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Synthesis of a tetrasaccharide repeating unit of O-antigenic polysaccharide of *Salmonella enteritidis* by use of unique and odorless dodecyl thioglycosyl donors

Sang-Hyun Son [a], Chiharu Tano [a], Tetsuya Furuike [b], Nobuo Sakairi [a,*]

[a] *Graduate School of Environmental Science, Hokkaido University, Kita-ku, Sapporo 060-0810, Japan*
[b] *Department of Chemical and Materials Engineering, Faculty of Chemistry, Materials and Bioengineering, and High Technology Research Center, Kansai University, Suita, Osaka 564-8680, Japan*

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ABSTRACT

The first total synthesis of a unique tetrasaccharide repeating unit of lipopolysaccharide from *Salmonella enteritidis* has been accomplished by assembly of dodecyl thioglycosides. The crucial key steps were preparation of a rare branched dideoxy sugar, D-tyvelose (3,6-dideoxy-D-*arabino*-D-hexose) and sequential regioselective glycosylation at 2,3-positions of a central D-mannose residue **5** with D-tyvelose **6** and D-galactose donors **7**.

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Salmonella enteritidis is known as a virulence food-borne enteric human pathogen,[1] which colonize inert food contact surfaces to form biofilms.[2] Like other Gram-negative bacteria, it has lipopolysaccharide (LPS), which consists of anchoring lipid A and O-antigenic polysaccharide, at the outer membrane. Isolation and characterization of its lipopolysaccharide (LPS) have been extensively studied to understand the molecular mechanisms of *Salmonella* infection, adherence, and biofilm formation on inert surfaces.[3] Recently, it was reported that O-antigen polysaccharide of *S. enteritidis* has a repeating tetrasaccharide structure, →3)-α-D-Gal*p*-(1→2)-[α-D-Tyv*p*-(1→3)]-α-D-Man*p*-(1→4)-α-L-Rha*p*-(1→ (**1**).[4] Its unusual feature is the occurrence of a rare dideoxy sugar, D-tyvelose (3,6-dideoxy-D-*arabino*-D-hexose), which was deduced to play an important role in pathogenesis.[5] Furthermore, central D-mannose residue in the repeating tetrasaccharide has three different monosaccharides at 1,2,3-positions. Due to its structural uniqueness and biomedical potential, we undertook to synthesize oligosaccharides related to **1**. Herein, we report the first total synthesis of a methyl glycoside **2** of the repeating unit (see Fig. 1).

Key problems needing to be addressed in the synthesis of **2** were preparation of a tyvelose donor and introduction of three monosaccharide moieties into the central mannose residue by efficient glycosylation methods. Retrosynthetic analysis shown in Scheme 1 suggested that sequential regioselective glycosylation of a disaccharide acceptor **5** having two free hydroxyl groups with D-tyvelose **6** and D-galactose donors **7** provides the target tetrasaccharide **2**. The sterically less hindered equatorial hydroxyl group at C-3′ would be the first glycosylation site. Furthermore, **5** was to be synthesized from a mannosyl donor **4** and known methyl rhamnoside acceptor[6] **3**. In all glycosylation reactions required for the construction of **2**, we planned to use our recently developed dodecyl thioglycosyl donor,[7] of which advantage is that they are able to prepare under almost odorless conditions.

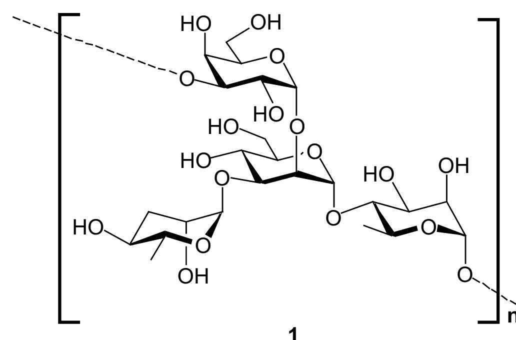

Figure 1. The structure of O-antigenic polysaccharide isolated from *Salmonella enteritidis*.

According to the synthetic plan mentioned above, we initiated to prepare three dodecyl thioglycoside donors **4**, **6**, and **7** as depicted in Scheme 2. Saponification of the acetylated thiomannopyranoside[7] **8** and subsequent O-benzylidenation and O-benzoylation afforded the fully protected derivative **4** in 71% yield. For the construction of α-mannosidic linkage, benzoyl group with neighboring group participation was anticipated to be the best choice to protect the C2 hydroxyl group, and it can be readily converted to free hydroxylic sites on the key intermediate **5** for the installation of the branched tyvelose moiety and galactose moiety. Moreover, **4**

* Corresponding author. Tel./fax: +81 11 706 2257.
E-mail address: nsaka@ees.hokudai.ac.jp (N. Sakairi).

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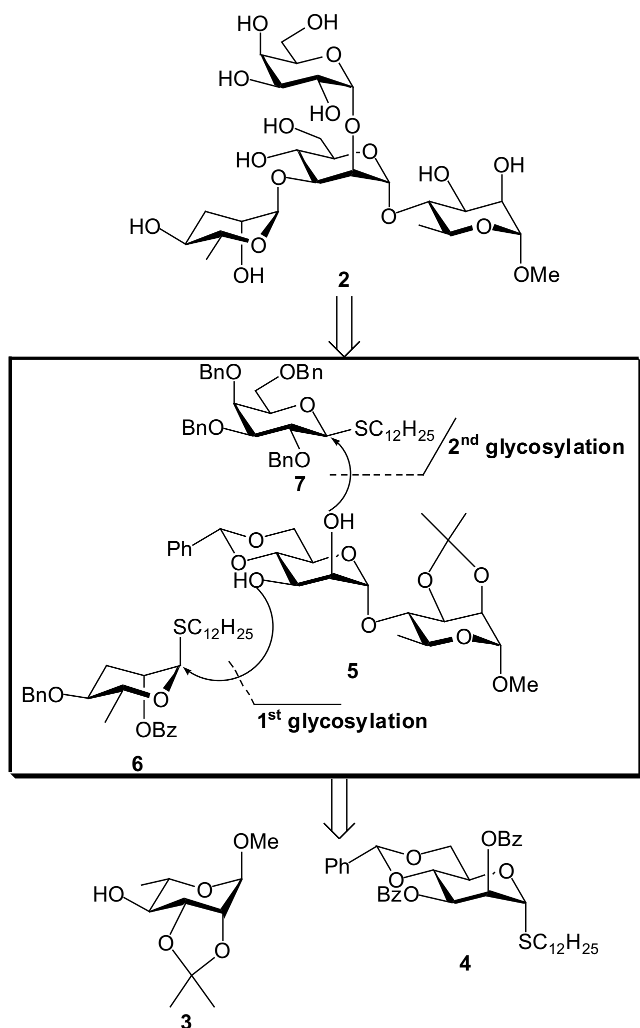
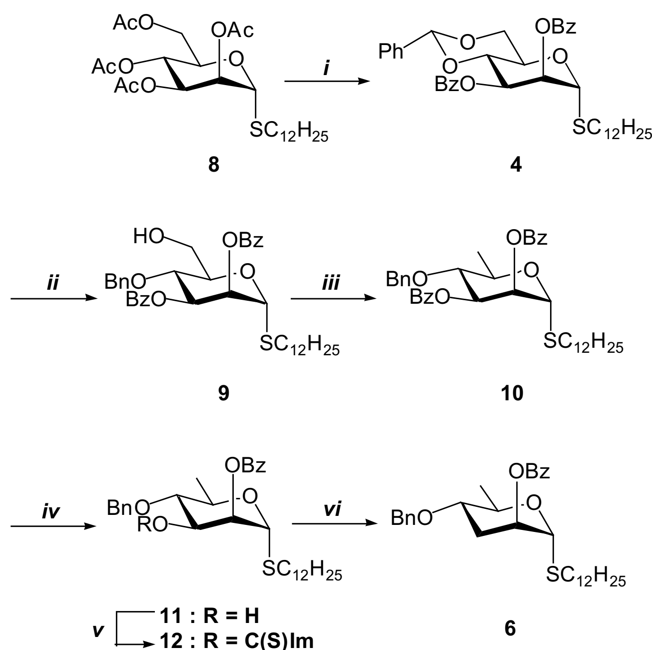


Scheme 1. Retrosynthetic analysis.

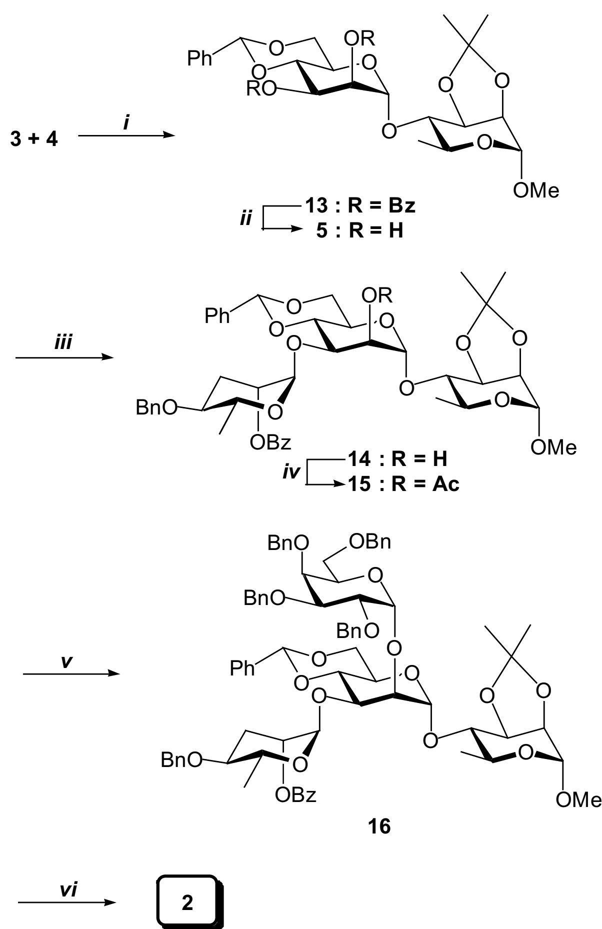
was also considered to be a precursor of the tyvelose donor **6**. Thus, the benzylidene acetal in **4** was regioselectively opened with $BH_3{\cdot}Me_2NH–BF_3{\cdot}Et_2O$ in dichloromethane furnished the primary alcohol[8] **9** in 82% yield. Next deoxygenation seemed to be a crucial step in our synthesis, because **9** has a reducible thioacetal function at the anomeric center. For deoxygenation of the primary alcohol,[9] **9** was subjected to O-tosylation followed by reduction with $NaBH_4$ in DMF to provide the dodecyl thio D-rhamnoside **10** in a yield of 81%. The second deoxygenation[10] at C3 was performed after transformation into a monobenzoate **11** in three steps including de-O-benzoylation, formation of an orthoester, and its acid-catalyzed ring opening. The resulting kinetically preferred axial benzolate **11** obtained in an overall yield of 79% was converted into thiocarbonylimidazole intermediate **12**, which was subjected to radical reduction with Bu_3SnH[10b,c] in toluene to give the tyvelose donor **6** in 82% (see Scheme 3).

Assembly of these thioglycosyl donors toward **2** was performed by use of thiophyllic reagents as the promoters. The first coupling of **3** and **4** proceeded at −20 °C to 0 °C in the presence of molecular sieves and a promoter, *N*-iodosuccinimide and catalytic triflic acid (NIS–TfOH),[11] giving the disaccharide[12] **13** in 90% yield. Subsequently, two benzolyl groups at 2′,3′-positions of **13** were removed by Zemplén method to give the disaccharide acceptor[12] **5** in 96% yield. Using this diol acceptor, we examined sequential and regio-



Scheme 2. Preparation of D-mannose and D-tyvelose donors. Reagents and conditions: (i) (a) NaOMe/MeOH, rt, 3 h; (b) $PhCH(OMe)_2$, cat. CSA, DMF, 50 °C, 4 h; (c) BzCl, DMAP, pyridine, 50 °C, 3 h, 71% over three steps; (ii) $BH_3{\cdot}Me_2NH$, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C, 1 h, 82% (Ref. 8); (iii) (a) TsCl, DMAP, pyridine, 0 °C to 50 °C, overnight; (b) $NaBH_4$, DMF, 70 °C, 3 h 81% over two steps (Ref. 9); (iv) (a) NaOMe/MeOH–CH_2Cl_2, rt, 3 h; (b) $PhC(EtO)_3$, cat. CSA, CH_2Cl_2, rt, 3 h; (c) 80% AcOH in H_2O, 80 °C, 2 h, 79% over three steps (Refs. 10b and c); (v) $C(S)Im_2$, imidazole, $(CH_2Cl)_2$, 80 °C, 18 h, 98%; (vi) Bu_3SnH, AIBN, toluene, reflux, 30 min, 82% (Ref. 10).

selective glycosylation in order to shorten the synthetic route to **2**. For selective glycosylation at the equatorial hydroxyl group at 3′-position, we used excess amount of the diol acceptor **5**. Thus, a mixture of **5** (0.25 mmol) and the tyvelose donor **6** (0.125 mmol) was activated with NIS–TfOH at −40 °C. As a result, the desired trisaccharide[12] **14** was obtained with completed α-stereoselectivity in excellent yield. The presence of the newly introduced α-(1→3) linkage between D-tyvelose and D-mannose units in **14** was unambiguously ascertained by ^{1}H COSY NMR spectroscopy after conversion of the acetylated derivative[12] **15**. Careful examination of the spectrum showed H-2 protons for the mannose residue at lower magnetic field (δ 5.21) as a double doublet which was assigned by a cross-peak between H-1 and H-2 protons. The regioselectivity is explainable by the facts that the equatorial 3′-hydroxyl group is less hindered than the axial 2′-hydroxyl group and that presence of bulky L-rhamnose residue at the anomeric position of the mannose residue reduced the reactivity of neighboring 2′-hydroxyl group. Furthermore, comparison of decoupling and non-decoupling HSQC spectra revealed signals of anomeric carbons at δ 99.0 ($J_{C1',H1'}$ = 175.9 Hz, C-1′), 97.8 ($J_{C1,H1}$ = 170.0 Hz, C-1), and 97.7 ($J_{C1'',H1''}$ = 172.9 Hz, C-1″), suggesting **15** had all α-glycosidic linkages. The next coupling between the donor **7** and the trisaccharide **14** was performed using a powerful promoter of thioglycosides so far reported, because of the prediction of the extremely poor reactivity of **14**. Thus, the donor **7** was pre-activated with the 1–benzenesulfinyl piperidine and triflic anhydride (BSP–Tf_2O)[13] combination in the presence of 2,6-di-*tert*-butyl-4-methylpyridine (DTBP) in dichloromethane at −78 °C, and then treated with the acceptor **14**. Interestingly, desired tetrasaccharide[12] **16** was isolated in 72% yield with complete α-stereoselectivity. No trace of the β-anomer was detected by both chromatographic and NMR spectroscopic analyses of the crude reaction mixture. Similar results had been obtained previously with fully benzylated



Scheme 3. Construction of tetrasaccharide **2** by assembly of dodecyl thioglycosides. Reagents and conditions: (i) NIS, TfOH, CH_2Cl_2, MS4 Å, −20 °C to 0 °C, 30 min, 90%; (ii) NaOMe/MeOH–CH_2Cl_2, rt, 3 h, 96%; (iii) **6**, NIS, TfOH, CH_2Cl_2, MS4 Å, −40 °C, 30 min, 92%; (iv) Ac_2O, pyridine; (v) **7**, BSP, Tf_2O, DTBM, CH_2Cl_2, MS4 Å, −78 °C, 72%; (vi) (a) 80% AcOH in H_2O, 50 °C, 24 h; (b) NaOMe/MeOH, rt, 6 h; (c) Pd/C, MeOH–H_2O–AcOH, H_2, rt, 24 h, 76% over three steps.

thiogalactosyl donors using sulfonium triflate per-activation procedure.[14]

Finally, global deprotection of **16** was successfully carried out by a three-step procedure that involved the hydrolysis of the acetal protecting groups by treatment with aqueous AcOH, transesterification of the *O*-benzoyl groups with sodium methoxide in methanol, and catalytic hydrogenolysis over Pd/C to remove the *O*-benzyl groups. The unprotected tetrasaccharide[12] **2** thus obtained was characterized spectroscopic analyses. Representative ^{1}H and ^{13}C NMR chemical shift data of the synthetic tetrasaccharide **2** is in excellent agreement with the reported data[4] of the natural polysaccharide **1**.

In conclusion, total synthesis of a tetrasaccharide repeating unit of O-antigenic polysaccharide of *S. enteritidis* was achieved by assembly of dodecyl thioglycosyl donors. Our finding such as regioselective and sequential glycosylation 2,3-positions of core mannose residue would make it possible to provide easily various oligosaccharides analogous of **2**, which are useful substrates for biological examinations toward its infection control.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.06.097.

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12. Representative ^{1}H NMR, and other physical data of compounds **13**, **5**, **14**, **15**, **16**, and **2**.
Compound **13**: $[\alpha]_D^{20.0}$ −94.3 (*c* 1.00, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.81 (dd, 1H, $J_{3',4'} = 3.4$ Hz, H-3′), 5.65 (dd, 1H, $J_{2',3'} = 3.5$ Hz, H-2′), 5.12 (s, 1H, H-1′), 4.88 (s, 1H, H-1), 1.37 (d, 3H, J = 6.1 Hz, H-6); HRMS (FAB) calcd for $C_{37}H_{41}O_{12}$ $[M+H]^+$: 677.2593; found, 677.2592.
Compound **5**: $[\alpha]_D^{20.0}$ −43.9 (*c* 1.00, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.90 (s, 1H, H-1′), 4.85 (s, 1H, H-1), 1.27 (d, 1H, J = 6.3 Hz, H-6); HRMS (FAB) calcd for $C_{23}H_{33}O_{10}$ $[M+H]^+$: 469.2068; found, 469.2075.
Compound **14**: $[\alpha]_D^{21.8}$ +38.6 (*c* 1.00, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.01–7.18 (m, 15H, CHarom), 5.59 (s, 1H, PhC*H*), 5.31 (s, 1H, H-2″), 5.28 (s, 1H, H-1″), 4.95 (s, 1H, H-1′), 4.84 (s, 1H, H-1), 4.62 (d, 1H, J = 11.0 Hz, PhCH_2), 4.47 (d, 1H, J = 11.6 Hz, PhCH_2), 4.31–4.28 (m, 1H, H-6′a), 4.25–4.18 (m, 1H, H-6′b), 4.16–4.02 (m, 5H, H-2, 3, 2′, 3′, 4′), 3.89–3.85 (m, 1H, H-5″), 3.84–3.78 (m, 1H, H-5′), 3.69–3.62 (m, 1H, H-5), 3.49–3.44 (m, 1H, H-4″), 3.58–3.42 (m, 1H, H-4), 3.36 (s, 3H, OMe), 2.53 (s, 1H, OH), 2.40–2.34 (m, 1H, H-3″), 2.04–1.96 (m, 1H, H-3″), 1.52 (s, 3H, CH_3), 1.35 (d, 3H, J = 6.6 Hz, H-6″), 1.33 (s, 3H, CH_3), 1.27 (d, 3H, J = 6.6 Hz, H-6); HRMS (FAB) calcd for $C_{43}H_{53}O_{14}$ $[M+H]^+$: 793.3430; found, 793.3437.
Compound **15**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.97–7.22 (m, 15H, CHarom), 5.63 (s, 1H, PhC*H*), 5.26 (br d, 1H, H-2″), 5.21 (dd, 1H, $J_{2',3'} = 3.5$ Hz, H-2′), 5.16 (s, 1H, H-1″), 4.91 (d, 1H, $J_{1',2'} = 1.2$ Hz, H-1′), 4.85 (s, 1H, H-1), 4.62 (d, 1H, J = 11.6 Hz, PhCH_2), 4.47 (d, 1H, J = 11.6 Hz, PhCH_2), 4.34 (dd, 1H, $J_{3',4'} = 9.5$ Hz, H-3′), 4.27 (dd, 1H, $J_{5',6'b} = 4.2$ Hz, H-6′b), 4.18–4.06 (m, 4H, H-2, 3, 4′, 6′a), 3.87–3.76 (m, 2H, H-5′, 5″), 3.70–3.61 (m, 1H, H-5), 3.55–3.33 (m, 2H, H-4, 4″), 3.35 (s, 3H, OMe), 2.33–2.26 (m, 1H, H-3″), 2.18 (s, 3H, acetyl), 2.08–1.92 (m, 1H, H-3″), 1.51 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 1.32 (d, 3H, J = 6.3 Hz, H-6″), 1.31 (d, 3H, J = 6.3 Hz, H-6); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 170.3, 165.4, 138.2, 137.3, 133.2, 130.1, 129.8, 128.7, 128.4, 128.4, 128.1, 127.9, 127.7, 126.0, 109.2, 101.3, 99.0 ($J_{C1',H1'} = 175.9$ Hz, C-1′), 97.8 ($J_{C1,H1}$ = 170.0 Hz, C-1), 97.7 ($J_{C1'',H1''} = 172.9$ Hz, C-1″), 81.2, 78.7, 76.7, 76.0, 74.9, 72.1, 71.4, 71.0, 70.8, 68.8, 68.7, 64.6, 63.8, 54.9, 29.3, 28.1, 26.4, 21.0, 18.2, 17.4.
Compound **16**: $[\alpha]_D^{21.0}$ +34.1 (*c* 1.00, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.62 (d, 1H, $J_{1''',2'''} = 3.8$ Hz, H-1‴), 5.29 (s, 1H, H-1″), 4.93 (s, 1H, H-1′), 4.83 (s, 1H, H-1), 4.39 (dd, 1H, $J_{3',4'} = 10.4$ Hz, H-3′), 4.17 (dd, 1H, $J_{2',3'} = 5.0$ Hz, H-2′), 2.29–2.23 (m, 1H, H-3″), 1.90–1.84 (m, 1H, H-3″), 1.32 (d, 3H, J = 6.1 Hz, H-6″), 1.18 (d, 3H, J = 6.1 Hz, H-6); HRMS (FAB) calcd for $C_{77}H_{87}O_{19}$ $[M+H]^+$: 1315.5836; found, 1315.5829.
Compound **2**: ^{1}H NMR (300 MHz, D_2O, 333 K): (selected data) δ 5.25 (d, 1H, $J_{1',2'} = 1.5$ Hz, H-1′), 5.21 (d, 1H, $J_{1''',2'''} = 3.7$ Hz, H-1‴), 4.90 (br d, 1H, H-1),4.70 (d, 1H, $J_{1'',2''} = 1.4$ Hz, H-1″), 4.18–3.52 (m, 29H), 3.40 (s, 3H, OMe), 2.08–2.01 (m, 1H, H-3″), 1.90–1.78 (m, 1H, H-3″), 1.34 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6), 1.28 (d, 3H, $J_{5',6'} = 6.2$ Hz, H-6′); ^{13}C NMR (75 MHz, D_2O): δ 104.1, 104.0, 103.5, 102.5, 84.4, 81.8, 80.1, 76.3, 74.3, 73.2, 73.1, 72.1, 72.1, 71.5, 70.2, 70.2, 70.0, 69.1, 64.0, 63.3, 57.6, 19.9, 19.6; HRMS (ESI) calcd for $C_{25}H_{44}O_{18}$ $[M-H^+]^-$: 631.2455; found, 631.2452.

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