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Chain-growth polyglycosylation: synthesis of linker-equipped mannosyl oligomers

Heiko J. Schuster, Balakumar Vijayakrishnan, Benjamin G. Davis *

Department of Chemistry, University of Oxford, Chemistry Research Laboratory, Mansfield Road, OX1 3TA Oxford, United Kingdom

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ABSTRACT

Direct syntheses of acetylated poly-mannosides can be achieved in one-step starting from a fully acetylated thioglycoside mannosyl donor using a polymerization-type strategy under the correct conditions. Under conditions that allow polymer growth from non-reducing to reducing end (N→R), different acceptor alcohols can be used as the 'terminating acceptors' to install different linkers at the reducing terminus. The efficiency is dependent on substituents of the linker, its length, temperature and choice of Lewis acid activator.

1. Introduction

As part of glycoproteins, so-called 'high mannose' glycans play fundamental roles in cells such as during protein biosynthesis (e.g., modulating folding, transport, enzymatic degradation) and in interactions (e.g., mediating adhesion, signalling).[1–4] These contain oligosaccharidic mannosyl 'arms' or 'caps', often mannobioside (α-D-Man-(1→2)-D-Man) and mannotrioside (α-D-Man-(1→2)-α-D-Man-(1→2)-D-Man) that are also determinants of pathogenicity of viruses such as the human immunodeficiency virus 1 (HIV-1),[5,6] bacteria (*Mycobacterium tuberculosis*) and protozoan parasites of the genus *Leishmania*.[7] Direct synthetic access to these fragments can be more difficult since the hydroxyl at C-2 is not accessible[8–12] by regioselective glycosylations.[13] Consequently, published syntheses typically require a minimum of 6–8 steps to form the mannosidic union between two moieties, often with bulky, atom-inefficient *O*-benzyl protected disaccharides.[14–19] A low yielding enzymatic access (3%) has also been reported.[20]

Glycosyl donors with 2-*O*-acyl protection give di-/tri-oxolenium ions upon activation (Fig. 1). These can, in principle, react directly with a nucleophile to form glycoside or reaction may proceed via an orthoester intermediate.[21] Orthoesters can also be rearranged to the desired glycosides or act as glycosyl donors themselves.[22,23] Under certain circumstances, they can also open in such a fashion that the 2-*O*-acyl group is transferred to the acceptor alcohol. Both of these latter reactions are often undesired side-reactions during glycosylations leading to, for example, *O*-acetyl migration (*trans*-acetylation) from the donor to the acceptor.[24–26] Nevertheless, we considered that such orthoesters, if generated from the corresponding thioglycosides in situ, theoretically showed a strong synthetic potential for sequential glycosylation at position 2-OH given that they and corresponding tri/dioxoleniums might act as *both* donors and acceptors (electrophiles and nucleophiles). Control of these ambident glycosyl moieties to show both of these characters under the appropriate conditions, might therefore allow a possible polymerization-type manifold. In principle, such a manifold could be made to 'grow'[27] sufficiently by requiring auto-activation to allow the formation of poly-mannosides of controllable length before termination by a chosen reducing end alcohol that possesses only acceptor ability. Here, we investigate the influence of the choice of Lewis acid, donor–acceptor ratio and scope of alcohol 'terminators' in first attempts to test this novel concept of what might be termed 'chain growth'[27] polyglycosylation. In addition, an initial systematic study of parameters offers first insights into the proposed mechanism involved in the generation of an intermediate O-2 mannosyl acceptor and observed acetyl transfer to alcohol.

2. Results and discussion

2.1. Testing polymannosylation

Acetylated thioglycoside **1** was chosen as a model glycosyl donor; such and similar donors have been reported to occasionally

* Corresponding author. Tel.: +44 1865 275 652.
E-mail address: ben.davis@chem.ox.ac.uk (B.G. Davis).

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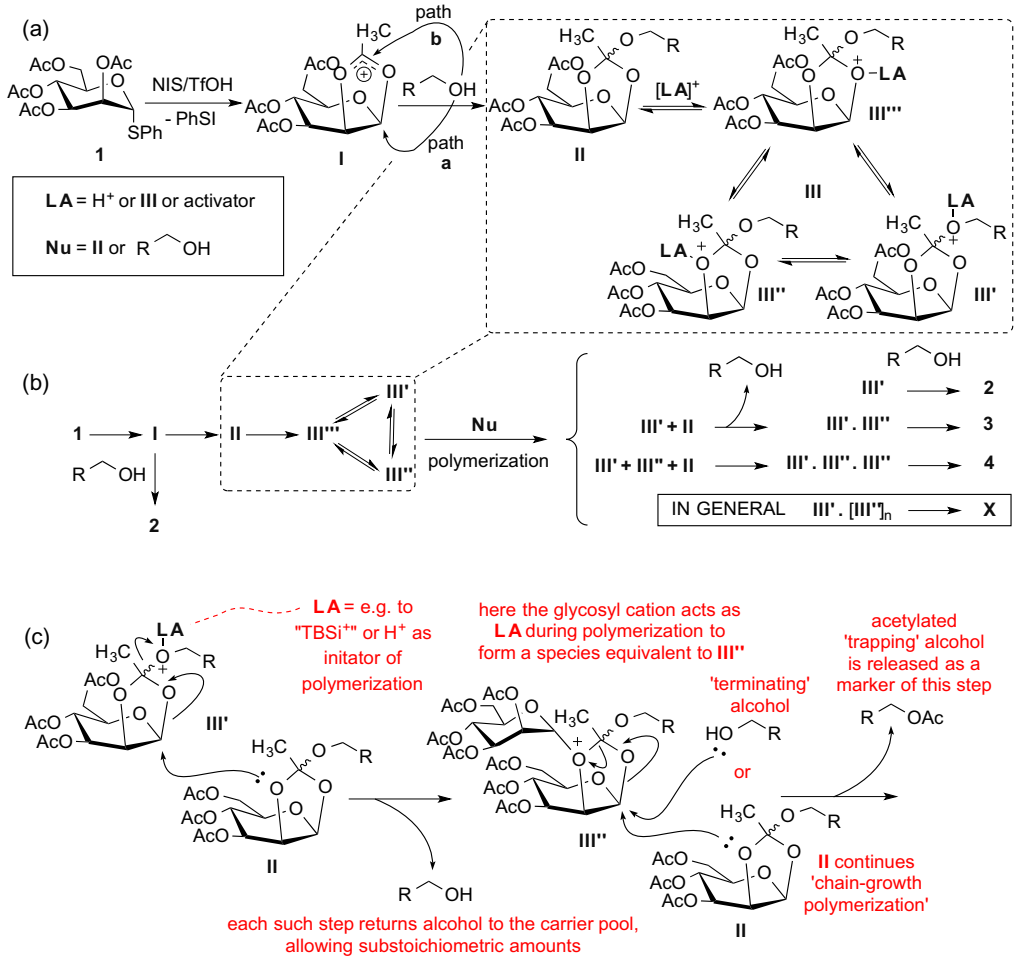

Figure 1. Suggested mechanism for 'chain growth polyglycosylation' leading to the formation of the mannoside oligomers from fully acetylated thioglycoside donor **1**. (a) The initiation and the stabilization of key ambident intermediate **III**; (b) the overall polyglycosylation manifold leading to oligosaccharides (dotted box corresponds to dotted box in panel (a)); (c) an example of one polymerization cycle. **LA** = Lewis acid; **Nu** = nucleophile.

produce 2-OH products[28] or transacetylated acceptors alcohols.[29] We reasoned that this implied orthoester formation and that this donor was therefore be a putative source of the key intermediates needed for the suggested approach. As expected, glycosylations with an excess of primary alcohol acceptor (2 equiv), such as typically used for discrete monomannoside syntheses, gave good yields of the monosaccharide (Table 1). However, we were delighted to see that glycosylation by thioglycoside **1** of functionalized alkyl alcohols (ROH) under varied NIS/TfOH activator conditions (Table 1 and Scheme 1) gave not only the expected monosaccharide products but also varying amounts of disaccharide and trisaccharide; this was a vital early sign that the polymerization manifold was potentially accessible.

2.2. Mechanistic analysis of the synthesis of mannoside oligomers

A plausible, mechanistic explanation for the formation of the α-1→2 linkage in oligomers (considered here for mannobiosides) involves an orthoester **II** (Fig. 1). In the case of thioglycoside, intermediate **II** may be formed from an attack of the acceptor alcohol not at the anomeric centre (pathway a) but the central carbon of the initially generated dioxolenium ion **I** (pathway b) (Fig. 1a). This process is then likely reversible as the orthoester **II** can then be attacked by the Lewis acid at the OR group or at O-1 leading to the formation of intermediate **III**, which can collapse back to **II**. If the incoming alcohol attacks the anomeric carbon of **I** or **III** then that leads to the formation of glycoside, for example, monosaccharide **2**. When sufficient stabilization is provided through Lewis acid coordination in intermediate **III**, we posited that a 'chain growth polyglycosylation' (Fig. 1b and c) might be initiated leading to the formation of disaccharide (**3**) and trisaccharide (**4**) *et cetera*. This idea was further supported by the observation by us and others[22,23,30,31] of acetylated acceptor alcohol as a marker of glycoside formation (Fig. 1c).

Next, this hypothesis was tested experimentally by the addition of varying equivalents of alcohol (as the putative terminator of polyglycosylation, Fig. 1b and c). This had a direct and marked effect on the formation of increased amount of mannoside oligomers (Table 1). Polyglycosylation would be terminated by the addition of such an acceptor alcohol to the anomeric centre of the intermediate **III**″, thereby eliminating the acetylated alcohol. Alternatively, this intermediate can be attacked by another orthoester **II** leading to the formation of higher mannosides (e.g., trisaccharide product **4**) through a polymerization that continues to 'grow'. When the acceptor alcohol employed in the reaction was reduced this second path is relatively favoured. Distributions obtained were also consistent with this mechanism: even with majority di- or tri-saccharide as product, traces of tetramannoside were also observed by MS when less than stoichiometric quantities of acceptor alcohol were employed for the glycosylation reaction. The rate of each individual pathway is directly determined by the amounts