, lH, H-r), δ4.16 (dd, lH, *J* = 10.2, 9.3 Hz, H-4), δ5.33 (m, 2H, H6a & H-6b'), δ4.09 (m, lH, H-3), δ5.86 (s, lH, H-22), δ4.95 (d, lH, *J* = 18.1 Hz, H-21), δ4.79 (d, 1H, *J* = 18.1 Hz, H-21), δ0.80 (s, 3H, $^{18}CH_3$), 0.94 (s, 3H, $^{19}CH_3$), δ3.49 (m, 1 H, H-12), δ2.01, 2.06, 2.10, 2.14 (s, 12H, -4 OAc)
Anal. Calcd. for $C_{37}H_{52}O_{14}$: C, 61.64 % ; H, 7.28%
Found : C, 61.59% , H, 7.25%

Strophanthidin 3-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranoside) (15) (scheme 2)

To a solution of **11** (75 mg, 0.214 mmol) in dry CH_2Cl_2 was added anhydrous K_2CO_3 (31.20 mg) and trichloroacetonitrile (0.214 ml). The suspension was stirred and worked up as in **18** and **22** and the oily residue of trichloroacetimidate donor **12** without further purification was used immediately for glycosidation. A solution of strophanthidin (40mg) and **12** (35 mg, 0.877 mmol) were dissolved in dry CH_2C1_2 (16.33 ml). TMS-OTf (0.011 ml, 0.677 meq) was added and the reaction mixture was stirred at 0°C for 1 hr under an atmosphere of N_2. The reaction mixture was filtered through celite and the residue washed with CH_2C1_2. Combined filtrate and washings were concentrated and purified by column chromatography which gave **15** (13 mg, 39%) as a crystalline product. m.p. l76-177°C, $[\alpha]_D^{25}$ +18.7, (c, 0.5 MeOH).

^{1}HNMR: δ4.10 (m, 2H, H5', H-3), δ4.27 (m, lH, H-4'), δ5.18 (t, lH, *J* = 9.0 Hz, H-2'), δ4.81 (d, lH, *J* = 18.3 Hz, H-21), δ4.91 (d, lH, *J* = 18.3 Hz, H-21), δ5.10 (dd, 1H, *J* = 3.3, 6.9 Hz, H-3'), δ5.52 (m, 2H, H-6a' & H-6b'), δ5.69 (d, lH, *J* = 8.4 Hz, H-l'), δ5.89 (s, lH, H-22), δ0.88 (s, 3H, $^{18}CH_3$), δ9.99 (s, 1H,CHO), δ1.96, 2.04, 2.09, 2.15(s,12H, -4 OAc)
FAB MS: 734[M1$^+$, 758 [M+Na+H]$^+$,796[M+Na+K]$^+$, 405[Genin+H]$^+$, 33l[M-Genin]$^+$
Anal. Calcd. for $C_{37}H_{50}O_{15}$: C, 60.46 % , H, 6.87%
Found : C, 60.40% ; H, 6.81%

Digoxigenin 3-*O*-(2,3,4,6-Tetra-*O*-acctyl-β-D-galactopyranoside (18) (scheme3)

A solution of **digoxigenin** (40 mg) and **12 (**35 mg, 0.87) were dissolved in dry CH_2C1_2 (16.33 ml). TMS-OTf (0.011 ml, 0.677 meq) was added and the reaction mixture was stirred at 0°C for 1 hr under an atmosphere of N_2. The reaction

mixture was filtered through celite and the residue washed with CH_2C1_2. Combined filtrate and washings were concentrated and purified by column chromatography which gave **18** (12 mg, 35%) as a crystalline product. m.p. 261-263°C, $[\alpha]_D^{25}$ -55.5° (c, 1.0 MeOH).

[1]HNMR(300MHz): δ4.14 (m, 2H, H-5' & H-3), δ4.34 (m, lH, H-4'), δ5.47 (t, lH, *J* = 9.1 Hz, H-2'), δ4.87 (d, lH, *J* = 18.1 Hz, H-21), δ4.92 (d, lH, *J* = 18.1 Hz, H-21), δ5.18 (dd, lH, *J* = 6.9, 3.9 Hz, H-3'), δ5.44 (m, 2H, H-6a' & H-6b'), δ4.14 (m, lH, H-3), δ5.94 (s, lH, H-22), δ0.81 (s, 3H, $^{18}CH_3$), δ0.96 (s, 3H, $^{19}CH_3$), δ3.39 (m, lH, H-12), δ5.49 (d, lH, H-l', *J* = 8.4 Hz), δ1.98, 2.04, 2.09, 2.14 (s, 12H, -4 OAc).
FAB MS: 721$[M+H]^+$, 743 $[M+Na]^+$, 782$[M+Na+K]^+$, 391$[Gemn+H]^+$, 331$[M-Genin]^+$
Anal. Calcd. for $C_{37}H_{52}O_{14}$: C, 61.64 % , H, 7.28%
Found : C, 61.60% ; H, 7.27%

Acetyl-2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl(l→6)-2,3,4-tri-*O*-acetyl-D-glucopyranoside (20) (Scheme-4)
Conventional acetylation of D-gentiobiose (100 mg) with Pyr : Ac_2O (2.5 ml : 2.5 ml) gave and column chromatography of the residue gave **20** (80 mg) as an anomeric mixture on the basis of [1]H NMR.

[1]HNMR: δ6.41 (d, 1H, H-1, *J* = 3.1 Hz, S-1), δ5.68 (d, 1H, H1', *J* = 8.1 Hz, S-1), δ5.19 (dd, 2H, H-2", S2, H-2', Sl, *J* = 9.3, 8.1 Hz), δ5.45 (dd, lH, H-3, *J* = 9.9 Hz, S-l), δ4.99 (bt, lH, H4', *J* = 9.9 Hz, S-l), δ3.80 (m, lH, H-5', S-l), δ3.95 (dd, lH, H-5, *J* = 11, 5.4 Hz, S-1), δ3.58 (dd, 1H, H-6b', *J* = 11.1, 7.8 Hz, S-1) δ4.53 (d, 1H, H-1', *J* = 6.9 Hz, S-2), δ5.19 (2t, 2H, H-3, *J* = 9.6 Hz & H-4", *J* = 9.3 Hz, S2) δ3.68 (m, lH, H-5,S2), δ4.14 (dd, lH, H6b", *J* = 12.3, 4.8 Hz, S-2), δ4.24 (dd, lH, H-6a", *J* = 12.3, 7.8 Hz, S-2), δ2.06, 2.02, 2.02, 2.03, 2.07, 2.09, 211 (7s, 24H, -8OAc)
Anal. Calcd. for $C_{28}H_{38}0_{19}$: C, 49.55% ; H, 5.65%
Found : C, 49.49 % ; H, 5.60%

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl(l→6)-2,3,4-tri-O-acetyl-D-glucopyranose (21) (Scheme-4)
To a stirred solution of ethylenediamine (0.0048 ml) in THF was added glacial acetic acid (0.0044 ml) followed by the compound **20** (40 mg) dissolved in THF (1.5 ml). The reaction mixture was stirred for 24 hr at room temperature. Evaporation of the reaction mixture under reduced pressure yielded **21**. Column chromatography of the residue gave the Hepta acetate **21** (32 mg) as a mixture of anomers as seen in its [1]H NMR spectrum

[1]HNMR: δ5.43 (d, lH, H1', *J* = 3.1 Hz, S-l), δ4.89 (d, 1H, H1, *J* = 8.4 Hz, S-l), δ4.97 (dd, 2H, S2, H2, *J* = 9.6, 8.1 Hz, S-l) δ5.41 (t, lH, H3', *J* = 9.6 Hz, S-l) δ5.12, (bt, lH, H-4', *J* = 9.9 Hz, S-l) δ3.87 (m, lH, H-5', S-l) δ3.41 (dd, lH, H-6a, *J* = 11.0, 5.4 Hz, S-l) δ3.60 (dd, lH, H6b, *J* = 11.0, 7.8 Hz, S-l) δ4.58 (d, lH, Hl", *J* = 7.8 Hz, S-2), δ5.18 (2t, 2H, H3", *J* = 9.6 Hz, & H4", *J* = 9.3 Hz, S-2), δ3.71 (m, lH, H5", S-2) δ4.28 (dd, lH, H6a", *J* = 12.1, 7.5 Hz, S-2), δ4.17 (dd, lH, H6b", *J* = 12.1, 4.5 Hz, S-2) δ2.01, 2.03, 2.04, 2.07, 2.08, 2.10 (6s, 21H, -7OAc).
Anal. Calcd. for $C_{26}H_{36}O_{18}$: C, 28.94% ; H, 7.96%
Found : C, 28.83 % ; H, 7.93%

Strophanthidin 3-*O*-[2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl(l→6)-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside] (23) (Scheme-5)
To a solution of **21**(100 mg, 0.125 mmol) in dry CH_2C1_2 was added anhydrous K_2CO_3 (15.34 mg) and trichloroactonitrile (0.125 ml). The reaction mixture was stirred at room temperature for 48 hr under an atmosphere of N_2. The mixture was filtered over celite, washed with CH_2Cl_2 (10ml) and the filtrate concentrated

1

under reduced pressure and the oily residue of gentibiosyltrichloroacetimidate **22** was used immediately for glycosidation. **A** solution of **strophanthidin** (40 mg) and **22** (50 mg, 0.62 mmol) were dissolved in dry CH_2Cl_2 (**10** ml) and the mixture was brought to 0°C under an atmosphere of N_2. TMS-OTf (0.12 ml, 0.71 meq) dissolved in CH_2C1_2 was added to the solution and the reaction mixture stirred for 1 hr at 0°C. The mixture was filtered through celite and the residue washed with CH_2C1_2. Combined filtrate and washings were concentrated and column chromatography of the residue gave **23** (20 mg, 57%), m.p.119- 121°C, $[\alpha]_D^{25}$ +23.8° (c, 0.4 $CHCl_3$).

^{1}HNMR: δ3.54 (dd, 1H, H-6b', *J* = 11.1, 7.5 Hz, S-l), δ3.65 (m, 1H, H-5", S2), δ3.95 (dd, 1H, H6a', *J* = 11.1, 4.8 Hz, S-1), δ3.79 (m, 1H, H5, S-1), δ4.28 (dd, 1H, H6a', *J* = 12.3, 7.8 Hz, S-2), δ4.09 (dd, lH, H6b", *J* = 12.3, 5.4 Hz, S-2), δ4.52 (d, 1H, *J* = 7.1 Hz, Hr, S-2), δ4.82 (d, lH, *J* = 18.2 Hz, H-21), δ4.97 (d, 1H, *J* = 18.2 Hz, H-21), δ5.06 (bt, 1H, H-4', *J* = 9.3 Hz, S-1), δ4.09 (m, 1H, H-3), δ5.11 (dd, 2H, H2', Sl & H2", S-2, *J* = 9.6, 8.1 Hz), δ5.19 (2t, 2H, H-3", *J* = 9.6 Hz & H-4", *J* = 9.1 Hz, S-2), δ5.41 (bt, 1H, *J* = 9.9 Hz, H-3', S-l), δ5.69 (d, 1H, H-1', *J* = 8.1 Hz, S-1), δ5.89 (s, 1H, H-22), δ0.88 (s, 3H, $^{18}CH_3$), δ9.89 (s, 1H, CHO)

FABMS: 1022$[M]^+$, 1045$[M+Na]^+$, 692[Genin+Sl]$^+$, 427[Genin+Na]$^+$, 443[Genin+K]$^+$, 466 [Genin+K+Na+H]$^+$, 33l[M$^+$-Genin+S-l]$^+$, 659[S-l+S-2+Na]$^+$.
Anal. Calcd. For $C_{49}H_{66}O_{23}$: C, 57.50% ; H, 6.51%
Found : C, 56.99% ; H,6.49%

Digoxigenin3-*O*-[2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosy(l→6)-2,3,4-tri-O-acetyl-β-D-glucopyranoside] (24) (Scheme-6)

A solution of **digoxigenin** (40 mg) and **22** (50 mg, 0.62 mmol) in dry CH_2C1_2 (10 ml) was brought to 0°C under an atmosphere of N_2. TMS-OTf (0.12ml, 0.71meq) dissolved in CH_2C1_2 was added to the solution and the reaction mixture stirred for lhr at 0°C. The mixture was filtered through celite and the residue washed with CH_2C1_2. Combined filtrate and washings were concentrated and column chromatography of the residue gave **24** (16 mg, 48%) m.p. 135-137°C, $[\alpha]_D^{25}$ -44.5° (c, 1.5 MeOH).

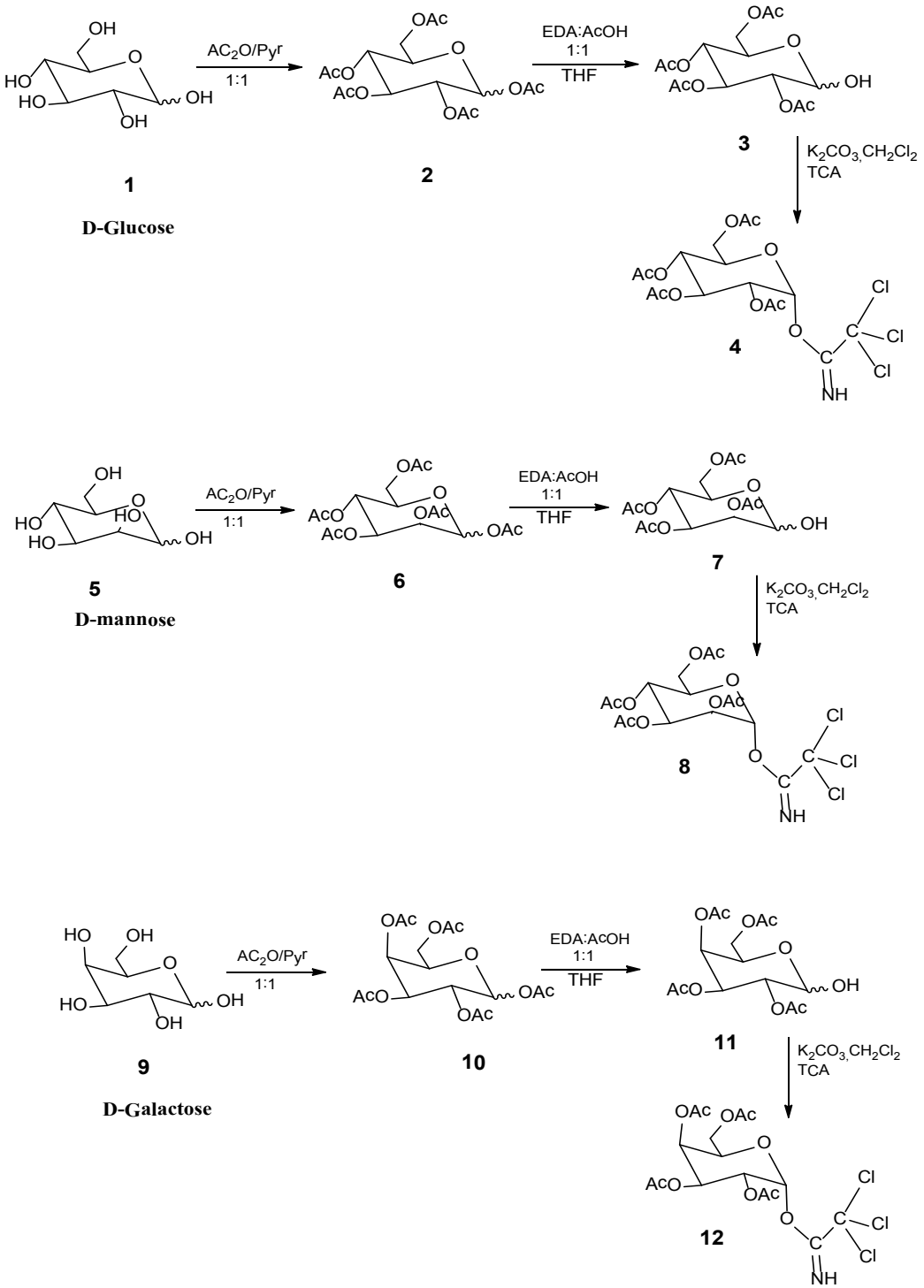
^{1}HNMR: δ3.48 (m, lH, H12), δ3.54 (dd, lH, H6b', *J* = 12.1, 7.5 Hz, S-l), δ3.65 (m, lH, H5", S2), δ3.76 (m, lH, H5', S-l), δ3.94 (dd, lH, H6a', *J* = 12.1, 4.5 Hz, S-l), δ4.06 (m, lH, H3), δ4.09 (dd, lH, H6b", *J* = 12.0, 4.5 Hz, S-2), δ4.28 (dd, lH, H6a", *J* = 12.0, 7.8 Hz, S-2), δ4.52 (d, 1H, *J* = 7.1 Hz, H1", S-2), δ4.83 (d, 1H, *J* = 18.1 Hz, H21), δ4.91(d, lH, *J* = 18.1 Hz, H21), δ5.02 (bt, lH, H4', *J* = 9.1 Hz, S-l), δ5.10 (dd, 2H, H-2', Sl, H2", S2, *J* = 9.6, 8.1 Hz), δ5.18 (2t, 2H, H3", *J* = 9.6 Hz, & H4", *J* = 9.4 Hz, S-2), δ5.41 (bt, lH, *J* = 9.6 Hz, H3',S-l), δ5.66 (d, lH, Hl', *J* = 8.4 Hz, S-l), δ5.93 (s, lH, H22), δ0.80 (s, 3H, $^{18}CH_3$), δ0.96 (s, 3H, $^{19}CH_3$), δ1.99, 2.01, 2.03, 2.04, 2.07, 2.08, 2.10 (7s, 21H, -7OAc).

FABMS:1008$[M]^+$,103l$[M+Na]^+$,1047$[M+K]^+$, 759[Genin+Sl+Na]$^+$, 677[Genin+S-l-H]$^+$, 391 [Genin+H]$^+$, 331[M$^+$-Genin+S-l], 659[S-l+S-2+Na]$^+$.
Anal. Calcd. for $C_{49}H_{68}O_{22}$: C, 58.31% ; H, 6.80%
Found : C,58.28 % ; H, 6.79%

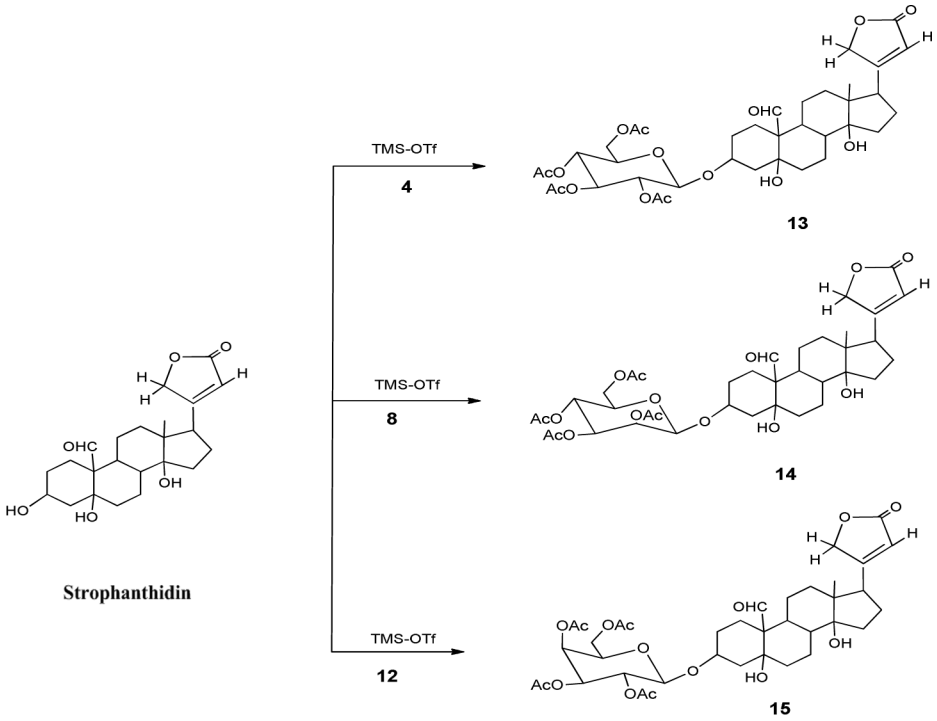
3. RESULTS AND DISCUSSIONS

3.1. Synthesis of mono glycosides of strophanthin and digoxigenin

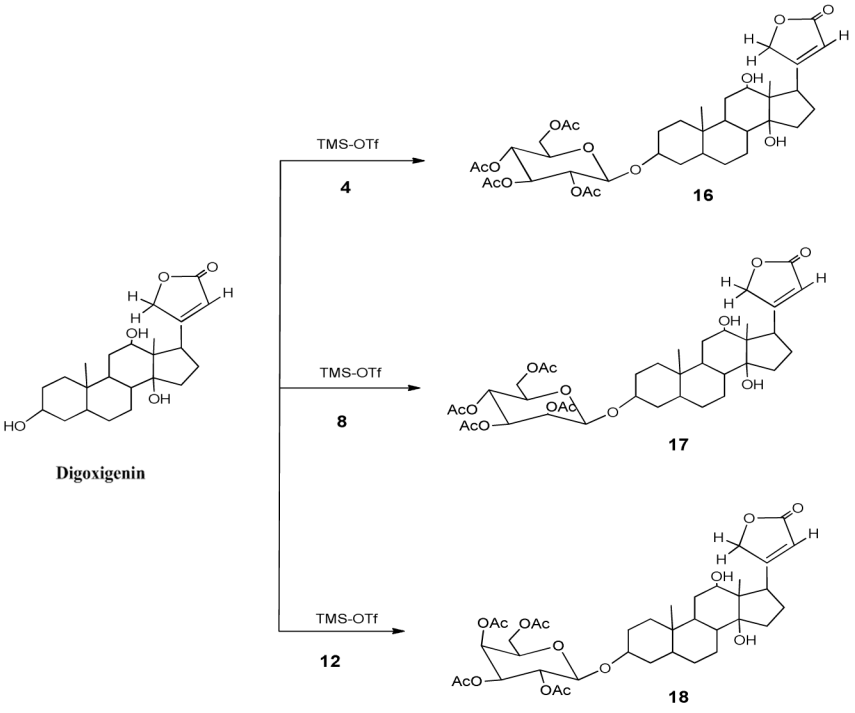
For the purpose of synthesizing the mono glycosides of strophanthidin[13] and digoxigenin[13] our first synthetic strategy was targeted at the synthesis of the per acetylated derivatives **2**, **6** and **10** (Scheme-1) of glucose (**1**), mannose (5) and galactose (9) by treating them with a 2:1 mixture of pyridine and acetic anhydride. For the


AC2O/Pyr
1:1
EDA:AcOH
1:1
THF
K2CO3,CH2Cl2
TCA
1
D-Glucose
2
3
4
5
D-mannose
6
7
8
9
D-Galactose
10
11
12

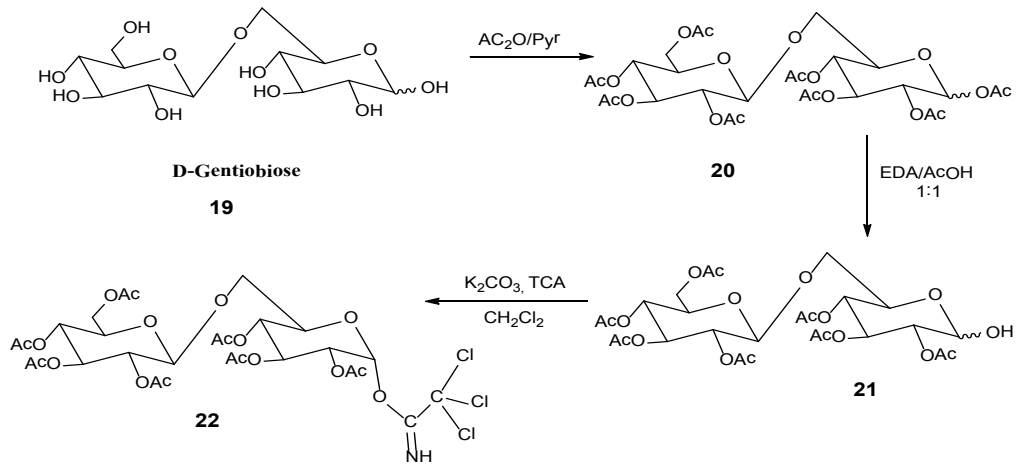
SCHEME-1



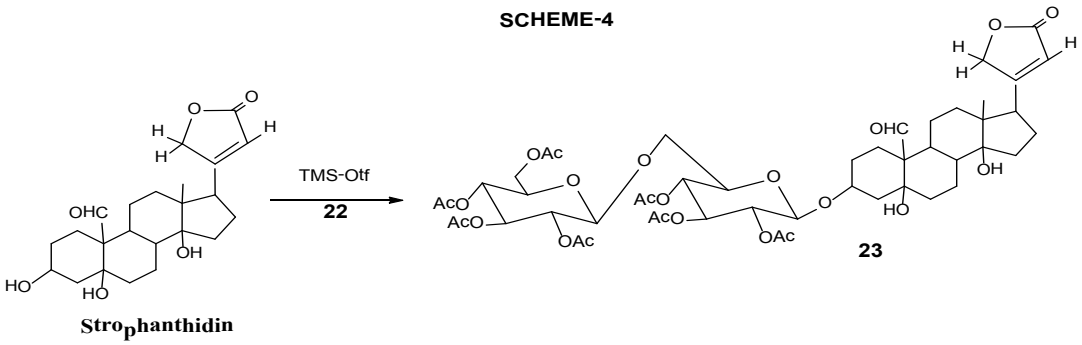
SCHEME-2



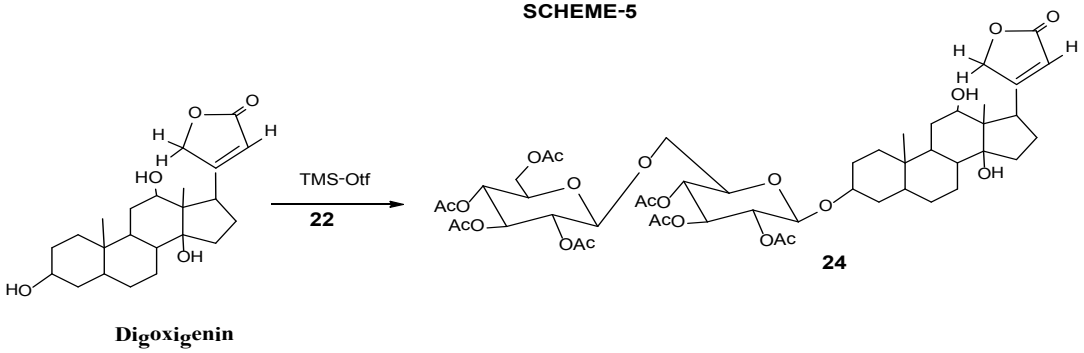
SCHEME-3



SCHEME-4



SCHEME-5



SCHEME-6

conversion of these per acetylated compounds into the active donors **4, 8** and **12** (Scheme-1) the compounds **2, 6** and **10** were subjected to selective deacetylation at the anomeric position using equimolar quantities of ethylenediamine and acetic acid followed by their treatment with TCA in the presence of anhyd. K_2CO_3 For the synthesis of the desired cardiac glycosides each of the active donors **4, 8** and **12** were condensed with equimolar quantities of the acceptors strophanthidin and digoxigenin respectively using TMS-OTf[14-17] as Lewis acid catalyst. The glycosidic linkage in the cardiac glycoside **13** Strophanthidin 3-O-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside) (Scheme-2) m.p. 165-166°C $[\alpha]_D^{25}$ +11.3° (lit.[18] m.p. 166-167°C, $[\alpha]_D^{25}$ +11.6°), was confirmed to be 1→ 3 by the ^{1}H NMR spectrum which showed a downfield shift to δ5.73 of the anomeric proton signal of the sugar moiety along with the downfield shift of the C-3 proton of strophanthidin to δ4.26. Moreover, the nature of the glycosidic linkage between the sugar moiety and the genin in **13** was shown to be β by the ^{1}H NMR spectrum as the downfield shifted doublet of the anomeric proton of the sugar moiety had a large coupling constant of 8.1 Hz. All the physical constants (m.p, $[\alpha]_D^{25}$) of this glycoside i.e. Strophanthidin 3-O-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside) was in accordance with the reported values. Appearence of the highest mass ion peaks at 796, 758 and 734 corresponding to $[M+Na+K]^+$, $[M+Na+H]$ and $[M]^+$ in the FAB mass spectrum of **15** Strophanthidin 3-O-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranoside) (Scheme-2) m.p. 176-177°C $[\alpha]_D^{25}$ +18.7°, confirmed its molecular formula $C_{37}H_{50}O_{15}$ Similarly the molecular weight of **18** Digoxigenin 3-O-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranoside) (Scheme-3) m.p. 261-263°C $[\alpha]_D^{25}$ -55.5° was confirmed to be 720 by the appearance of the highest mass ion peaks at m/z 782$[M+Na+K]^+$, 743 $[M+Na]^+$ and 721$[M+H]^+$ in the FAB mass spectrum of **18** The nature of the glycosidic linkages in **15** Strophanthidin 3-O-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranoside), **16** Digoxigenin 3-O-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside) (Scheme-3) m.p. 195-198°C $[\alpha]_D^{25}$ -33.3° (lit.[17] m.p. 194-199°C, $[\alpha]_D^{25}$ -33°) and **18** Digoxigenin 3-O-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranoside) was shown to be 1→3 β linked by the ^{1}H NMR spectra of these cardiac glycosides, which contained downfield shifted doublets at **δ**5.69, 5.72 and 5.49 respectively with large coupling constants (8.4Hz) for the anomeric proton of the sugar moieties, along with downfield shifted signals for the C-3 methine proton of the genins. Where as the nature of the glycosidic linkage in **14** Strophanthidin 3-O-(2,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranoside) (Scheme-2) m.p. 259- 263°C $[\alpha]_D^{25}$ +78.7° and **17** Digoxigenin 3-O-(2,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranoside) (Scheme-3) m.p. 277-279°C $[\alpha]_D^{25}$ -48.7° respectively was shown to be α in their respective ^{1}H NMR spectra which consisted of downfield shifted signals for H-3 of the genin moiety at **δ**4.08 and 4.09 respectively, along with downfield shifted anomeric signals (J=negligible) at **δ**5.86 and 5.99 respectively for the anomeric protons. The possibility of the sugar being linked to the hydroxyl group at C-12 of digoxigenin in the cardiac glycosides **16, 17** and **18** was ruled out as the multiplet for H-12 remained unshifted in the ^{1}H NMR spectra of **16, 17** and **18.**

3.2. Synthesis of di glycosides of strophanthidin and digoxigenin

The synthetic endeavors carried out to synthesize the di-glycosides of strophanthidin and digoxigenin required gentiobiose (Scheme-4) to be acetylated with acetic anhydirde and pyridine to obtain **20** which was then deacetylated at the anomeric position by E.D.A. and AcOH (1:1). **21** was then converted into the active trichloroacetimidate donor **22** by T.C.A. which was then linked to strophanthidin

and digoxigenin by TMS-OTf method of glycosidation to get **23** Strophanthidin 3-O-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl(l→6)-2,3,4-Tri-*O*-acetyl-β-D-glucopyranoside) (Scheme-5) m.p. 119-121 °C $[\alpha]_D^{25}$ +23.8° and **24** Digoxigenin 3-O-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl(l→6)-2,3,4-tri-O-acetyl-β-D-glucopyranoside) (Scheme-**6**) m.p. 135-137°C **$[\alpha]_D^{25}$ -44.5°.** The FAB mass spectrum of **23** contained the highest mass ion peaks at m/z 1045 and **1022** corresponding to $[M+Na]^+$ and $[M]^+$ thereby confirming the molecular formula $C_{49}H_{66}O_{23}$ of **23.** The ^{1}H NMR spectrum of **23** contained a downfield shifted multiplet at **δ**4.09 for H-3 of strophanthidin along with a downfield shifted doublet at **δ**5.69 for the anomeric proton of the S-l glucosyl moiety in gentiobiose indicating the presence of a 1→3 glycosidic linkage in **23.** The large value of the coupling constant (8.1 Hz) for the anomeric doublet confirmed the linkage in **23** to be β type. The FAB mass spectrum of **24** showed the highest mass ion peaks at m/z 1031 as [M+Na]+ and 1008 as $[M]^+$ confirming the molecular weight as 1008 for molecular formula $C_{49}H_{68}O_{22}$, while the glycosidic linkage between the sugar and the aglycon was ascertained as a 1→3 β glycosidic linkage. Which was based on the downfield shifted signal of H-3 of the genin and a downfield shifted doublet at δ5.66 with a large coupling constant (8.4Hz) for the anomeric proton of S-l, whereas H-12 of the genin remained unshifted and appeared as a multiplet at δ 3.48, confirming the involvement of 3- hydroxyl of the genin.

4. Conclusion

With the endeavor to synthesize some novel cardiac glycosides for cardiotonic activity we have synthesized six monoglycosides comprising of digoxigenin and strophanthidin and two diglycosides of the same aglycon with gentiobiose using TMS-OTF method. The structures of synthetically prepared compounds were confirmed by C, H analysis and ^{1}HNMR.

5. Acknowledgement

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