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Note

Synthesis of bivalent glycoclusters containing GlcNAc as hexasaccharide mimetics. Bactericidal activity against *Helicobacter pylori*

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ABSTRACT

The Cu(I) catalysed cycloaddition reaction of azides and alkynes has been used to generate a series of divalent GlcNAc clusters with both α and β configurations. These glycoclusters can be considered as potential mimetics of an anti *Helicobacter pylori* hexasaccharide as they present two GlcNAc residues grafted onto a core scaffold. Two bivalent compounds based on α-O-GlcNAc were identified that selectively reduced the viability of *H. pylori*. These compounds showed activity towards different strains of *H. pylori* (Pu4 vs P12). The activity of the oligosaccharide mimetics is speculated to be due to the GlcNAc residues being able to adopt spatial arrangements accessible to the anti *H. pylori* hexasaccharide which may be important for activity.

Helicobacter pylori is one of the commonest infections of mankind. It colonizes the gastric mucosa of more than 50% of the world's population and is a leading cause of duodenal ulceration, gastric carcinoma and gastric malignant lymphoma of mucosa-associated lymphoid tissue.[1] However, most infected individuals are asymptomatic which suggests that the host defence mounted against the organism is capable of limiting the pathology caused by the infection. *H. pylori* is rarely found in deeper portions of the gastric mucosa, where O-glycans are expressed which contain terminal α-1,4-linked *N*-acetylglucosamine. Lee et al. have shown that these O-glycans have antimicrobial activity against *H. pylori*.[2] They inhibit bacterial cholesterol α-glucosyltransferase, thereby preventing the biosynthesis of α-glucosyl cholesterol, a major constituent of the cell wall of *H. pylori*.[2,3] Thus, unique α-GlcNAc derivatives could have potential in protecting hosts from *H. pylori* infection. An anti *H. pylori* hexasaccharide with α-1,4-GlcNAc capped glycans has been synthesized.[4] The nitrophenol glycoside of GlcNAc is known to also inhibit *H. pylori* growth. Promise has been identified for 1-deoxynojirimycin and castanospermine as inhibitors of α-glucosyl cholesterol.[5] We hypothesized that bivalent glycoclusters based on α-GlcNAc could have activity against *H. pylori*. This is based on the structure of the anti *H. pylori* hexasaccharide[4] (Fig. 1) which could be viewed as being comprised of two α-GlcNAc residues grafted onto a core tetrasaccharide scaffold. We designed and synthesized a series of bivalent GlcNAc derivatives (**1**–**8**, Fig. 1) where two GlcNAc residues are grafted to non carbohydrate scaffolds as glycocluster[6] mimetics of the oligosaccharide and report on their bactericidal activity against *H. pylori*. Although the native hexasaccharide contains the α-glycosidic linkage we included compounds where the GlcNAc residues also contain β-linkages.

The synthesis of **1**–**3** began from β-azide **9**, which was prepared from *N*-acetyl-D-glucosamine as previously described.[7] The bispropargyl derivative **10** was prepared[8] from 1,4-dihydroxybenzene and similar conditions were used to give **11** and **12** from the appropriate dihydroxylated aromatic precursors. Then the azide **9** was coupled with the bisacetylenes **10**–**12** using the copper catalysed azide–alkyne cycloaddition reaction (CuAAC). Hence reaction with Cu(II)SO_4 in the presence of sodium ascorbate in 1:1 MeOH–H_2O and subsequent de-O-acetylation gave **1**–**3** (Scheme 1).

The alkyne precursor **13**[9] was prepared (26%) by adding acetyl chloride to propargyl alcohol at 0 °C, with *N*-acetyl-D-glucosamine (**14**) being subsequently added to the mixture at room temp.[10,11] Then alkyne **13** was reacted with the azide **9** using the CuAAC reaction giving the divalent compound **4** after de-O-acetylation of the intermediate (60%, two steps, Scheme 2).

The azide **15** (76%) was prepared from *N*-acetyl-D-glucosamine in two steps (Scheme 3). Firstly 2-bromoethanol was heated in the presence of **14** and acetyl chloride at 70 °C for 3 h.[12] The intermediate obtained was subsequently treated with sodium azide and tetrabutylammonium iodide at 60 °C overnight to give **15** (>97%).

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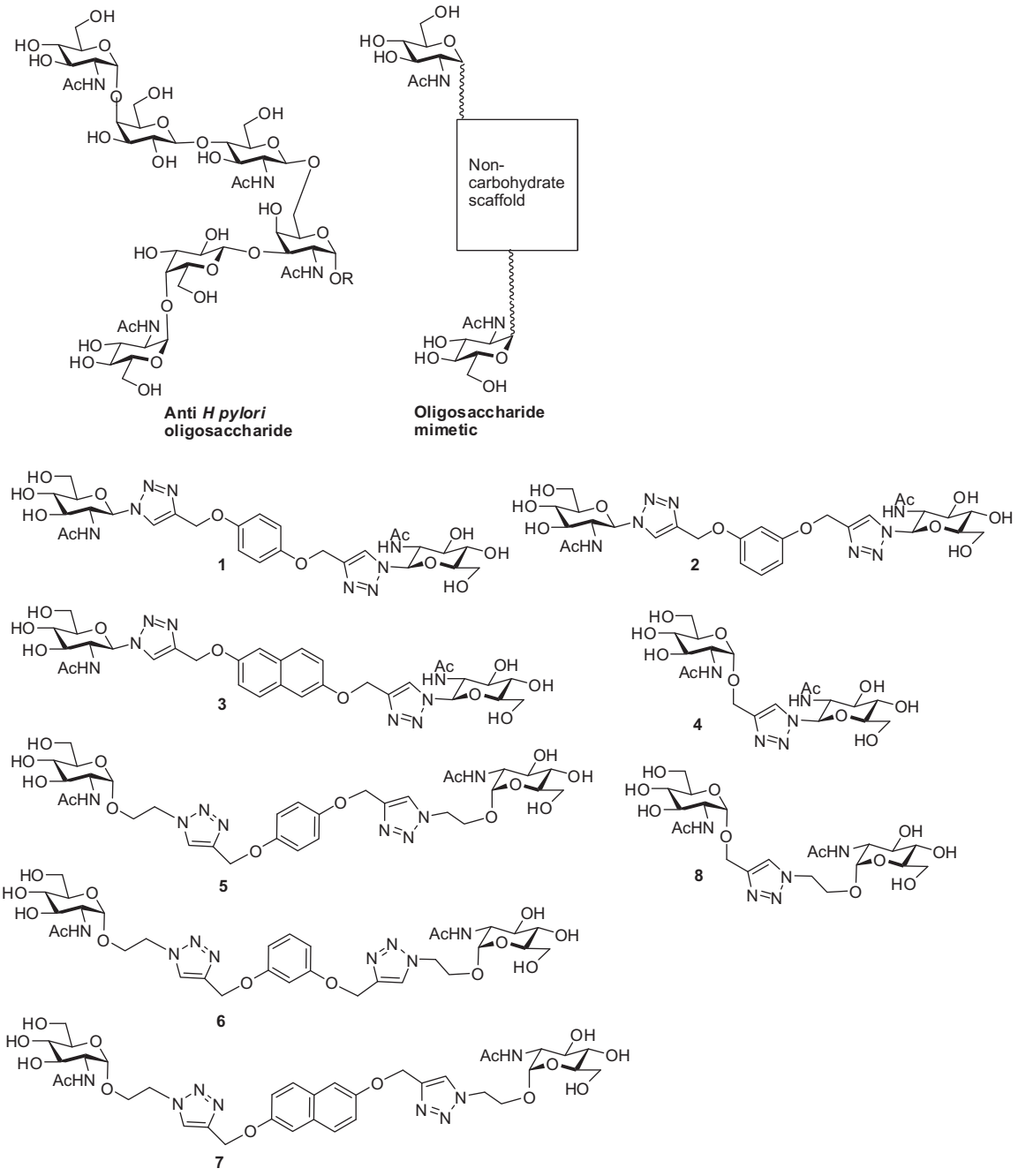
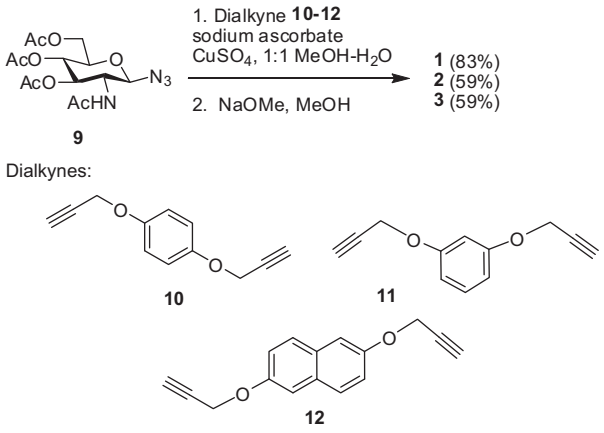


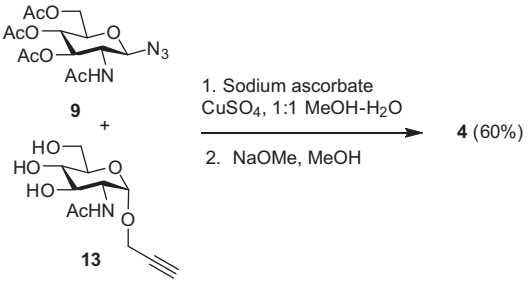
Figure 1. Rationale for selection of bivalent structures based on different scaffolds and structures of **1**–**8**.

This azide was then subjected to the CuAAC reaction with dialkynes **10**–**12** and gave the desired bivalent clusters **5**–**7** in acceptable yields. The direct reaction of **13** with **15** was carried out and gave **8** but it could not be purified sufficiently. Therefore **15** was acetylated to give **16** and the CuAAC reaction with **13** followed by deacetylation gave **8** in higher purity (NMR) than from the direct reaction of **15**.

All bivalent clusters were tested against *H. pylori*. The direct bactericidal effect of compounds on *H. pylori* strain Pu4 (Fig. 2) was tested by measuring the number of viable organisms present after exposure to the compounds. The bivalent α-O-GlcNAc derivative **5** was found to be the most toxic compound ($P = 0.0001$) from the collection of compounds tested, being significantly more potent at a concentration of 1 mM than the other GlcNAc derivatives tested at the same concentration. The compound was not active at a concentration of 0.75 mM. The compounds were also evaluated for their ability to inhibit growth of a second strain of *H. pylori*, strain P12 (Fig. 3). In this case the bivalent α-O-GlcNAc derivative **6** was the most potent inhibitor ($p < 0.0001$) at 1 mM. Compound **6** was also inactive at 0.75 mM. While the concentrations at which **5** and **6** show activity are high, it is the case that high concentrations of core O-glycan structures are also required. Monosaccharides and oligosaccharides were evaluated at concentrations of 0.125 mM to 1 mM previously by Lee et al.[2] This assay measured the ability of compounds to inhibit *H. pylori* growth over a period of 5 days and showed that a pentasaccharide, GlcNAcα1→4Galβ1→4GlcNAcβ1→6(Galβ1→3)GalNAcα1→octyl, which lacks a terminal GlcNAc residue of the hexasaccharide (Fig. 1)[4] was the most potent of the compounds tested, inhibiting growth significantly at concentrations of 0.5 mM or higher.



Scheme 1. Synthesis of **1**–**3**.



Scheme 2. Synthesis of **4**.

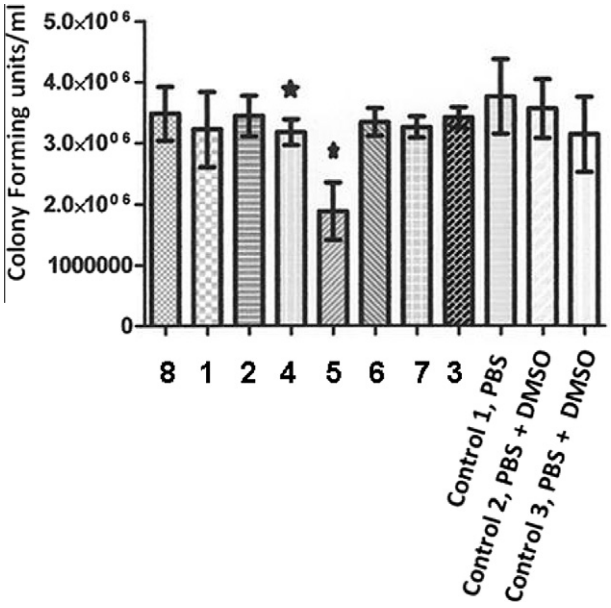
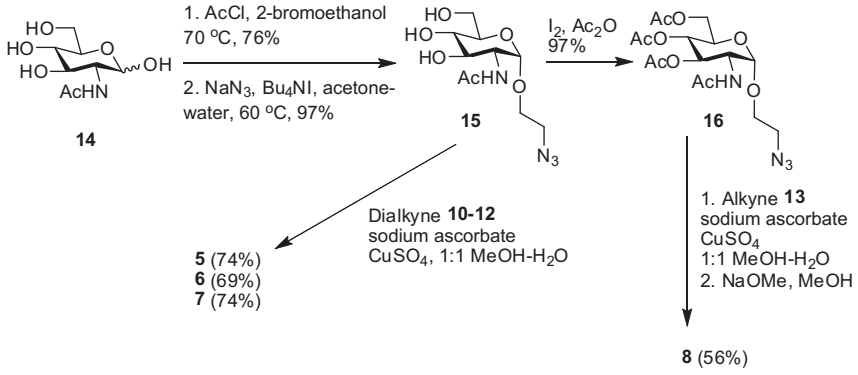


Figure 2. The effect of GlcNAc containing glycoclusters **1**–**8** on viability of *H. pylori* strain Pu4. Bacteria were exposed to the different compounds at a concentration of 1 mM. or to control diluent solutions for 1 h. Bacterial viability was assessed by enumerating the number of CFU present in the solutions. Results are presented as the mean result of three separate experiments ± the standard deviation of the mean. * = Statistically significant ($p < 0.05$).

The distances between the GlcNAc residues in extended conformations of **1**–**8** would be expected to vary with longer distances between GlcNAc compounds being accessible to compounds **5**–**7** when compared to other more constrained structures. The antibactericidal activity is speculated to be related to the distance between the GlcNAc residues, which may be optimum in **5**/**6**. Molecular modelling was used to investigate if this was in principle possible. Hence a model of the anti *H. pylori* oligosaccharide in an extended conformation was generated. This was then overlapped with a model of compound **5** (Fig. 4) which was generated to have the spacing between the GlcNAc residues (Fig. 4). In these models the distance between the anomeric carbon atoms of the GlcNAc residues is ~20 Å. This indicates that the core scaffold in **5** can in principle mimic the core tetrasaccharide scaffold in the natural hexasaccharide. Similarly a model of compound **6** was generated (Fig. 4) which showed it could mimic the hexasaccharide. To date there is no information about the bioactive conformation of the hexasaccharide, which could more specifically direct glycomimetic design.

In summary, the Cu(I) catalysed variation of the cycloaddition reaction of azides and alkynes[13,14] has simply and successfully been used to generate a series of readily synthesized divalent GlcNAc-containing glycoclusters, which can be considered mimics of an anti *H. pylori* hexasaccharide. Two bivalent compounds based on α-O-GlcNAc were identified from the series that selectively reduced the viability of *H. pylori*. It is interesting that there is activity towards different strains of *H. pylori* (Pu4 vs P12) for different compounds. The activity of the oligosaccharide mimetics could be due to the ability of the GlcNAc residues to adopt spatial arrangement which is accessible to the anti *H. pylori* hexasaccharide. One referee indicated that oxidative dealkylation of hydroquinone as found in **5**, might occur in vivo and increase cytotoxicity. In such a scenario **5** or a related compound would be bi-functional. This could be the basis of a strategy to target more effectively *H. pylori* infection as part of future work. Aside from the application described herein the glycoclusters based on GlcNAc would be interesting to evaluate against other lectins.[15]



Scheme 3. Synthesis of **5**–**8**.

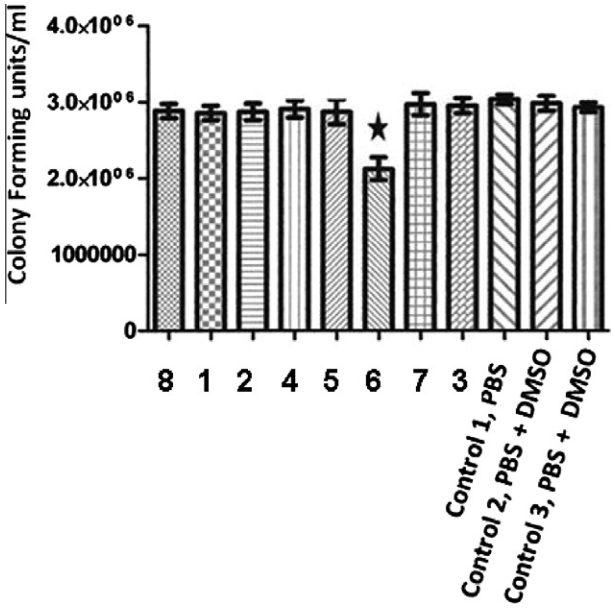


Figure 3. The effect of GlcNAc containing glycoclusters (**1**–**8**) on viability of *H. pylori* strain P12. Bacteria were exposed to the different compounds at a concentration of 1 mM. or to control diluent solutions for 1 h. Bacterial viability was assessed by enumerating the number of CFU present in the solutions. Results are presented as the mean result of three separate experiments ± the standard deviation of the mean. * = Statistically significant ($p < 0.05$).

1. Experimental

1.1. General

Optical rotations were determined at the sodium D line at 20 °C. NMR spectra were recorded with 400 & 500 MHz spectrometers. Chemical shifts are reported relative to internal Me_4Si in $CDCl_3$ (δ 0.0), HOD for D_2O (δ 4.80) for ^1H and $CDCl_3$ (δ 77.0) for ^{13}C. ^1H NMR signals were assigned with the aid of COSY and TOCSY. ^{13}C NMR signals were assigned with the aid of DEPT, HSQC and HMBC. Coupling constants are reported in hertz. The IR spectra were recorded using a thin film between NaCl plates. Mass spectral data were in positive and/or negative mode as indicated in each case. Thin layer chromatography (TLC) was performed on aluminium sheets pre-coated with silica gel and spots visualized by UV and charring with H_2SO_4–EtOH (1:20), vanillin, iodine, or cerium molybdate. Preparative TLC was carried out using analtech silica gel HLF 20 × 20 cm. Flash chromatography was carried out with silica gel 60 (0.040–0.630 mm) and using a stepwise solvent polarity gradient correlated with TLC mobility. Chromatography solvents were used as obtained from suppliers. Dichloromethane, MeOH and THF reaction solvents were used as obtained from a Pure Solv™ Solvent Purification System. Anhydrous acetonitrile, DMF, pyridine and toluene were used as obtained from Sigma–Aldrich.

1.2. 1,4-Bis((1-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzene 1

To a mixture of *p*-bispropargyloxybenzene **10** (8 mg, 0.04 mmol), the azido sugar **9** (34 mg, 0.09 mmol) in MeOH–water (1:1 v/v, 0.5 mL), sodium ascorbate (2 mg, 8×10^{-3} mmol) and Cu(II)SO_4 (7 mg, 4×10^{-3} mmol) were subsequently added. The mixture was stirred at room temp overnight. The solvent was removed under diminished pressure and chromatography of the residue (CH_2Cl_2–MeOH, 10:1) gave the protected glycocluster as a white solid (36 mg, 94%); ^1H NMR (500 MHz, DMSO): δ 8.13 (m, 2H, C*H*=C), 6.92 (4H, aromatic H), 6.12 (d, 2H, J = 10.0 Hz, H-1), 5.43 (t, 2H, J = 10.0 Hz, H-3), 5.17 (t, 2H, J = 10.0 Hz, H-4), 5.09 (s, 4H, CH=CCH_2), 4.65 (apparent q, 2H, J = 9.5 Hz, H-2), 4.24 (dd, 2H, J = 5.5 Hz, J = 13.0 Hz, H-6a), 4.10 (m, 4H, overlapping signals of H-6b and H-5), 2.04, 2.00 (s, CH_3); ^{13}C NMR (125 MHz, DMSO): δ 170.1, 170.0, 169.8, 169.3 (C=O), 152.7 (aromatic C), 143.7 (CH=*C*), 122.6 (*C*H=C), 115.9 (aromatic CH), 85.5 (C1), 74.3 (C5), 72.7 (C3), 68.3 (C4), 62.2 (CH=C–CH_2), 61.9 (C6), 52.5 (C2), 22.7, 20.7, 20.6, 20.5 (each CH_3). This intermediate (36 mg, 0.04 mmol) was dissolved in dry MeOH (5 mL) and freshly prepared NaOMe in MeOH (0.5 mL of 1 M) was added and the mixture was stirred at room temp overnight. The reaction was neutralized to pH 7 using 10% HCl at 0 °C. The MeOH was removed under diminished pressure. Chromatography of the residue (CH_2Cl_2–MeOH, 10:1, then 5:1, then MeOH) gave **1** (20 mg, 88%) as a white solid; IR ν_{max}/cm^{-1}: 3298, 2922, 1649 (C=O), 1560, 1511, 1415, 1234, 1211, 1105, 1046, 896; $[\alpha]_D$ −19.5° (*c* 1.06, CH_3OH); ^1H NMR

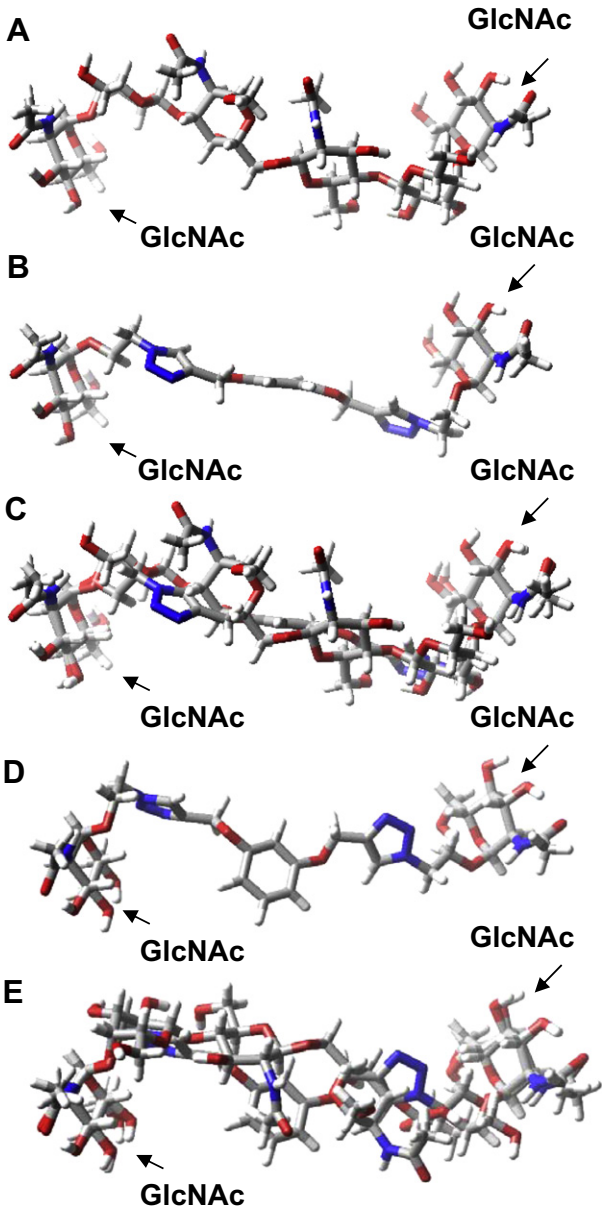


Figure 4. Models of: (A) anti *H. pylori* oligosaccharide; (B) compound **5**; (C) overlapped hexasaccharide and compound **5**; (D) compound **6**; (E) overlapped hexasaccharide and compound **6**.

(500 MHz, D_2O): δ 8.17 (s, 2H, CH=C), 6.93 (s, 4H, aromatic CH), 5.78 (d, 2H, J = 9.5 Hz, H-1), 5.16 (s, 4H, CH=C–CH_2), 4.19 (t, 2H, J = 10.0 Hz, H-2), 3.87 (dd, 2H, J = 13.0, 2.0 Hz, H-6b), 3.77–3.673(m, 8H, overlapping signals of H-6b, H-3, H-4, H-5), 1.84 (s, 6H, CH_3); ^{13}C NMR (125 MHz, D_2O) δ 173.8 (C=O), 151.9 (aromatic C), 143.5 (CH=C), 123.8 (CH=C), 116.8 (aromatic CH), 86.2 (C1), 78.9, 73.5, 69.2 (C3, C4 and C5), 61.7 (CH=C–CH_2), 60.3 (C6), 55.1 (C2), 21.4 (CH_3). ESI-HRMS: calcd for $C_{28}H_{37}N_8O_{12}$ 677.2531, found m/z 677.2553 $[M-H]^-$.

1.3. 1,3-Bis((1-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzene 2

To a mixture of *m*-bispropargyloxybenzene **11** (9 mg, 0.05 mmol), the azido sugar **9** (35 mg, 0.09 mmol) in MeOH–water (2 mL, 1:1 v/v) sodium ascorbate (2 mg, 9.5×10^{-3} mmol) and Cu(II)SO_4 (1 mg, 4.7×10^{-3} mmol) were subsequently added and the mixture was stirred at room temp overnight. The solvent was removed under diminished pressure and chromatography of the residue (CH_2Cl_2–acetone, gradient elution, 5:2 to 1:1) gave the protected intermediate as a white solid (28 mg, 60%); IR ν_{max}/cm^{-1}: 3265, 1745 (C=O), 1668, 1530, 1375, 1228, 1039; ^{1}H NMR (500 MHz, CD_3CN): δ 8.09 (s, 2H, CH=C), 7.23 (t, 1H, J = 8.5 Hz, aromatic CH), 6.69 (t, 1H, J = 2.0 Hz, aromatic CH), 6.64 (dd, 2H, J = 8.5, 2.5 Hz, aromatic CH), 6.59 (d, 2H, J = 9.5 Hz, NH), 6.13 (d, 2H, J = 10.0 Hz, H-1), 5.45 (t, 2H, J = 10.0 Hz, H-3), 5.18 (6H, overlapping signals of H-4 and CH=CCH_2), 4.59 (q, 2H, J = 9.5 Hz, H-2), 4.23–4.12 (m, 6H, overlapping signal of H-6 and H-5), 2.04, 2.01, 2.00, 1.63 (s, CH_3); ^{13}C NMR (125 MHz, CD_3CN) δ 170.4, 170.2 (2s), 169.8 (C=O), 159.7 (aromatic C), 143.9 (CH=C), 130.3 (aromatic CH), 122.9 (CH=C), 107.9, 102.0 (aromatic CH), 85.5 (C1), 74.6 (C5), 72.3 (C3), 68.3 (C4), 61.9 (C6), 61.5 (CH=C-CH_2), 53.0 (C2), 21.9, 20.1, 20.0, 20.0 (each CH_3); ESI-HRMS calcd for $C_{40}H_{49}N_8O_{18}$ 929.3165, found m/z 929.3168 $[M-H]^-$. The title compound **2** was obtained (98%) after deacetylation of this intermediate according to procedure used to give **1**; IR ν_{max}/cm^{-1}: 3265, 2924, 1658 (C=O), 1593, 1374, 1035, 898; $[\alpha]_D$ +1° (*c* 0.50, CH_3OH–H_2O, 1:1); ^{1}H NMR (500 MHz, D_2O): δ 8.10 (s, 2H, CH=C), 7.12 (apt t, 1H, J = 8.0 Hz, aromatic CH), 6.54–6.51 (overlapping signals, 3H, aromatic CH), 5.69 (d, 2H, J = 10.0 Hz, H-1), 5.08 (s, 4H, CH=CCH_2), 4.10 (t, 2H, J = 10.0 Hz, H-2), 3.77 (dd, 2H, J = 12.5, 1.5 Hz, H-6b), 3.50–3.58 (m, 6H, overlapping signals of H-3, H-4, H-5 and H-6a), 1.55 (s, 6H, CH_3); ^{13}C NMR (125 MHz, D_2O): δ 173.9 (C=O), 158.3 (aromatic C), 143.4 (CH=C), 130.5 (aromatic CH), 123.8 (CH=C), 108.6, 103.0 (aromatic CH), 86.2 (C1), 76.9, 73.5 (C3 and C4), 69.2 (C5), 60.9 (CH=CCH_2), 60.4 (C6), 55.2 (C2), 21.4 (CH_3); ESI-HRMS calcd for $C_{28}H_{37}N_8O_{12}$ 677.2531, found m/z 677.2531 $[M-H]^-$.

1.4. 2,6-Bis((1-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl)methoxy)naphthalene 3

To a mixture of 2,6-bispropargyloxynaphthalene **12** (11 mg, 0.05 mmol), the azido sugar **9** (34 mg, 0.09 mmol) in MeOH–water (1:1 v/v, 2 mL) sodium ascorbate (2.0 mg, 9.1×10^{-3} mmol) and Cu(II)SO_4 (1 mg, 4.7×10^{-3} mmol) were subsequently added. The mixture was stirred at room temp overnight. The solvent was removed under diminished pressure and chromatography of the residue (CH_2Cl_2–acetone, gradient elution of 5:2 to 1:1) gave the protected intermediate as a white solid (26 mg, 60%); ^{1}H NMR (500 MHz, CF_3CD_2OD): δ 7.97 (s, 2H, *CH*=C), 7.64 (d, 2H, J = 9.0 Hz, aromatic CH), 7.19 (s, 2H, aromatic CH), 7.11 (d, 2H, J = 8.5 Hz, aromatic CH), 5.96 (d, 2H, J = 9.5 Hz, H-1), 5.43 (t, 2H, J = 9.5 Hz, H-3), 5.22 (t, 2H, J = 10.0 Hz, H-4), 5.17 (s, 4H, CH=C–CH_2), 4.45 (t, 2H, J = 10.0 Hz, H-2), 4.18 (s, 4H, H-6), 3.97 (d, 2H, J = 10.0 Hz, H-5), 1.99, 1.97, 1.96, 1.63 (each CH_3); ^{13}C NMR (125 MHz, CF_3CD_2OD) δ 175.7, 175.4, 174.7, 174.1 (each C=O), 156.8 (aromatic C), 146.4 (CH=C), 132.3 (aromatic C), 130.5 (aromatic CH), 124.6 (CH=C), 120.9, 109.9 (aromatic CH), 87.9 (C1), 76.7 (C5), 74.2 (C3), 70.4 (C4), 63.9 (C6), 62.9 (CH=C–CH_2), 55.7 (C2), 22.7, 20.9, 20.9, 20.8 (each CH_3); ESI-HRMS calcd for $C_{44}H_{53}N_8O_{18}$ 981.3478; found m/z 981.3460 $[M+H]^+$. The title compound **3** (19 mg, 97%) was obtained after deacetylation according to the procedure used for the preparation of **1**; $[\alpha]_D$ −50° (*c* 0.09, DMSO); ^{1}H NMR (500 MHz, DMSO): δ 8.31 (s, 2H, *CH*=C), 7.87 (d, 2H, J = 9.5 Hz, NH), 7.75 (d, 2H, J = 9.0 Hz, aromatic CH), 7.44 (d, 2H, J = 2.0 Hz, aromatic CH), 7.17 (dd, 2H, J = 9.0, 2.5 Hz, aromatic CH), 5.74 (d, 2H, J = 10.0 Hz, H-1), 5.26 (dd, 4H, J = 17.0, 5.5 Hz, CH=C–CH_2), 4.09 (apt q, 2H, J = 9.5 Hz, H-2), 3.71 (dd, 2H, J = 10.5, 5.5 Hz, H-6a), 3.56 (m, 2H, H-3), 3.50–3.43 (m, 4H, overlapping signals of H-6b and H-5), 3.29 (m, 2H, H-4), 2.09 (s, CH_3); ^{13}C NMR (125 MHz, DMSO) δ 169.0 (C=O), 154.4 (aromatic C), 142.4 (CH=C), 129.3 (aromatic CH), 128.2 (aromatic C), 123.1 (CH=C), 118.8, 107.4 (aromatic CH), 85.9 (C1), 80.0 (C5), 73.8 (C3), 69.8 (C4), 61.5 (CH=C–CH_2), 61.1 (C6), 54.9 (C2), 22.6 (CH_3); ESI-HRMS calcd for $C_{32}H_{39}N_8O_{12}$ 727.2687; found m/z 727.2684 $[M-H]^-$.

1.5. Progargyl 2-acetamido-2-deoxy-α-D-glucopyranoside 13

Acetyl chloride (1.1 mL, 15.4 mmol) was added dropwise to propargyl alcohol (4 mL), under N_2 and at 0 °C. *N*-Acetyl-D-glucosamine **14** (1.0 g, 1.36 mmol) was then added at room temp. The reaction mixture was stirred at 70 °C for 3 h. Solid $NaHCO_3$ was added until the pH was 7 and the suspension was filtered through celite and washed several times with MeOH. The solvent was removed under diminished pressure and chromatography ($CHCl_3$–MeOH, 10:1 and 5:1) of the residue gave the previously known **13**[16] as a white solid (0.30 g, 26%); ^{1}H NMR (CD_3OD, 400 MHz): δ 4.92 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.26 (broad AB d, 2H, J = 12 Hz, OCH_2), 3.93 (dd, 1H, J = 3.6 Hz, J = 10.8 Hz, H-2), 3.81 (dd, 1H, J = 2.0 Hz, J = 12.0 Hz, H-6), 3.61–3.70 (m, 2H), 3.57 (ddd, 1H, J = 2.4 Hz, J = 5.6 Hz, J = 10.0 Hz, H-5), 3.36 (apt t, 1H, J = 9 Hz, H-4), 2.83 (t, 1H, J = 1 Hz, CCH), 1.98 (s, CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 172.4 (C=O), 95.8 (C1), 79.2 (CCH), 73.0 (C5), 71.3 (C3), 70.9 (C4), 61.2 (C6), 53.9 (CH_2), 53.7 (CH), 21.2 (CH_3).

1.6. 4-(2-Acetamido-2-deoxy-α-D-glucopyranosyloxymethyl)-1-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-1*H*-1,2,3-triazole 4

To a mixture of **13** (21 mg, 0.08 mmol), the azido sugar **9** (28 mg, 0.07 mmol) in MeOH–water (1:1 v/v, 0.5 mL) sodium ascorbate (1 mg, 5×10^{-3} mmol) and Cu(II)SO_4 (0.5 mg, 3×10^{-3} mmol) were subsequently added. The mixture was stirred at room temp overnight. The solvent was removed under diminished pressure and chromatography of the residue (CH_2Cl_2–MeOH, 10:1) gave the protected intermediate as a white solid (34 mg, 72%); ^{1}H NMR (CD_3OD, 500 MHz): δ 8.26 (s, 1H, CH=C), 6.10 (d, 1H, J = 10.0 Hz, H-1′), 5.47 (t, 1H, J = 10.0 Hz, H-3′), 5.22 (t, 1H, J = 10.0 Hz, H-4′), 4.88 (d, 1H, J = 3.5 Hz, H-1), 4.81 (d, 1H, J = 12.0 Hz, CH=C–*CH(H)*), 4.60 (d, 1H, J = 12.0 Hz, CH=C–*CH(*H*)*), 4.56 (d, 1H, J = 10.0 Hz, H-2′), 4.32 (dd, 1H, J = 5.0 Hz, J = 12.5 Hz, H-6′a), 4.16 (m, 2H, overlapping signals of H-6′b and H-5′), 3.93 (dd, 1H, J = 3.5 Hz, J = 11.0 Hz, H-2), 3.84 (br d, 1H, J = 12.0 Hz, H-6), 3.66 (m, 3H, overlapping signals of H-6, H-5 and H-3), 3.35 (t, 1H, J = 9.0 Hz, H-4), 2.05, 2.04, 2.01, 1.73 (each CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 172.3, 172.0, 170.8, 170.3, 169.8 (each C=O), 144.4 (C=CH), 122.6 (C=*CH*), 96.7 (C1), 85.7 (C1′), 74.6 (C5′), 72.8 (C3), 72.3 (C3′), 71.4 (C5), 70.9 (C4), 68.2 (C4′), 61.7 (C6′), 61.3 (C6), 59.6 (*CH*$_2$–C=CH), 53.8 (C2), 53.3 (C2′), 21.2, 21.0, 19.2, 19.1, 19.0 (each CH_3); ESI-HRMS calcd for $C_{25}H_{36}$ N_5O_{14} 630.2259; found m/z 630.2284 $[M-H]^-$. The title compound **4** (15.9 mg, 83%) was obtained from the deacetylation of the intermediate according to the procedure used to prepare **1**;

IR ν_{max}/cm^{-1}: 3270, 2924, 1650, 1555, 1376, 1314, 1097, 1034, 950; $[\alpha]_D$ +50.3° (*c* 0.94, CH_3OH); ^1H NMR (CD_3OD, 500 MHz): δ 8.16 (s, 1H, C=*CH*), 5.73 (d, 1H, *J* = 10.0 Hz, H-1′), 4.86 (d, 1H, *J* = 3 Hz, H-1), 4.74 (d, 1H, *J* = 12.0 Hz, *CH*HC≡CH), 4.54 (d, 1H, *J* = 12.0 Hz, CH*H*C≡CH), 4.15 (apt t, 1H, *J* = 10.0 Hz, H-2′), 3.83 (3H, overlapping signals, H-2, H-6′b and H-6), 3.70 (dd, 1H, *J* = 5.0 Hz, *J* = 12.5 Hz, H-6′a), 3.64 (2H, overlapping signals, H-6 and H-3′), 3.53–3.29 (5H, overlapping signals of H-3, H-5, H-5′, H-4′, H-4), 1.90, 1.73 (each s, each CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 172.3, 172.1 (each C=O), 143.8 (*C*≡CH), 122.5 (C=*CH*), 96.5 (C1), 86.8 (C1′), 79.8 (C5′), 74.2 (C3′), 72.8 (C5), 71.4 (C3), 71.0 (C4), 70.0 (C4′), 61.4 (C6′), 60.9 (C6), 59.6 (*CH*$_2$-C≡CH), 55.4 (C2′), 53.8 (C2), 21.2, 21.1 (each CH_3); ESI-HRMS calcd for $C_{19}H_{30}N_5O_{11}$ 504.1942; found *m/z* 504.1950 $[M-H]^-$.

1.7. 2-Azidoethyl 2-acetamido-2-deoxy-α-D-glucopyranoside

Acetyl chloride (0.33 mL, 4.61 mmol) was added dropwise to 2-bromoethanol (3 mL), under N_2 and at 0 °C. *N*-Acetyl-D- glucosamine (0.3 g, 1.36 mmol) was then added at room temp. The reaction mixture was stirred at 70 °C for 3 h and then solid $NaHCO_3$ was added until the pH was 7 and the suspension was filtered through celite, washing with MeOH. The solvent was removed under diminished pressure and chromatography ($CHCl_3$–MeOH, 8:1and 5:1) of the residue gave the bromide containing intermediate as light brown solid (0.34 g, 76%); ^1H NMR (CD_3OD, 500 MHz): δ 4.87 (d, 1H, $J_{1,2}$ = 4.0 Hz, H-1), 3.99 (m, 1H, *m* OCH*H*CH_2), 3.89 (dd, 1H, *J* = 4.5 Hz, *J* = 13.5 Hz, H-2), 3.79 (m, 2H, overlapping signals of H-6 and OCH_2CH_2), 3.68 (m, 2H, overlapping signal of H-6′ and H-3), 3.58 (m, 2H, OCH_2CH_2), 3.36 (m, 1H, H-5), 3.30 (t, 1H, *J* = 2.0 Hz, H-4), 2.00 (s, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 172.3 (C=O), 97.5 (C1), 72.7 (C3), 71.3 (C5), 68.0 (OCH_2CH_2), 61.2 (C6), 54.0 (CH), 47.6 (CH), 30.3 (OCH_2CH_2), 21.3 (CH_3); ESI-HRMS calcd for $C_{10}H_{18}$ NO_6Br 350.0215; found *m/z* found: 350.0204 $[M+Na]^+$. To this bromide (0.26 g, 0.78 mmol) in acetone–water (1:1, 6 mL), NaN_3 (0.30 g, 4.68 mmol) and Bu_4NI (0.29 g, 0.78 mmol) were subsequently added. The light brown solution was heated at reflux overnight. The solvent was removed under diminished pressure and chromatography (CH_2Cl_2–MeOH, 8:1 and 5:1) of the residue gave the title azide **15** as light brown solid (0.24 g, 99%); ^1H NMR (500 MHz, CD_3OD): δ 4.86 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1), 3.90 (m, 2H, overlapping signals of OCH_2CH_2 and H-2), 3.83 (d, 1H, *J* = 12.0 Hz, H-6), 3.70 (m, 2H, overlapping signals of H-6′ and H-5), 3.61 (2H, overlapping signals of OCH_2CH_2 and H-3), 3.47 (t, 2H, *J* = 4.5 Hz, OCH_2CH_2), 3.37 (m, 1H, H-4), 2.00 (s, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 172.4 (C=O), 97.4 (C1), 72.6 (C3), 71.3 (C5), 70.8 (C4), 66.4 (OCH_2CH_2), 61.2 (C6), 53.9 (C2), 50.3 (OCH_2CH_2), 21.4 (CH_3). ESI-HRMS calcd for $C_{10}H_{17}$ N_4O_6 289.1148; found *m/z* found: 289.1157 $[M-H]^-$.

1.8. 1,4-Bis((1-(2-(2-acetamido-2-deoxy)-α-D-glucopyranosyloxyethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzene 5

To a mixture of p-bispropargyloxybenzene **10** (6 mg, 0.03 mmol), the azido sugar **15** (19 mg, 0.06 mmol) in MeOH–water (1:1 v/v, 0.5 mL) sodium ascorbate (1 mg, 6×10^{-3} mmol) and Cu(II)SO_4 (0.5 mg, 3×10^{-3} mmol) were subsequently added. The mixture was stirred at room temp overnight. The solvent was removed under diminished pressure and chromatography (CH_2Cl_2–MeOH, 5:1 then MeOH) of the residue gave **5** as a gel (17 mg, 74%); IR ν_{max}/cm^{-1}: 3287, 2923, 1647, 1552, 1504, 1376, 1207, 1084, 1027, 829; $[\alpha]_D$ +83° (*c* 0.93, CH_3OH); ^1H NMR (500 MHz, CD_3OD): δ 8.12 (s, 2H, CH=C), 6.96 (broad s, 4H, aromatic CH), 5.12 (s, 4H, CH=C*CH*$_2$), 4.78 (d, 2H, *J* = 2.0 Hz, H-1), 4.65 (m, OCH_2CH_2), 4.09 (m, 2H, OCH_2CH_2), 4.09 (m, 4H, overlapping signals of OCH_2CH_2 and H-2), 3.58–3.76 (5H, overlapping signals H6b, H-6a, H-3), 3.35 (s, 2H, H-4), 3.31 (s, 2H, H-5), 1.96 (s, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 172.2 (C=O), 152.9 (C), 143.9 (CH), 124.4 (CH), 115.6 (C), 97.3 (C1), 72.7 (C5), 71.3 (C3), 70.4 (C4), 65.8 (OCH_2), 61.7 (CH=C*CH*$_2$), 61.1 (C-6), 53.8 (C2), 49.8 (OCH_2CH_2), 21.3 (CH_3); ESI-HRMS calcd for $C_{32}H_{45}N_8O_{14}$ 765.3055; found *m/z* found: 765.3088 $[M-H]^-$.

1.9. 1,3-Bis((1-(2-(2-acetamido-2-deoxy)-α-D-glucopyranosyloxyethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzene 6

The title compound **6** (38 mg, 69%) was obtained from **11** and **15** according to the same procedure used to give **5**; IR ν_{max}/cm^{-1}: 3306, 2926, 1732, 1636, 1603, 1461, 1380, 1229, 1145, 1085, 1031, 768; $[\alpha]_D$ +71.7° (*c* 0.63, H_2O); ^1H NMR (500 MHz, D_2O): δ 8.03 (s, 2H, CH=C), 7.16 (t, 1H, *J* = 8.5 Hz, aromatic CH), 6.59 (dd, 2H, *J* = 8.5, 1.5 Hz, aromatic H), 6.55 (broad s, 1H, aromatic H), 5.08 (s, 4H, CH=C*CH*$_2$), 4.60 (d, 2H, *J* = 3.5 Hz, H-1), 4.53 (broad s, 4H), 3.95 (m, 2H), 3.69–3.75 (m, 4H), 3.54 (m, 4H, H-6), 3.49 (t, 2H, *J* = 10.0 Hz, H-3), 3.29 (t, 2H, *J* = 9.5 Hz, H-4), 3.04 (br s, 2H, H-5), 1.82 (s, 6H, CH_3); ^{13}C NMR (125 MHz, D_2O): δ 174.0 (C=O), 158.6 (aromatic C), 143.3 (CH=*C*), 130.6 (aromatic CH), 125.3 (*CH*=C), 108.3, 102.7 (aromatic CH), 96.7 (C1), 71.9 (C5), 70.8 (C3), 69.5 (C4), 65.7 (OCH_2CH_2), 61.0 (CH=C*CH*$_2$), 60.2 (C6), 53.4 (C2), 50.0 (OCH_2CH_2), 21.8 (CH_3). ESI-HRMS calcd for $C_{32}H_{45}N_8O_{14}$ 765.3055; found *m/z* found: 765.3055 $[M-H]^-$.

1.10. 2,6-Bis((1-(2-(2-acetamido-2-deoxy)-α-D-glucopyranosyloxyethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)naphthalene 7

The title compound **7** (12 mg, 74%) was obtained from **12** and **15** according to the procedure used for the preparation of **5**; IR ν_{max}/cm^{-1}: 3307, 2926, 1730, 1646 (C=O), 1603 (NH), 1510, 1393, 1225, 1116, 1031, 852; $[\alpha]_D$ +79° (*c* 0.66, H_2O); ^1H NMR (500 MHz, D_2O): δ 7.79 (s, 2H, CH=C), 7.38 (d, 2H, *J* = 9.0 Hz, aromatic CH), 6.96 (s, 2H, aromatic CH), 6.90 (d, 2H, *J* = 8.5 Hz, aromatic CH), 4.89 (s, 4H, CH=C*CH*$_2$), 4.51 (d, 2H, *J* = 3.5 Hz, H-1), 4.37 (broad s, 4H), 3.83 (m, 2H), 3.66 (m, 2H), 3.61 (m, 2H), 3.52 (m, 4H, H-6), 3.47 (t, 2H, *J* = 10.5 Hz, H-3), 3.28 (t, 2H, *J* = 9.5 Hz, H-4), 3.02 (m, 2H, H-5), 1.79 (s, 6H, CH_3); ^{13}C NMR (125 MHz, D_2O) δ 173.9 (C=O), 154.0 (aromatic C), 143.3 (CH=*C*), 129.5 (aromatic C), 128.5 (aromatic CH), 125.0 (*CH*=C), 118.8, 107.8 (aromatic CH), 96.7 (C1), 71.9 (C5), 70.8 (C3), 69.5 (C4), 65.6 (O-*CH*$_2$CH$_2$), 60.8 (CH=C*CH*$_2$), 60.2 (C6), 53.3 (C2), 49.9 (CH_2), 21.8 (CH_3); ESI-HRMS calcd for $C_{36}H_{49}N_8O_{14}$ 817.3368; found *m/z* found: 817.3355 $[M+H]^+$.

1.11. Propargyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranoside 16

Iodine (1.8 mg, 6.9 μmol) was added to a stirred suspension of **15** (30 mg, 0.12 mmol) in acetic anhydride (1 mL) at room temp. The mixture was then diluted with CH_2Cl_2 (2 mL) and 10% $Na_2S_2O_3$ (2 mL) was added and the mixture left to stir for 40 min. It was then filtered and the filtrate was removed. Chromatography of the residue (CH_2Cl_2–MeOH, 20:1) gave **16** as a light yellow solid (40 mg). ^1H NMR (500 MHz, $CDCl_3$): δ 5.74 (d, 1H, *J* = 9.5 Hz, NH), 5.22 (t, 1H, *J* = 9.5 Hz, H-3), 5.15 (t, 1H, *J* = 10.0 Hz, H-4), 5.04 (d, 1H, *J* = 4.0 Hz, H-1), 4.39 (ddd, 1H, *J* = 3.5 Hz, *J* = 9.5 Hz, *J* = 10.5 Hz, H-2), 4.28 (dd, 2H, *J* = 2.5 Hz, *J* = 6.0 Hz, O*CH*$_2$CCH), 4.24 (dd, 1H, *J* = 4.5 Hz, *J* = 12.5 Hz, CH_2), 4.11 (dd, 1H, *J* = 2.0 Hz, *J* = 12.0 Hz, CH_2), 4.00 (ddd, 1H, *J* = 2.5 Hz, *J* = 4.0 Hz, *J* = 10.0 Hz, H-5), 2.49 (apt t., 1H, *J* = 2.0 Hz, CC*H*), 2.10, 2.03, 2.02, 1.96 (each s, each CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.2, 170.6, 170.0,

169.2 (each C=O), 96.2 (C1), 78.1 (CH_2CCH), 75.4 (CCH), 71.0 (C3), 68.3 (C5), 68.0 (C4), 61.8 (C6), 55.3 (CH_2CCH), 51.6 (C2), 23.1, 20.7, 20.6, 20.5 (CH_3).

1.12. 1-(2-(2-Acetamido-2-deoxy-α-D-glucopyranosyl)oxyethyl)-4-((2-acetamido-2-deoxy-α-D-glucopyranosyl)oxymethyl)-1*H*-1,2,3-triazole 8

The acetylated intermediate was obtained from alkyne **13** and **16** (70%) as described above. IR ν_{max}/cm^{-1}: 3346.3, 1747, 1660, 1545, 1373, 1238, 1043; $[\alpha]_D$ +112° (*c* 0.51, CH_3OH); ^1H NMR (500 MHz, CD_3OD): δ 5.04 (dd, 1H, *J* = 11.0, 9.5 Hz, CH), 4.85 (t, 1H, *J* = 10.5 Hz, CH), 4.80 (d, 1H, *J* = 3.5 Hz, H-1), 4.71 (d, 1H, *J* = 12.5 Hz, CH_2), 4.72 (d, 1H, *J* = 4.5 Hz, H-1′), 4.60 (t, 2H, *J* = 5.0 Hz, CH_2), 4.55 (d, 1H, *J* = 12.5 Hz, CH_2), 4.11 (dd, 1H, *J* = 11.0, 3.5 Hz, CH), 4.06 (dd, 1H, *J* = 12.0, 4.5 Hz, CH_2), 4.00 (dt, 1H, *J* = 11.5, 6.0 Hz, CH_2), 3.92 (dd, 1H, *J* = 12.5, 2.0 Hz, CH_2), 3.85 (dt, 1H, *J* = 11.0, 4.5 Hz, CH_2), 3.82 (dd, 1H, *J* = 10.5, 4.0 Hz, CH), 3.74 (dd, 1H, *J* = 12.0, 2.0 Hz, CH_2), 3.61–3.51 (m, 3H, overlapping signals of 2 × CH and 1H of CH_2), 3.44 (dq, 1H, *J* = 10.5, 2.5 Hz, CH), 3.26 (t, 1H, *J* = 9.5 Hz, CH), 1.95, 1.90, 1.86, 1.86, 1.85 (s, 15H, CH_3). ^{13}C NMR (125 MHz, CD_3OD): δ 172.2, 172.0, 170.9, 170.6, 169.8 (C=O), 143.9 (CH=*C*), 124.6 (*CH*=C), 97.1 (C1′), 96.6 (C1), 72.8, 71.4, 70.9, 70.7, 68.6, 67.7 (CH), 66.0, 61.6, 61.4, 59.7 (CH_2), 53.8, 51.3 (CH), 49.6 (CH_2), 21.2, 21.1, 19.2, 19.2, 19.1 (CH_3); ESI-HRMS calcd for $C_{27}H_{40}N_5O_{15}$ 674.2521; found *m/z* found: 674.2523 $[M-H]^-$. The title compound **8** (10.9 mg) was obtained (80%) after de-O-acetylation of this intermediate; IR ν_{max}/cm^{-1}: 3297, 2922, 1650, 1550, 1376, 1323, 1229, 1118, 1034; $[\alpha]_D$ +136° (*c* 0.09, CH_3OH); ^1H NMR (500 MHz, CD_3OD): δ 4.88 (d, 1H, *J* = 3.5 Hz, H-1), 4.82 (d, 1H, *J* = 13.0 Hz, CH_2), 4.78 (d, 1H, *J* = 3.5 Hz, H-1′), 4.65 (t, 2H, *J* = 5.5 Hz, CH_2), 4.61 (d, 1H, *J* = 12.0 Hz, CH_2), 4.09 (dt, 1H, *J* = 11.5, 4.5 Hz, CH_2), 3.92 (dd, 1H, *J* = 10.5, 3.0 Hz, CH), 3.87–3.83 (m, 3H, overlapping signals of CH and CH_2), 3.76 (dd, 1H, *J* = 11.5, 2.0 Hz, CH_2), 3.71–3.60 (m, 4H, overlapping signals of 2 × CH and CH_2), 3.54 (t, 1H, *J* = 9.0 Hz, CH), 3.36–3.29 (m, 2H, overlapping signals of 2 × CH), 3.23–3.20 (m, 1H, CH), 1.98, 1.96 (each s, each CH_3). ^{13}C NMR (125 MHz, CD_3OD) δ 172.3, 172.2 (C=O), 143.9 (CH=*C*), 124.4 (*CH*=C), 97.4 (C1′), 96.6 (C1), 72.8, 72.7, 71.3, 71.3, 70.9, 70.7 (each CH), 65.7, 61.4, 61.2, 59.8 (each CH_2), 53.8, 53.7 (each CH), 49.8 (CH_2), 21.3, 21.2 (each CH_3). ESI-HRMS calcd for for $C_{21}H_{34}N_5O_{12}$ 548.2204; found *m/z* found: 548.2204 $[M-H]^-$.

1.13. Assay to test the bactericidal activity of test compounds

H. pylori strains PU4, and P12 were used in this study. Bacteria were grown on Columbia blood agar containing 7% (v/v) defibrinated horse blood for 48 h at 37 °C under microaerophilic conditions generated using Campygen gaspaks. Bacteria were harvested from agar plates and suspended in brain heat infusion broth containing 10% (v/v) foetal calf serum. The OD_{600nm} of the bacterial suspension was adjusted to 0.5 and 100 μl aliquots were added to the wells of a 96 well microtitre plate. Stock solutions of the test compounds were dissolved in PBS (Dulbecco's formula A, pH 7.3) or PBS containing DMSO. The test compounds were added to the wells containing the bacteria so that the final concentration of the compound was 1 mM. PBS and PBS containing DMSO were added to wells to act as controls. Bacteria and test solutions were incubated together for one hour at 37 °C under microaerophilic conditions after which time serial dilutions of the bacterial suspensions were prepared. 100 μl volumes of the dilutions were spread on Columbia blood agar plates which were incubated at 37 °C under microaerophilic conditions for up to 5 days. The number of viable organisms (colony forming units per ml) present in each solution was calculated. Each assay was performed in duplicate on three separate occasions.

1.14. Molecular modelling

A model of the anti *H. pylori* hexasaccharide was built using Maestro version 6.0 (Schrödinger Inc., LLC, New York, USA). Constraints were then applied during energy minimization using Macromodel version 8.5 (Schrödinger Inc.) so as to generate an extended conformation for the hexasaccharide where the GlcNAc residues were constrained at a distance of ~20 Å. Minimization (gas phase) was carried out using the OPLSAA force field & PRCG method. A model of **5** was then built with the GlcNAc residues constrained in the same spatial arrangement as the extended hexasaccharide. Then minimization was carried out with constraints applied to all GlcNAc atoms and allowing the remainder of the structure, the scaffold, to minimize. This led to generation of the structures shown in Figure 4.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carres.2012.07.011.

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