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A SYNTHETIC OCTASACCHARIDE MIMICS THE NATIVE, O-SPECIFIC DETERMINANT OF THE *Shigella dysenteriae* TYPE 1 LIPOPOLYSACCHARIDE

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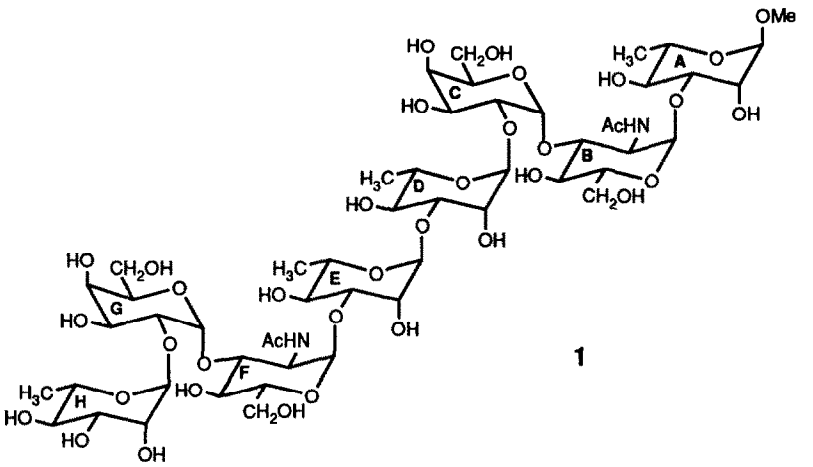
Abstract: The chemical synthesis of a linear octasaccharide, composed of two contiguous repeating units of the O-specific determinant of *Shigella dysenteriae* type 1 is described. ^{1}H-NMR data indicate that this octasaccharide possesses conformational features of the native polysaccharide.

Shigella dysenteriae type 1 is a human pathogen causing dysentery with a high incidence of mortality.[1] The virulence of this pathogen requires full expression of its lipopolysaccharide (LPS): indirect evidence suggests that the O-specific polysaccharide (O-SP) is also a protective antigen in humans.[1] Its LPS contains tetrasaccharide **A** as the repeating unit of the O-SP region.[2] It is an attractive assumption that protective, anti O-

-3)-α-L-Rha*p*-(1,2)-α-D-Gal*p*-(1,3)-α-D-Glc*p*NAc-(1,3)-α-L-Rha*p*-(1- **A**

SP antibodies might also be elicited by using smaller fragments of the native, O-SP.[3] Oligosaccharides (OSs) that mimick the properties of the native O-SP define the minimum structural requirements of such immunodeterminants. We are studying the synthesis of OSs possessing extended chain-length related to **A**.



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As part of this project, we describe a synthetic route to octasaccharide methyl glycoside **1**, which corresponds to two contiguous repeating units of the O-SP of the LPS of *S. dysenteriae* type 1.[4,5]

The synthesis of the octasaccharide **1** is based on assembly of two tetrasaccharides (**36** and **41**). These tetrasaccharides correspond to units A-D[6] and E-H[6] and were combined to provide a protected derivative of **1**. Tetrasaccharide acceptor **36**, and donor **41** were assembled in a stepwise manner using heterofunctional

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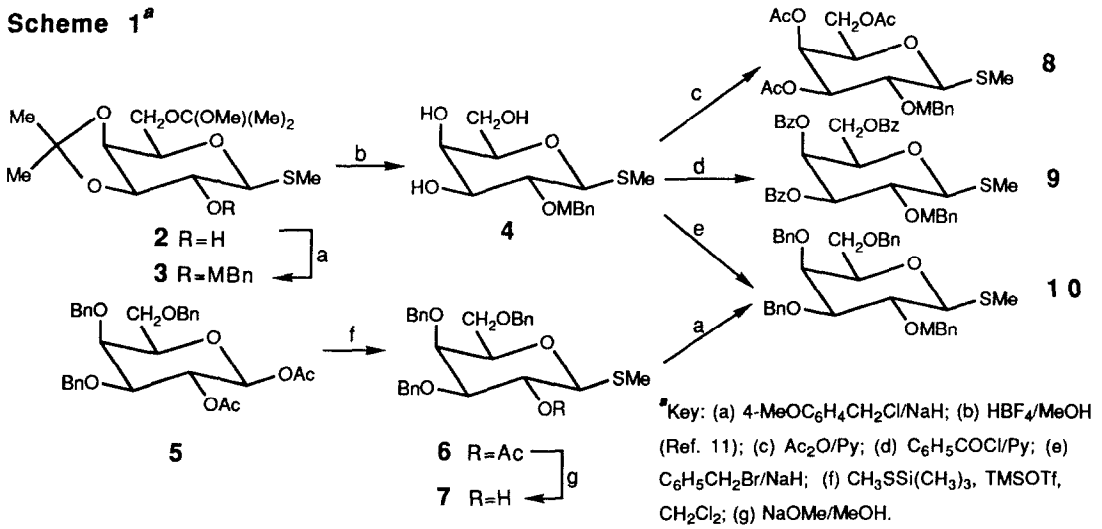
D-galacto-, D-gluco-, and L-rhamnopyranoside derivatives, the syntheses of which are described next.

D-Galactose synthons (Scheme 1) Thioglycosides **8-10** were selected as galactosyl donors.[7] Their common feature is the non-participating, 4-methoxybenzyl group at *O*-2. This type of protection allows the stereoselective formation of a 1,2-*cis* interglycosidic linkage upon activation of the anomeric center by thiophilic reagents. The sensitivity of the 4-methoxybenzyl group to oxidation[8] permits selective unmasking in the presence of a variety of protective groups which is a favourite feature for a multifunctional oligosaccharide intermediate. Triol[4,9] **4** (Scheme 1) was obtained from mixed acetal[10] **2** in two steps using the fully protected thiogalactoside[11] **3** (64 %) and converted directly to acyl- [**8** (92 %) and **9** (90 %)] and benzyl-protected thiogalactoside donors [**10** (93 %)] in conventional reactions. An alternative route to **10** utilized diacetate[12] **5** which was converted[13] to thioglycoside **6** (82 %) ($CH_3SSi(CH_3)_3$, TMSOTf, CH_2Cl_2) followed by deacetylation to give alcohol **7** (83 %) which was then 4-methoxybenzylated at *HO*-2 to provide **10** (85 %).

Scheme 1[a]

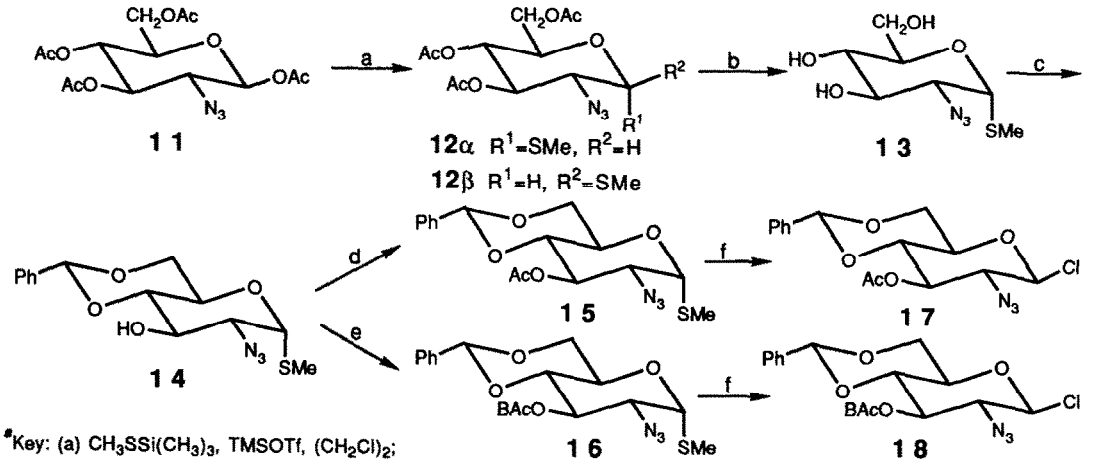


[a]Key: (a) 4-$MeOC_6H_4CH_2Cl$/NaH; (b) HBF_4/MeOH (Ref. 11); (c) Ac_2O/Py; (d) C_6H_5COCl/Py; (e) $C_6H_5CH_2Br$/NaH; (f) $CH_3SSi(CH_3)_3$, TMSOTf, CH_2Cl_2; (g) NaOMe/MeOH.

N-Acetyl-D-glucosamine synthons (Scheme 2) Tetraacetate[14] **11** was the starting material for the glucosamine synthons **15-18.** It was converted[13] to thioglycoside **12** ($CH_3SSi(CH_3)_3$, TMSOTf, $(CH_2Cl)_2$) (93 %, **12α/12β** 4:1). Routine transformation of **12α** by way of alcohols **13** and **14** [(i) de-*O*-acetylation, (ii) benzylidene acetal-formation, (iii) acylation)] provided acetate **15** and bromoacetate **16** respectively: these can be used as donors, either directly, or after conversion to crystalline glucosyl chlorides **17** and **18.**

D-Rhamnose synthons (Scheme 3) The intermediate for residue D was rhamnosyl donor **28** (ref. 15, 16), first prepared by Pavliak *et al.*[16] Its 2-*O*-benzoyl group anchimerically assists the stereocontrolled formation of a 1,2-*trans* interglycosidic linkage, and the bromoacetyl group permits selective unmasking of *HO*-3 without compromising the other linkages. Compounds **19** (ref. 17) and **20** (ref. 18) were first converted[19,20] to intermediate, cyclic orthoesters **21** and **22,** which were then benzoylated at *HO*-4. Regioselective opening[19,21] of the orthoester ring with acetic acid provided the 2,4-dibenzoates **23** and **24** [69-88 %

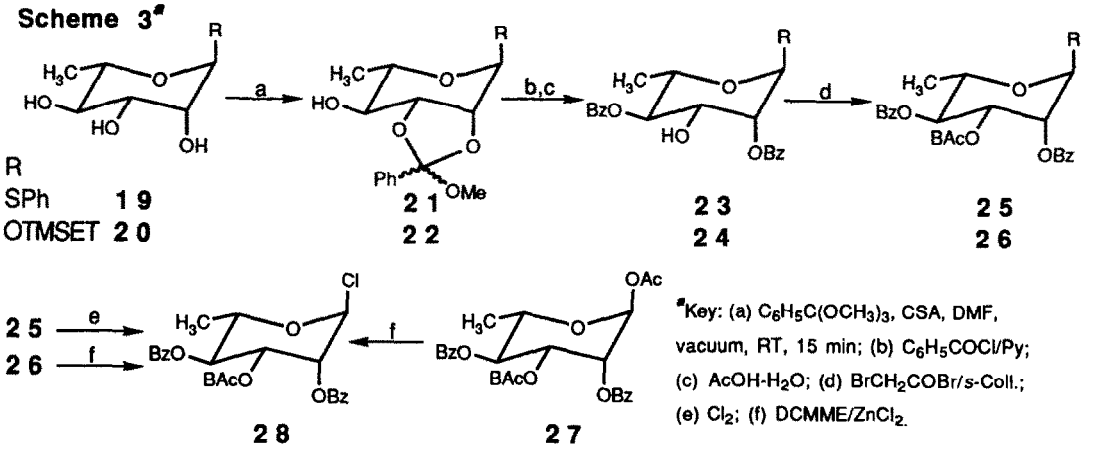
Scheme 2[a]



[a]Key: (a) $CH_3SSi(CH_3)_3$, TMSOTf, $(CH_2Cl)_2$; (b) NaOMe/MeOH; (c) $C_6H_5CH(OMe)_2$, CSA; (d) Ac_2O/Py; (e) $BrCH_2COBr$/*s*-Coll.; (f) Cl_2.

yields for three steps (a-c)]. Subsequent bromoacetylation at *HO*-3 yielded the fully protected rhamnosides **25** and **26**. Thioglycoside **25**, and 2-(trimethylsilyl)ethyl glycoside **26**, were converted to chloride **28** directly by chlorine and α,α-dichloromethyl methyl ether (DCMME)/$ZnCl_2$[22] respectively. Alternatively, chlorination (DCMME/$ZnCl_2$) of acetate **27** provided rhamnosyl donor **28** in 98 % yield.

Scheme 3[a]

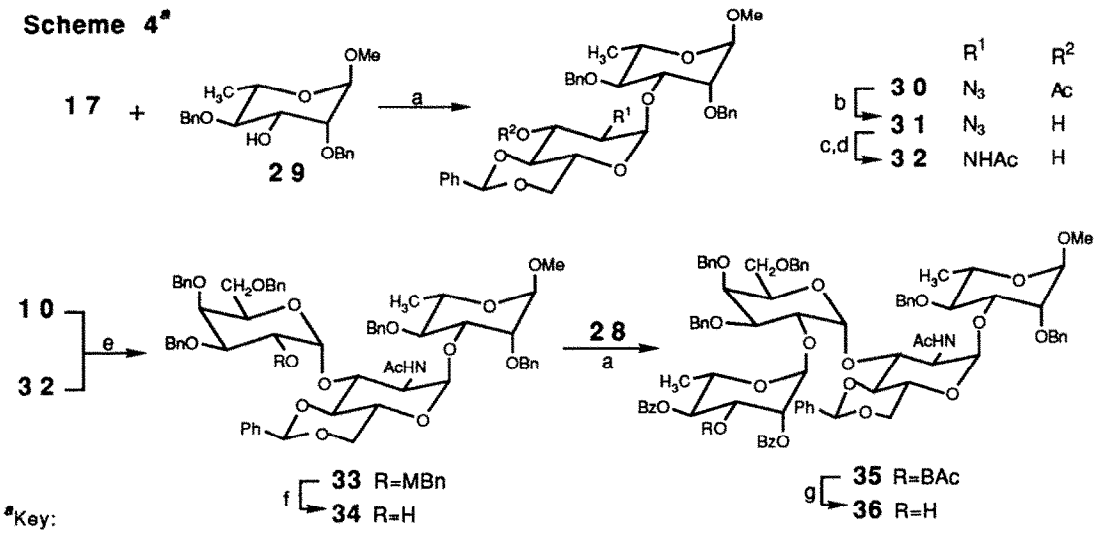


[a]Key: (a) $C_6H_5C(OCH_3)_3$, CSA, DMF, vacuum, RT, 15 min; (b) C_6H_5COCl/Py; (c) AcOH-H_2O; (d) $BrCH_2COBr$/*s*-Coll.; (e) Cl_2; (f) DCMME/$ZnCl_2$.

Stereoselective coupling of glucosamine donor **17** with the previously described alcohol[23] **29** (AgOTf) afforded disaccharide **30** in 65 % yield (Scheme 4). Removal of the acetyl group (NaOMe) gave alcohol **31** which was converted into acceptor **32** by azide-reduction[24] ($NiCl_2/H_3BO_3$-$NaBH_4$) followed by *N*-acetylation (79 %). Glycosylation of **32** with **10** (MeOTf, ether) provided trisaccharide **33** (87 %) which was treated[8a] with

DDQ to provide alcohol **34** (57 %). Reaction of rhamnosyl chloride **28** with nucleophile **34** (AgOTf) gave fully protected tetrasaccharide **35** (94 %) which was de-bromoacetylated to provide the acceptor **36** (94 %).

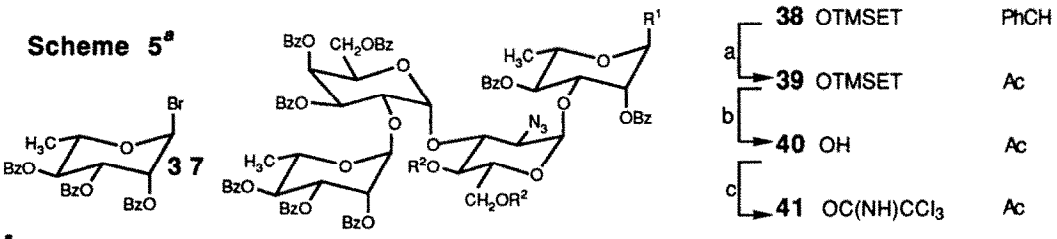
Scheme 4[a]



[a]Key: (a) AgOTf/2,6-di-^{t}Bu-4-Me-Py, CH_2Cl_2; (b) NaOMe; (c) $NiCl_2/H_3BO_3$; (d) Ac_2O; (e) MeOTf, ether; (f) DDQ; (g) $CS(NH_2)_2$

Compounds **9**, **18**, **24**, and **37** were used for the *stepwise* synthesis of tetrasaccharide **38** employing conditions for glycosylation and deprotection similar to those used for the synthesis of **36** (61 % yield for five steps). Sequential replacement of the benzylidene by acetyl groups (**38**→**39**), hydrolytic removal of the trimethylsilylethyl group[22] (**39**→**40**) and imidate formation[25] afforded tetrasaccharide donor **41** (75 % for three steps).

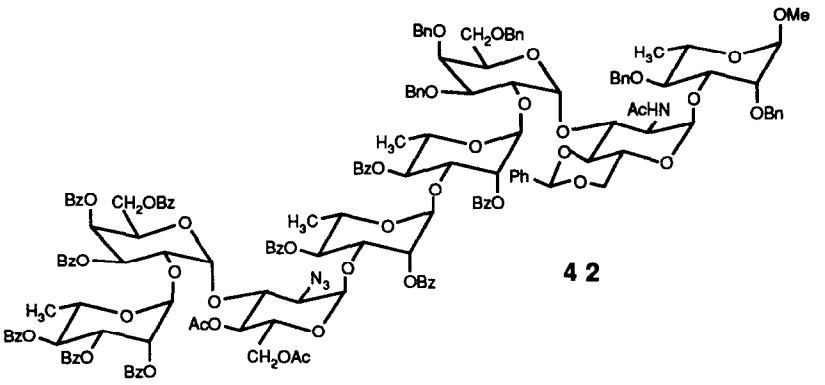
Scheme 5[a]



[a]Key: (a) AcOH-H_2O; (b) DCMME/$ZnCl_2$; (c) CCl_3CN, DBU.

Combination of tetrasaccharide acceptor **36** and donor **41** ($BF_3.Et_2O$)[25] gave fully protected octasaccharide **42** (69 %).[26] Its routine transformation by azide reduction ($NiCl_2/H_3BO_3$), N-acetylation (Ac_2O), de-O-acylation (NaOMe) and debenzylation (H_2/Pd-C), gave the free octasaccharide methyl glycoside **1**.

The chemical shifts of the anomeric protons in octasaccharide **1**, hexasaccharide[27] **43**, pentasaccharide[27] **44**, and tetrasaccharides[27] **45**, **46** depend on the length of the OS (Table).



42

43 α-L-Rha*p*-(1,3)-α-L-Rha*p*-(1,2)-α-D-Gal*p*-(1,3)-α-D-Glc*p*NAc-(1,3)-α-L-Rha*p*-(1,3)-α-L-Rha*p*-OMe

44 α-L-Rha*p*-(1,3)-α-L-Rha*p*-(1,2)-α-D-Gal*p*-(1,3)-α-D-Glc*p*NAc-(1,3)-α-L-Rha*p*-OMe

45 α-L-Rha*p*-(1,2)-α-D-Gal*p*-(1,3)-α-D-Glc*p*NAc-(1,3)-α-L-Rha*p*-OMe

46 α-D-Gal*p*-(1,3)-α-D-Glc*p*NAc-(1,3)-α-L-Rha*p*-(1,3)-α-L-Rha*p*-OMe

Table ^{1}H-NMR chemical shifts (ppm) of the anomeric protons of the O-specific polysaccharide of *Shigella dysenteriae* type 1 and oligosaccharides **1**, and **43-46**[a]

	Chemical shift[b]								
Compound	Residue								
	Rha	Gal	GlcN	Rha	Rha	Gal	GlcN	Rha	Rha
O-SP (A)	**5.11**	**5.60**	**5.04**	**5.11**	**5.05**	**5.60**	**5.04**	**5.11**	**5.05**
1	5.08	**5.60**	**5.04**	**5.11**	**5.05**	**5.60**	4.99	4.71	
43				5.08	**5.05**	**5.60**	**5.04**	5.05	4.66
44				5.07	**5.05**	**5.60**	4.99	4.71	
45					5.09	**5.61**	5.00	4.73	
46						5.42	**5.04**	5.07	4.66

[a]In D_2O at 296K , int. acetone δ=2.225 ppm. [b]Chemical shifts for the anomeric protons of the O-SP and for those in the oligosaccharides which coincide with the corresponding resonances of the O-SP are shown **in boldface**.

The chemical shift of only one anomeric proton in each of compounds **45** and **46** is identical with that of the corresponding proton in the O-SP. That number of common resonances is two for the O-SP/pentasaccharide **44** and three only for the O-SP/hexasaccharide **43** *i.e.* none of the OSs **43-46** embodies a *complete* repeating unit sequence of four residues whose conformation mimics that of the corresponding fragment in the O-SP. In octasaccharide **1**, the chemical shifts of the anomeric protons of five consecutive residues coincide with the corresponding resonances of the O-SP. It is probable that in **1** the fragment consisting of residues C-G resembles the conformation of the O-SP. Thus octasaccharide **1** is expected to be a valuable hapten for the preparation of synthetic antigens, which is in progress in these laboratories.

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6. For designation of the individual glycose residues, see formula **1**.
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12. Pozsgay, V.; Jennings, H.J. *Synthesis* **1990**, 724.
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19. This approach employs the chemistry originally suggested by Garegg, P.J. and Hultberg, H. (*Carbohydr. Res.* **1979**, *72*, 276), with the important modification described in Ref. 20.
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27. Manuscript in preparation.

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