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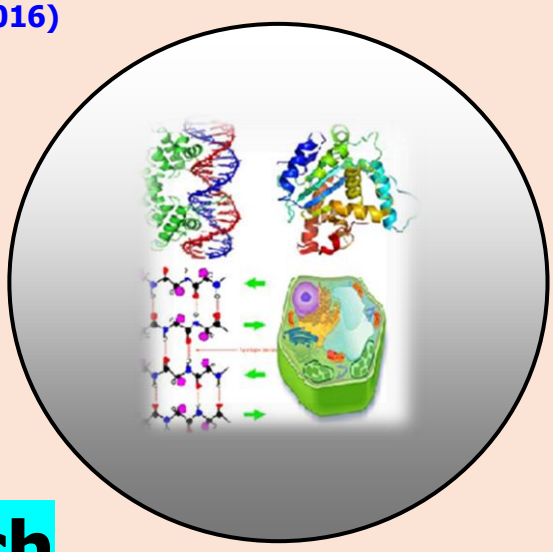

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Dr. Ruchi Saxena
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RESEARCH PAPER

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Synthetic 1-O-allyl-α-D-glucopyranoside and its Derivatives as a Presumptive Safeguard against *Helicobacter pylori*

***Ruchi Saxena and Seema Singh**

***Department of Chemistry, Nari Shiksha Niketan, P.G. College, Lucknow, U.P., India**
Department of Zoology, Nari Shiksha Niketan, P.G. College, Lucknow, U.P., India

ABSTRACT

The bacterium (H. pylori) is responsible for persistent gastric disorders in 50% of the world's population. Stomach cancers due to H. pylori represent 5.5% of all cancers worldwide with 25% infection-associated malignancies. This high virulent factor of H. pylori has compelled WHO to declare it as Class-1 carcinogen. To eradicate it, Standard Triple Therapy (STT) is established in clinical practice, but antibiotic resistance and evasion of the hosts' immune response by H. pylori has rendered the prevalent therapies ineffective. In the cell wall of H. pylori α-D-glucopyranoside and acyl-α-D-glucopyranoside are present, which combine with the cholesterol of the host, catalysed by the enzyme Cholesterol-α-glucosyl transferase(CHLαGcT) to evade the immune response. The exploitation or modulation of the host cholesterol has to be targeted to eradicate H. pylori infections. The activities of CHLαGcT are inhibited by synthetic O-linked oligosaccharides and monosaccharides, which can be used for prevention against H. pylori.

For this purpose, α-D-glucopyranoside and its derivatives were synthesized with allyl linker arms and conjugated to a carrier Bovine Serum Albumin (BSA) using reductive ozonolysis followed by reductive amination to yield the 1-O-ethylimine-N-lysyl-BSA-α-D-glucopyranoside(Allyl-α-D-glucopyranoside-oxylysyl-BSA), 1-O-ethylimine-N-lysyl-BSA-2,3-di-O-methyl-α-D-glucopyranoside(Allyl-2,3-di-O-methyl-α-D-glucopyranoside-oxylysylBSA) (13) and 1-O-ethylimine-N-lysyl-BSA-2,3-di-O-benzyl-α-D-glucopyranoside(Allyl-2,3-di-O-benzyl-α-D-glucopyranoside-oxylysyl-BSA). Purification of compounds was done by gel-filtration and thereafter dialysed. The percentage of sugar bound to BSA was determined by phenol-sulphuric acid method. The antigenic activity of the three carbohydrate BSA conjugates was tested in rabbits by Enzyme Linked Immunosorbent Assay (ELISA) and Ouchterlony techniques.

Results showed a positive yield of IgG class of antibodies in dilutions of neat, 1/10,1/20 and 1/100 against the compound 1-O-ethylimine-N-lysyl-BSA-α-D-glucopyranoside(Allyl-α-D-glucopyranoside). This compound may be a lead for the development of a glycoconjugate vaccine against H. pylori.
Key-words: H. Pylori, gastritis, glucopyranoside, conjugate, Bovine Serum Albumin (BSA) and Enzyme Linked Immunosorbent Assay (ELISA).

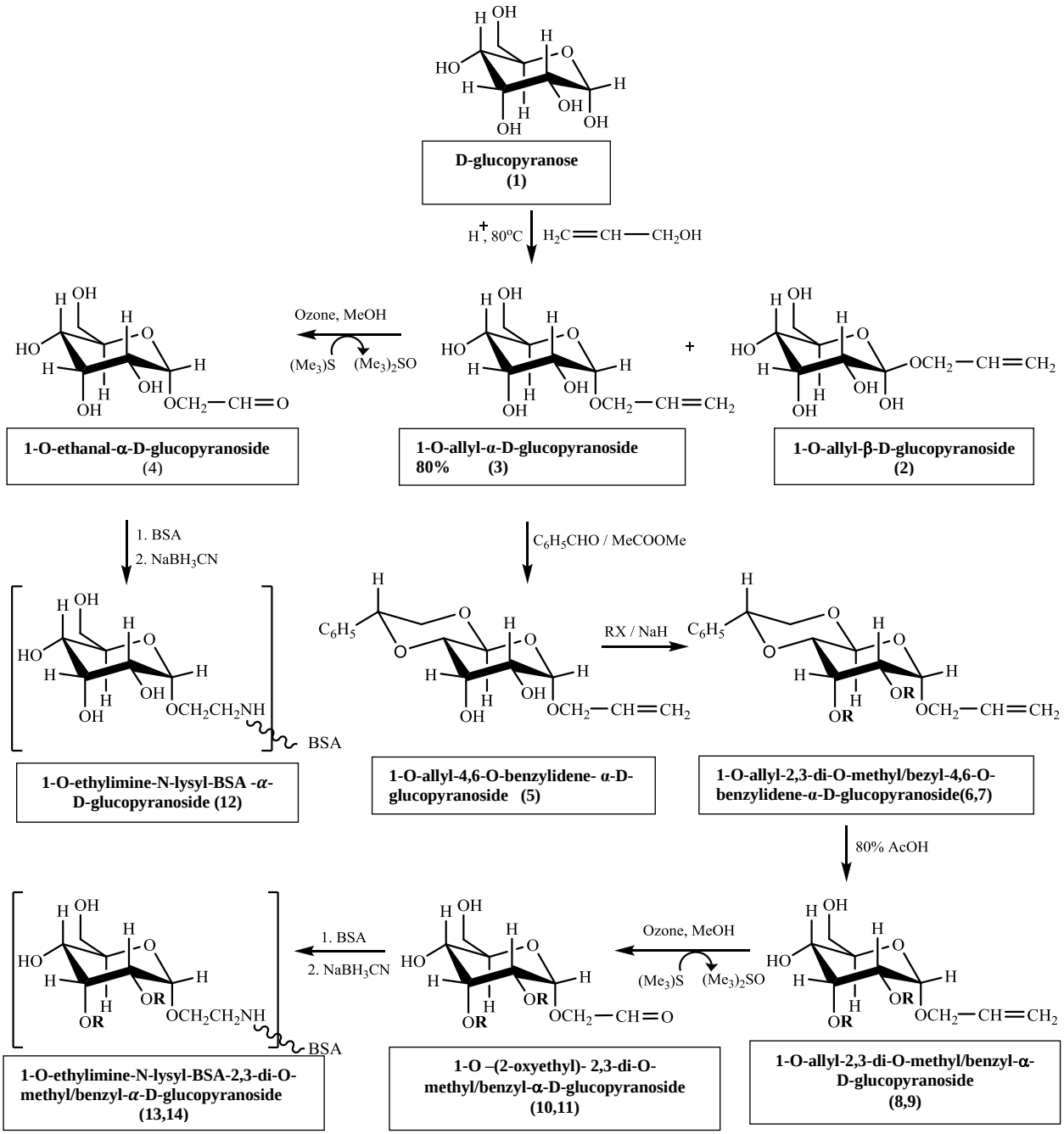
INTRODUCTION

Helicobacter pylori (*H. pylori*) is a gram-negative, flagellated, spiral shaped, microaerophilic bacterium belonging to the genus *Spirillum*. It was initially isolated from the gastric mucous membrane of a patient suffering from chronic gastritis by Marshall and Warren in 1983, who received the noble prize (2005) in Physiology for this work(Warren *et al*,1983; Megraud, 2005). This bacterium causes infection worldwide, its prevalence among adults in many developing countries is approximately 80% and in developed countries is 50%. *H. pylori* related stomach cancer represents 5.5% of all cancers worldwide and 25% of all infection-associated malignancies (James *et al*, 2017). The pathogenesis of *H. pylori* leads to gastritis, Peptic Ulcer Disease (PUD), duodenal ulcer, gastric adenocarcinoma and gastric Mucosa-Associated Lymphoid Tissue (MALT) lymphoma. It is also related to non-digestive conditions like neurological disorders, iron deficient anaemia, cardiovascular, metabolic and kidney diseases. The morbidity due to *H. pylori* infections has compelled the World Health Organization (WHO) to classify it as Class-1 carcinogen with a risk of stomach cancers (Tytgat, 2011).

The clinical practice against *H. pylori* infections is a Standard Triple Therapy(STT) by i)Proton Pump Inhibitor(PPI), ii) Clarithromycin and iii) Amoxicillin but it is not fully successful due to antibiotic resistance (Kim *et al*, 2015). The primary cause of the antibiotic resistance in *H. pylori* is attributed to the evasion of immune response by glucosylation of the host cholesterol (Wunder *et al*, 2006) and by the emergence of point mutations. To prevent *H. pylori* infections treatment should be based on the inhibition of cholesterol glucosylation and making it accessible to the immune system of the patient followed by antibiotics to mediate the bacterial killing. Therefore, in the present study, monosaccharide BSA conjugates of 1-O-ethylimine-N-allyl-α-D-glucopyranoside(Allyl-α-D-glucopyranoside), 1-O-ethylimine-N-allyl-2,3-di-O-methyl-α-D-glucopyranoside(Allyl-2,3-di-O-methyl-α-D-glucopyranoside) and 1-O-ethylimine-N-allyl-2,3-di-O-benzyl-α-D-glucopyranoside(Allyl- 2,3-di-O-benzyl-α-D-glucopyranoside) were synthesized as antigen. Their antigenic activity was tested in rabbits by ELISA and Ouchterlony techniques for protective efficacy against *H. pylori*(Aspinal, 1992).

MATERIAL AND METHODS

D-glucopyranoside was used as a starting material to synthesize the carbohydrate epitopes 1-O-ethylimine-N-allyl-α-D-glucopyranoside,1-O-allyl-2,3-di-O-methyl-α-D-glucopyranoside, 1-O-allyl-2,3-di-O-benzyl-α-D-glucopyranoside by protection and deprotection according to the following scheme (Fig 1) (Aspinal, 1992;Brimacombe, 1973).



R = —$CH_2C_6H_5$; RX = $C_6H_5CH_2Br$ BSA= Bovine serum albumine

R = —CH_3 ; RX = CH_3I Me= CH_3

Figure 1. Scheme for Synthesis of carbohydrate epitopes.

1. Synthesis of 1-O-allyl-α-D-glucopyranoside (3) $C_9H_{18}O_6$:

5gm of α-D-glucopyranose(1) was heated with 15ml of allyl alcohol in presence of cation resin I.R.120[H^+](2.5gm) for 18hours at 70^{0}C. The reaction mixture was filtered in hot to yield (3).

Yield = 80%; MP = 96^{0}C; $[\alpha]_D$ = +131 (c = 0.3CH_3OH)

^{1}HNMR at 90MHz in DMSO(D_8)(ppm)

δ 6.00-5.65(m,1H,H-2'); δ 5.38-5.31, δ5.04-4.90(m,2H,H-3'); δ 4.55(d,1H,H-1,J=4Hz); δ4.00-3.75(m,3H,H-4,H-6); δ3.54-2.99(m,5H,H-2,H-3,H-5,H-1').

2. Synthesis of 1-O-allyl-4,6-O-benzylidene-α-D-glucopyranoside(5) $C_{16}H_{20}O_6$:

5gm of (3) was reacted under reduced pressure with 7ml(1.5eq) of benzaldehyde-dimethyl-acetal and 25mg of p-toluene-sulphonic-acid in 40ml dimethyl-formamide(DMF). On completion of the reaction, 8gm of sodium bicarbonate was added. The workup was done with chloroform and was washed in sequence with saturated solution of sodium carbonate, brine and water. The washed chloroform layer was dried over anhydrous sodium sulphate then filtered and dried to yield (5).

Yield = 66%; MP = 116-119°C; $[\alpha]_D$ = +31.9(c = 0.06CH_3OH)

^{1}HNMR at 60MHz (Varian EM- 360L spectrometer) in $CDCl_3$(ppm)

δ 7.27-7.06 (m, 5H, aromatic); δ 6.00-5.65 (m,1H,H-2'); δ 5.18(s, 1H, methine of acetal);
δ 5.38-5.31, δ 5.04-4.90 (m, 2H, H-3'); δ 4.55 (d,1H, H-1,J = 4Hz); δ 4.55-4.32 (m, 3H, H-4, H-6); δ 3.80-3.72(m,1H,H-5); δ 3.61-3.20 (m, 4H, H-2, H-3, H-1').

3. Synthesis of 1-O-allyl-2,3-di-O-methyl-4,6-O-benzylidene-α-D-glucopyranoside(6) $C_{18}H_{24}O_6$:

500mg(2.5meq) of (5) was dissolved in 10ml of tetra hydro furan(THF) and stirred with 116mg of NaH(2.5meq) at room temperature and then cooled to 0^0C.Thereafter 0.3ml CH_3I (4meq) was added dropwise and the mixture was stirred for 1.5 hours and lastly methanol was added. The reaction mixture was concentrated and worked up with CH_3Cl and washed sequentially with saturated solution of sodium carbonate, brine and water. The washed chloroform layer was dried over anhydrous sodium sulphate, filtered and dried to yield (6).

Yield = 74%, $[\alpha]_D$ = +42.8 (c = 0.03CH_3OH)

^{1}HNMR at 90MHz in $CDCl_3$ (ppm)

δ 7.20-7.08(m,5H,aromatic); δ 5.90-5.50(m,1H, H-2'); δ 5.31(s,1H, methine of acetal);
δ 5.35-5.29, δ 5.04-4.90 (m, 2H, H-3'); δ 4.80(d,1H, H-1, J=4Hz); δ 4.35-3.70 (m,4H, H-4, H-5, H-6); δ3.52-3.49 (m,1H, H-3); δ3.42 (s, 3H,OCH_3); δ3.31(s,3H, OCH_3);
δ3.20-3.00 (m, 3H, H-2, H-1').

4. Synthesis of 1-O-allyl-2,3-di-O-methyl-α-D-glucopyranoside(8) $C_{11}H_{20}O_6$:

2-3 drops of 80% CH_3COOH was added to a solution of 250gm of (6) in CH_3Cl and heated to 80^0C for 1hour and concentrated. The reaction mixture was concentrated and worked up with CH_3Cl and washed sequentially with saturated solution of sodium carbonate, brine and water. The washed chloroform layer was dried over anhydrous sodium sulphate, filtered and dried to yield (8).

Yield = 78%, $[\alpha]_D$ = +88.09(c = 0.2CH_3OH)

^{1}HNMR at 90MHz in$CDCl_3$ (ppm)

δ 5.93-5.61(m,1H,H-2'); δ5.35-5.30, δ5.22-5.04 (m,2H,H-3');δ 4.91(d,1H,H-1, J=4Hz); δ4.30-4.00 (m, 2H, H-6); δ 3.80-3.70 (m,1H,H-3); δ3.56 (s,3H, OCH_3);
δ 3.53-3.46(m,3H, H-2, H-1'); δ3.40(s,3H,OCH_3); δ 3.21-3.02 (m, 2H, H-4, H-5).

5. Synthesis of 1-O-allyl-2,3-di-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside(7) $C_{30}H_{32}O_6$:

500mg(2.5meq)of(5)was dissolved in 10ml of freshly prepared tetra hydro furan(THF) and stirred with 116mg of NaH(2.5meq) at room temperature and then cooled to 0^0C.Thereafter 0.4 ml benzyl bromide (4meq) was added dropwise and the mixture was stirred for 1.5hours at room temperature and finally 10ml methanol was added. The reaction mixture was concentrated and worked up with CH_3Cl and washed sequentially with sodium carbonate, brine and water. The washed chloroform layer was dried over anhydrous sodium sulphate, filtered and dried to yield (7).

Yield: 58%, $[\alpha]_D$ = +10.1(c=0.1CH_3OH)

^{1}HNMR at 90 MHz in $CDCl_3$ (ppm)

δ 7.40-7.10(m,15H, aromatic); δ 5.91-5.60 (m,1H, H-2'); δ 5.45(s,1H, methine of acetal); δ 5.35-5.29, δ 5.22-5.04 (m, 2H, H-3'); δ4.70(d,1H, H-1); δ4.22-3.82(m, 4H,H-4, H-6); δ 3.95-3.82 (m, 2H, H-2, H-3;) δ 3.75-3.38 (m, 6H,OCH_2).

6. 1-O-allyl-2,3-di-O-benzyl-α-D-glucopyranoside (9) $C_{23}H_{28}O_6$:

2-3 drops of 80% CH_3COOH was added to a solution of 250gm of (7) in CH_3Cl and heated to 80^0C for 1hour and concentrated. The reaction mixture was concentrated and worked up with CH_3Cl and washed sequentially with sodium carbonate, brine and water. The washed chloroform layer was dried over anhydrous sodium sulphate, filtered and dried to yield (9).

Yield = 52%,$[\alpha]_D$ = +76.05(c=0.4 CH_3OH)

^{1}HNMR at 90 MHz in $CDCl_3$ (ppm)

δ 7.32-7.02(m,10H,aromatic); δ 6.01-5.52(m,1H,H-2'); δ 5.29-5.20, δ5.11-4.92(m, 2H, H-3'); δ 4.65(d,1H,H-1, J=4Hz); δ4.19-3.80 (m, 4H, H-4, H-5, H-6); δ 3.82-3.51(m, 6H,OCH_2); δ 3.50-3.30(m, 2H, H-2, H-3).

7. Synthesis of carbohydrate bearing epitope with immunogenic carrier (BSA)(Bernstein, 1980).

1-O-ethylimine-N-lysy-lBSA-α-D-glucopyranoside (12)

1-O- ethylimine-N-lysy-lBSA-2,3-di-O-methyl-α-D-glucopyranoside (13)

1-O- ethylimine-N-lysy-lBSA-2,3-di-O-benzyl-α-D-glucopyranoside (14)

30mg of each 1-O-allyl-α-D-glucopyranoside(3), 1-O-allyl-2,3-di-O-methyl-α-D-glucopyranoside(8) and 1-O-allyl-2,3-di-O-benzyl-α-D-glucopyranoside(9) were dissolved separately in 50ml CH_3OH, treated with O_3 at -78^0C for 10minutes and 0.2ml of Dimethyl sulphide was added to each solution and brought to room temperature to yield the corresponding aldehydes [1-O-(2-oxyethyl)-α-D-glucopyranoside(4); 1-O-(2-oxyethyl)-2,3-di-O-methyl-α-D-glucopyranoside(10);1-O-(2-oxyethyl)-2,3-di-O-benzyl-α-D-glucopyranoside(11)]. 35mg BSA dissolved in 8ml of 0.2M sodium phosphate buffer (pH=7.8) was added to 25mg of each aldehyde in presence of 35mg sodium cyanoborohydride and kept at 37^0C for 72 hours to yield the corresponding 1-O- ethylimine-N-lysyl-BSA-α-D-glucopyranoside(12); 1-O-ethylimine-N-lysyl-BSA-2,3-di-O-methyl-α-D-glucopyranoside(13); 1-O-ethylimine-N-lysylBSA-2,3-di-O-benzyl-α-D-glucopyranoside (14).

Purification was brought about by gel-filtration through Sephadex G-25. Presence of sugars was confirmed by phenol sulphuric acid test and by absorbance at 490nm.

The BSA conjugates (glycoprotein) fractions were separated by absorbance at 280nm from the carbohydrate rich fractions. The percentage of monosaccharide per BSA molecule was determined as:
7.22% of 1-O-allyl-α-D-glucopyranoside(3) was present in 1-O- ethylimine-N-lysylBSA-α-D-glucopyranoside(12).
5.69% of 1-O-allyl-2,3-di-O-methyl-$\boldsymbol{\alpha}$-D-glucopyranoside(8) was present in 1-O-ethylimine-N-lysylBSA-2,3-di-O-methyl-α-D-glucopyranoside(13).
4.3%of 1-O-allyl-2,3-di-O-benzyl-α-D-glucopyranoside(9) was present in1-O-ethylimine-N-lysylBSA-2,3-di-O-benzyl-α-D-glucopyranoside (14).
The glycoprotein fractions were dialysed against distilled water to remove phosphate ions and freeze dried.

Biological Activity
Antibody formation in New Zealand White strain Rabbits (Oryctolagus cuniculus) was tested by two techniques (ELISA and Ouchterlony)(Hornbeck *et al,* 2017; Lequin *et al,* 2005).
12 New Zealand White strain of Rabbits (*Oryctolagus cuniculus*), 6 months old, weighing between 3 to 3.5kgs were housed in standardized animal house at Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS), Lucknow. They were provided food and water *ad libitum.* All the procedures on animals were reviewed and approved by the ethical committee. Pre-immune serum on day zero was collected from the rabbits and stored at -20^0 C to be used as control for Ouchterlony and ELISA techniques. 1ml of Carbohydrate BSA conjugant was mixed with 1ml of complete Freund's adjuvant to yield a colloidal paste by repeated drawing in and out by a syringe. This colloidal paste was then injected at 10 sites intradermally along the back and the hind limbs. Total of four injections were given at 2 weeks interval to these animals i.e. on day 0, 14, 28 and 42. On day 0 and 14 the injections of carbohydrate BSA conjugates given were mixed with complete Freund's adjuvant, whereas the subsequent injections given on day 28 and 42 were mixed with equal quantities of incomplete Freund adjuvant. A test bleed were drawn from the marginal ear vein of the rabbits, ten days after the fourth injection i.e. on day fifty-two. The test bleed was tested for antibody formation. Serum was separated from the test blood samples. Tests were carried out with the following strengths\ dilutions of the sera: neat, 1/10,1/20 and 1/100 and the titres of antibodies were evaluated. No precipitation reaction was observed in Ouchterlony technique. Melting points were recorded by Boetius micro melting point apparatus. Optical rotations were measured with Perkin Elmer 241 polarimeter in 1dm tube (Macdonald *et al*, 1969). The ^{1}HNMR spectra were recorded with 90 MHz Perkin Elmer R-32 spectrometer in $CDCl_3$ using TMS (Tetra Methyl Silane) as the internal standards (Lowicki *et al,* 2013).Sugars were visualised on Thin Layer Chromatography (TLC) (with 50% H_2SO_4 on the adsorbent Silica gel-G BDH (Ovodov *et al*, 1967) Silica gel 60-120 mesh (BDH) was used for column chromatography by Duncan's Method (Altenau, 1966). Sephadex G25 Sigma was used for gel filtration (O'Fagain *et al,* 2017). Carbohydrate estimation was done by Phenol Sulphuric acid reagent. Phosphate buffer pH 7.8 was used (Nielsen, 2017).

RESULTS
(1) 1-O-allyl-α-D-glucopyranoside(Allyl-α-D-glucopyranoside) (3) as the major product ($C_9H_{18}O_6$; yield-80%; MP-138^0C; $[\alpha]_D$ = +131; c=0.3 CH_3OH)

(2) 1-O-allyl-β-D-glucopyranoside(Allyl-β-D-glucopyranoside)(2) in traces (kinetically controlled product).
(3) 1-O-(2-oxyethyl)-α-D-glucopyranoside(4) was unstable.
(4) 1-O-ethylimine-N-lysyl-BSA-α-D-glucopyranoside(12).
(5)1-O-allyl-4,6-O-benzylidene-α-D-glucopyranoside(Allyl-4,6-O-benzylidene-α-D-glucopyranoside) (5)Yield = 66%; MP = 116-119°C; $[\alpha]_D$ = +31.9(c = 0.06CH_3OH).
(6)1-O-allyl-2,3di-O-methyl-4,6-O-benzylidene-α-D-glucopyranoside(Allyl-2,3di-O-methyl-4,6-O-benzylidene-α-D-glucopyranoside)(6) Yield = 74%, $[\alpha]_D$ = +42.8 (c = 0.03CH_3OH).
(7)1-O-allyl-2,3di-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside (Allyl-2,3di-O-methyl-4,6-O-benzylidene-α-D-glucopyranoside) (7) Yield: 58% , $[\alpha]_D$ = +10.1(c=0.1CH_3OH)
(8)1-O-allyl-2,3-di-O-methyl-α-D-glucopyranoside**(**Allyl-2,3-di-O-methyl-α-D-glucopyranoside.($C_{11}H_{20}O_8$; yield-78%; MP-96^{0}C; $[\alpha]_D$= +88.09; c=0.2 CH_3OH)
(9)1-O-allyl-2,3-di-O-benzyl-α-D-glucopyranoside (9) Yield: 58% , $[\alpha]_D$ = +10.1(c=0.1CH_3OH).
(10) 1-O-(2-oxyethyl)-2,3-di-O-methyl-α-D-glucopyranoside (10) was unstable.
(11) 1-O-(2-oxyethyl)-2,3-di-O-benzyl-α-D-glucopyranoside (11)was unstable

Synthesis of carbohydrate bearing epitope with immunogenic carrier (BSA)
The percentage of monosaccharide per BSA molecule:
(12)1-O-ethylimine-N-lysyl-BSA-α-D-glucopyranoside(Allyl-α-D-glucopyranoside-oxylysyl BSA)(12)-7.22% binding
(13) 1-O-ethylimine-N-lysyl-BSA-2,3-di-O-methyl-α-D-glucopyranoside
(Allyl-2,3-di-O-methyl-α-D-glucopyranoside-oxylysyl BSA) (13)-5.69% binding
(14)1-O-ethylimine-N-lysylBSA-2,3-di-O-benzyl-α-D-glucopyranoside-(Allyl-2,3-di-O-benzyl-α-D-glucopyranoside-oxylysyl BSA)(14)-4.3% binding

Biological Activity
Out of the three serums separated from the test bleed samples, the synthetic 1-O-ethylimine-N-lysyl-BSA-α-D-glucopyranoside (Allyl-α-D-glucopyranoside-oxylysl-BSA)(12),on preliminary testing in ELISA gave positive results for immunoglobulin M(IgM) class of antibodies when tested in rabbits in dilutions of neat, 1/10, 1/20 and 1/100. While there was no precipitation reaction observed with any of the three sera in the Ouchterlony technique.

DISCUSSION

Mode of Infection by *H. pylori*
H. pylori adapts itself by secreting large amounts of enzyme urease to hydrolyse the endogenous urea, thereby neutralizing the acidic pH of the gut. The neutral pH facilitates its binding to the gastric epithelial cells via bacterial adhesins by recognizing specific carbohydrate residues, such as the Lewis b blood group antigen and sialyl dimeric Lewis X. Analysis of these specific carbohydrate residues present in *H. pylori* lipids by mass spectrometry and nuclear magnetic resonance established their structures as cholesteryl-α-D-glucopyranoside (α-CG)), cholesteryl-acyl-α-glucoside (cholesteryl-6-O-tetradecanoyl-α-D-glucopyranoside (α-CAG)) and cholesteryl-phosphatidyl-α-glucoside (cholesteryl-6-O-phosphatidyl-α-D-glucopyranoside(α-CPG)). Thus the cell wall of *H. pylori* consists of 1.6% cholesterol and 25% cholesteryl glucosides of the total lipids respectively.

Defence mechanisms of the host against the pathogenesis of *H. pylori*

The gland mucous cells of the deeper layer produce mucins having terminal α-1,4-linked-GlcNAc residues attached to core 2branched O-glycans [GlcNAcα1→ 4Galβ1→ 4GlcNAcβ1→ 6(GlcNAcα1→ 4Gal-β1→3) GalNAcα→Ser/Thr], (α1,4-GlcNAc-capped O-glycans) which protect against H. pylori by restricting the activities of Cholesterol-α-glucosyl transferase (CHLαGcT). In-vitro studies, suggest that α-1,4-GlcNAc-capped O-glycans protect against *H. pylori* infection and inhibit its growth. The in-vivo studies too revealed that the activities of CHLαGcT were inhibited to certain extent, by synthetic O-linked oligosaccharides and monosaccharides (Motohiro *et al,* 2009).

Inspite of the mucins secreted for protection several virulence factors from *H. pylori* are able to exploit or modulate cholesterol to gain a foothold in the host niche. For the synthesis of CLGs, cholesterol is needed as the bacteria cannot synthesize cholesterol on its own. *H. pylori* growing in the human gastric mucosa synthesizes CLGs, by taking up cholesterol available in that environment. Therefore *H. pylori* infection in the gastric mucosa persisted by evasion of the immune response of the host. The enzyme, cholesterol-α-glucosyltransferase is identified to be responsible for cholesterol glucosylation in macrophages, hence protecting *H. pylori* from phagocytosis and reducing the antigen specific T-cell responses.

Synthesis Of carbohydrate bearing epitopes

To generate this protective immunity, alternative immune response is needed to be activated, or immune evasion pathways need to be targeted. For this three ∝-olefinic glycosides 1-O-allyl-α-D-glucopyranoside (Allyl-α-D-glucopyranoside), 1-O-allyl-2,3-di-O-methyl-α-D-glucopyranoside(Allyl-2,3-di-O-methyl-α-D-glucopyranoside) and 1-O-allyl-2,3-di-O-benzyl-α-D-glucopyranoside(Allyl2,3-di-O-benzyl-α-D-glucopyranoside) were synthesized and conjugated through their allyl linker arm with an immunogenic carrier (BSA) to form immunogens.

The introduction of 2-Carbon chain spacer arm or the allyl linker arm to D-glucopyranose yielded **1-O-allyl-α-D-glucopyranoside**(3)(Allyl-α-D-glucopyranoside) as a major product together with a kinetically controlled product 1-O-allyl-β-D-glucopyranoside(2) (Allyl-β-D-glucopyranoside) in traces. **1-O-allyl-2,3-di-O-methyl/benzyl-α-D-glucopyranoside**(6,7) were synthesized from **1-O-allyl-α-D-glucopyranoside**(3) by protecting C-4 and C-6 position by benzylidene. Methyl /benzyl groups were introduced at the unprotected C-2 and C-3 hydroxyl groups, under basic conditions to avoid hydrolysis of base sensitive benzylidene group by treatment of CH_3I in presence of NaH benzylidene. Deprotection of C-4 and C-6 positions was done by 80% acetic acid at 80^0C to yield the compound **1-O-allyl-2,3-di-O-methyl/benzyl-α-D-glucopyranoside** (8,9) (Allyl-2,3-di-O-methyl-α-D-glucopyranoside). Compounds 3-9 were characterised by 90 MHz ^{1}H NMR spectroscopy.

1-O-allyl-2,3-α-Dglucopyranoside(3), 1-O-allyl-2,3-di-O-methyl-α-D-glucopyranoside(8) and 1-O-allyl-2,3-di-O-benzyl-α-D-glucopyranoside(9) having the allyl linker arms on ozonolysis formed unstable aldehydes which on reductive amination, in presence of sodium cyanoborohydride at pH7.8 linked to the lysines of BSA forming the immunogens.

Immunogenicity

Antibody formation in New Zealand White strain Rabbits (*Oryctolagus cuniculus*) was tested by two techniques (ELISA and Ouchterlony)(Hornbeck, 2017; Lequin, 2005). Out of the three carbohydrate bearing epitopes only 1-O-ethylimine-N-lysyl-BSA-α-D-glucopyranoside (12)

(Allyl-α-D-glucopyranoside-oxylysl-BSA gave positive results for immunoglobulin M (IgM) class of antibodies. Negative results with the Ouchterlony technique are not surprising because the technique is sensitive for immunoglobulin G(IgG) class of antibodies and the earliest antibodies to form are usually of the IgM class; the IgG antibodies develop on prolonged exposure to antigen.

The results of ELISA are encouraging and it is strongly possible to develop carbohydrate antigens by testing in the *H. pylori* infected hosts for the eradication of this microorganism to prevent gastric disorders.

CONCLUSION

The carbohydrates epitopes of the conjugates-(1)Allyl-α-D-glucopyranoside, (8)Allyl 2,3-di-O-methyl-α-D-glucopyranoside and (9)Allyl 2,3-di-O-benzyl-α-D-glucopyranoside, α-D-glucopyranoside bear resemblance with the oligosaccharides and monosaccharides in the cell wall of *H. pylori*. Out of these three synthetic carbohydrate conjugates, (1)Allyl-α-D-glucopyranoside oxylysl BSA, on preliminary testing in ELISA gave positive results for IgG class of antibodies when tested in rabbits. The results of ELISA are encouraging as it could possibly give a lead to develop carbohydrate antigens for the eradication of *H. pylori* to prevent gastric disorders.

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Corresponding author: Dr. Ruchi Saxena, Department of Chemistry, Nari Shiksha Niketan P.G. College, Lucknow, U.P., India
Email: ruchisaxena65@gmail.com