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Chemical Synthesis of Cyclic Galactooligofuranosides Isolated from Enzymatic Degradation Products of Cell Wall Arabinogalactan of *Mycobacterium tuberculosis*

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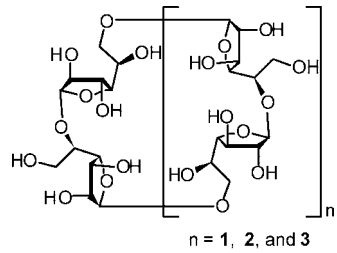
ABSTRACT



Synthesis of cyclic tetra-, hexa- and octasaccharides containing alternating (1→5)-β- and (1→6)-β-galactofuranosyl linkages has been achieved by intramolecular cycloglycosylation of corresponding linear sugars and by cyclooligomerization of 1,6-linked and 1,5-linked disaccharides. In particular, cyclooligomerization of the (1→6)-β-galactofuranosyl disaccharide provides an efficient way to secure all three cyclic sugars in one operation.

In recent years, a great deal of effort has been devoted to the elucidation of the cell wall structure[1] of *Mycobacterium tuberculosis, the causative agent of human tuberculosis, due to the emergence of multidrug resistant strains.*[2] One of the major structural components of the cell wall of *M. tuberculosis* is the complex of mycolic acid, arabinogalactan, and peptidoglycan (mAGP complex), which plays a crucial role in the survival and pathogenicity of *M. tuberculosis*. Active structural investigations have revealed the structural relationship of arabinogalactan (AG) with other parts of the mAGP complex and the detailed structure of AG, which consists of D-arabinan and D-galactan.[3] Ethambutol, an effective antituberculosis drug, inhibits the polymerization step of D-arabinan biosynthesis[4] while the D-galactan moiety was suggested to be essential for the growth and viability of

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(2) For multi-drug resistance of *M. tuberculosis* strains, see: (a) Nettleman, M. D. *JAMA* **2005**, *293*, 2788–2790. (b) Long, R. *CMAJ* **2000**, *163*, 425–428. (c) Pablos-Mendez, A; Raviglione, M. C.; Laszlo, A.; Binkin, N.; Rieder, H. L.; Bustreo, F.; Cohn, D. L.; Weezenbeek, C. S. B, L.; Kim S., J.; Chaulet, P.; Nunn, P. *N. Eng. J. Med.* **1998**, *338.*, 1641–1649.

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(4) (a) Takayama, K.; Kilburn, J. O. *Antimicrob. Agents Chemother.* **1989**, *33*, 1493–1499. (b) Mikusov, K.; Slayden, R. A.; Besra, G. S.; Brennan, P. J. *Antimicrob. Agents Chemother.* **1995**, *39*, 2484–2489.

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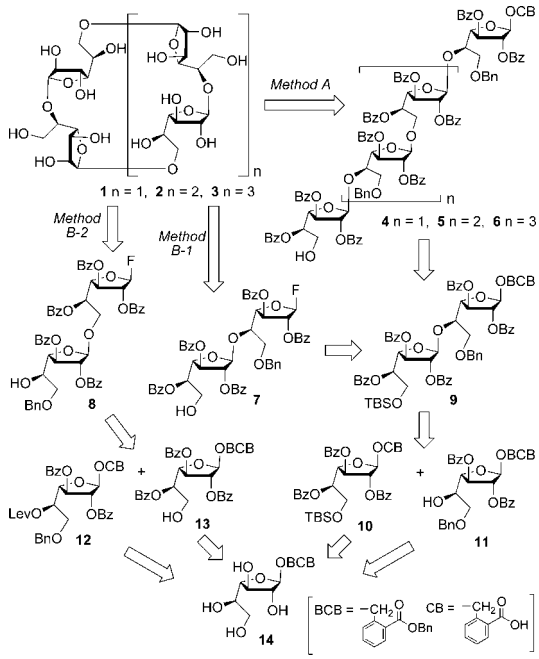
mycobacteria, and thus, the Gal*f* metabolism is a potential target for development of new antituberculosis drugs.[5] During the structural study of AG, Brennan and co-workers unexpectedly identified novel cyclic galactooligosaccharides **1**, **2**, and **3** among the degradation products of AG by extracellular enzymes isolated from the bacterium *Cellulomonas* sp.[6] Compounds **1**−**3** are attractive synthetic targets due to their structural uniqueness, their potential ability to form inclusion complexes, and their potential inhibitory activities on the Gal*f* metabolism of *M. tuberculosis*. Although there have been reports on synthetic cyclic galactofuranosides by Kochetkov and co-workers,[7] they all contain a single type of the Gal*f* linkage such as (1→6)-*β*-, (1→3)-*β*-, or (1→5)-*β*-linkage, while compounds **1**−**3** have alternating (1→5)-*β*- and (1→6)-*β*-Gal*f* linkages. Neither their molecular geometries nor their capabilities to form inclusion complexes have been investigated probably due to low yields in the Kochetkov's synthesis of the cyclic sugars.

Herein, we describe synthesis of cyclic sugars **1**−**3** by employing two different methodologies, one by intramolecular cycloglycosylation of linear oligosaccharides **4**−**6** (method A in Scheme 1) and another by cyclooligomerization of (1→5)-*β*-disaccharide **7**(method B-1) and (1→6)-*β*-

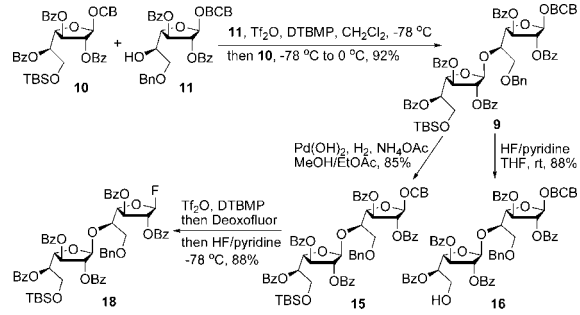
Scheme 1



disaccharide **8** (method B-2). The latent-active glycosylation strategy employing 2′-(benzyloxycarbonyl)benzyl (BCB) glycosides and 2′-carboxybenzyl (CB) glycosides[8] would be used here extensively for the construction of linear oligosaccharides and their cyclization. Retrosynthesis of **4**−**6** leads to disaccharide **9**, and further analysis of disaccharide **7**−**9** provides monosaccharides **10**−**13**, which would be synthesized from a common starting materail **14**.

The synthesis commenced with preparation of four monosaccharide building blocks **10**−**13** from known compound **14**[9] (see the Supporting Information). We decided to synthesize at first each of the target molecules **1**−**3** by cyclization of linear sugars **4**−**6**, respectively (method A). Coupling of **10** with **11** was carried out by sequential addition of Tf_2O and **10** to a solution of **11** in the presence of 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) in CH_2Cl_2 at −78 °C to give desired *β*-disaccharide **9** as shown in Scheme 2.

Scheme 2



Compound **9** was converted into disaccharide donor **15** by selective hydrogenolysis and into disaccharide acceptor **16** by desilylation. Coupling of **15** and **16**, however, afforded desired tetrasaccharide **17** in only 27% yield along with the self-condensed ester[8b] of **15** in 52% yield. The poor results with the glycosyl donor **15** in the glycosylation of **16** led us to examine other glycosyl donors such as glycosyl fluorides. We have previously shown that CB glycosides could be readily converted into glycosyl fluorides by treatment with Tf_2O and HF/pyridine[9] or Tf_2O and (diethylamino)sulfur trifluoride (DAST).[10] Both HF/pyridine and DAST with Tf_2O, were not quite satisfactory for the conversion of **15** into galactosyl fluoride **18**, while a combination of Tf_2O, bis(2-methoxyethyl)aminosulfur trifluoride (Deoxofluor),[11] and HF/pyridine cleanly converted **15** into **18**.[12]

Glycosylation of **16** with the fluoride donor **18** using $SnCl_2$ and $AgClO_4$ in ether[13] at −10 °C afforded exclusively the desired *β*-tetrasaccharide **17** in 62% yield (Scheme 3).

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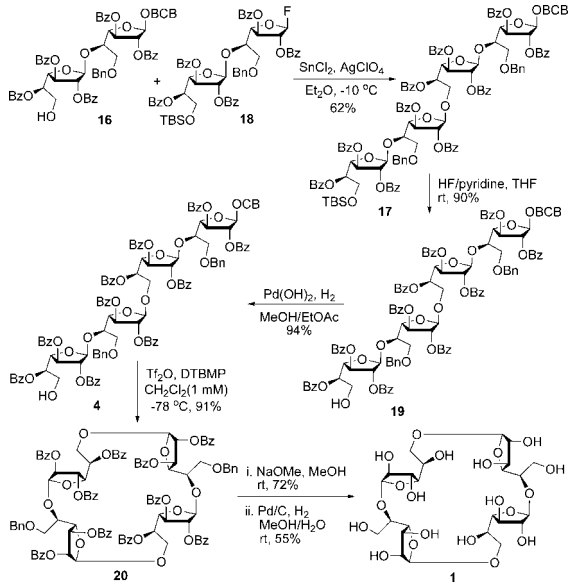
(10) Lee, Y. J.; Baek, J. Y.; Lee, B.-Y.; Kang, S. S.; Park, H.-S.; Jeon, H. B.; Kim, K. S. *Carbohydr. Res.* **2006**, *341*, 1708–1716.

Scheme 3

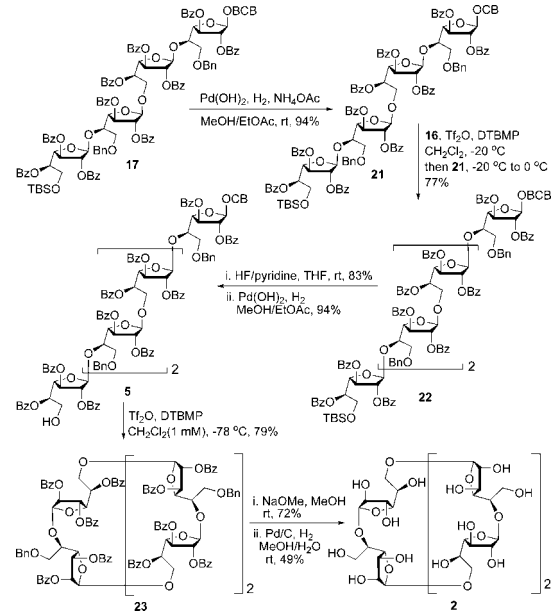


Compound **17** was then transformed to alcohol **19** by desilylation and subsequent selective hydrogenolysis of **19** afforded CB tetrasaccharide **4** bearing both glycosyl donor and acceptor functions. Cycloglycosylation of **4** in CH_2Cl_2 (1.0 mM) employing Tf_2O in the presence of DTBMP at −78 °C afforded cyclic tetrasaccharide **20** in 91% yield. Removal of benzoyl groups of **20** by NaOMe and remaining benzyl groups by hydrogenolysis provided fully deprotected cyclic sugar **1**. Anomeric carbon chemical shifts at δ 105.1, 105.5, 105.9, and 106.5 of the linear tetrasaccharide **4** clearly indicated that its four glycosyl linkages were in the β-configuration. On the other hand, the ^{13}C NMR spectrum of the cyclic tetrasaccharide **20** showed only two anomeric carbon peaks at δ 106.7 and 106.8 due to its symmetric nature. Two anomeric proton signals at δ 5.06 (d, $J = 1.2$ Hz) and 5.16 and two anomeric carbon peaks at δ 107.6 and 106.8 for **1** also indicated that all glycosyl bonds are β-linkages.[9,14]

The BCB tetrasaccharide **17** was transformed to CB tetrasaccharide **21** by selective hydrogenolysis (Scheme 4). Sequential addition of Tf_2O and **21** to a solution of disaccharide acceptor **16** in the presence of DTBMP in CH_2Cl_2 at −20 °C afforded exclusively the desired β-hexagalactoside **22** in 77% yield. Desilylation of **22** and subsequent hydrogenolysis of the resulting BCB glycoside afforded CB hexasaccharide **5** bearing both glycosyl donor and acceptor functions. Cycloglycosylation of **5** was carried out in dilute solution (1.0 mM in CH_2Cl_2) −78 °C to afford cyclic hexasaccharide **23** in 79% yield. Removal of the benzoyl groups of **23** and subsequent hydrogenolysis of benzyl groups afforded cyclic hexagalatofuranoside **2**. The 400 MHz NMR spectra of **2** in $CDCl_3$ showed two anomeric proton signals at δ 5.07 and 5.25 as singlets and two anomeric carbon peaks at δ 109.6 and 110.7.

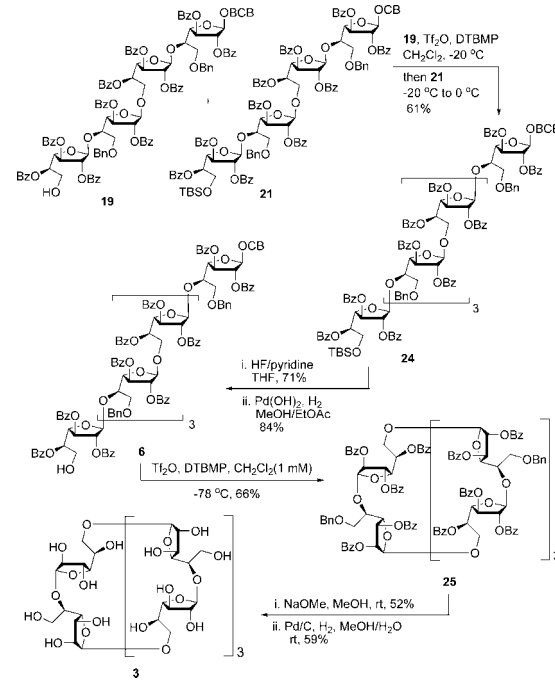
Linear octasaccharide **24** was prepared in 61% yield by glycosylation of tetrasaccharide **19** with CB tetrasaccharide **21** using Tf_2O in the presence of DTBMP at −20 °C

Scheme 4



(Scheme 5). Removal of TBS group in **24** followed by selective hydrogenolysis gave CB octasaccharide **6**. Cy-

Scheme 5

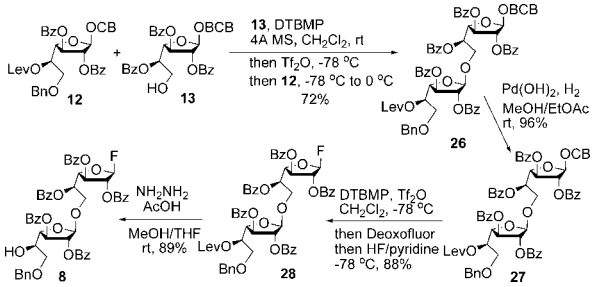


cloglycosylation of **6** by treatment with Tf_2O in dilute CH_2Cl_2 afforded desired cyclic octasaccharide **25** in 66% yield. Deprotection of the 20 benzoyl groups of **25** by NaOMe followed by hydrogenolysis of benzyl groups provided deprotected cyclic octasaccharide **3**.

We then performed synthesis of cyclic oligosaccharides **1**−**3** directly from disaccharides **7** and **8** by cyclooligomer-

ization (method B). The 1,5-linked disaccharide **7** was obtained by desilylation of **18** (see the Supporting Information) and the 1,6-linked disaccharide **8** was prepared starting from **12** and **13** as shown in Scheme 6. Thus, glycosylation

Scheme 6



of **13** with **12** gave BCB disaccharide **26**, which was then converted into CB disaccharide **27** by hydrogenolysis. After conversion of **27** into digalactosyl fluoride **28** using Tf_2O, Deoxofluor, and HF/pyridine, the levulinyl group of **28** was removed to give the disaccharide **8**.

Cyclooligomerizations of **7** and **8** were accomplished employing $SnCl_2$ and $AgClO_4$ as promoters at a few different concentrations in ether as shown in Table 1. The reaction with the 1,5-linked disaccharide **7** afforded cyclic tetrasaccharide **20** as the major product and cyclic hexasaccharide **23** as the minor product without generation of cyclic octasaccharide **25** (entries 1−3 in Table 1). On the other hand, cyclooligomerization of 1,6-linked disaccharide **8** provided not only **20** and **23** but also a substantial amount

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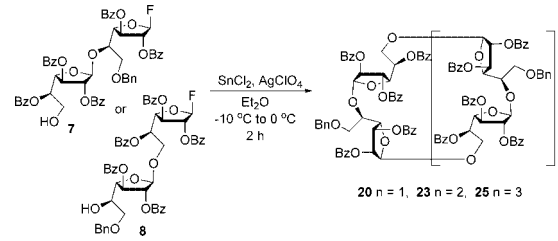
(12) A proposed mechanism for the conversion of **15** into **18** is as follows. Addition of Tf_2O to **15** would give a glycosyl triflate mixed anhydride, and then upon addition of Deoxofluor, the triflate would be displaced by the fluoride anion of Deoxofluor to provide a glycosyl carboxy fluoride intermediate. Finally, HF/pyridine would facilitate the lactonization of the carboxy fluoride to generate a glycosyl oxocarbenium ion, which would readily react with fluoride ion to afford glycosyl fluoride **18**. In fact, we were able to isolate the glycosyl carboxy fluoride intermediate.

(13) For $SnCl_2/AgClO_4$ as the promoter for glycosyl fluoride, see: Mukaiyama, T.; Murai,Y.; Shoda, S. *Chem. Lett.* **1981**, 431.

(14) Gelin, M.; Ferrieres, V.; Plusquellec, D. *Eur. J. Org. Chem.* **2000**, 1423–1431.

Table 1. Cyclooligomerization of Disaccharides **7** and **8**



			product (yield, %)		
entry	disaccharide	conc (mM)	**20**	**23**	**25**
1	**7**	1	70	3	0
2	**7**	4	69	8	0
3	**7**	20	69	15	0
4	**8**	4	23	23	16
5	**8**	20	28	32	16
6	**8**	40	20	31	18

of **25** (entries 4−6). Especially, the reaction at 40 mM concentration afforded **25** in up to 18% yield. Thus, we have established a way to secure substantial amounts of all three cyclic sugars **1**, **2**, and **3** for further studies.

In summary, synthesis of natural cyclic oligosaccharides **1**−**3** has been achieved. In particular, cyclooligomerization of (1→6)-β-disaccharide **8** provides efficiently all three cyclic sugars **1**−**3** in one operation. Studies on inclusion complexes and the selective functionalization of these cyclic sugars are currently underway.

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Supporting Information Available: Experimental procedures, characterization data, and copies of ^1H and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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