Carbohydrate Research 352 (2012) 18–22

Contents lists available at SciVerse ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Synthesis of a tetrasaccharide analog corresponding to the repeating unit of the *O*-polysaccharide of *Salmonella enterica* O59: unexpected stereo outcome in glycosylation

Abhijit Sau [a], Rajib Panchadhayee [a], Debjani Ghosh [b], Anup Kumar Misra [a,*]

[a] Bose Institute, Division of Molecular Medicine, P-1/12, C.I.T. Scheme VII M, Kolkata 700054, India
[b] Scottish Church College, 1&3 Urquhart Square, Kolkata 700006, India

ARTICLE INFO

Article history:
Received 11 November 2011
Received in revised form 27 January 2012
Accepted 31 January 2012
Available online 10 February 2012

Keywords:
Salmonella enterica
Glycosylation
O-Polysaccharide
Stereoselectivity
Tetrasaccharide

ABSTRACT

Convergent synthesis of a tetrasaccharide analog corresponding to the repeating unit of the O-polysaccharide of *Salmonella enterica* O59 is presented. A thioglycoside disaccharide donor was prepared by the glycosylation of two thioglycosides by tuning their relative reactivity. An unexpected stereochemical outcome was observed in a glycosylation using an ethyl 2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-bensylidene-thiogalactoside donor, where the alpha-galactoside was formed in spite of the presence of the 2-*O*-acetyl participating group.

1. Introduction

Salmonella is a Gram-negative facultative rod-shaped bacterium in the family of Enterobacteriaceae, commonly known as 'enteric' bacteria.[1] *Salmonella enterica* is responsible for the food borne gastrointestinal infections causing salmonellosis in animals and humans.[2] *Salmonella* live in the intestinal tracts of warm and cold blooded animals. In general, *Salmonella enterica* infects cattle and poultry, which act as the reservoir of infections to humans.[3] Approximately 1.5 million cases of salmonellosis (common symptoms are enteric fever and acute gastroenteritis) and a significant number of deaths caused due to this infection are noted annually in the developed and developing countries.[4] *Salmonella* species are divided into a large number of serotypes based on the structure of their cell wall lipopolysaccharides (*O*-antigen) and the flagellar structures (*H*-antigen).[5,6] However, only few of them are responsible for the infections in humans. Recently, Perepelov et al. reported the structure of the cell wall *O*-polysaccharide of *Salmonella enterica* O59,[7] which contains two D-glucosamine, one D-galactose and one L-rhamnose moieties (Fig. 1).

Several reports in the recent past elaborately demonstrated the useful role of *O*-antigenic polysaccharides in the development of carbohydrate based therapeutics.[8,9] In order to have better understanding of the pathogenic role of the *O*-antigenic polysaccharide of *Salmonella enterica* O59, several immunochemical experiments should be carried out requiring a large quantity of the tetrasaccharide. Since isolation of the polysaccharide fragments from the natural source can not meet the requirement, a concise chemical synthetic strategy for the synthesis of the tetrasaccharide and its close analogs is highly desirable. As a part of the ongoing program on the synthesis of complex oligosaccharides from the bacterial origin, synthesis of the tetrasaccharide repeating unit (Fig. 2, structure 1) corresponding to the *O*-antigenic lipopolysaccharide of *Salmonella enterica* O59 strain has been undertaken. However, during the course of the synthesis of the target tetrasaccharide **1**, an unusual stereochemical outcome of one of the glycoside formations was observed. Although using a thiogalactoside donor with a 2-*O*-participating group still the disaccharide formed was found to be alpha-linked. Continuing from this unexpected disaccharide derivative a tetrasaccharide analog **2** (Fig. 2) related to the *O*-antigenic lipopolysaccharide of *Salmonella enterica* O59 strain was synthesized.

→4)-α-L-Rha*p*-(1→3)-β-D-Glc*p*NAc-(1→2)-β-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→

Figure 1. Structure of the repeating unit of the *O*-polysaccharide of *Salmonella enterica* O59.

* Corresponding author. Tel.: +91 33 2569 3240; fax: +91 33 2355 3886.
E-mail address: akmisra69@gmail.com (A.K. Misra).

0008-6215/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2012.01.026

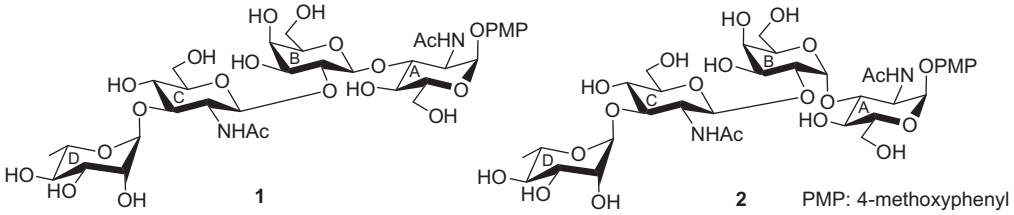


Figure 2. Chemical structure of the alpha –p-methoxybenzyl glycoside of the tetrasaccharide repeating unit of the *O*-polysaccharide of *Salmonella enterica* O59 (**1**) and structure of the synthesized tetrasaccharide **2** related to tetrasaccharide **1**.

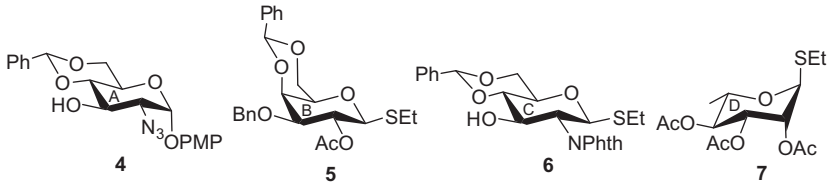
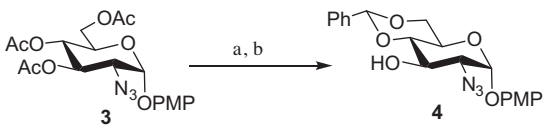


Figure 3. Monosaccharide intermediates used for the synthesis of compound **2**.

2. Results and discussion

In the initial attempt to synthesize compound **1**, a number of suitably functionalized monosaccharide intermediates **3**,[10] **4**, **5**,[11] **6**[12], and **7**[12] (Fig. 3) were prepared from the commercially available reducing sugars using earlier reported reaction conditions. A convergent strategy involving [2+2] glycosylation has been adopted for the synthesis of compound **1**. Compound **4** was prepared in 77% yield from compound **3** using a two step sequence involving de-acetylation and iodine catalyzed 4,6-*O*-benzylidene acetal formation[13] (Scheme 1). Iodonium ion promoted stereoselective glycosylation[14] of compound **4** with thioglycoside **5** using *N*-iodosuccinimide (NIS) and trifluoromethane sulfonic acid (TfOH) furnished compound **8** in 77% yield having a newly formed 1,2-*cis* glycosyl linkage. Formation of 1,2-*cis* glycoside was confirmed from its spectral analysis [signals at δ 5.68 (d, J = 4.0 Hz, H-1_B), 5.52 (d, J = 3.5 Hz, H-1_A) in the ^{1}H NMR and δ 98.6 (C-1_A), 97.4 (C-1_B) in the ^{13}C NMR spectra]. Formation of compound **8** with 1,2-*cis* glycosyl linkage using 2-O-acetylated D-galactosyl thioethyl glycoside donor was unexpected due to the well known concept that the presence of an acyl protection group on 2-OH of a glycosyl donor commonly induces the exclusive formation of a 1,2-*trans* glycoside because of the neighboring group participation effect.[15,16] Using other 2-O-acetylated glycosyl donors (e.g., thiophenyl glycoside, trichloroacetimidate derivative) showed similar unexpected stereo outcome of the glycosylation. In a separate experiment, reaction of the same glycosyl acceptor with ethyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside, exclusively furnished 1,2-*trans* glycosylated product (80%). In both galactosyl thioglycoside donors 4,6-*O*-benzylidene acetal is present, but the difference in 3-O-substituents (benzyl and acetyl) directs the stereochemical outcome of the glycosylation product. This observation indicated that the presence of 3-*O*-benzyl ether in the thioglycoside donor controls the stereo-outcome of the glycosylations. It is presumed that a five-member twisted boat transition state may form during the activation of the thioglycoside with NIS–TfOH, which forces the nucleophile to approach from the 1,2-*cis* orientation and thus furnishes 1,2-*cis* glycosylated product. In earlier reports by Satoh et al.,[17] Manabe et al.,[18] Oscarson et al.[19] and Crich et al.[20] it was considered that *endo*-cyclic post glycosylation anomerization plays the pivotal role in the formation of 1,2-*cis* glycosylation products. Recently, Oscarson and co-workers[21] reported the anomerization of disaccharides by kinetic measurements. However, in this particular case such possibilities may be excluded since the functional groups present in the glycosyl donor are different. Although the formation of compound **8** was unexpected and can not be used for the synthesis of the target tetrasaccharide **1**, we planned to use compound **8** in the preparation of an analogous tetrasaccharide **2** related to the target compound **1**. Analogous compounds are very important in evaluating their biological potential in comparison to the original compounds. Thus compound **8** was deacetylated using sodium methoxide to give disaccharide derivative **9** in quantitative yield.



Scheme 1. Reagents and conditions: (a) 0.1 M CH_3ONa, CH_3OH, room temperature, 2 h; (b) benzaldehyde dimethyl acetal, I_2, CH_3CN, 50 °C, 8 h, 77%.

In another experiment, stereoselective glycosylation of thioglycoside **6** and thioglycoside **7** in the presence of a combination of NIS–TfOH[14] furnished disaccharide derivative **10** in an 81% yield. Appearance of signature peaks in the NMR spectra [signals at δ 5.37 (d, J = 10.5 Hz, H-1_C), 4.51 (br s, H-1_D) in the ^{1}H NMR and δ 97.3 (C-1_D), 81.9 (C-1_C) in the ^{13}C NMR spectra] confirmed the formation of compound **10**. Although both compounds **6** and **7** are thioethyl glycosides and can be activated by the combination of NIS–TfOH, the selective activation of compound **7** was achieved because of the fact that deoxy sugars are more highly reactive than the amino sugars, which has been established in several earlier reports by Wong and co-workers using relative reactivity values.[22,23] Presence of a deactivating *N*-phthalimido group at C-2 position of compound **6** induces disarming effect to act as glycosyl acceptor in the presence of a comparatively activated 6-deoxy-L-sugar derived thioglycoside **7**. This kind of selective activation has also been documented by Demchenko and co-workers[24] and Bols and co-workers[25] considering super armed/disarmed glycosylation approach. Iodonium ion promoted stereoselective glycosylation of disaccharide **9** with disaccharide **10** using NIS–TfOH combination furnished tetrasaccharide derivative **11** in a 74% yield. Formation of compound **11** was supported by it's spectral analysis [signals at δ 5.55 (d, J = 4.0 Hz, H-1_B), 5.48 (d, J = 3.5 Hz, H-1_A), 5.39 (d, J = 8.0 Hz, H-1_C), 4.45 (d, J = 1.5 Hz, H-1_D) in the ^{1}H NMR and δ