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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.[1] Later, trehalose glycolipids were primarily isolated from the cell wall of *Mycobacterium*, *Corynebacterium*, and *Norcadia*,[2] and were recognised as an essential part of the pathogenesis of these bacteria.[3] Trehalose glycolipids are believed to protect the bacteria from harsh conditions in hosts and environments[4] 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 *Mycobaterium tuberculosis in vivo*.[5] Thus, the roles of these compounds remain undetermined and were hypothesised to be species specific.[6] Moreover, previous studies reported that trehalose glycolipids modulate the immune response of the host;[7] 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-D-glucopyranosyl α,β-trehalose isolated from *Streptococus faecalis*,[2] only α,α-trehalose glycolipids, such as maradolipid (**1**), trehalose monomycolate (**2**), lipooligosaccharide (**3**), 2,3-diacyl-α-D-trehalose (DAT) (**4**), Ac_2SGL (**5**), sulfolipid-I (**6**), and polyacyltrehalose (PAT) (**7**) are observed in nature (Figure 1). Among them, **1** and **2** and their analogues are known to present antitumor activities.[5f] 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 (**8**) 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 **9**, but differentiating the two identical glucose units is considerably challenging.

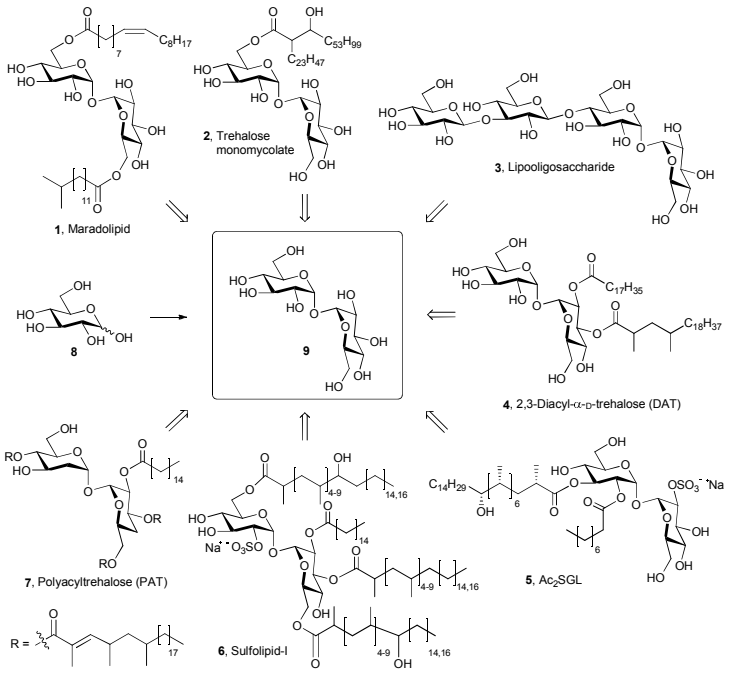
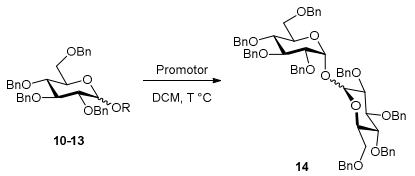


Figure 1. Some trehalose-derived natural products.

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 **10–13** to furnish the trehalose derivative **14**, producing high yields; however, the selectivity for the 1,1-α,α-linkage through the self-coupling of **10–12** (Entries 1–5) or through donor-acceptor coupling (Entry 6) was poor to

Table 1. Synthesis of trehalose**14** from glucosides.[8]



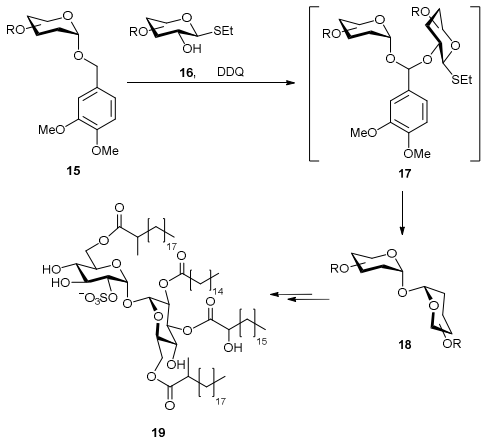
Entry	Glucoside	Promotor	T (°C)	Yield (αα:αβ)
1	**10**, R= H	Tf_2O	-70	97% (2:1)[8a]
2	**10**	TMSOTf	rt	64% (1:2)[8b]
3	**10**	TMSOTf	rt	76% (3:1)* [8c]
4	**11**, R= $SO_3^-NHMe_3^+$	BF_3-Et_2O	0	77% (1:2)[8d]
5	**12**, R= TMS	TMSOTf	rt	96% (1:1)[8e]
6	**10** and **13**, R= TCA**	TMSOTf	rt	91% (1:1)[8f]

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

moderate.[8] Bertozzi *et al.* applied the intramolecular aglycone delivery (IAD) method to solve the selectivity problem of the 1,1-α,α-linkage (Scheme 1).[9] The 3,4-dimethoxybenzyl (DMB) group on glycoside **15** was oxidised using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to yield a benzylic cation, which was trapped by the *C*2 alcohol of thioglycoside **16**. Acetal **17** was further activated using a promoter (MeOTf, 2,6-di-tert-butyl-4-methylpyridine) to afford the asymmetric trehaloside **18** as a single stereoisomer from **15** 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 *C*2 alcohol) and other protecting groups according to the suitability of protecting group patterns, challenging trehalose desymmetrisation can be obviated.

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 *O*6 and *O*6' of trehalose (**9**) can be temporarily protected by the trityl groups, and the remaining hydroxyl groups can be benzylated. Symmetric esterification from **20** was achieved under appropriate conditions to furnish **21** with an overall yield of 48%–60% (Scheme 2).[10] The slight difference in the reactivity of the *C*6 and the *C*6' hydroxyl groups was the key for the desymmetric etherification of **20** to **22**.[11] 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.[10,11] 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** from unprotected **9** in two steps.[12] Oleic acid was activated using *O*-

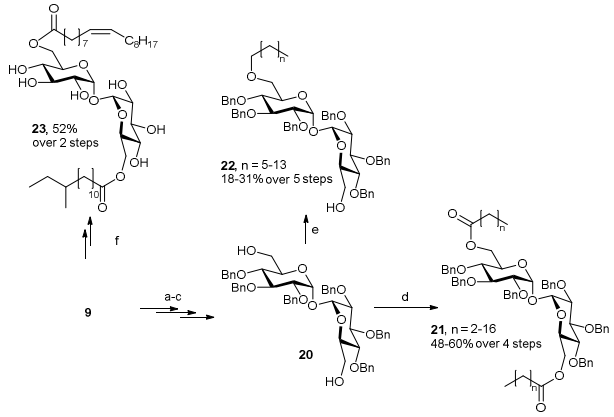


Scheme 1. 1,1-α,α-glycosidic bond construction via the intramolecular aglycone delivery pathway.

(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) and was then attached to the *O*6 of **9**. The 6-monoester was the major product, and the esterification can be repeated similarly to produce an asymmetric 6,6'-trehalose diester **23** in a 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**), Ac_2SGL (**5**), sulfolipid-I (**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).[13] The second acylation was achieved using a microwave-DCC procedure to obtain the product in 15 min in a yield of 68%. After desilylation, *C*2'-sulfation was conducted using SO_3-pyridine in dry DMF,[13,14] and after debenzylidenation, the target molecule **26** was obtained in a total yield of 9%.[13] Moreover, this strategy was used to synthesise Ac_2SGL **5** from **25** in a yield of 19%.[15] Because four lipid chains are present on the *C*2, *C*3, *C*6, and *C*6' of sulfolipid-I **6**, 4,6,4',6'-debenzylidenation must be performed under an acidic condition. After 6,6'-diesterification, desilylation, and sulfation,

Scheme 2. Synthesis of 6,6'-diester trehalose**21**, **23**, and 6-

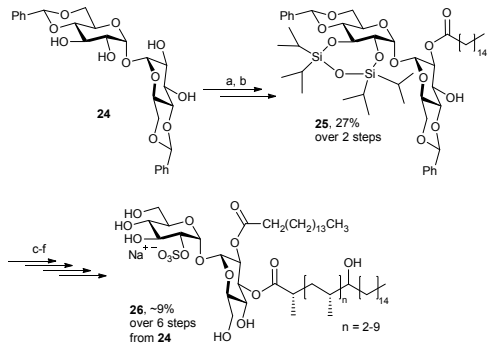


monoether trehalose **22**. a) TrCl, pyridine; b) BnBr, NaH, DMF; c) TFA/Et_3SiH or conc. HCl, DCM; d) fatty acid, EDC, DMAP, pyridine; e) alkyl halide, NaH, TBAB, DMF; f) fatty acids, TBTU, pyridine, repeat.

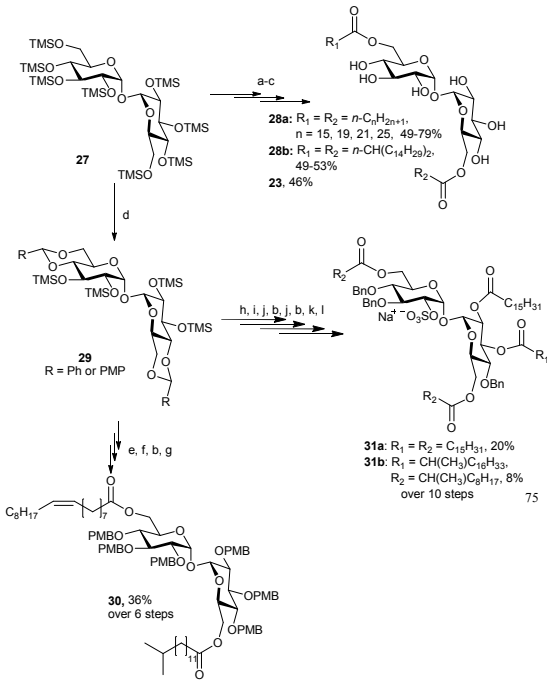
the synthetic **6** was obtained from **25** in a 2% yield after more than 6 steps.[16]

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-hexatrimethylsilyl trehalose formation, both the *O*6 and the *O*6' TMS groups were selectively removed using acetic acid at 8–10 ˚C or using K_2CO_3 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*-methoxylbenyl 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 *O*3,*O*3'-dibenzylation and selective *O*2'-*tert*-butyldimethylsilylation, followed by *O*3-debenzylation. Stepwise esterification at *O*2 and *O*3 was followed by removing the two benzylidene groups and the installation of the palmitoyl groups at *O*6 and *O*6'. Finally, *C*2'-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-hexamethyldisilazane (HMDS) 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'-dibenzylation, *O*3,*O*3'-benzylation, *O*2,*O*2'-acetylation, and *O*2,*O*2'-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-*O*6-phosphorylation could occur in the



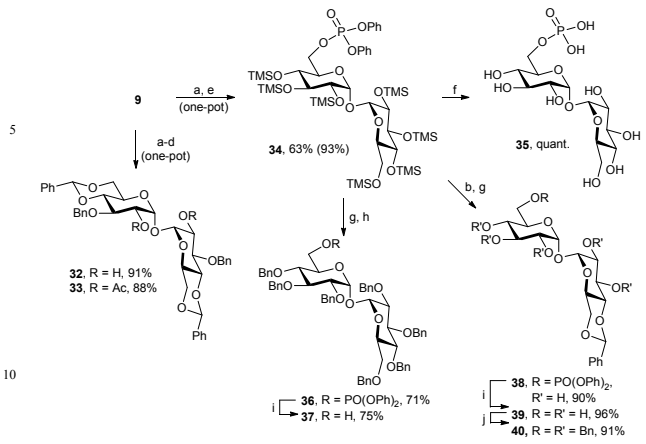
Scheme 3. Synthesis of diacylated trehalose sulphates**26**. a) $CH_3(CH_2)_{14}COCl$, DMAP, pyridine; b) $TIPSCl_2$, pyridine; c) fatty acid, DCC, pyridine, μW; d) n-Bu_4NF in THF; e) SO_3-pyridine, DMF; f) 1.7% $H_2SO_{4(aq)}$/$CHCl_3$/MeOH.



Scheme 4. Various treholose glycolipids synthesized from the per-*O*- trimethylsilylated tehalose (**9**). a) K_2CO_3 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, Et_3SiH, DCM/MeCN; i) TBDMSOTf, 2,6-lutidine, DCM; j) HCO_2NH_4, Pd/C, MeOH; k) n-Bu_4NF in THF; l) SO_3-pyridine, pyridine.

trimethylsilylated-trehalose **27** mediated reaction to produce **34** in a 93% recovered yield. No *O*6,*O*6'-diphosphate 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 PtO_2 and H_2 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 *C*6-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 treholse through regioselective *O*6-phosphorylation. a) *cat*. TMSOTf, HMDS, DCM; b) PhCHO, *cat*. TMSOTf; c) PhCHO, *cat*. TMSOTf; d) n-Bu_4NF or Ac_2O, *cat*. TMSOTf; e) $(PhO)_2POCl$, pyridine; f) H_2, PtO_2, 75% EtOH; g) Amberlite 120 H^+, MeOH; h) TriBOT/TfOH 1,4-dioxane; i) $NaNO_2$, DMF, μW; j) NaH, BnBr, 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 *C*6 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 desymmetrising 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

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