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Strategies for Desymmetrising Trehalose to Synthesise Trehalose Glycolipids

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The desymmetrisation and regioselective protection of trehalose are major challenges in the chemical synthesis of biologically essential trehalose glycolipids. We reviewed the literature on desymmetrising trehalose to synthesise trehalose glycolipids and highlighted an efficient regioselective 6-O-phosphorylation method that can be applied to synthesise asymmetric trehalose glycolipids.

Trehalose glycolipids were first extracted from Mycobacterium tuberculosis in 1933 by Anderson and Newman, who described them as “the neutral fat from the human tubercle bacillus which is soluble in cold acetone is, therefore, not glycerides but a complex ester of fatty acid with trehalose”; however, the structure had not yet been defined. Later, trehalose glycolipids were primarily isolated from the cell wall of Mycobacterium, Corynebacterium, and Norcadia, and were recognised as an essential part of the pathogenesis of these bacteria. Trehalose glycolipids are believed to protect the bacteria from harsh conditions in hosts and environments and are essential for the virulence of these bacteria. Therefore, the bioactivity and potential of trehalose glycolipids have attracted increasing attention. In contrast to the results obtained in vitro, recent studies have indicated that not all trehalose glycolipids are essential for the virulence of Mycobacterium tuberculosis in vivo. Thus, the roles of these compounds remain undetermined and were hypothesised to be species specific. Moreover, previous studies reported that trehalose glycolipids modulate the immune response of the host; however, the mechanism remains unclear. Additional studies are required to determine the functions and SARs of these compounds and to use them as vaccine adjuvants. Trehalose glycolipids are relatively rare in nature, being observed only in mycobacteria and related groups; consequently, synthesising trehalose glycolipids enables the mechanisms of diseases such as tuberculosis and diphtheria to be understood. Excluding 3-O-α,α-glucopyranosyl α,β-trehalose isolated from Streptococcus faecalis, only α,α-trehalose glycolipids, such as maradolipid, trehalose monomycolate, lipooligosaccharide, 2,3-diacylα,α-trehalose (DAT), Ac₂SGL, sulfolipid-I, polyacyltrehalose (PAT) are observed in nature (Figure 1).

Among them, 1 and 2 and their analogues are known to present antitumor activities. Although the stereoselective construction of the 1,1,α,α-glycosyl linkage and the regioselective esterifications of trehalose can be achieved enzymatically, the high costs and inaccessibility to unnatural trehalose glycolipids make chemical synthesis a more practical method for obtaining these target molecules. However, trehalose is symmetric, and trehalose glycolipids are typically asymmetric. Therefore, synthesis strategies can be divided into two major categories. The first strategy begins with two glucose units, but forming a 1,1,α,α-glycosyl linkage is much more difficult than forming typical glycosidic bonds. The second strategy begins with trehalose, but differentiating the two identical glucose units is considerably challenging.

Synthesis from glucose

To form the 1,1,α,α-linkage of trehalose, as shown in Table 1, some researchers have begun by using glucosides to furnish the trehalose derivative, producing high yields; however, the selectivity for the 1,1,α,α-linkage through the self-coupling of entries 1–5 or through donor-acceptor coupling (Entry 6) was poor to...
Table 1. Synthesis of trehalose from glucosides.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Glucoside</th>
<th>Promoter</th>
<th>T (°C)</th>
<th>Yield (α:αβ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10, R= H</td>
<td>TfO</td>
<td>-70</td>
<td>97% (2:1)</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>TMSOTf</td>
<td>rt</td>
<td>64% (1:2)</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>TMSOTf</td>
<td>rt</td>
<td>76% (3:1)</td>
</tr>
<tr>
<td>4</td>
<td>R= SO3NMe3</td>
<td>BF3-EtO</td>
<td>0</td>
<td>77% (1:2)</td>
</tr>
<tr>
<td>5</td>
<td>12, R= TMS</td>
<td>TMSOTf</td>
<td>rt</td>
<td>96% (1:1)</td>
</tr>
<tr>
<td>6</td>
<td>10 and 13, R= TCA**</td>
<td>TMSOTf</td>
<td>rt</td>
<td>91% (1:1)</td>
</tr>
</tbody>
</table>

*4 A molecular sieve was used in reaction. ** TCA = trichloroacetimidate.

Moderate. Bertozzi et al. applied the intramolecular aglycone delivery (IAD) method to solve the selectivity problem of the 1,1-α,α-linkage (Scheme 1). The 3,4-dimethoxybenzyl (DMB) group on glucoside was oxidized using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to yield a benzylic cation, which was trapped by the C2 alcohol of thioglycoside. Acetal was further activated using a promoter (MeOTf, 2,6-di-tert-butyl-4-methylpyridine) to afford the asymmetric trehalose as a single stereoisomer from in a yield of 68%-88%. Using this method, the protecting group patterns of trehalose derivatives can be determined prior to coupling. Although additional synthetic steps are required to prepare both of the building blocks (the first bearing an anomeric DMB group and the second bearing a C2 alcohol) and other protecting groups according to the suitability of protecting group patterns, challenging trehalose desymmetrization can be obviated.

Scheme 1. 1,1-α,α-glycosidic bond construction via the intramolecular aglycone delivery pathway.

Scheme 2. Synthesis of 6,6'-diester trehalose from unprotected 9 in two steps. Oleic acid was activated using O-

Synthesis from trehalose

Many chemists use commercially available trehalose even if the regioselective protection between the two glucose units is a major problem. By taking advantage of the more reactive primary alcohol, the O6 and O6' of trehalose (9) can be temporarily protected by the trityl groups, and the remaining hydroxyl groups can be benzylated. Symmetric esterification from was achieved under appropriate conditions to furnish 21 with an overall yield of 48%-60% (Scheme 2). The slight difference in the reactivity of the C6 and the C6' hydroxyl groups was the key for the desymmetric etherification of 20 to 22. The benzyl groups enabled trehalose 20 to dissolve easily in organic solvents; however, they rendered the coupling of longer alkyl or lipid chains at the O6 or O6' and the subsequent debenzylation reaction difficult. Thus, protecting-group-free strategies are optimal although they provide low yields. Grindley et al. achieved the synthesis of asymmetric 6,6'-trehalose diester 23 in an yield of 52%, even though the reaction time is considerably long.

In addition, 4,6,4',6'-dibenzylidenated trehalose 24 was prepared to synthesise DAT (4), AcSGL (5), sulfolipid (6), and their derivatives. Dibenzylidenated trehalose 24 was selectively palmitoylated using palmitoyl chloride and DMAP in pyridine within 25 h, and 2,3-silylation was subsequently conducted to form 25 in a yield of 27% in two steps (Scheme 3). The second acylation was achieved using a microwave-DCC procedure to obtain the product in 15 min in a yield of 68%. After desilylation, C2'-sulfation was conducted using SO2-pyridine in dry DMF, and after debenzylation, the target molecule 26 was obtained in a total yield of 9%. Moreover, this strategy was used to synthesise AcSGL 5 from 25 in a yield of 19%. Because four lipid chains are present on the C2, C3, C6, and C6' of sulfolipid, 6, 4,6,4'-debenzylation must be performed under an acidic condition. After 6,6'-diesterification, desilylation, and sulfation,
the synthetic 6 was obtained from 25 in a 2% yield after more than 6 steps. 16

To solve the poor solubility problem of free trehalose (9) in organic solvents, fully trimethylsilylated trehalose 27 is typically prepared by combining trimethylsilyl chloride with triethylamine or pyridine. 17-21 To benefit 6,6'-diester-hexamethyldisilazyl trehalose formation, both the O6 and the O6' TMS groups were selectively removed using acetic acid at 8–10 °C or using K2CO3 at 0 °C in pyridine. Because of the instability of the TMS groups under acidic conditions, the TMS groups were easily removed after esterification to obtain diesters 23, 28a, and 28b (Scheme 4). 17,18 In addition, per-O-trimethylsilylated trehalose 27 was used in 4,6,4',6'-dibenzylidenation to yield 29, of which the TMS ethers are commonly transformed further into other more stable protecting groups such as benzyl or p-methoxymethyl groups before subsequent glycosylation, esterification, or sulfation reactions in an acidic or basic environment. Kulkarni et al. recently achieved the regioselective openings of one of the two benzylidene rings by carefully controlling the solvent, temperature, and amounts of reagents. 19 From 27, the diester 30 was obtained in a 36% yield in six steps. This method was recently used to prepare a 4-azido derivative from 29. 19 Beau et al. modified 29 by conducting O3,3'-dibenzylation and selective O2',6'- tert-butyldimethylsilylation, followed by O3-debenzylation. Stepwise esterification at O2 and O3 was followed by removing the two benzylidene groups and the installation of the palmitolmyl groups at O6 and O6'. Finally, C2'-sulfated 31a and 31b were respectively prepared from 27 in 20% and 8% yields in 10 steps. 20,21

In our study, we conducted per-O-trimethylsilylation by using 1,1,1,3,3,3-hexamethylidisilazane (HMDMS) as the silylating reagent and TMSOTf as the catalyst, thus obviating the production of a multiequivalent amount of solid ammonium salts. Thus, without requiring further purification, 4,6,4',6'-dibenzyl, O3,3'-benzyl, O2, O2'-acetylation, and O2, O2'-desilylation could be conducted from trehalose (9) directly to provide 32 or 33 in a one-pot manner in yields of 91% and 88%, respectively. 22 Furthermore, we recently focused on the biologically essential trehalose 6-phosphate (35), 23 and reported a simple two-step procedure for preparing 35 from trehalose (9). 24 Only mono-O6-phosphorylation could occur in the

Scheme 3. Synthesis of diacylated trehalose sulphates 26. a) CH2(CH2)3COCl, DMAP, pyridine; b) TIPSCI2, pyridine; c) fatty acid, DCC, pyridine, μW; d) n-Bu4NF in THF; e) SO3-pyridine, DMF; f) 1.7% H2SO4/w/CHCl3/Methanol.

Scheme 4. Various trehalose glycolipids synthesized from the per-O- trimethysilylated trehalose (9). a) K2CO3 or diluted AcOH; b) fatty acid, DCC or EDC, DMAP, pyridine; repeat; c) Dowex-H'; d) aromatic aldehyde, cat. acid, DCM, then TBAF; e) PMBCl, NaH, DMF; f) DIBAL, toluene; g) repeat f and b; h) PhCHO, cat. acid, Et3SiH, DCM/McCN; i) TBDMSOTf, 2,6-lutidine, DCM; j) HCOONa, Pd/C, MeOH; k) n-Bu4NF in THF; l) SO3-pyridine, pyridine.

trimethylsilylated-trehalose 27 mediated reaction to produce 34 in a 93% recovered yield. No O6, O6'-diphosphosphate side product could be isolated even when the reaction time was prolonged or an increased amount of diphenylphosphate chloride was used. The fully deprotected 35 was efficiently produced in a quantitative yield after the diphenyl group was hydrogenolysed and the TMS groups were hydrolysed after the treatment with PtO2 and H2 in 75% aqueous ethanol (Scheme 5). To extend the usefulness of the asymmetric trehalose derivative 34 in synthesising trehalose glycolipids, we removed the diphenylphosphate group by using sodium nitrite under a microwave-assisted condition. The compound 34 was desilylated by treating it with acidic resin and consecutively per-O-benzylated it using 2,4,6-tris(benzyloxy)-1,3,5-triazine (TriBOT) under an acidic condition (71%). The diphenylphosphate group was subsequently removed to yield the asymmetric C6-alcohol 37 (75%) in three steps. Moreover, the functional group transformation from 6-O-phosphate-4,6-O-benzylidene 38 to 39 occurred smoothly in a 96% yield under the aforementioned condition; thus, asymmetric 39 and 40, trehalose derivatives containing only one 4,6-O-benzylidene group, could be prepared easily.

In summary, as indicated in Scheme 5, the method enabled trehalose 6-phosphate 35 to be prepared in only two steps in an excellent overall yield and enabled the synthetically highly useful asymmetric 36–40 to be prepared after the
**Scheme 5.** Desymmetrisation of trehalose through regioselective O6-phosphorylation. a) cat. TMSOTf, HMDS, DCM; b) PhCHO, cat. TMSOTf; c) PhCHO, cat. TMSOTf; d) n-BuNF or Ac2O, cat. TMSOTf; e) (PhO)2POCl, pyridine; f) H2, PtO2, 75% EtOH; g) Amberlite 120 H+, MeOH; h) TriBOT/TiOH 1,4-dioxane; i) NaNO3, DMF, µW; j) NaH, BuBr, DMF.

diphenylphosphate group was removed and the protecting groups were modified further. Moreover, we observed that the strong inductive effect of the phosphate group on the C6 can create a difference in reactivity between the two glucose units of 34. We are currently using this method to differentiate each hydroxyl group of trehalose (9). We believe that desymmetrisation of trehalose from 34 constitutes a new approach to obtaining asymmetric trehalose glycolipids, such as trehalose monomycolate, trehalose tetraester, trehalose pentaester, and sulfolipid-I, thus facilitating additional mechanism studies and further vaccine development.

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**Notes and references**