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Synthesis of the tetrasaccharide glycoside moiety of Solaradixine and rapid NMR-based structure verification using the program CASPER

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ABSTRACT

The major glycoalkaloid in the roots of *Solanum laciniatum* is Solaradixine having the branched tetrasaccharide β-D-Glc*p*-(1→2)-β-D-Glc*p*-(1→3)[α-L-Rha*p*-(1→2)]-β-D-Gal*p* linked to O3 of the steroidal alkaloid Solasodine. We herein describe the synthesis of the methyl glycoside of the tetrasaccharide using a super-armed disaccharide as a donor molecule. A 2-(naphthyl)methyl protecting group was used in the synthesis of the donor since it was tolerant to a wide range of reaction conditions. The 6-*O*-benzylated-hexa-*O*-*tert*-butyldimethylsilyl-protected β-D-Glc*p*-(1→2)-β-D-Glc*p*-SEt donor, which avoided 1,6-anydro formation, was successfully glycosylated at O3 of a galactoside acceptor molecule. However, subsequent glycosylation at O2 by a rhamnosyl donor was unsuccessful and instead a suitably protected α-L-Rha*p*-(1→2)-β-D-Gal*p*-OMe disaccharide was used as the acceptor molecule together with a super-armed β-D-Glc*p*-(1→2)-β-D-Glc*p*-SEt donor in the glycosylation reaction, to give a tetrasaccharide in a yield of 55%, which after deprotection resulted in the target molecule, the structure of which was verified by the NMR chemical shift prediction program CASPER.

1. Introduction

Saponins are glycosides with one or more sugars in their structure and the aglycone is either a triterpene, a steroid or a steroidal alkaloid.[1–5] The oligosaccharide portion is typically made from a limited number of different monosaccharides, viz., D-glucose, D-galactose, D-glucuronic acid, D-xylose, L-arabinose or L-rhamnose, and linked to O3 of the aglycone (sapogenin);[6] the number of oligosaccharides possible to form is still very large. These amphiphilic compounds are secondary metabolites widely spread in the plant kingdom and several have been found in marine animals.[7,8] Saponins are biologically active compounds that can be used in pharmacological applications owing to their cytotoxic effects as well as their immunostimulatory, anti-inflammatory, antiviral and hypoglycemic activities among others.[9–15]

Members of the plant family Solanaceae include, *inter alia*, eggplant, tomato and potato.[16] Glycoalkaloids, as well as oligosaccharides and saponins, from these plants have anticarcinogenic properties inhibiting cell growth both in culture and in vivo. *Solanum laciniatum* produces glycoalkaloids that exert various biological activities. Several different glycoalkaloids are found in the leaves, berries and roots of this species. Besides trisaccharide-containing saponins, larger glycoalkaloids are present in the roots, having Solasodine (Fig. 1) as the sapogenin, viz., Solashabanine having a tetrasaccharide with a terminal β-(1→6)-linked glucosyl residue, Solaradixine (**1b**) containing a tetrasaccharide but instead with a terminal β-(1→2)-linked glucosyl residue (Fig. 1) and Solaridine comprising a pentasaccharide with a terminal β-(1→6)-linked glucosyl residue attached to the β-(1→2)-linked glucosyl residue of Solaradixine.[17]

Synthesis of saponins and oligosaccharide glycosides[18–20] thereof facilitates corroboration of their structures, chemical modification of functional groups to study their impact and effects in biological environments as well as enabling sufficient amounts of material for in vitro as well as in vivo studies.[21–27] Herein we describe, to the best of our knowledge, for the first time, the synthesis of the branched tetrasaccharide β-D-Glc*p*-(1→2)-β-D-Glc*p*-(1→3)[α-L-Rha*p*-(1→2)]-β-D-Gal*p*-OMe (**1**) corresponding to the glycoside moiety of the glycoalkaloid Solaradixine from *S. laciniatum* and its rapid structure verification based on unassigned ^{1}H and ^{13}C NMR spectra as implemented in the computer program CASPER.

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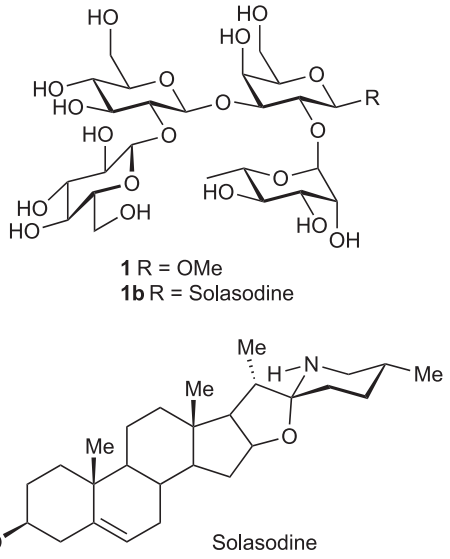
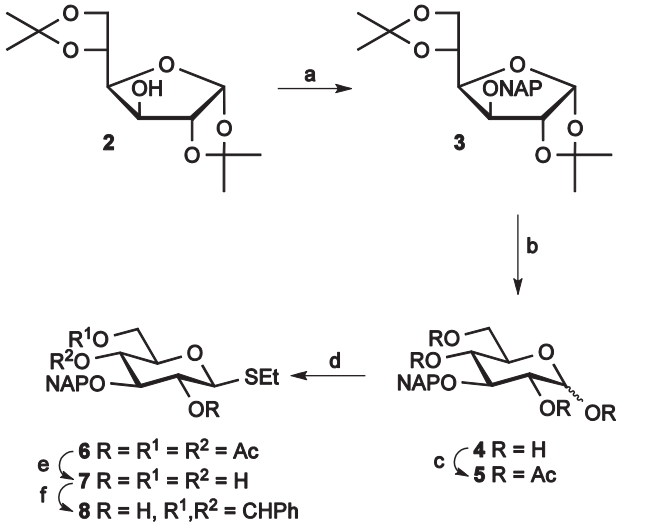


Fig. 1. Schematic of methyl α-L-rhamnopyranosyl-(1→2)[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3)]-β-D-galactopyranoside (**1**), the glycoalkaloid Solaradixine (**1b**) and Solasodine.



Scheme 1. Formation of thioglycoside acceptor **8**. Reagents and conditions: (a) NAPBr, NaH, DMF, 3 h, 90%; (b) 2 M HCl, EtOH, 80 °C, 4 h, 80%; (c) Ac_2O, pyridine, 90%; (d) $BF_3{\cdot}Et_2O$, EtSH, $CHCl_3$, 3 h, 68%; (e) 1 M NaOMe, MeOH, 16 h, 88%; (f) $PhCH(OMe)_2$, CSA, CH_3CN, 2 h, 55 °C, 82%.

2. Results and discussion

2.1. Tetrasaccharide synthesis

In the synthesis of the branched target tetrasaccharide **1** three glycosidic linkages are to be formed. Realizing that β-(1→2)-linked glucosyl-containing disaccharides have been utilized as donors in glycosylation reactions with very high β-anomeric selectivity,[28] we envisioned that this could be a suitable donor, especially since super-armed monosaccharide donors had been used for glycosylation reactions at O4 of a protected glucosyl acceptor,[29] i.e., at a secondary carbon atom. If this strategy were to be chosen, a suitably protected methyl galactopyranoside could be used as an acceptor, followed by a second glycosylation reaction by a rhamnosyl donor molecule or one could use a preformed disaccharide acceptor, which had been made by glycosylation of a rhamnosyl donor to O2 of the methyl galactopyranoside.

Formation of a suitably protected monosaccharide, which has a thioethyl group to be used as the leaving group in the glycosylation reaction and that can be employed as the acceptor molecule in order to form the β-(1→2)-linked donor disaccharide utilized a 2-naphthylmethyl (NAP) protecting group at O3, which was introduced to diisopropylidene glucose (**2**) using sodium hydride and NAPBr (Scheme 1) to give compound **3**.

The NAP protecting group was chosen since it can be selectively removed in the presence of *O*-benzyl groups and that it is stable to strong acids and bases.[30] Subsequent removal of the isopropylidene groups by hydrochloric acid treatment gave **4**, which was *O*-acetylated using pyridine and acetic anhydride to give **5**. The donor functionality was introduced by treatment with $BF_3{\cdot}Et_2O$ and ethanethiol to give **6**, which was *O*-deacetylated under Zémplen conditions[31] resulting in **7**, followed by protection of O4 and O6 with a 4,6-*O*-benzylidnene group using benzaldehyde dimethyl acetal and camphor sulfonic acid for its installation, to give compound **8**. The different transformations from **2** to **8**[32] were carried out in good to excellent yields ranging between 68% and 90% leading to a selectively protected monosaccharide having both donor and acceptor functionalities in place.

Formation of the β-(1→2)-linkage between the two orthogonally protected glucosyl donors[33] employed a glycosylation strategy in which a trimethylsilyl triflate-promoted coupling[28] of the known trichloroacetimidate donor **9**[34] with acceptor **8** gave disaccharide **10** in 75% yield (Scheme 2).

It has been noted by Boons and co-workers that thioethyl migration from an acceptor to a donor molecule can occur.[35] Herein we were able to suppress this side-reaction by increasing the polarity of the solvent used, i.e., by employing a mixture of DCM and toluene in equal proportions. The 4,6-*O*-benzylidene group was regioselectively opened in a good yield (68%) using $BH_3{\cdot}NMe_3$ and $AlCl_3$ in THF[36] to give **11** with a 6-*O*-benzyl group, suitably positioned to suppress 1,6-anhydro formation in the subsequent glycosylation reaction (vide infra). Deprotection of **11** using DDQ cleaved selectively the NAP group[37] and furnished diol **12** in 85% yield, followed by O-deacetylation using sodium methoxide in methanol to give compound **13** in 91% yield. The super-armed disaccharide donor **14** was then obtained in 82% yield from **13**, using *tert*-butyldimethylsilyl triflate as the reagent for the O-silylation reaction.[29,38] The arming effect of the donor as a result of the steric crowding due to the *tert*-butyldimethylsilyl (TBS) protecting groups[39–42] resulted in a conformational switch from standard $^4C_1$ chair conformation for the pyranose rings of the two glucosyl residues to $^3S_1$ skew-like conformations, based on, *inter alia*, $J_{H1,H2}$=5.1 Hz, $J_{H1',H2'}$=5.8 Hz, $J_{H2,H3}$<2 Hz and $J_{H2',H3'}$<2 Hz, in good agreement with NMR data for the corresponding hepta-TBDMS-protected glucosyl-containing disaccharide donor,[28] and a 3,4-di-*O*-TBDPS-protected glucosyl donor.[40]

With the disaccharide donor in hand the known monosaccharide methyl 4,6-*O*-benzylidene-α-D-galactopyranoside **15**[43] was regioselectively protected at O2 by an *O*-acetyl group using acetic anhydride and pyridine to give compound **16**, albeit in a low yield (Scheme 3).

The β-(1→3)-linkage between the disaccharide donor **14** and the relatively unreactive acceptor **16** was facilitated by using Tf_2O-DMDS[44] as the promoter system. Several activators were tried including NIS/TfOH, NIS/AgOTf, NIS/TMSOTf and MeOTf at different temperatures, but only Tf_2O-DPS and Tf_2O-BSP gave similar results to Tf_2O-DMDS, which was judged to be the best reagent, in the presence of the sterically hindered base DTBMP. The reaction resulted in the formation of a mixture of trisaccharides, *viz.*, the