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PAPER

Synthesis of 1,2,3-triazole linked galactopyranosides and evaluation of cholera toxin inhibition†

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We report the synthesis of a series of bivalent 1,2,3-triazole linked galactopyranosides as potential inhibitors of cholera toxin (CT). The inhibitory activity of the bivalent series was examined (ELISA) and the series showed low inhibitory activity (millimolar IC_{50}s). Conversely, the monomeric galactotriazole analogues were strong inhibitors of cholera toxin (IC_{50} = 71–75 μM).

Introduction

Glycoconjugates are important as molecular probes for the interrogation of biological processes, biomolecular communication events and related processes. Glycoconjugates are rich in structural and functional diversity and involve protein–carbohydrate interactions that play important roles in biology (*i.e.* immune function and fertilization) and various disease states.[1–3] There are early events in the infectious cycles of many bacteria, viruses, mycoplasma and parasites that involve carbohydrate-mediated recognition of the host by the pathogen.[1] The ability to control or prevent these events from occurring *via* the use of small or multivalent ligands is highly desirable.

In recent years, the Cu^{I}-catalysed 1,3-dipolar cycloaddition of terminal azides and alkynes (the Huisgen reaction), which is commonly abbreviated as CuAAC (Cu^{I}-catalysed azide-terminal alkyne cycloaddition), has emerged in glycoconjugation chemistry for linking carbohydrate moieties to desired scaffolds.[4,5] CuAAC yields 1,4-disubstituted-1,2,3-triazoles in a regiospecific and usually efficient fashion and was independently discovered by the laboratories of Sharpless and Meldal.[6–8] Since the designation of the CuAAC transformation by Sharpless as a 'click' chemical reaction, the 1,4-disubstituted-1,2,3-triazole ring has emerged as an important heterocycle in medicinal and material chemistry.[7] This is largely attributed to the mild conditions under which the cycloaddition usually proceeds, strong functional group tolerance, stereospecificity, high yield, ease of isolation and purification, and biocompatibility.[4,9] The versatility of the transformation has been demonstrated in the synthesis of glycoconjugates, where anomeric glycotriazoles have been readily prepared from azido sugars in high yields and excellent anomeric purity.[10–14] The choice of ligation ameliorates often difficult conditions associated with glycosidic bond-forming reactions[15] and the cycloaddition proceeds readily with *O*-protected sugars as well as with those containing free hydroxyl groups.[15–23]

It was reasoned that the use of galactotriazoles facilitates the rapid and efficient synthesis of galactoconjugates in a regiospecific and stereoselective fashion. We sought to devise the synthesis of *C*- and *N*-linked galactotriazoles to enable access to suitable galactoconjugates that inhibit cholera toxin (CT). CT is the enterotoxin secreted by *Vibrio cholerae* and is the agent responsible for the symptoms presented by cholera-infected patients.

Cholera is a severe diarrheal disease associated with infection by the gram-negative bacterium *V. cholerae* and is often correlated to high infective and mortality rates. Since 1961 there have been approximately 5 million recorded cases of cholera infection, with over 250 000 deaths.[24] CT is of the AB_5 class of toxins,[25] comprised of a catalytic A-subunit and multiple B-subunits that bind specifically to the ganglioside G_{M1}; a complex glycolipid found in the epithelial cytoplasmic membrane.[24,26] A large association constant (k_a = 1.05×10^6 M^{-1}) for the oligosaccharide-G_{M1}:B pentamer complex[26] permits the receptor mediated endocytosis of the toxic A subunit. Cellular internalization of the A-subunit activates adenyl cyclase, leading to the disruption of the normal sodium influx, thereby effecting an uncontrolled loss of intracellular electrolytes into the intestinal lumen. This action leads to the establishment of severe diarrheal symptoms and onset of dehydration.[24] Crystallographic studies have led to the extensive characterisation of the recognition of G_{M1} by the pentameric CTB-subunits.[27,28] These studies reveal that the terminal galactose of the G_{M1} oligosaccharide binds to a shallow binding epitope on each B subunit.[26,29] The intrinsic dissociation constant for this terminal galactose in the CTB:G_{M1}

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† Electronic supplementary information (ESI) available: Experimental procedures for compounds **3**, **4**, **6**, **8**, **10**, **12–18**, **20**, **21**, **24**, **27–30**, **36**, **37**, **39–42** and bioassay procedures. ^{1}H and ^{13}C NMR spectra for compounds **7–12**, **19–23**, **25**, **26**, **31–37**, **41** and **42**. CCDC reference numbers 833575 and 833576. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c1ob06317k

complex is around 50 mM.[30] Thus the recognition of CT by G_{M1} represents a target for therapeutic intervention and an opportunity to design antagonists as prophylactic and therapeutic drugs against the symptoms of infection by *V. cholerae*.

Classical approaches to CT anti-infective therapies have relied on small molecule inhibitors that reversibly bind to the B subunit (forming an association complex), which disrupts host cell surface recognition. Such molecules are generally derivatives of the entire G_{M1} oligosaccharide unit[31] or mimics of the terminal binding galactose and/or sialic acid residues of G_{M1}, and include conformationally restricted G_{M1} mimics.[32–37] For the small molecule antagonists, galactose itself has been shown to have a low but specific affinity for CT (IC_{50} = 45 mM).[38,39] These design strategies, however, suffer from difficulties relating to complex oligosaccharide synthesis and consequently unfavourable economic considerations (in the case of G_{M1} mimics), in addition to the relatively low binding affinities of galactose derivatives. In the latter case, the poor affinities are largely due to the inherent weak interaction of protein–carbohydrate binding.

In contrast, multivalent antagonists have resulted in significant improvement in inhibitory activity relative to monovalent analogues.[40,41] Multivalent antagonists employ multiple copies of an inhibitor to take advantage of the symmetric distribution of binding epitopes on the pentameric B subunit, thereby effecting the simultaneous occupation of the binding sites on the CTB subunit. The simplest multivalent inhibitors are bivalent derivatives and their general structure is shown in Fig. 1. Bivalent inhibitors consist of two terminal galactose groups, a core and an appropriate linker. Work by Hol and Fan[27] established that bivalent inhibitors can be classified as "spanning", where the galactose binding can reach the required binding epitopes on CT, or as "non-spanning", where the galactose can not reach adjacent galactose binding sites (in the latter case, the bivalent linkers can bind in an intermolecular fashion, in which the second unbound galactose binds to a second CT molecule). The length of the chosen linker will determine whether or not the bivalent inhibitor will be spanning or non-spanning. Generally, a small molecule lead compound, such as *m*-nitrophenyl-α-D-galactopyranoside (MNPG), is attached to a bivalent scaffold that enables the affinity for CT to be enhanced significantly. A series of non-spanning bivalent ligands based on MNPG were observed to have a gain in activity of two orders of magnitude in comparison to monovalent MNPG.[27] This gain in activity was greater than that expected for the presence of an additional copy of a monovalent ligand, which could be a result of steric blocking playing a role in the competitive surface receptor binding inhibition process.[27] Some pseudo-bivalent linkers have shown non-specific binding interactions between the linkers and CT.[42] It was observed that these non-specific interactions were enhanced as the linker lengths were increased. Other bivalent inhibitors have been synthesized with galactose, lactose, G_{M1}, G_{M2}, or G_{M1} derivatised headgroups that exhibited substantial gains in affinity in comparison to the corresponding monovalent ligands.[43–47] Some of the most potent bivalent CT inhibitors possess improved affinity for CT over that of G_{M1}, which illustrates the importance of increasing the valence of the inhibitors.

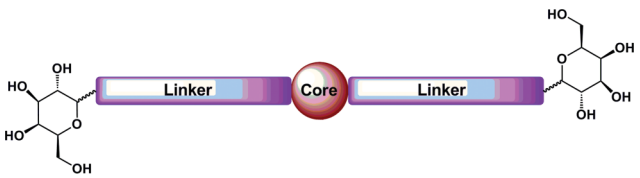
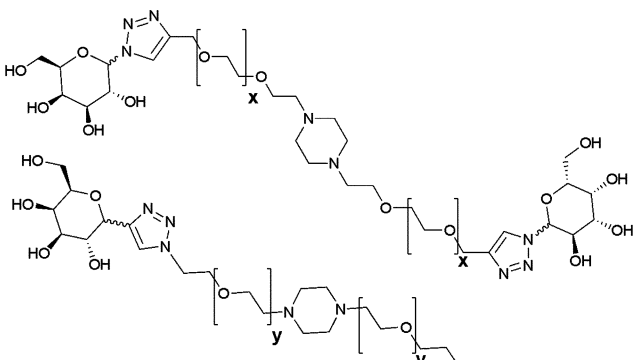


Fig. 1 General structure of bivalent CT inhibitors.

The incorporation of triazoles adjacent to galactose and sialic acid scaffolds, *via* CuAAC reactions, has been utilized in developing small molecules, polymer-based heterobifunctional ligands, glycopeptides, and glycopolypeptides against CT that have shown improvement in activity in comparison to galactose and small molecule derivatives.[22,23,30] This body of work suggests that the triazole is a suitable isostere for the *m*-nitrophenyl group found in MNPG derivatives. This approach is particularly attractive owing to the synthetic accessibility of triazoles under mild conditions relative to those required for the installation of the *m*-nitrophenyl group in glycoside forming reactions. The mild conditions may provide greater stereochemical control enabling the isolation of either pure α- or β-configured galactosides and perhaps a reduced requirement for OH-protecting groups, thereby reducing the number of synthetic steps. We believe these considerations will allow rapid access to a library of derivatives, enabling optimization of bivalent compounds following biological assay.

We now report the synthesis and biological evaluation of bivalent 1,2,3-triazole linked galactopyranosides. The general structure of the bivalent CT inhibitors reported herein is shown in Fig. 2. They comprise the following design elements: 1. piperazine core, 2. polyethylene glycol (PEG) linker, 3. pendant galactotriazole units. The piperazine scaffold was chosen on the basis that MNPG derivatised propylpiperazine analogues have been shown to have improved activity against CT.[27,48] The PEG linker groups were considered to be essential for water solubility. The length of the PEG oligomers was varied within the series to enable access to *spanning* and *non-spanning* derivatives.



β-Galactotriazoles

11 x = 2; β-***N*-GTPEG3**
12 x = 3; β-***N*-GTPEG4**
23 y = 2; β-***C*-GTPEG3**
26 y = 3; β-***C*-GTPEG4**

α-Galactotriazoles

35 y = 2; α-***C*-GTPEG3**
32 y = 3; α-***C*-GTPEG4**

Fig. 2 Target bivalent galactotriazole CT ligands.

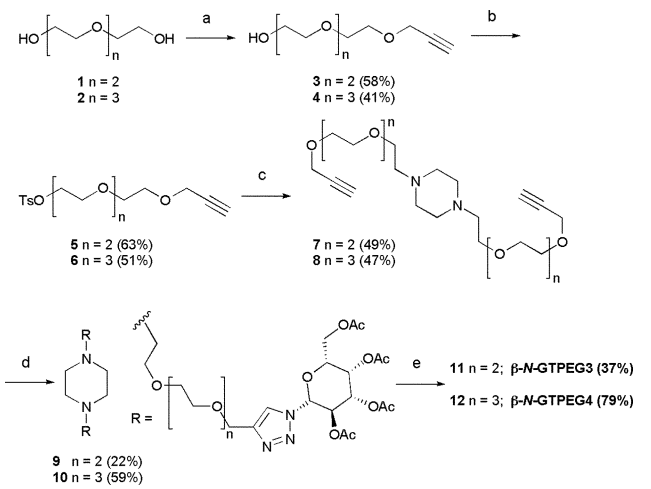
It is well established that *N*- and *C*-glycosides are stable and do not possess the enzymatic lability of traditional *O*-glycosides.[5,49–51] A survey of multivalent galactotriazole-linked CT inhibitors

previously reported,[22,23,30] reveals that they contain metabolically unstable amide bonds and enzymatically labile *O*-glycosidic linkages. As we envisage the bivalent inhibitors to comprise an adjunct therapy, in which oral administration would serve to reduce the symptoms of the toxin following *V. cholerae* infection, these derivatives are required to have prolonged stability in the gastric environment. It was therefore of interest to prepare both *N*- and *C*-galactotriazoles. It was of further interest to develop syntheses which preserved the stereochemistry at the anomeric position. This would be achievable by constructing galactose derivatives with either a terminal alkyne or an azide group at the anomeric position. These could then undergo cycloaddition with the appropriately functionalised bi-functional cross-linker.

Results and discussion

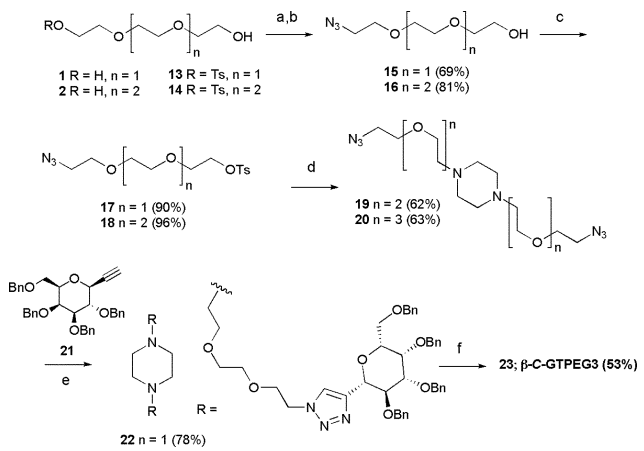
1. Synthesis of bivalent inhibitors.

***β*-N-*Galactotriazoles* [*β*-N-*GTPEG3* (*11*), *β*-N-*GTPEG4* (*12*)].** Scheme 1 shows the synthesis for target bivalent compounds β-*N*-GTPEG3 (**11**) and β-*N*-GTPEG4 (**12**). The synthesis was initiated by the installation of the propargyl moiety on the appropriate ethylene glycol unit *via* the use of propargyl bromide (Scheme 1), affording the intermediates **3** and **4** in moderate yields (58% and 41%, respectively).[52,53] Mono-tosylation gave the activated alkynyl-ethyleneglycols[52] (**5** and **6**), which enabled double alkylation of piperazine to yield the bis-adducts **7** and **8**. The 1,3-dipolar cycloaddition of 1-β-azido-2,3,4,6-tetra-*O*-acetyl-D-galactose[54] with **7** furnished the bis-galactotriazole (**9**), although in a low yield. Subsequent deprotection of the methyl ethers afforded the target dimeric compound **11** (β-*N*-GTPEG3). Compound **12** (β-*N*-GTPEG4) was prepared in a similar fashion, however with improved yields in the cycloaddition step, which may be attributed to the increased water solubility derived from the additional polyethylene glycol unit.

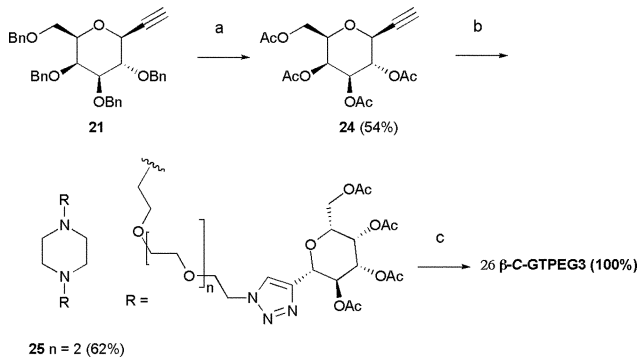


Scheme 1 *Reagents and conditions*: **a** NaH, propargyl bromide, THF; **b** DMAP, Et_3N, TsCl, THF; **c** piperazine, NaH, 75 °C, THF; **d** Cu(0), 1-β-azido-2,3,4,6-tetra-*O*-acetyl-D-galactose, 2 : 1 *t*-BuOH : H_2O; **e** MeOH, K_2CO_3.

***β*-C-*Galactotriazoles* [*β*-C-*GTPEG3* (*23*), *β*-C-*GTPEG4* (*26*)].** Attention was then turned to the preparation of the corresponding *C*-linked galactotriazoles, in which the anomeric alkynyl-glycoside replaced the respective azido-glycoside. The synthesis of the target compounds is shown in Schemes 2 and 3, respectively. For the synthesis of the target compounds [β-*C*-GTPEG3 (**23**) and β-*C*-GTPEG4 (**26**)], the preparation of the piperazinyl-azides (**19** and **20**) was required. Accordingly, triethylene and tetraethylene glycol were both mono-tosylated to afford tosylates **13** and **14**.[55–59] These were then treated with sodium azide to furnish the mono-azides **15** and **16**.[56,60] Subsequent tosylation of the remaining alcohol[61] to give **17** and **18** provided sufficient activation to permit the double nucleophilic addition of piperazine that resulted in the production of **19** and **20** in good yields. The cycloaddition of 3,7-anhydro-4,5,6,8-tetra-*O*-benzyl-1,1,2,2-tetradehydro-1,2-D-glycero-L-mannooctitol[62,63] (**21**) to PEG3-azide (**19**), proceeded smoothly giving adduct **22**. This was followed by hydrogenolysis to afford the target compound β-*C*-GTPEG3 (**23**). The complete removal of the benzyl protecting groups for the analogous tetraethylene glycol compound of **22** proved difficult by hydrogenolysis. This problem was overcome through conversion of the *O*-benzyl groups in **21** (Scheme 3) to the corresponding *O*-acetates *via* the use of acetic anhydride and trimethylsilyl triflate, which yielded compound **24**.[62] The crystal structure for **24** was solved (Fig. 3) and comparison with other galactoacetylenes is discussed (*vide infra*). The click reaction was carried out under the same conditions as described for β-*C*-GTPEG3 (**23**) and the



Scheme 2 *Reagents and conditions*: **a** Et_3N, TsCl, CH_2Cl_2; **b** EtOH, NaN_3, 85 °C; **c** Et_3N, TsCl, CH_2Cl_2; **d** piperazine, NaH, THF, −78 °C/rt/75 °C; **e** **21**, **19**, Cu(0), TBTA, 2 : 1 *t*-BuOH : H_2O; **f** 5% Pd/C, H_2, EtOH.



Scheme 3 *Reagents and conditions*: **a** Ac_2O, TMSOTf; **b** **20**, **24**, Cu(0), TBTA, 1 : 1 : 1 DMSO : H_2O : CH_2Cl_2; **c** MeOH, K_2CO_3.

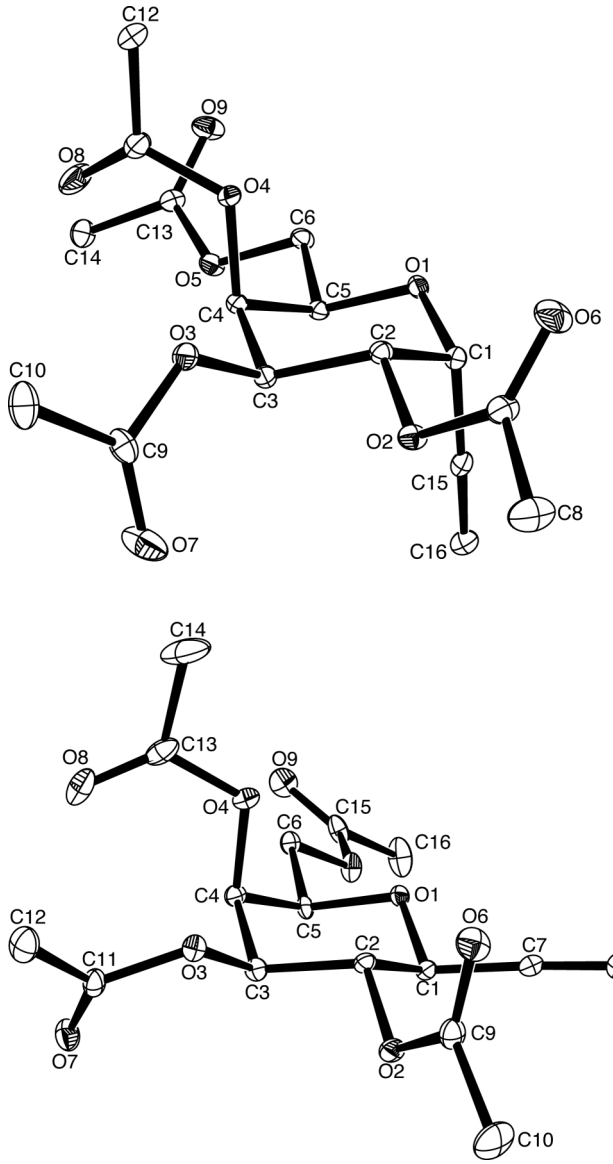
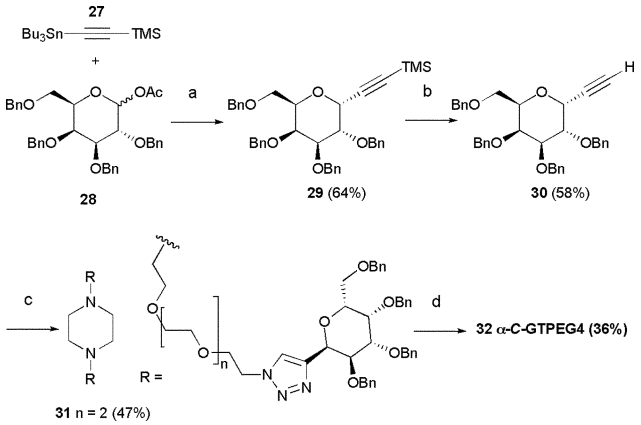


Fig. 3 Thermal ellipsoid plot for α- and β-alkynes **33** and **24**, respectively. Ellipsoids are at the 20% probability level.

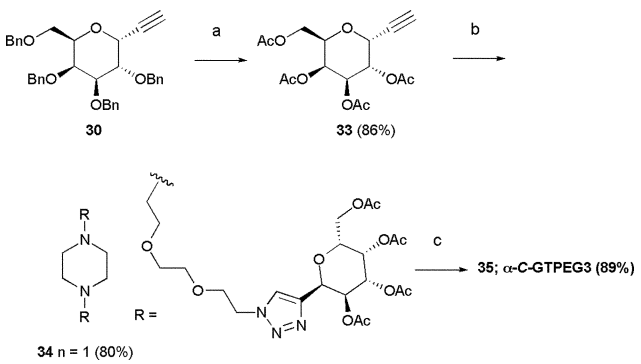
acetate removal proceeded smoothly with methanolic potassium carbonate affording β-*N*-GTPEG4 (**26**) in a quantitative conversion. Thus, β-*N*-GTPEG4 (**26**) was obtained and critical to its success was the method of transferring the *O*-benzyl protecting groups of **21** to *O*-acetate in a single step procedure.

***α*-C-*Galactotriazoles* [*α*-C-*GTPEG3* (*35*), *α*-C-*GTPEG4* (*32*)].** Following the preparation of the corresponding β-*C*-linked galactotriazoles, the synthesis of the α-anomeric derivatives [α-*C*-GTPEG3 (**35**) and α-*C*-GTPEG4 (**32**)] was performed. The syntheses of the target compounds are shown in Schemes 4 and 5. In order to obtain these α-*C* linked bivalent inhibitors (**35** and **32**) the synthesis of the α-alkynyl galactose **30** was required, which was accomplished by coupling TMS-protected acetylenylbutylstannane (**27**) with acetyl 2,3,4,6-tetra-*O*-benzyl-D-galactopyranoside (**28**), followed by basic trimethylsilyl deprotection of **29**.[64,65] The cycloaddition of the tetraethylene glycoyl azide (**20**) with the α-alkynyl galactose **30** was straightforward in the presence of



Scheme 4 *Reagents and conditions*: **a** TMSOTf, CH_2Cl_2; **b** 1 M NaOH, CH_3OH/CH_2Cl_2; **c** **20**, Cu(0), TBTA, 2 : 1 : 1 *t*-BuOH : H_2O : CH_2Cl_2; **d** 10% Pd/C, H_2, EtOH : AcOH.

Cu(I) [generated from Cu(0)] and tris(benzyltriazolylmethyl)amine (TBTA), followed by debenzylation to give the target compound α-*C*-GTPEG4 (**32**) with a 36% yield for the final step (Scheme 4). Application of this synthesis to the second target in this series, α-*C*-GTPEG3 (**35**) was unsuccessful owing to incomplete benzyl deprotection in the final step of the sequence. However, this problem was ameliorated by conversion of the 2,3,4,6-tetra-*O*-benzyl-protected alkynyl galactose (**30**) to the corresponding 2,3,4,6-tetra-*O*-acetate (**33**) (Scheme 5). The crystal structure of **33** was solved and is shown in Fig. 3. It should be noted that the Ac_2O/TMSOTf mediated conversion of the α-alkynyl 2,3,4,6-tetra-*O*-benzyl-protected galactose **30** to **33** has not been previously reported and **33** was obtained in a yield of 86%. The acetate deprotection proceeded smoothly to give α-*C*-GTPEG3 (**35**) with an overall yield of 12% starting from D-galactose.



Scheme 5 *Reagents and conditions*: **a** Ac_2O, TMSOTf; **b** **19**, Cu(0), TBTA, 1 : 1 : 1 DMSO : H_2O : CH_2Cl_2; **c** MeOH, K_2CO_3.

The thermal ellipsoid plot for **33** establishes this derivative to have the α-configuration, while **24** has the β-configuration. The C1–C7 bond distance in the α-alkyne **33** (1.481(2) Å) is significantly longer than the corresponding distance in the β-alkyne **24** (1.459(3) Å) and the previously reported β-alkyne **21**[63] for which the corresponding bond distance is 1.462(4) (Fig. 4). This bond lengthening is consistent with the presence of (n_O-σ*CC) orbital interactions between the ring oxygen lone pair and the antibonding orbital of the C1–C7 bond and is a clear example of the anomeric effect.[66,67] The differences between the C1–C7 bond

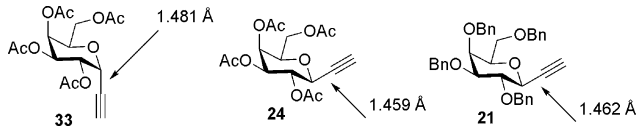


Fig. 4 Comparison of the C1–C7 bond distances for the α- and β-alkynes (**33**, **24**, **21**[63]).

distances for **33** and **24** are not as pronounced as those observed for 2,3,4,6-tetraacetate-β-D-glucopyranosyl azide [1.460 Å (C1–N distance)] and 2,3,4,6-tetraacetate-α-D-glucopyranosyl azide [1.510 Å (C1–N distance)], which indicates that the anomeric effect is much stronger for the more electronegative azides compared to the acetylenes.[68]

2. Cholera toxin inhibition assay

With the synthesis of the bivalent galactotriazoles complete, the inhibitory activity of the derivatives against the binding of 10 ng mL^{-1} cholera toxin (CT) to its cell surface receptor (G_{M1}) was determined as described previously.[69] The results from the biological analysis are shown in Table 1. The β-*N*-linked galactotriazoles, β-*N*-GTPEG3 (**11**) and β-*N*-GTPEG4 (**12**) showed minimal inhibitory potency (0–11% at concentrations of 11–12 mM), suggesting that they would be no more potent than D-galactose (IC_{50} = 45 mM).[38] We next examined the *C*-linked galactosides, compounds β-*C*-GTPEG3 (**23**) and β-*C*-GTPEG4 (**26**), the regioisomers for β-*N*-GTPEG3 (**11**) and β-*N*-GTPEG4 (**12**). These possess a 1,4-disubstituted triazole, however the glycosidic linkage is at the 4-position of the triazole ring (*cf.* the 1-position for β-*N*-GTPEG3 (**11**) and β-*N*-GTPEG4 (**12**)). The IC_{50} values were 0.51 mM and ≫ 2.5 mM for β-*C*-GTPEG3 (**23**) and β-*C*-GTPEG4 (**26**), respectively. It should be noted that β-*C*-GTPEG3 (**23**) is more potent than galactose by almost two orders of magnitude (Table 1). This significant improvement in activity of β-*C*-GTPEG3 (**23**) in comparison to β-*N*-GTPEG3 (**11**) and β-*N*-GTPEG4 (**12**) could be attributed to β-*C*-GTPEG3 (**23**) possessing a *C*-glycoside bond of the sugar, which results in the atoms of the triazole group being optimally arrayed. It is therefore surprising that β-*N*-GTPEG4 (**12**), with a similar structure, does not mirror the potency of β-*C*-GTPEG3 (**23**). By considering the overall improvement in biological activity of β-*C*-GTPEG4 (**26**) and β-*C*-GTPEG3 (**23**) in comparison to β-*N*-GTPEG3 (**11**), and β-*N*-GTPEG4 (**12**), it can be deduced that one of the key components of these CT inhibitors is the triazole ring, and that the most active compounds are the ones originating from the alkyne group on the sugar and not the azide group. This lack of activity could be due to the beta anomeric configuration of the products, which may thus adopt an incorrect conformation in solution, preventing access to the active site in CT. The triazole ring may also be forcing the galactose to be in an incompatible conformation that results in no observable activity.

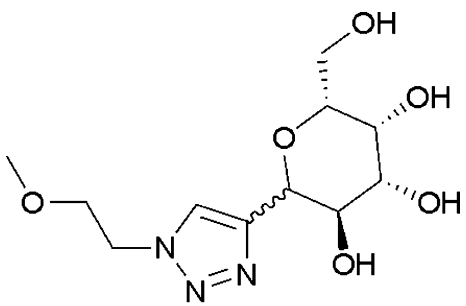
Table 1 Data for inhibition of binding of cholera toxin to its receptor[a]

Entry	Triazole	% Inhib.	IC_{50} (μM)	Gain over galactose[b]
1	**11**	0		—
2	**12**	11 ± 3		—
3	**23**		506 ± 42	89
4	**26**	19 ± 12		
5	**35**	8 ± 4		
6	**32**	16 ± 3		
7	**36**		75 ± 5	600
8	**37**		71 ± 4	634

[a] Data are quoted as mean (arithmetic for % inhibition, geometric for IC_{50}) ± standard error of the mean from 2–5 experiments (n = 1 for compound **11**). [b] Based on galactose IC_{50} = 45 mM.[38]

We next considered inhibitory activity based on the anomeric configuration. As a result the alpha analogues of β-*C*-GTPEG3 (**23**) and β-*C*-GTPEG4 (**26**) were synthesized [α-*C*-GTPEG3 (**35**) and α-*C*-GTPEG4 (**32**)] and biologically tested. These compounds did not bind strongly to CT, and as a result IC_{50} values could not be obtained, *i.e.* it seemed that the two bivalent alpha analogues α-*C*-GTPEG3 (**35**) and α-*C*-GTPEG4 (**32**) were less active than their beta counterparts. This result was surprising since the majority of active compounds against CT are alpha configured. Disappointingly, inhibitory activity based on the anomeric configuration was not observed for any of the analogues.

The lack of activity led to the consideration that the triazole-sugar head group is not a suitable isostere for the MNPG functionality. In order to test this last hypothesis, two small molecules were synthesized [β-*C*-GT (**36**) and α-*C*-GT (**37**)] (Fig. 5) which comprise the triazole-sugar head group with a single PEG unit. The synthesis for β-*C*-GT (**36**) and α-*C*-GT (**37**) is detailed in the supporting information. The *C*-linked galactotriazoles were chosen for comparison owing to the improved activity relative to the *N*-linked galactotriazole derivatives, which is evidenced in Table 1.



36; β-*C*-GT
37; α-*C*-GT

Fig. 5 Monovalent inhibitors.

Both compounds were found to be potent inhibitors of cholera toxin binding, with IC_{50} values in the low micromolar range. Both of these analogues possessed better activity in comparison to MNPG (IC_{50} = 0.72 mM)[38] by one order of magnitude and are of a comparable potency with respect to each other. In addition β-*C*-GT (**36**) and α-*C*-GT (**37**) were respectively 600 and 634 times more potent than galactose (see Table 1). This suggests that the triazole is indeed a suitable isosteric substitute for MNPG, whether as the beta or alpha glycoside, although from these results it suggests that CT does have a slight preference for the galactose to be an alpha anomer. The significant improvement in the biological activity of the monovalent compounds β-*C*-GT (**36**) and α-*C*-GT (**37**) compared with the bivalent series is counter-intuitive.

It is plausible that the PEG chains could be coiling around the sugar groups in solution and thus preventing the desired extended conformation in the assay. This suggests that the next generation of bivalent inhibitors should include rigid spacer groups between the triazole ring and the PEG chain to inhibit intramolecular hydrogen bonding of the PEG chain oxygen atoms to the sugar hydroxyls. We anticipate that the highly modular synthetic approach adopted will enable the rapid synthesis of such derivatives.

Conclusions

A series of potential bivalent CT inhibitors was successfully synthesized; the key to the synthesis was the 'click' reaction that yielded the corresponding 1,2,3-triazole linked galactopyranosides. It was observed that these triazole galactopyranosides could be enantioselectively prepared in both monovalent and bivalent systems. On the whole there was not much difference between the alpha and beta analogues for inhibition of the binding of cholera toxin to its receptor, despite α-*C*-GT (**37**) (alpha configured) being the most potent molecule described herein, with an IC_{50} of 71 μM. These 1,2,3-triazole linked galactopyranosides can effectively act as an isostere for the MNPG group due to β-*C*-GT (**36**) and α-*C*-GT (**37**) being more potent than MNPG by an order of magnitude. There would need to be some general improvements in the efficiency of the synthesis before a viable cholera prophylactic/therapeutic drug could be obtained from such a method. Compound α-*C*-GT (**37**) is a potent small molecule CT inhibitor that can serve as an improved lead for the development of a multivalent CT inhibitor.

Experimental section

General methods

All non-aqueous reactions were performed under an atmosphere of dry nitrogen or argon, unless otherwise specified. CH_2Cl_2 and Et_2O were distilled from CaH_2 in a recycling still. THF was dried with $LiAlH_4$ and then distilled from potassium metal in a recycling still. Et_3N was freshly distilled from CaH_2 under N_2. Reaction progress was monitored *via* thin-layer chromatography (TLC) using kieselgel 60 F_{254} plates (aluminium backed) and ethyl acetate/hexane or CH_2Cl_2/MeOH. Aluminium oxide 60 F_{254} neutral (Type E) plates were also used with ethyl acetate/hexane. TLC plates were visualised using a 254 nM UV lamp and/or a 10% w/v molybdophosphoric acid/EtOH stain or permanganate stain consisting of $KMnO_4$ (3.0 g), K_2CO_3 (20 g) and 5% w/v aqueous NaOH (5 mL) in H_2O (300 mL). Flash column chromatography was performed using silica gel (silica gel 60, 230–400 mesh ASTM) or aluminium oxide GF_{254} (Type 60/E) as the stationary phase and ethyl acetate/hexane or CH_2Cl_2/MeOH mixtures as the mobile phase. ^{1}H, ^{13}C, DEPT, ^{1}H/^{13}C-HSQC, ^{1}H/^{13}C-HMBC and ^{1}H/^{1}H-COSY nuclear magnetic resonance (NMR) spectra were obtained on a Bruker AV-300 spectrometer (^{1}H at 300.13 MHz and ^{13}C at 75.47 MHz, respectively) and a Bruker AV-500 spectrometer (^{1}H at 500.19 MHz and ^{13}C at 125.78 MHz, respectively). Proton and carbon chemical shifts are reported as δ values in parts per million (ppm) and are relative to residual solvent; coupling constants (J) are in Hz. Infrared (IR) spectra were recorded on a Shimadzu IRPrestige-21 Fourier-Transform infrared spectrometer. Samples were analysed using either sodium chloride plates (NaCl) or attenuated total reflection (ATR), and absorptions are given in wavenumbers (cm^{-1}). Low resolution mass spectra were obtained on a Bruker Daltronics Esquire 6000 Ion-Trap mass spectrometer employing electrospray ionisation (ESI) with 40 eV cone voltage; samples were run in 0.1% formic acid. High resolution mass spectra were obtained on a Waters Q-TOF II, employing electrospray ionisation (ESI) with 35 eV cone voltage, using lock spray and sodium iodide as a reference sample. High performance liquid chromatography (HPLC) was carried out using a GBC instrument with a photodiode array detector. Analytical HPLC was carried out with an Alltech Apollo C_{18} 5 μm 150 × 4.6 mm column. Semi-preparative HPLC was carried out with a Grace Apollo C_{18} 5 μm 250 × 10 mm column. Optical rotation ([α]) values were determined using an Atago Polax-2L polarimeter with a 1 dm path length glass cell using the sodium D line (589 nm) at the temperature specified.

2-[2-(2-Prop-2-ynoxyethoxy)ethoxy]ethyl-4-methylbenzene sulfonate (5). Compound **3** (3.08 g, 16.4 mmol) was dissolved in 170 mL dry THF (170 mL) to which was added TsCl (3.43 g, 18.0 mmol), Et_3N (6.80 mL, 49.1 mmol), and DMAP (80 mg, 0.65 mmol). The reaction mixture was allowed to stir at room temperature for 24 h and then the solvent was removed *in vacuo*. The residue was dissolved in EtOAc and washed with water. The combined organic layers were dried over $MgSO_4$, concentrated *in vacuo*, and then purified by column chromatography on silica gel eluting with 33–60% EtOAc : hexane to yield **5** as a colourless oil {3.52 g, 63% (lit.[52] 87%)}. Data for compound **5** were identical to literature values.[52]

1,4-Bis-{2-[2-(2-prop-2-ynyloxyethoxy)ethoxy]ethyl}piperazine (7). Piperazine (124 mg, 1.4 mmol) was dissolved in dry THF (15 mL), to which was added sodium hydride (60% dispersion in oil, 234 mg) and the reaction mixture was stirred at room temperature for 2.5 h. A solution of toluene-4-sulfonic acid 2-[2-(2-prop-2-ynyloxy-ethoxy)-ethoxy]-ethyl ester **5** (980 mg, 2.86 mmol) in dry THF (3 mL + 10 mL rinse) was added. This mixture was then heated at 75 °C (reflux) for 24.5 h, cooled, diluted with CH_2Cl_2 and the organic layers were washed with water. The combined organic extracts were dried over $MgSO_4$ and concentrated under vacuum to yield a crude oil. The crude oil was dry loaded onto silica and purified by column chromatography eluting initially with EtOAc, which was the followed with a 50% CH_2Cl_2 : MeOH that contained 1% Et_3N. Compound **7** was obtained as a brown oil (298 mg, 49%). IR (ATR, cm^{-1}) 3246, 2916, 2868, 2818, 2112, 1456, 1327, 1304, 1244, 1092, 1032, 1011, 943, 841, ^{1}H NMR δ ($CDCl_3$, 300 MHz), 4.09 (4H, d, J = 2.4 Hz), 3.58–3.52 (20H, m), 2.57–2.53 (12H, m), 2.36 (2H, t, J = 2.3 Hz); ^{13}C NMR δ ($CDCl_3$, 75 MHz), 79.4, 74.4, 70.3, 70.09, 70.14, 68.8, 68.5, 58.1, 57.3 and 52.9. MS-ESI: m/z $[M + H]^+$ for $C_{22}H_{39}N_2O_6^+$ calculated 427.3; observed 427.3. HRMS-EI: m/z $[M]^+$ for $C_{22}H_{38}N_2O_6^+$, calculated 426.2730; observed 426.2718.

[(2*R*,3*S*,4*S*,5*R*,6*R*)-3,5-Diacetoxy-2-(acetoxymethyl)-6-[(1*R*)-4-[2-[2-[2-[4-[2-[2-[2-[[1-[(2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-triacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]triazol-4-yl]methoxy]ethoxy]ethoxy]ethyl]piperazin-1-yl]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]tetrahydropyran-4-yl] acetate (9). Compound **7** (298 mg, 0.7 mmol) was suspended in a 2 : 1 *t*-BuOH : H_2O mixture (3 mL)

to which was added β-D-galactopyranosyl azide 2,3,4,6-tetra-*O*-acetate (575 mg) and Cu(0) mesh (444 mg). The reaction was allowed to stir vigorously for 23 h at room temperature. Then it was diluted with CH_2Cl_2 and filtered through a bed of celite. The filtrate was washed with water and the organic layer was dried over Na_2SO_4 and concentrated to yield a crude oil. The crude oil was dry loaded onto a silica column and purified by column chromatography eluting with MeOH. Compound **9** was obtained as a light brown oil (181 mg, 22%). $[\alpha]_D^{20}$ −4.0 (*c* 1.7, DCM); IR (NaCl, cm^{-1}) 2923, 2874, 2831, 1749, 1454, 1370, 1221, 1092, 1063, ^{1}H NMR δ ($CDCl_3$, 300 MHz), 7.78 (2H, s), 5.80 (2H, d, J = 9.0 Hz), 5.51–5.45 (4H, m), 5.20 (2H, dd, J = 3.3, 10.5 Hz), 4.62 (4H, s), 4.18–4.07 (6 H, m), 3.60–3.51 (20 H, m), 2.53–2.49 (12H, m), 2.15 (6H, s), 1.97 (6H, s), 1.93 (6H, s), 1.81 (6H, s); ^{13}C NMR δ ($CDCl_3$, 75 MHz), 170.2, 169.9, 169.7, 168.9, 145.8, 121.0, 86.1, 73.9, 70.7, 70.5, 70.4, 70.3, 69.7, 68.9, 67.8, 66.8, 64.4, 61.1, 57.7, 53.4, 20.6, 20.4 and 20.2. MS-ESI: m/z [M + H]$^+$ for $C_{50}H_{77}N_8O_{24}^+$ calculated 1173.5; observed 1173.4. HRMS-ESI: m/z [M + H]$^+$ for $C_{50}H_{77}N_8O_{24}^+$, calculated 1173.5051; observed 1173.5088.

(2*R*,2′*R*,3*R*,3′*R*,4*S*,4′*S*,5*R*,5′*R*,6*R*,6′*R*)-6,6′-(4,4′-(2,2′-(2,2′-(2,2′-(Piperazine-1,4-diyl)bis(ethane-2,1-diyl))bis(oxy)bis(ethane-2,1-diyl))bis(oxy)bis(ethane-2,1-diyl))bis(oxy)bis(methylene)bis(1*H*-1,2,3-triazole-4,1-diyl))bis(2-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol) (11). Compound **9** (113 mg, 0.1 mmol) was dissolved in distilled methanol (4 mL), to which was added potassium carbonate (1.2 mg). The reaction was stirred at room temperature for 24 h after which the solvent was removed under vacuum to yield a crude oil. Purification was carried out by column chromatography eluting with 0–1% Et_3N : MeOH to give **11** as a colourless oil (26 mg, 32%). $[\alpha]_D^{20}$ +9.0 (*c* 0.79, MeOH); IR (ATR, cm^{-1}) 3372, 2914, 2877, 2833, 2833, 1656, 1643, 1633, 1454, 1444, 1350, 1308, 1234, 1079, 1056, 1019, ^{1}H NMR δ (CD_3OD, 300 MHz), 8.23 (2H, s), 5.59 (2H, d, J = 9.0 Hz), 4.66 (4H, s), 4.16 (2H, t, J = 9.3 Hz), 3.99 (2H, d, J = 3.0 Hz), 3.85–3.58 (28H, m), 2.57 (12H, t, J = 5.7 Hz); ^{13}C NMR δ (CD_3OD, 75 MHz), 146.2, 123.9, 90.2, 80.0, 75.3, 71.5, 71.4, 71.3, 70.8, 70.4, 69.6, 65.0, 62.5, 58.6 and 54.0. MS-ESI: m/z [M + H]$^+$ for $C_{34}H_{61}N_8O_{16}^+$ calculated 837.4; observed 837.4. HRMS-ESI: m/z [M + H]$^+$ for $C_{34}H_{61}N_8O_{16}^+$, calculated 837.4206; observed 837.4241.

1,4-Bis[2-[2-(2-azidoethoxy)ethoxy]ethyl]piperazine (19). To a solution of piperazine (41 mg, 0.47 mmol) in dry THF (4 mL) was added sodium hydride (60% dispersion in oil, 77 mg). The reaction mixture was cooled to −78 °C and stirred for 1 h before a solution of 2-[2-(2-azidoethoxy)ethoxy]ethyl 4-methylbenzenesulfonate **17** (318 mg, 0.96 mmol) in THF (3 mL + 3 mL rinse) was added dropwise. The resulting mixture was allowed to stir at −78 °C for 3 h. The reaction mixture was then allowed to warm up to room temperature and refluxed at 75 °C for 15.5 h. The reaction mixture was diluted with DCM and washed with water. The aqueous extracts were then backwashed with DCM and the combined organic extracts were dried over $MgSO_4$, and concentrated *in vacuo* to yield crude material that was purified by column chromatography on silica gel eluting initially with 33–100% EtOAc : hexane, which was followed with 1% Et_3N : MeOH to yield compound **19** as a yellow oil (116 mg, 62%). IR (NaCl, cm^{-1}) 2933, 2871, 2813, 2106, 1456, 1348, 1303, 1119, ^{1}H NMR δ ($CDCl_3$, 300 MHz), 3.66–3.58 (16H, m), 3.36 (4H, t, J = 5.1 Hz), 2.63–2.60 (12H, m);^{13}C NMR δ ($CDCl_3$, 75 MHz), 70.6, 70.4, 70.0, 68.6, 57.5, 53.1 and 50.7. MS-ESI: m/z [M – N_6H + Na]$^+$ for $C_{16}H_{31}N_2O_4Na^+$ calculated 338.2; observed 338.3. HRMS-ESI: m/z [M + H]$^+$ for $C_{16}H_{33}N_8O_4^+$, calculated 401.2625; observed 401.2607.

1-(2-(2-(2-(4-((2*S*,3*S*,4*R*,5*S*,6*R*)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)tetrahydro-2*H*-pyran-2-yl)-1*H*-1,2,3-triazol-1-yl)-ethoxy)ethoxy)ethyl)-4-(2-(2-(3-(4-((2*S*,3*S*,4*R*,5*S*,6*R*)-3,4,5-tris-(benzyloxy)-6-(benzyloxymethyl)tetrahydro-2*H*-pyran-2-yl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethyl)piperazine (22). Compound **20** (116 mg, 0.29 mmol) was suspended in a 2 : 1 : 1 mixture of *t*-BuOH : H_2O : DCM (6 mL), to which was added **21** (325 mg, 0.61 mmol), Cu(0) (184 mg, 2.90 mmol), and TBTA (3.1 mg, 0.006 mmol). The reaction mixture was allowed to stir vigorously at room temperature for 23 h after which it was diluted with DCM, and then filtered through a bed of celite to remove the excess copper. The filtrate was then washed with H_2O, dried over Na_2SO_4, and concentrated *in vacuo* to yield crude material. The crude material was dry loaded onto alumina and purified by column chromatography eluting initially with 14% EtOAc : hexane that was followed by 9–17% MeOH : EtOAc to yield compound **22** as a colourless oil (340 mg, 78%). $[\alpha]_D^{20}$ +17 (*c* 0.72, DCM); IR (ATR, cm^{-1}) 3088, 3063, 2869, 2243, 1733, 1652, 1607, 1496, 1454, 1363, 1308, 1208, 1105, 911, ^{1}H NMR δ ($CDCl_3$, 300 MHz), 7.61 (2H, s), 7.34–7.17 (36H, m), 7.02–7.00 (4H, m), 4.96 (2H, d, J = 11.7 Hz), 4.74–4.62 (8H, m), 4.51–4.24 (14H, m), 4.05 (2H, d, J = 2.4 Hz), 3.80–3.69 (8H, m), 3.58–3.43 (16H, m), 2.65–2.53 (12H, m);^{13}C NMR δ ($CDCl_3$, 75 MHz), 145.8, 138.7, 138.33, 138.26, 137.8, 128.3, 128.1, 128.0, 127.94, 127.90, 127.7, 127.6, 127.5, 127.4, 123.6, 84.4, 78.4, 74.9, 74.6, 73.9, 73.4, 72.4, 70.4, 70.1, 69.4, 68.7, 57.4, 53.0 and 50.1. MS-ESI: m/z [M + H]$^+$ for $C_{88}H_{105}N_8O_{14}^+$ calculated 1497.8; observed 1497.6. HRMS-ESI: m/z [M + H]$^+$ for $C_{88}H_{105}N_8O_{14}^+$, calculated 1497.7750; observed 1497.7703.

(2*R*,3*R*,4*R*,5*R*,6*S*)-2-(Hydroxymethyl)-6-[1-[2-[2-[2-[2-[4-[2-[2-[2-[4-[(2*S*,3*R*,4*R*,5*R*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydropyran-2-yl]triazol-1-yl]ethoxy]ethoxy]ethyl]piperazin-1-yl]-ethoxy]ethoxy]ethyl]triazol-4-yl]tetrahydropyran-3,4,5-triol (23). Compound **22** (159 mg, 0.11 mmol) was dissolved in EtOH (5 mL, required heating) and acetic acid (1 mL). To this mixture was added 5% Pd/C catalyst (610 mg). The reaction flask was evacuated and the atmosphere was replenished with hydrogen gas. This was repeated three times. The reaction was allowed to proceed at room temperature under an atmosphere of hydrogen for 3 d. The palladium catalyst was then removed by passing the reaction mixture through a bed of celite. The filtrate was evaporated to yield **22** (43.6 mg, 53%) as a yellow/brown oil. ^{1}H NMR δ (D_2O, 300 MHz), 8.19 (2H, s), 4.69 (4H, d, J = 4.5 Hz), 4.56 (2H d, J = 9.9 Hz), 4.10 (2H, d, J = 3.0 Hz), 4.03–3.98 (8H, m), 3.89–3.87 (4H, m), 3.83 (2H, d, J = 3.3 Hz), 3.79–3.67 (16H, m), 3.15–3.07 (8H, m); ^{13}C NMR δ (D_2O, 75 MHz), 144.8, 124.6, 78.9, 73.7, 73.5, 69.9, 69.3, 69.0, 68.8, 68.3, 64.9, 60.9, 55.4, 49.9 and 49.7. MS-ESI: m/z [M + H]$^+$ for $C_{32}H_{57}N_8O_{14}^+$ calculated 777.4; observed 777.3. HRMS-ESI: m/z [M + Na]$^+$ for $C_{32}H_{56}N_8NaO_{14}^+$, calculated 799.3814; observed 799.3799.

[(2*R*,3*S*,4*R*,5*S*,6*S*)-4,5-Diacetoxy-2-(acetoxymethyl)-6-[1-[2-[2-[2-[2-[4-[2-[2-[2-[2-[4-[(2*S*,3*S*,4*R*,5*S*,6*R*)-3,4,5-triacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]triazol-1-yl]ethoxy]ethoxy]ethoxy]ethyl]piperazin-1-yl]ethoxy]ethoxy]ethoxy]ethyl]triazol-4-yl]-tetrahydropyran-3-yl] acetate (25). Compounds **24** (26 mg, 0.07 mmol) and **20** (16 mg, 0.03 mmol) were combined and dissolved in 1 : 1 : 1 DCM : DMSO : H_2O (3 mL). To this reaction mixture TBTA (0.2 mg, 0.1 eq) was added, followed by Cu(0) (0.2 mg, 0.1 eq). The reaction mixture was stirred at room temperature for 16 h and worked up as for compound **22**. The crude material was dry loaded and purified by column chromatography on silica gel eluting initially with 20–100% EtOAc : hexane that was followed with 6–100% MeOH : EtOAc to yield compound **25** as a colourless oil (24 mg, 62%). $[\alpha]_D^{20}$ +23 (*c* 1.2, DCM); IR (NaCl, cm^{-1}) 2876, 2112, 1748, 1636, 1456, 1435, 1371, 1231, 1092, 1053, ^1H NMR δ ($CDCl_3$, 300 MHz), 7.75 (2H, s), 5.49–5.39 (4H, m), 5.13 (2H, dd, J = 10.2, 3.3 Hz), 4.73 (2H, d, J = 10.2 Hz), 4.51 (4H, t, J = 5.1 Hz), 4.12–4.06 (6H, m), 3.86–3.81 (4H, m), 3.61–3.58 (20H, m), 2.75–2.62 (12H, m), 2.15 (6H, s), 2.00 (6H, s), 1.96 (6H, s), 1.83 (6H, s); ^{13}C NMR δ ($CDCl_3$, 75 MHz), 170.4, 170.2, 170.1, 144.2, 123.3, 74.7, 73.8, 72.0, 70.6, 70.4, 70.3, 69.3, 68.5, 67.6, 61.5, 57.4, 52.9, 50.4, 20.7 and 20.6. MS-ESI: m/z $[M + H]^+$ for $C_{52}H_{81}N_8O_{24}^+$ calculated 1201.5; observed 1201.4. HRMS-ESI: m/z $[M + H]^+$ for $C_{52}H_{81}N_8O_{24}^+$, calculated 1201.5364; observed 1201.5344.

(2*R*,3*R*,4*R*,5*R*,6*S*)-2-(Hydroxymethyl)-6-[1-[2-[2-[2-[2-[4-[2-[2-[2-[2-[4-[(2*S*,3*R*,4*R*,5*R*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)-tetrahydropyran-2-yl]triazol-1-yl]ethoxy]ethoxy]ethoxy]ethyl]piperazin-1-yl]ethoxy]ethoxy]ethoxy]ethyl]triazol-4-yl]tetrahydropyran-3,4,5-triol (26). Compound **26** was obtained by the same procedure as used for compound **11** with the following modifications. Compound **25** (22 mg, 0.02 mmol) was dissolved in distilled MeOH (3 mL) to which was added a catalytic amount of potassium carbonate. The reaction was stirred at room temperature for 19 h. Compound **26** was obtained as a pale brown oil (15.8 mg, 100%). ^1H NMR δ (D_2O, 300 MHz), 8.18 (2H, s), 4.70 (4H, t, J = 5.1 Hz), 4.51 (2H, d, J = 9.9 Hz), 4.09 (2H, d, J = 3.0 Hz), 4.04–3.98 (6H, m), 3.89 (2H, t, J = 6.3 Hz), 3.83–3.77 (6H, m), 3.70–3.65 (20H, m), 2.62 (12H, t, J = 5.7 Hz); ^{13}C NMR δ (D_2O, 75 MHz), 144.8, 124.7, 78.8, 73.8, 73.5, 69.9, 69.2, 69.1, 69.0, 68.7, 68.4, 67.1, 60.8, 56.0, 51.6 and 49.7. MS-ESI: m/z $[M + H]^+$ for $C_{36}H_{65}N_8O_{16}^+$ calculated 865.5; observed 865.3. HRMS-ESI: m/z $[M + Na]^+$ for $C_{36}H_{64}N_8NaO_{16}^+$, calculated 887.4338; observed 887.4357.

1,4-Bis[2-[2-[2-[2-[4-[(2*R*,3*S*,4*R*,5*S*,6*R*)-3,4,5-tribenzyloxy-6-(benzyloxymethyl)tetrahydropyran-2-yl]triazol-1-yl]ethoxy]ethoxy]ethyl]piperazine (31). Compound **31** was obtained *via* the same procedure as that used for compound **25** with the following modifications: compound **30** (434 mg, 0.79 mmol), compound **20** (187 mg, 0.38 mmol), TBTA (9 mg, 0.4 eq.), Cu(0) (1.1 mg, 0.04 eq.) and a 1 : 1 : 1 mixture of DCM : DMSO : H_2O (9 mL) were used. The reaction was allowed to proceed at room temperature for 17.5 h and the crude material was dry loaded onto silica and purified *via* column chromatography eluting with 0–17% MeOH : EtOAc containing 1% Et_3N to yield compound **31** as a pale yellow oil (512 mg, 84%). $[\alpha]_D^{20}$ +28 (*c* 1.41, DCM); IR (NaCl, cm^{-1}) 3028, 2870, 2106, 1722, 1495, 1452, 1275, 1096, ^1H NMR δ ($CDCl_3$, 300 MHz), 7.70 (2H, s), 7.28–7.11 (40H, m), 5.26 (2H, d, J = 4.2 Hz), 4.74–4.40 (18H, m), 4.30–4.26 (2H, m), 4.18–4.15 (2H, m), 4.08 (4H, q, J = 3.6 Hz), 3.82 (4H, t, J = 5.1 Hz), 3.64–3.53 (24H, m), 3.39–3.33 (2H, m), 2.60 (12H, br s); ^{13}C NMR δ ($CDCl_3$, 75 MHz), 144.7, 138.19, 138.16, 137.94, 137.89, 127.9, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2, 127.1, 124.2, 73.8, 73.2, 73.1, 72.8, 72.6, 70.33, 70.25, 70.19, 70.15, 70.09, 69.9, 69.1, 68.1, 67.1, 66.9, 57.1, 52.7 and 49.8. MS-ESI: m/z $[M + H]^+$ for $C_{92}H_{113}N_8O_{16}^+$ calculated 1585.8; observed 1585.3. HRMS-ESI: m/z $[M + H]^+$ for $C_{92}H_{113}N_8O_{16}^+$, calculated 1585.8275; observed 1585.8262.

(2*R*,3*R*,4*R*,5*R*,6*R*)-2-(Hydroxymethyl)-6-[1-[2-[2-[2-[2-[4-[2-[2-[2-[2-[4-[(2*R*,3*R*,4*R*,5*R*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)-tetrahydropyran-2-yl]triazol-1-yl]ethoxy]ethoxy]ethoxy]ethyl]piperazin-1-yl]ethoxy]ethoxy]ethoxy]ethyl]triazol-4-yl]tetrahydropyran-3,4,5-triol (32). Compound **32** was obtained by the same procedure as used for compound **23** with the following modifications: compound **31** (143 mg, 0.09 mmol), 10% Pd/C catalyst (71 mg), glacial acetic acid (8 mL) were used. The reaction mixture was stirred for a total of 7 d, and toluene was used to azeotropically remove the glacial acetic acid (×3). Compound **32** was obtained as a light brown oil (28 mg, 36%). ^1H NMR δ (D_2O, 300 MHz), 8.19 (2H, s), 5.36 (2H, d, J = 6.0 Hz), 4.68 (4H, t, J = 4.5 Hz), 4.30–4.25 (2H, m), 4.17–4.00 (6H, m), 3.80–3.65 (28H, m), 2.73 (12H, br s); ^{13}C NMR δ (D_2O, 75 MHz), 142.6, 125.9, 73.3, 71.3, 69.9, 69.8, 69.2, 69.0, 68.5, 68.4, 67.3, 66.5, 60.6, 60.0, 55.9, 51.1 and 49.6. MS-ESI: m/z $[M + H]^+$ for $C_{36}H_{65}N_8O_{16}^+$ calculated 865.5; observed 865.3. HRMS-ESI: m/z $[M + H]^+$ for $C_{36}H_{65}N_8O_{16}^+$, calculated 865.4519; observed 865.4540.

[(2*R*,3*S*,4*R*,5*S*,6*R*)-3,4,5-Triacetoxy-6-ethynyl-tetrahydropyran-2-yl]methyl acetate (33). Compound **30** (851 mg, 1.55 mmol) was dissolved in acetic anhydride (46 mL) and the solution was cooled to 0 °C before trimethylsilyl triflate (1.88 mL, 10.4 mmol) was added dropwise. The reaction mixture was then allowed to warm to room temperature and stirred for 7 d. The solution was then cooled to 0 °C and quenched by the cautious addition of a saturated solution of $NaHCO_3$. Dilution of the reaction mixture with EtOAc was followed by washing the organic layer with a solution of $NaHCO_3$, then water, and then brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* with silica gel. The crude material was added to the top of a silica gel column and eluted with 66–90% EtOAc : hexane. Compound **33** was obtained as a light yellow solid (473 mg, 86%). $[\alpha]_D^{20}$ +93 (*c* 0.94, DCM); IR (NaCl, cm^{-1}) 2965, 2114, 1748, 1371, 1219, 1087, ^1H NMR δ ($CDCl_3$, 300 MHz), 5.43 (1H, dd, J = 3.3, 1.2 Hz), 5.33 (1H, dd, J = 10.5, 3.3 Hz), 5.18–5.12 (1H, m), 5.04 (1H, dd, J = 5.7, 2.1 Hz), 4.38 (1H, td, J = 6.6, 0.9 Hz), 4.13–4.01 (2H, m), 2.57 (1H, d, J = 2.4 Hz), 2.11 (3H, s), 2.06 (3H, s), 2.02 (3H, s), 1.97 (3H, s); ^{13}C NMR δ ($CDCl_3$, 75 MHz), 170.3, 170.1, 170.0, 169.9, 78.0, 76.5, 69.6, 68.5, 67.7, 66.7, 65.8, 61.5, 20.7, 20.62, 20.57 and 20.5. MS-ESI: m/z $[M + Na]^+$ for $C_{16}H_{20}NaO_9^+$ calculated 379.1; observed 379.1. MS-EI: m/z $[M]^+$ for $C_{16}H_{20}O_9^+$ calculated 356.1; observed 356.1. HRMS-EI: m/z $[M]^+$ for $C_{16}H_{20}O_9^+$, calculated 356.1107; observed 356.1095.

[(2*R*,3*S*,4*R*,5*S*,6*R*)-4,5-Diacetoxy-2-(acetoxymethyl)-6-[1-[2-[2-[2-[2-[4-[2-[2-[2-[2-[4-[(2*R*,3*S*,4*R*,5*S*,6*R*)-3,4,5-triacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]triazol-1-yl]ethoxy]ethoxy]ethoxy]ethyl]-piperazin-1-yl]ethoxy]ethoxy]ethyl]triazol-4-yl]tetrahydropyran-3-yl] acetate (34). Compound **34** was obtained *via* the same procedure as that used for compound **25** with the following

modifications: compound **19** (53.8 mg, 0.13 mmol), compound **33** (101 mg, 0.28 mmol), TBTA (7.1 mg, 0.1 eq.), Cu(0) (0.9 mg, 0.1 eq), and a 1 : 1 : 1 mixture of DCM : DMSO : H_2O (3 mL) was used. The reaction was allowed to proceed at room temperature for 21 h and the crude material was dry loaded onto silica and purified *via* column chromatography eluting with 10–100% MeOH : EtOAc to yield compound **34** as a colourless oil (120 mg, 80%). $[\alpha]_D^{20}$ +65 (*c* 2.9, DCM); IR (NaCl, cm^{-1}) 2961, 2874, 2816, 2104, 1748, 1639, 1549, 1456, 1369, 1227, 1049, ^1H NMR δ ($CDCl_3$, 300 MHz), 7.72 (2H, s), 5.93 (2H, dd, J = 9.3, 3.3 Hz), 5.53 (2H, dd, J = 3.3, 1.5 Hz), 5.49–5.43 (4H, m), 4.54–4.46 (6H, m), 4.09–4.03 (4H, m), 3.84 (4H, t, J = 5.1 Hz), 3.55–3.53 (12H, m), 2.57–2.51 (12H, m), 2.13 (6H, s), 2.03 (12H, s), 2.00 (6H, s);^{13}C NMR δ ($CDCl_3$, 75 MHz), 170.4, 170.2, 170.0, 169.7, 142.4, 125.2, 70.5, 70.1, 69.3, 68.8, 68.2, 68.1, 68.0. 67.6, 61.4, 57.6, 53.4, 50.2 and 20.6. MS-ESI: m/z $[M + K]^+$ for $C_{48}H_{72}KO_{22}^+$ calculated 1151.4; observed 1151.7. HRMS-ESI: m/z $[M + H]^+$ for $C_{48}H_{73}N_8O_{22}^+$, calculated 1113.4839; observed 1113.4825.

(2*R*,3*R*,4*R*,5*R*,6*R*)-2-(Hydroxymethyl)-6-[1-[2-[2-[2-[2-[4-[2-[2-[2-[4-[(2*R*,3*R*,4*R*,5*R*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydropyran-2-yl]triazol-1-yl]ethoxy]ethoxy]ethyl]piperazin-1-yl]-ethoxy]ethoxy]ethyl]triazol-4-yl]tetrahydropyran-3,4,5-triol (35). Compound **35** was obtained by the same procedure used for compound **11** with the following modifications. Compound **34** (45 mg, 0.04 mmol) was dissolved in distilled MeOH (3 mL) to which was added potassium carbonate (0.2 mg). The reaction was stirred at room temperature for 17 h. Compound **35** was obtained as a thick colourless oil (28 mg, 89%). ^1H NMR δ (D_2O, 300 MHz), 8.18 (2H, s), 5.39 (2H, d, J = 6.3 Hz), 4.67 (4H, t, J = 4.5 Hz), 4.29 (2H, dd, J = 10.2, 6.3 Hz), 4.12 (2H, dd, J = 9.9, 3.3 Hz), 4.07 (2H, d, J = 3.3 Hz), 4.02 (4H, t, J = 4.8 Hz), 3.82–3.62 (18H, m), 2.62 (12H, t, J = 5.7 Hz); ^{13}C NMR δ (D_2O, 75 MHz), 142.6, 125.9, 73.4, 69.9, 69.8, 69.3, 68.9, 68.5, 68.4, 67.4, 67.1, 60.6, 56.0, 51.6 and 49.6. MS-ESI: m/z $[M + H]^+$ for $C_{32}H_{57}N_8O_{14}^+$ calculated 777.4; observed 777.3. HRMS-ESI: m/z $[M + H]^+$ for $C_{32}H_{57}N_8O_{14}^+$, calculated 777.3994; observed 777.3975.

Biological studies

The binding of cholera toxin (CT) to its cell surface receptor (G_{M1}) was determined as described previously.[69] In brief, a solution of G_{M1} in phosphate-buffered saline (PBS) was used to coat wells of a Nunc-Immuno Maxisorp 96-well plate (100 µL per well) overnight at room temperature. After this and subsequent incubations, the plate was washed 3 times with PBS. The wells of the plate were initially incubated for one hour with 360 µL blocking buffer (0.25% bovine serum albumin in PBS–0.05% Tween 20) to block non-specific binding sites, and then with 100 µL CT (1–100 ng mL^{-1} in blocking buffer), 100 µL goat anti-CT antibody in blocking buffer, and 100 µL rabbit anti-goat alkaline phosphatase conjugate in blocking buffer. These incubations were at room temperature for one hour, except for CT (1.5 h). Finally, the plate received an extra wash with distilled water, and 100 µL alkaline phosphatase substrate (*para*-nitro phenol) in Tris buffer was added. The rate of change of absorbance at 405 nm was measured over 10 min using a Bio-Tek Synergy HT plate reader (Bio-Tek, Winooski, VT, USA). Inhibition of the binding of CT to immobilised G_{M1} was determined by incubating the inhibitor with CT (total volume 130 µL) for 1 h in a disposable glass tube with gentle rocking on a rocking platform mixer (Ratek Instruments, Melbourne, Australia) during the incubation of the assay plate with 360 µL blocking buffer. A 100 µL aliquot from each test tube was then added to a well of the 96-well plate, after which the assay proceeded as normal. The amount of unbound CT was determined by reference to a control plot of rate of absorbance change *vs.* CT concentration that was performed on the same plate. The amount of unbound CT was plotted against the concentration of inhibitor. If the values of percent inhibition of binding approached or spanned 50%, the IC_{50} for inhibition of binding was calculated from a second-order polynomial fit to the data. Examples of a control plot and an inhibition plot are shown in the supplementary information.

Crystallography

Crystals of **33** and **24** were mounted in low temperature oil then flash cooled to 130 K using an Oxford low temperature device. Intensity data were collected at 130 K with an Oxford SuperNova X-ray diffractometer with CCD detector using Cu-Kα radiation for **33** (λ = 1.54184 Å) and Mo-Kα radiation (λ = 0.71073 Å) for **24**. Data were reduced and corrected for absorption.[70] The structures were solved by direct methods and difference Fourier synthesis using the SHELX suite of programs[71] as implemented within the WINGX[72] software. Thermal ellipsoid plots were generated using the program ORTEP-3.

Crystal data for 33

The crystals obtained for **33** were recrystallized from hexane–dichloromethane, mp 137–138 °C. $C_{16}H_{20}O_9$, M = 356.32, T = 130.0(2) K, λ = 0.71069, Orthorhombic, space group $P2_12_12_1$ a = 7.1296(2) b = 9.0495(4), c = 27.544(1) Å, V = 1777.1(1) $Å^3$, Z = 4, D_c = 1.332 mg M^{-3} μ(Cu-Kα) 0.943 mm^{-1}, F(000) = 752, crystal size 0.52 × 0.27 × 0.18 mm. 7003 reflections measured, 3448 independent reflections (R_{int} = 0.0123), the final R was 0.0360 [$I > 2\sigma(I)$] and w$R(F^2)$ (all data) was 0.0961.

Crystal data for 21

The crystals obtained for **24** were recrystallized from hexane–dichloromethane, mp 154–155 °C. $C_{16}H_{20}O_9$, M = 356.32, T = 130.0(2) K, λ = 0.71069, Monoclinic, space group C2, a = 18.1428(8) b = 8.3337(5), c = 11.7816(9) Å, V = 1776.1(2) $Å^3$, Z = 4, D_c = 1.333 mg M^{-3} μ(Mo-Kα) 0.110 mm^{-1}, F(000) = 752, crystal size 0.6 × 0.3 × 0.2 mm. 4376 reflections measured, 2620 independent reflections (R_{int} = 0.0260), the final R was 0.0374 [$I > 2\sigma(I)$] and w$R(F^2)$ (all data) was 0.0906.

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