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Rapid synthesis of oligomannosides with orthogonally protected monosaccharides†‡

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Sue-Ming Chang,[ab] Zhijay Tu,[b] Hau-Ming Jan,[ab] Jia-Fu Pan[b] and Chun-Hung Lin*[ab]

We developed a facile synthesis to yield orthogonally protected mannose building blocks with high overall yields. The protection/glycosylation steps can be carried out in a successive manner without purification of intermediate products. This developed synthesis led to formation of linear/branched tri-, penta- and heptasaccharides.

Oligomannosides are a major component of asparagine-linked oligosaccharides (*N*-glycans). In *N*-glycans, mannose is usually attached to either D-mannose or *N*-acetyl-D-glucosamine *via* α1,2-, α1,3-, α1,6- and β1,4-linkages. Many bioactive glycoconjugates also contain multiple mannose residues in correlation with their physiological activities, such as GPI anchors,[1] fungal cell wall mannans,[2] and high affinity sugar ligands of concanavalin A[3] and cyanovirin N.[4] Among various mannose-containing oligosaccharides, the 3,6-branched trimannosaccharide (Manαl,3[Manα1,6]Man) and pentamannoside [(Manαl,3[Manα1,6]Man)αl,6Man3,1αMan] units have been shown to exist in a wide range of important glycoproteins. For instance, *Mycobacterium tuberculosis* is the main cause of tuberculosis. The pathogen possesses mannose-rich glycophospholipids that were found to not only have the 1,6-linear trimannosaccharide, but also effectively avoid the immune surveillance.[5]

Since mannose residues can be assembled into a myriad of oligomannosides *via* formation of various glycosidic linkages, there are several concerns associated with the synthesis, such as regioselective masking and unmasking of hydroxyl groups, stereoselective glycosylation, and retrosynthetic analysis[6] (*i.e.* to determine what fragments of glycosides need to be prepared first). The synthetic efforts thus become labor intensive. Despite many reports on preparation of oligomannosides,[7] most of them do not employ orthogonal protecting groups,[8] which restricts the possibility of further derivatization. We herein report a general and practical method for facile preparation of two mannose building blocks that were orthogonally protected. Both building blocks were further derived to afford glycans **1–4** and **5** (the fully deprotected product of **4**) (Fig. 1) that are linear/branched tri-, penta- and heptasaccharides. The retrosynthetic analysis of **4** represents one example demonstrating how mannose building blocks **6** and **7** are applied for the glycan assembly.

Our development started with 6-*O*-*t*-butyldiphenylsilyl-1-thio-*p*-tolylmannopyranoside (**10**, Scheme 1),[9] which was treated with trimethyl orthobenzoate and a catalytic amount of camphor sulfonic acid to protect C2- and C3-hydroxyl groups with formation of orthoesters. Without purification, the product was acetylated with acetic anhydride and a catalytic amount of *N*,*N*-dimethylaminopyridine (DMAP) at *O*4, followed by the acidic hydrolysis with 2 N HCl to generate the protected mannoside (68% in three steps) with *O*2-benzoate and *O*4-acetate groups. Further reaction with levulinic acid, EDC and DMAP afforded the fully protected product (**11**) in 92% yield. Consistent with our previous development,[10] the conditions of these protection reactions are all compatible to be carried out sequentially without chromatography of intermediate products. Interrupted by removal of reaction solvent and simple workup, the conversion from compound **10** to **11** led to the total yield of 64% (Scheme 1, also entry 3 of Table 1). Likewise, compounds **6** and **7** can be obtained in 73% and 67% yields, respectively (entries 1 and 2 of Table 1). The consecutive syntheses of **6**, **7** and **11** have been operated at a scale of >15 g. This synthetic approach is flexible, allowing for different modifications to generate a large number of protected mannose monosaccharides. The aforementioned acetylation, for example, can be substituted with benzylation (see entry 2 of Table 1) or benzoylation (compound **SI-13** in Fig. S1, ESI‡) or others. Trimethyl orthoacetate is applicable to the protection of C2- and C3-hydroxyl groups, resulting in the formation of *O*2-acetate after the partial hydrolysis with 2 N HCl (**SI-6–SI-13**). Meanwhile, *O*3-levulinate ester can also be replaced with other ester- or ether-groups (*e.g.* **SI-9**).[11] So far we have prepared sixteen orthogonally protected mannosides that include compounds **6**, **7** and **11** (see Fig. S1, ESI‡).

[a] *Institute of Biochemical Sciences, National Taiwan University, Taipei 10617, Taiwan*

[b] *Institute of Biological Chemistry and the Genomics Research Center, Academia Sinica, No. 128, Academia Road Section 2, Nan-Kang, Taipei 11529, Taiwan. E-mail: chunhung@gate.sinica.edu.tw; Fax: +886-2-26514705*

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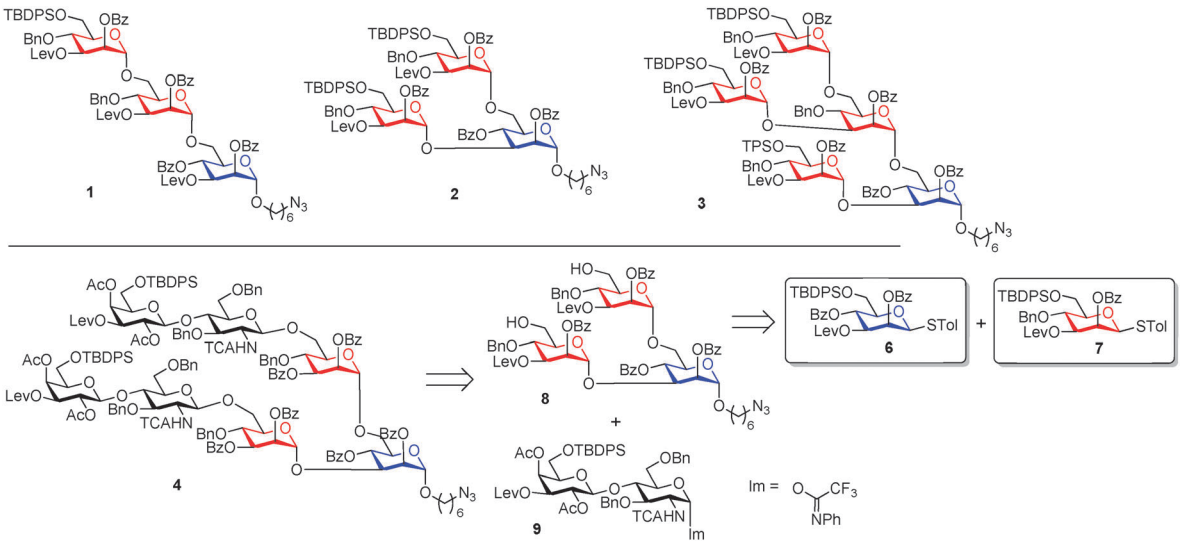
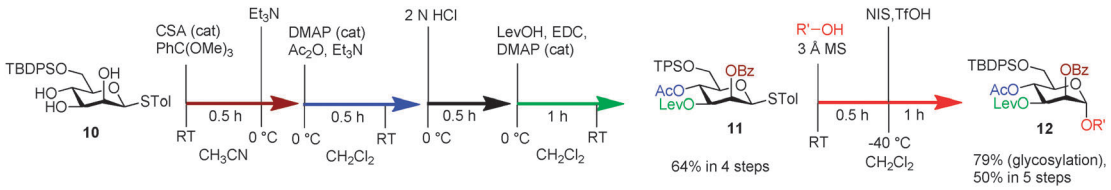


Fig. 1 Structures of target molecules **1–4** and the retrosynthetic analysis of **4** in which **6** (blue) and **7** (red) serve as the building blocks for the synthesis *via* proper deprotection and glycosylation steps. Blue and red colours help to demonstrate how **1–4** are derived from **6** and **7**.



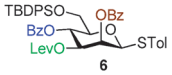
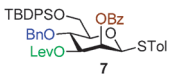
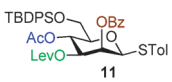
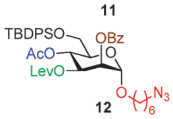
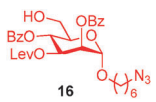
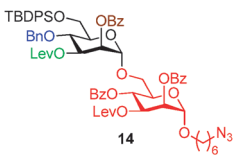
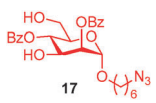
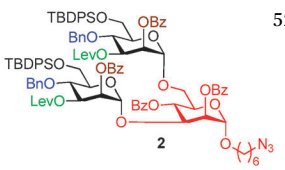
Scheme 1 Compound **10** was consecutively protected without purification of intermediate products to give mannoside **11**. The operation can include the subsequent glycosylation to obtain the final product with a high overall yield (see Table 1). Each arrow represents one reaction. Colours designate different reactions and the resulting groups.

Compounds **11** and **6** then served as glycosyl donors to couple with 6-azidohexanol in the presence of *N*-iodosuccinimide (NIS), triflic acid (TfOH) and 3 Å molecular sieves to afford the glycosylated products **12** and **13** in 71% and 79% yields, respectively (entries 4 and 5 of Table 1). Combining the glycosylation reaction with the aforementioned protection procedure, we successfully carried out five-step consecutive syntheses to generate products **12–14** and **2** in 50–52% total yields (corresponding to entries 4–7 of Table 1, respectively).

To prepare mannose oligosaccharides, treatment of compound **14** with tetrabutylammonium fluoride (TBAF) afforded **18** (84%) under acidic conditions (Scheme 2), and was followed by NIS–TfOH-promoted glycosylation with donor **7** to give linear trimannoside **1** (81%). Meanwhile, *O*3- and *O*3′-levulinate esters of **18** were hydrolyzed by using hydrazine monoacetate. The resulting product **19** (84%) then served as the acceptor for the triple glycosylation with donor **7** to produce the branched pentamannoside **3** (81%), forming one αl,6- and two αl,3-mannosidic linkages at the same time. Furthermore, *O*3b- and *O*3c-levulinate esters of trimannoside **2** were replaced by benzoate esters (see the conversion from **2** to **21** with a total 82% yield in Scheme 2). The subsequent deprotection of silyl ethers by HF–pyridine led to formation of product **8** (85%) that contains two unmasked primary alcohols. Compound **9**, previously employed in the synthesis of LacNAc-containing tetrasaccharide,[10] acted as the donor to transfer the Gal-βl,4-GlcNAc disaccharide to C6b- and C6c-hydroxyl groups of **8**. In the presence of TfOH and 3 Å molecular sieves, the glycosidation produced heptasaccharide **4** in 82% yield. The remaining reactions (from **4** to **5**) correspond to deprotection steps and the introduction of sulfates into C3 of two terminal Gal residues. These steps include (1) reduction of the *N*-trichloroacetyl group and the terminal azide to produce *N*-acetyl and the amino groups, respectively, (2) protection of the resulting amino group to give the benzyl carbamate (82% in two steps), (3) hydrolysis of the levulinate esters (85%), (4) sulfation of the resulting hydroxyl groups (80%), (5) removal of the TBDPS ethers, (6) hydrolysis of acetate and benzoate esters, and (7) hydrogenolysis to remove the benzyl groups (42% in three steps). The procedures are all listed in ESI.‡ Additionally, the structures of these synthesized saccharides (including target molecules **1–5**) were determined in detail by HRMS and several NMR methods, including ^{1}H–^{1}H COSY, ^{1}H–^{13}C HMQC, 1D-selective TOCSY and DEPT (see Fig. S2, ESI‡ (A–G) for a detailed explanation).

In summary, we developed a consecutive synthetic procedure to prepare mannose mono-, di- and trisaccharides. The sugars were orthogonally protected, allowing specific deprotection at desirable site(s) at a later stage, as well as fast assembly of glycans. This method also highlights three features valuable to the field of carbohydrate chemistry, including versatility (protecting groups at C2, C3 and/or C4-positions can be changed to increase the diversity), practicability (several compounds can be prepared at a scale of >15 g), and flexibility (several mentioned sugars are glycosyl donors and can be converted into acceptors after selective deprotection).

Table 1 Consecutive protection/glycosylation reactions. It is noted that protection products **6**, **7** and **11** can function as glycosyl donors or as acceptors after specific deprotection (*e.g.* conversion of **6** to **16**/**17**). Different colours are used to designate individual reactions

Entry	R_2O/RBr, base	R'OH (glycosidation)	Product	Isolated yield
1	Bz_2O, Et_3N	—	 **6**	73%
2	BnBr, NaH	—	 **7**	67%
3	Ac_2O, Et_3N	—	 **11**	64%
4	Ac_2O, Et_3N	 **15**	 **12**	50%
5	Bz_2O, Et_3N	 **15**	 **13**	52%
6	BnBr, NaH	 **16**	 **14**	50%
7	BnBr, NaH	 **17**	 **2**	52%

[a] Thiomannoside **10** (1.0 eq.) in CH_2CI_2 was treated with $PhC(OMe)_3$ (3.0 eq.), CSA. [b] Two methods were applied to protect the C_4-hydroxyl group. To form an ester, Ac_2O (or Bz_2O, 2.0 eq.) and Et_3N (3.0 eq.) in CH_2CI_2 were used. To form an ether, BnBr (2.0 eq.) and NaH (4.0 eq.) in THF were utilized. [c] The reaction mixture was treated with LevOH (1.5 eq.) and EDC (1.5 eq.) in CH_2CI_2. [d] For entries 4–7, 1.0 eq. of glycosyl donor (**11**, **6**, **7** or **7**) reacted with an acceptor [**15** (1.5 eq.), (**15** (1.5 eq.), **16** (0.5 eq.) or **17** (0.25 eq.), respectively] in CH_2CI_2 at −40 °C in the presence of NIS (2.0 eq.), TfOH (0.2 eq.) and 3 Å molecular sevies.

On the basis of this development, we have been preparing various fully deprotected oligomannosides (*e.g.* **5**) for examining their biological activities. The result will be reported in due course.

Notes and references

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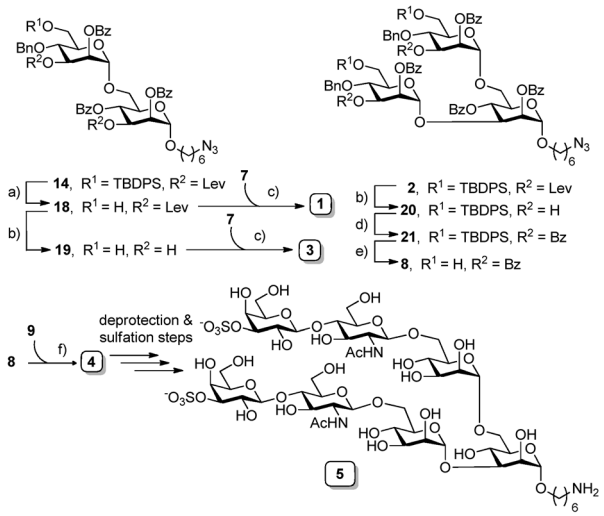

Reagents and conditions: a) TBAF/AcOH, THF, 0 °C, 84%; b) NH_2NH_2/AcOH, THF/MeOH (9:1), 84% (formation of **19**) or 86% (**20**); c) NIS, TfOH, 3 Å M.S., CH_2Cl_2, -40 °C, 75% (formation of **1**) or 81% (**3**); d) Bz_2O, Et_3N, DMAP, CH_2Cl_2, 95%; e) HF-pyridine, THF, 0 °C, 85%, f) TfOH, 3 Å M.S., CH_2Cl_2, -70 to -40 °C, 4 h, 82%. Sulfation and deprotection steps: please see ESI.‡
Abbreviations: TBAF (tetrabutylammonium fluoride), NIS (*N*-iodosuccinimide), TfOH (trifluoromethanesulfonic acid), M.S. (molecular sieves).

Scheme 2 Synthetic procedures to prepare oligomannosides **1**, **3**, **4** and **5**.

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11 Formation of allyl ether led to a lower yield due to a small extent of decomposition if acetate or benzoate existed in the same molecule.