Tetrahedron 67 (2011) 5902–5912

Contents lists available at ScienceDirect

Tetrahedron

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

Glycoclusters presenting lactose on calix[4]arene cores display trypanocidal activity

Eva Galante [a,b], Corrada Geraci [b], Sebastiano Sciuto [c], Vanessa L. Campo [a,d], Ivone Carvalho [d], Renata Sesti-Costa [e], Paulo M.M. Guedes [e], João S. Silva [e], Lionel Hill [f], Sergey A. Nepogodiev [a], Robert A. Field [a,*]

[a] Department of Biological Chemistry, John Innes Centre, Norwich Research Park, Norwich NR4 7 UH, UK
[b] Istituto di Chimica Biomolecolare, C.N.R., Via P. Gaifami, 18, I-95126, Italy
[c] Dipartimento di Scienze Chimiche, Università di Catania, Viale A. Doria 6, 95125 Catania, Italy
[d] Faculdade de Ciências Farmacêuticas de Ribeirão Preto, USP, Av. Café S/N, CEP 14040-903, Ribeirão Preto, SP, Brazil
[e] Faculdade de Medicina de Ribeirão Preto, USP, Av. Bandeirantes 3900, CEP 14049-900, Ribeirão Preto, SP, Brazil
[f] Department of Metabolic Biology, John Innes Centre, Norwich Research Park, Norwich NR4 7 UH, UK

ARTICLE INFO

Article history:
Received 29 March 2011
Received in revised form 3 June 2011
Accepted 20 June 2011
Available online 25 June 2011

Keywords:
Glycocluster
Calix[4]arene
Calixsugar
Multivalency
Trypanosoma cruzi

ABSTRACT

A new series of water-soluble tetravalent glycoclusters incorporating β-lactosyl residues attached to a central calix[4]arene core was synthesised using azide–alkyne Cu(I)-catalysed cycloaddition ('click chemistry'). Carbohydrate moieties were attached either to the upper or lower rim of rigid cone-shaped or partial cone macrocycles via 14–21 atom spacer arms. The glycoclusters with a C_4-symmetrical arrangement of β-lactosyl residues showed trypanocidal activity, with one of them showing comparable activity to established anti-trypanosomal drug benznidazole in *in vitro* anti-parasite assays.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Given the important roles played by carbohydrate–protein interactions in many biological recognition events,[1] development of new tools to facilitate understanding of these events at the molecular level remains an important challenge.[2] Since carbohydrate–protein interactions are generally weak, special efforts are often required to achieve tighter binding of carbohydrate-based ligands. One of the established approaches to such improved ligands is based on a concept of the cluster effect,[3] which is related to the number and the geometry of carbohydrate residues and also depends on their steric bulk, density and relative distance, as well as on the three-dimensional arrangement, which is determined by the nature of a central core or a scaffold. Application of the cluster effect concept in the field of synthetic carbohydrate chemistry has led to the design of a wide variety of multivalent neoglycoconjugates.[4]

Calix[4]arene[5] is an attractive scaffold for the construction of multivalent glycoconjugates due to the unique shape of its conformers, its synthetic versatility and low cost.[6] Each of the two distinct rims present in the cone-shaped conformer of calix[4]arene can be derivatised selectively using a variety of synthetic approaches, including Cu(I)-assisted 1,3-dipolar azide–alkyne cycloaddition (CuAAC),[7] leading to symmetrical glycoclusters bearing four[8] or two[8a,b,e,f,9] sugar residues. In addition, neoglycoconjugates with asymmetrically presented ligands can be accessed by means of selective chemical modifications of calix[4]arene locked in partial cone or 1,3-alternate conformations.[10] Through the changes of the conformation of the macrocyclic scaffold and variation of the type and length of a carbohydrate-calix[4]arene linkers one can achieve control over the most important characteristics of multivalent neoglycoconjugates—the three-dimensional arrangement of sugar residues. The possibility of such control in synthetic calixsugars is particularly valuable in the search for the best multivalent ligands for poorly defined receptors.

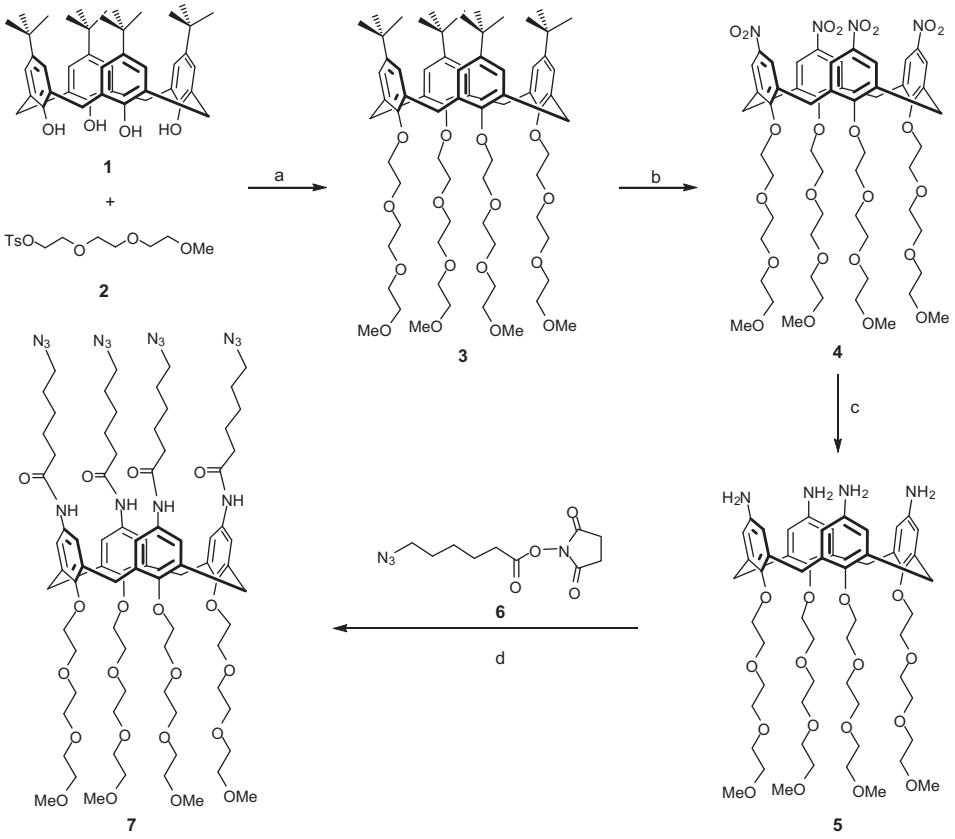
Lactose (galactose β-1,4-linked to glucose) and its derivatives have been shown to inhibit[11] the *trans*-sialidase from *Trypanosoma cruzi* (TcTS), the etiological agent of Chagas' disease.[12] Given the essential roles of TcTS in parasite infection and survival, this enzyme is a potential target for drug development. In the course of our work on TcTS substrate specificity and inhibitor development,[13] we identified

* Corresponding author. E-mail address: rob.field@bbsrc.ac.uk (R.A. Field).

0040-4020/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2011.06.065

a series of triazole-modified galactose derivatives that showed promising trypanocidal activity while only showing modest TcTS inhibition.[13b] Generally, monovalent compounds display rather weak inhibitory activity against TcTS.[14] Given the multi-copy, parasite cell surface presentation of TcTS, we were drawn to evaluate multivalent galactosides/lactosides as potential anti-*T. cruzi* agents.

In this paper we report the synthesis of a series of water-soluble lactose-containing calix[4]arene conjugates via Cu(I)-assisted 1,3-dipolar azide–alkyne cycloaddition (CuAAC—'click chemistry'). The spatial arrangement of β-lactosyl ligands in these neo-glycoconjugates is defined by (i) their presentation either on the top or the bottom rim of the macrocycle, and (ii) the variable length and structure of the linker between the core and the carbohydrate structure. The activity of these calixsugars towards the parasite *T. cruzi* is also reported.



Scheme 1. Reagents and conditions: (a) NaH, dry DMF, 75 °C, 17 h, 76%; (b) CH_3COOH, HNO_3, CH_2Cl_2, rt, 6 h, 70%; (c) H_2–Pd/C, CH_2Cl_2, 50 °C, 17 h, 95%; (d) CH_2Cl_2, rt, 17 h, 85%.

2. Results and discussion

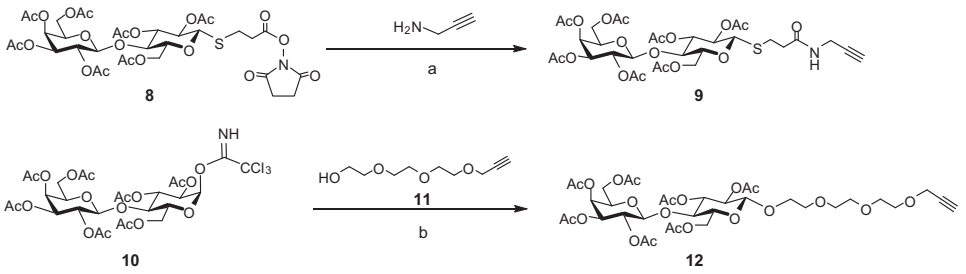
In order to prepare calixsugars modified on either the upper or lower rim, synthetic approaches were adopted that relied upon 'click' coupling of suitable azide and alkyne building blocks. Examples of both sugar alkynes and sugar azides were investigated in conjunction with the cognate azide- or alkyne-functionalised calixarene, respectively.

2.1. Conjugation of lactose residues to the upper rim of calix[4]arene

Functionalisation of the more open upper rim of calix[4]arene fixed in a rigid cone-shaped conformation allows the design of C_4-symmetric tetravalent structures. In these molecules pendant groups are present in all-*syn* orientation and relatively free from steric hindrance. In the case of multivalent glycomimetics such arrangement of attached saccharides could be advantageous for carbohydrate–protein interaction and a number of calixsugars have been prepared before with this in mind.[8e,f,15] A calix[4]arene building block suitable for attachment of acetylenic lactosides by means of copper-mediated azide–alkyne cycloaddition (CuAAC) was designed in the form of tetra-azide **7** (Scheme 1). The cone shape of target calixarenes (**1**–**5**) was fixed by functionalisation of the lower rim with methyl-capped triethylene glycol chains, which were also introduced to improve the water solubility of the final construct.

Alkylation of calix[4]arene **1** with tosylate **2**[16] was performed under standard conditions (NaH in DMF) and afforded compound **3**, which was subjected to *ipso*-nitration according to a known procedure.[17] As a result, compound **3** underwent a direct replacement of all *tert*-butyl groups with nitro groups giving derivative **4**, which was hydrogenated in the presence of a Pd–C catalyst affording the tetra-amino derivative **5**. Reaction of **5** with four molecules of *N*-succinimidoyl ester **6**[18] led to target azide-functionalised calix[4]arene **7** in 43% overall yield.

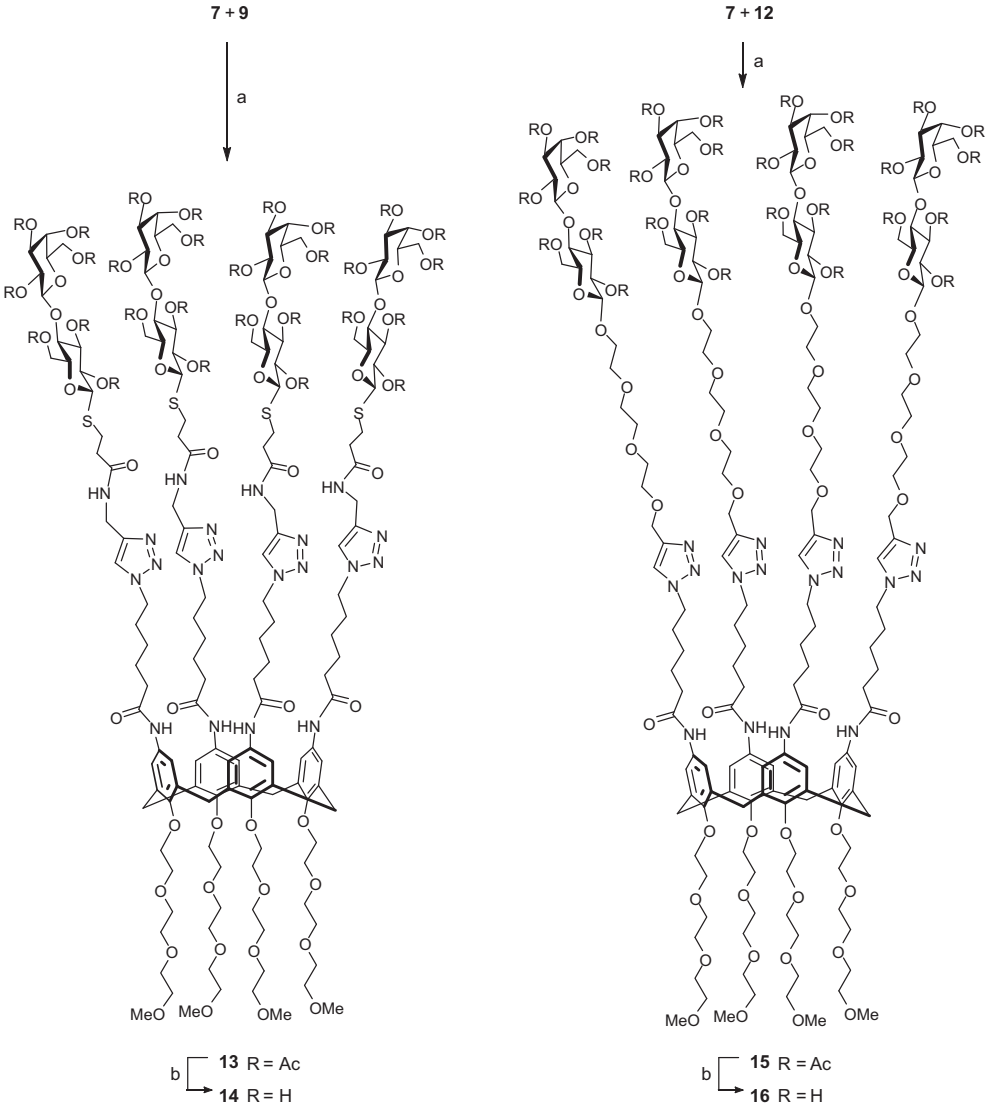
Two lactose derivatives bearing alkyne-terminated aglycones were prepared (Scheme 2). *S*-Linked lactoside **9** was synthesised in 62% yield by amide coupling of readily available *N*-succinimidoyl ester **8**[19] with propargylamine. Glycosidation of peracetylated α-lactose trichloroacetimidate **10**[20] with the monopropargyl ether of triethylene glycol, **11**,[21] in the presence of TMSOTf led to *O*-β-lactoside **12** in 50% yield. Attachments of alkynes **9** and **12** to calixarene core **7** were carried out using a Cu(I)-catalysed azido–alkyne cycloaddition approach under conditions previously used by us[22] and others[23] ($CuSO_4$/Na ascorbate, DMF, 70 °C, microwave reactor) and, which are suitable for fully protected carbohydrate



Scheme 2. Reagents and conditions: (a) CH_2Cl_2, rt, 17 h, 62%; (b) Me_3SiOTf, CH_2Cl_2, −30 °C 1 h and then rt for 17 h, 50%.

derivatives. The syntheses were performed in a microwave reactor in DMF solution at 70 °C in the presence of 0.2 equiv of $CuSO_4$ and 0.4 equiv of sodium ascorbate. The product was purified by silica gel chromatography, giving the tetra-lactosyl derivative **13** in 80% yield (Scheme 3).

the equatorial and axial protons of the methylene bridges of the macrocycle, and a singlet at δ 6.85 ppm, which corresponds to the aromatic protons of the macrocycle. The large positive $\Delta(\delta_{C4}-\delta_{C5})$ values (ca. 22 ppm) in ^{13}C NMR spectra of **13** indicated that all newly-formed 1,2,3-triazole rings had the desired 1,4-substitution



Scheme 3. Reagents and conditions: (a) $CuSO_4$, Na ascorbate, DMF, MW, 70 °C, 20 min, 80% for **13** and **15**; (b) 0.1 M NaOMe in MeOH, 2 h, rt, 81% and 79% for **14** and **16**, respectively.

The structural features of **13** were evident from the analysis of its NMR spectra. The fixed cone conformation and the C_4 symmetry of the calix[4]arene scaffold were confirmed by its ^1H NMR spectra, which had two doublets at δ 3.12 and 4.45 ppm, corresponding to [data for the isomeric 1,5-substituted triazoles $\Delta(\delta_{C4}-\delta_{C5})$ were expected be ca. −7 ppm].[8e] The original β-D-configuration of all glucopyranosyl residues in **13** was preserved, as expected and as confirmed by the large vicinal coupling constant ($J_{1,2}$ ~8.0 Hz)

observed in ^{1}H NMR spectra. Deacetylation of the carbohydrate components of **13** was achieved by the action of 0.1 M NaOMe in MeOH and gave fully deprotected tetrakis-lactoside **14** in 81% yield. The structure of **14** was confirmed by NMR spectroscopy and MS analysis, which revealed a peak at m/z=3516.5 corresponding to the $[M+Na]^+$ ion.

Multiple 1,3-dipolar cycloaddition reactions, under the same conditions, as described for glycocluster **13**, were employed for the synthesis of peracetylated tetravalent glycocalixarene **15**. The latter, obtained in the same yield as **13** (80%), was deacetylated under standard conditions (NaOMe–MeOH) to give compound **16**. Despite the presence of a large number of the overlapping signals for the methylene groups belonging to linkers, the analysis of ^{1}H and ^{13}C NMR spectra of **16** provided evidence for the correct structure: the regioselectivity of cycloaddition reactions [large $\Delta(\delta_{C4}-\delta_{C5})$= 19.1 ppm in ^{13}C NMR spectra] revealed all characteristic features of the calix[4]arene scaffolds (C_4 symmetry and cone conformation on the basis of doublets at δ 3.15 and 4.55 ppm corresponding to methylene bridges) and characteristic signals for the anomeric centres of the β-lactosyl residues (4.37 ppm, d, J=7.7 Hz, Gal H-1; 4.35 ppm, d, J=7.9 Hz, Glc H-1). MALDI-TOF MS of **16** showed a signal at m/z=3696.5 corresponding to the $[M+Na]^+$ ion.

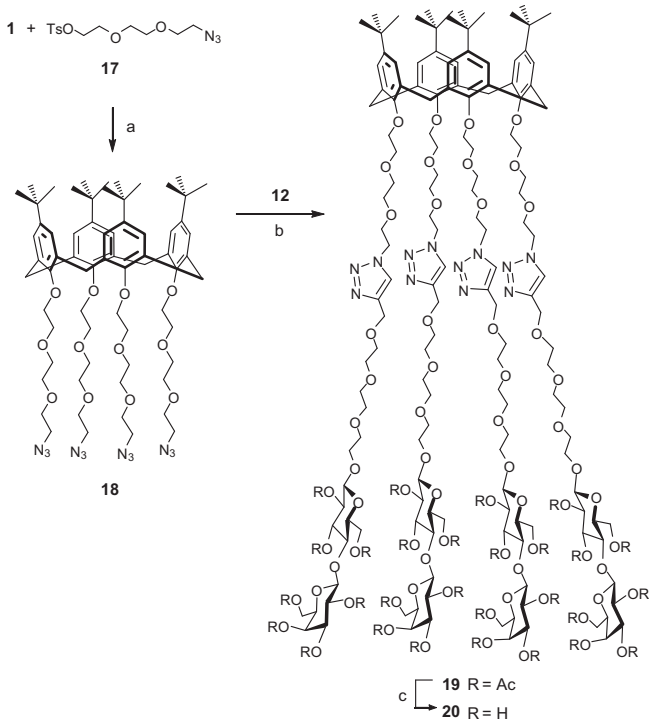
Attempts were also made to couple tetra-amine **5**, lacking additional 6-azidohexanamido linkers, with an excess of *N*-succinimidoyl ester **8** in order to synthesise analogues of glycocluster **13** lacking the aliphatic spacer and triazole fragments of the linker moiety. However this amide coupling resulted in a mixture of conjugation products in low overall yield (<20%) and consisting of di- (m/z=2953.7), tri- (m/z=3665.9) and tetra-substituted (m/z=4367.6) compounds, as revealed by analysis of $[M+Na]^+$ peaks in MALDI-TOF mass spectra. Clearly, cycloaddition conjugation procedures leading to **13** and **15** (Scheme 3) were much more efficient, but they can also benefit from the longer linkers connecting reactive azide functionalities to the macrocyclic core (compound **7**).

2.2. Lower rim modification of calix[4]arenes by attachment of alkyne 12 or azide 23

Conjugation of carbohydrate ligands to the lower rim of calix[4]arene is facilitated by the presence of phenolic OH groups, which can be used for the attachment of various types of linkers, functionalised either with azides or alkynes at their ends. Thus, alkylation of generic calix[4]arene **1** with ω-azidotriethyleneglycol tosylate **17**[21] produced tetra-azide derivative **18** in 50% yield. Compound **18** represents a suitable scaffold for multiple conjugations via Cu(I)-catalysed 1,3-dipolar cycloaddition (Scheme 4).

The reaction of tetra-azide **18** with a β-lactoside containing a terminal alkyne, as in **12**, was carried out under the same conditions as used for synthesis of **14** and **16**, albeit with lower efficiency. After silica gel chromatography, tetravalent calixsugar **19** was obtained in 52% yield. The lower coupling efficiency in this case was accounted for by more steric hindrance at the lower rim of **18**, which made formation of the lactoside tetramer product more difficult compared to calix[4]arene **7** bearing reactive azido functionalities on more open upper rim. Complete deacetylation of sugar residues in **19** under standard conditions led to tetravalent β-lactosyl-calix[4]arene glycocluster **20**. ^{1}H and ^{13}C NMR spectra of both protected (**19**) and deprotected (**20**) compounds confirmed the symmetry of these structures and the presence of solely 1,4-substituted triazole bridges. Sodium adducts of molecular ions in MALDI-TOF mass spectra corresponded to the expected masses of tetra-substituted structures **19** (m/z 4525.6) and **20** (m/z 3350.5), respectively.

Tetra-propargyl derivatives of calix[4]arene **21**[24] and its partial cone conformational isomer, **22**,[25] represent another type of core molecule suitable for the attachment of sugar ligands equipped



Scheme 4. Reagents and conditions: (a) NaH, DMF, 75 °C, 17 h, 50%; (b) $CuSO_4$, Na ascorbate, DMF, MW, 70 °C, 20 min, 52%; (c) 0.1 M NaOMe in MeOH, 2 h, rt, 92%.

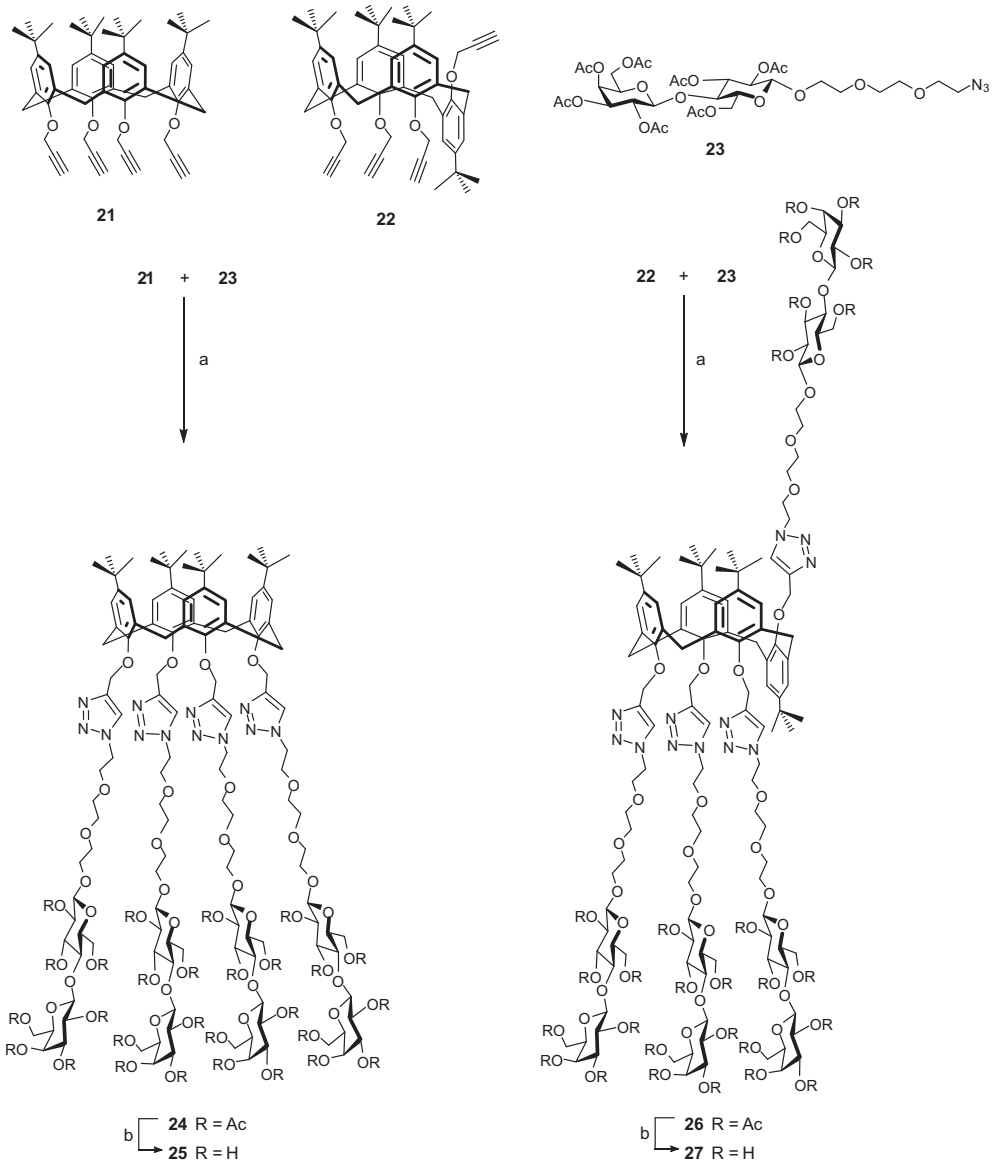
with azido groups.[26] Thus, Cu(I)-catalysed cycloaddition of **21** and **22** and known β-lactoside **23**[27] incorporating a N_3-terminated spacer-arm, under the conditions of microwave-assisted reaction described above, yielded conjugates **24** and **26**, which were isolated after silica gel chromatography purification in 55% and 66% yield, respectively (Scheme 5).

Analysis of its NMR spectra provided evidence for the structure of the C_4-symmetrical compound **24**, having characteristic doublets at 3.20 and 4.15 ppm corresponding to the equatorial and axial methylene bridge protons, respectively, of the calix[4]arene core. ^{13}C NMR spectra also showed 1,4-substitution of the 1,2,3-triazole rings, based on the large value of $\Delta(\delta_{C4}-\delta_{C5})$ (18.4 ppm). Deacetylation of sugar residues in **24** afforded unprotected cone-shaped calixsugar **25**, NMR data for which were consistent with the proposed structure. MALDI-TOF mass spectra of **24** and **25** revealed sodium adducts of their molecular ions at m/z 3996.9 and 3002.9, respectively.

The partial cone shape of the calix[4]arene scaffold of calixsugar **26** was reflected in the appearances of multiple signals of triazole and aromatic rings in its ^{1}H NMR spectra: singlets at δ 8.05, 7.85 and 7.83 ppm in a ratio 2:1:1 belonged to the triazole residues and signals at δ 7.05, 6.85 and 6.81 ppm and 6.36 in 1:1:1:1 ratio were assigned to aromatic protons. There were no detectable differences in chemical shifts of signals of lactose residues in ^{13}C NMR spectra of **26**, which appeared as a set of peaks assigned on the basis of the interpreted spectrum of precursor **23**. Deacetylation of sugar residues in **26** afforded unprotected partial cone-shaped calixsugar **27**, which was also characterised by NMR spectroscopy. MALDI-TOF mass spectra of **26** and **27** revealed sodium adducts of their molecular ions at m/z 3997.3 and 3002.7, respectively.

2.3. *In vitro* trypanocidal activities of calixsugars 14, 16, 20, 25 and 27

The five calixsugars prepared in this study were assessed for their activity against whole parasites. The trypanocidal activities of compounds **14**, **16**, **20**, **25** and **27** were evaluated against the host infectious trypomastigote form of *T. cruzi* Y strain. This was



Scheme 5. Reagents and conditions: (a) $CuSO_4$, Na ascorbate, DMF, MW, 70 °C, 20 min, 55% for **24** and 66% for **26**; (b) 0.1 M NaOMe in MeOH, rt, 2 h, 96% for **25** and 92% for **27**.

achieved by counting the number of motile forms of the parasite after treatment with compounds of interest at the range of concentrations, according to the protocol of Brener.[28] Benznidazole (**28**), the current front-line drug used to treat Chagas' disease, was employed as a positive control in these tests.[29] Results of parasite viability measured in this way are summarised in Fig. 1. The concentrations of compounds corresponding to 50% trypanocidal activity after 24 h of incubation are expressed as IC_{50try} (Table 1).

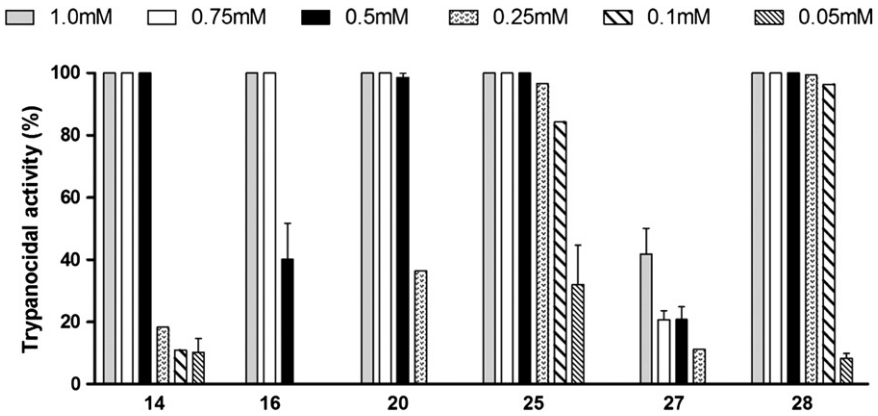


Fig. 1. Trypanocidal activities of calixsugars **14**, **16**, **20**, **25**, **27** and reference compound benznidazole **28**.

Table 1
Trypanocidal activities of compounds **14**, **16**, **20**, **25**, **27** and benznidazole **28** expressed as IC_{50Try}

Compound	IC_{50Try} (mM)
14	0.278±0.004
16	0.502±0.001
20	0.266±0.018
25	0.068±0.005
27	1.60±0.65
28	0.067±0.005

As shown in Fig. 1 and Table 1, of the calixsugars tested compound **25** displayed remarkably potent trypanocidal activity, resulting in 50% cell kill (IC_{50try}) at 62 μM concentration. In fact, this compound proved to be as effective under these assay conditions as the reference benznidazole **28** (IC_{50try} 67 μM). Other symmetrical tetravalent calixsugars also exhibited reasonable activities, decreasing in order **20**>**14**>**16**. In contrast, unsymmetrical partial cone compound **27** showed relatively low activity. Glycoclusters **14** and **20** showed lower IC_{50try} than the analogous compounds **16** and **25**, respectively, having longer linkers between the calix[4]arene core and the β-lactosyl residues. The location of carbohydrate residues with respect to the macrocycle core (i.e., on the top or bottom rim) seems to be less important.

In order to compare the trypanocidal activities of synthetic calixarene-based glycoclusters with their component parts (i.e., calixarene and tether-containing lactosides), similar trypanocidal tests were carried out with compounds **3**–**5**, **7**, **18**, **21**–**23**. In all of these cases the trypanocidal activity was at best marginal weak negative showing only marginal activities for some sample at >200 μM concentration. In addition, trypanocidal activity tests with β-lactoside **23**, representing a single spacer-armed component of glycoclusters **20**, **25** and **27**, were also carried out but no significant activity was detected at >200 μM concentration.

3. Conclusions

In summary, we have completed the synthesis of five new tetravalent calix[4]arene-based glycoclusters, employing 14–21 atom long linkers incorporating 1,2,3-triazole rings to present terminal β-lactosyl residues from a central macrocyclic core. The triazole rings were formed as a result of Cu(I)-catalysed azide–alkyne cycloaddition, which served as an efficient glycoconjugation reaction. We observed that the efficiency of these conjugations were essentially unaffected by the design of the calix[4]arene-based scaffold, which may have azide or alkyne reactive functional groups attached, via spacers, either to the upper or to the lower rims of the rigid-shape macrocycle.

Biological evaluation of the synthetic calixsugar glycoclusters **14**, **16**, **20**, **25** and **27** provided evidence for anti-parasite activity. Experiments with trypomastigote forms of *T. cruzi* revealed trypanocidal activity for all synthesised glycoclusters, with the highest potency shown by calixsugars having the all-*syn* orientation of saccharide moieties. Under these assay conditions, surprisingly the calixsugar **25** proved to be equipotent to the established anti-trypanosomal drug benznidazole. Bioassays with the glycocluster component parts showed no activity, supporting the notion that multivalent display of lactose ligands is key to efficacy.

As with our other recent work of sugar triazoles,[13b] the remarkable trypanocidal activity observed in this study is difficult to rationalise. While lactosides are indeed TcTs ligands/substrates, the biological function of this enzyme is associated with manipulation of parasite cell surface glycosylation. It is not clear that inhibition of this process would give rise to parasite kill *in vitro*, although inability to manipulate its surface glycosylation in vivo would be expected to render the parasite sensitive to the defences of the infected host. This suggests that the trypanocidal activity associated with calixsugar **25** may well not be associated with inhibitor binding to TcTS.

Though the role of multi-valency in the mechanism of trypanocidal activity of the calixsugars investigated in this study is not yet clear, the fact that it is dependent on the fine structure of the β-lactosyl glycoclusters presents the possibility of further improvement in design of such multivalent glycoconjugates. Studies addressing this point will be presented in due course.

4. Experimental section

4.1. General methods

All chemicals were purchased as reagent grade and used without further purification. *N*-benzyl-2-nitro-1-imidazolacetamide (benznidazole **28**) (Roche) was used as a reference drug in trypanocidal activity assays.

Solvents were dried according to standard methods. NaH was used as a 60% suspension in mineral oil. Reactions were monitored by thin layer chromatography (TLC) on 0.25 nm pre-coated silica gel plates (Whatman, AL SIL G/UV, aluminium backing) with the indicated eluents. Compounds were visualized under UV light (254 nm) and/or dipping in ethanol–sulfuric acid (95:5, v/v) or a solution of $CeSO_4$ (1% w/v)–phosphomolybdic acid (2.5% w/v) in 6% aqueous sulfuric acid, followed by heating the plate at 120 °C. Column chromatography was performed on a Biotage SP4 FLASH Chromatography system using 12 mm or 25 mm silica gel cartridges with the eluents indicated. The microwave-assisted reactions were carried out in a Biotage Initiator System, using sealed tubes.

^1H and ^{13}C NMR spectra were recorded on a JEOL Lambda spectrometer at 400 MHz and 100 MHz, respectively. Assignments were made with the aid of HSQC and COSY experiments. Numbering system used in assignment of NMR spectra for calix[4]arene derivatives is shown in Fig. 1. Optical rotations were measured at ambient temperature on a Perkin–Elmer model 341 polarimeter using a sodium lamp. Electrospray ionization mass spectra (ESI-MS) and MALDI-TOF-MS were obtained from the John Innes Centre metabolite analysis platform on a Thermo Finningan DecaXP^{plus} and a Bruker Ultraflex mass spectrometer, respectively, both operating in positive ionization mode.

*4.1.1. 5,11,17,23-Tetrakis(1,1-dimethylethyl)-25,26,27,28-tetrakis[2-(2-(2-methoxyethoxy)ethoxy)ethoxy]-calix[4]arene (**3**).* To a stirred mixture of *p-tert*-butylcalix[4]arene (**1**) (1.00 g, 1.54 mmol) and NaH (60% suspension, 440 mg, 10.8 mmol) in dry DMF (25 mL) was added tosylate **2**[16] (3.40 g, 10.8 mmol), the mixture was heated at 75 °C for 17 h and then poured into 1 N HCl solution (150 mL). The mixture was extracted with CH_2Cl_2, the combined organic phases were washed with water, dried over Na_2SO_4, filtered and solvents were removed under vacuum. Compound **3** was purified by column chromatography (EtOAc–MeOH 95:5) giving a colourless syrup (1.45 g, 76%). ^1H NMR ($CDCl_3$, 297 K): δ=6.76 (s, 8H, Ar), 4.42 (d, 4H, J=12.6 Hz, ArC*H*$_2$Ar), 4.10 (t, 8H, J=6.0 Hz, ArOC*H*$_2$), 3.94 (t, 8H, J=6.0 Hz, ArOCH$_2$C*H*$_2$O), 3.74–3.60 (m, 24H, OC*H*$_2$C*H*$_2$OC*H*$_2$CH$_2$OCH$_3$), 3.57–3.51 (m, 8H, C*H*$_2$OCH$_3$), 3.38 (s, 12H, OCH$_3$), 3.09 (d, 4H, J=12.6 Hz, ArC*H*$_2$Ar), 1.07 (s, 36H, *t*-Bu). ^{13}C NMR ($CDCl_3$, 297 K): δ=153.3 (C5, C11, C17, C23), 144.5 (C25, C26, C27, C28), 133.8 (C1, C3, C7, C9, C13, C15, C19, C21), 124.9 (C4, C6, C10, C12, C16, C18, C22, C24), 72.7 (ArOC*H*$_2$), 71.9 (C*H*$_2$OCH$_3$), 70.3, 70.4, 70.5, 70.6 (C*H*$_2$OCH$_2$C*H*$_2$OC*H*$_2$CH$_2$OCH$_3$), 58.9 (OCH$_3$), 33.6 (*C*(CH$_3$)$_3$), 31.3 (C(C*H*$_3$)$_3$), 30.9 (C2, C8, C14, C20). ESI-MS: M+NH_4^+, found 1251.7. $C_{72}H_{112}O_{16}NH_4$ requires 1250.83.

*4.1.2. 5,11,17,23-Tetranitro-25,26,27,28-tetrakis[2-(2-(2-methoxyethoxy)ethoxy)ethoxy]-calix[4]arene (**4**).* To a solution of compound **3** (1.45 g, 1.16 mmol) in CH_2Cl_2 (20 mL) was added

glacial CH_3COOH (20 mL) and fuming HNO_3 (5.3 mL); the mixture was stirred for 6 h at 20 °C. The reaction was quenched by addition of H_2O (120 mL) and the mixture was extracted with CH_2Cl_2 (3×100 mL). The organic layer was dried, and the solvents were removed under vacuum. Compound **4** was purified by column chromatography (EtOAc–MeOH 95:5) to give a colourless syrup (975 mg, 70%). ^{1}H NMR ($CDCl_3$, 297 K): δ=7.58 (s, 8H, Ar), 4.65 (d, 4H, J=12.6 Hz, ArCH_2Ar), 4.25 (t, 8H, J=8.0 Hz, ArOCH_2), 3.82 (t, 8H, J=8.0 Hz, ArOCH$_2$CH_2O), 3.63–3.53 (m, 24H, OCH_2CH_2OCH_2-CH$_2$OCH$_3$), 3.52–3.48 (m, 8H, CH_2OCH$_3$), 3.39 (d, 4H, J=12.6 Hz, ArCH_2Ar), 3.38 (s, 12H, OCH$_3$). ^{13}C NMR ($CDCl_3$, 297 K): δ=161.8 (C5, C11, C17, C23), 143.0 (C25, C26, C27, C28), 135.7 (C1, C3, C7, C9, C13, C15, C19, C21), 123.9 (C4, C6, C10, C12, C16, C18, C22, C24), 74.3 (ArOCH$_2$), 71.8 (CH$_2$OCH$_3$), 70.6, 70.5, 70.4, 70.3 (CH$_2$OCH$_2$-CH$_2$OCH$_2$CH$_2$OCH$_3$), 58.9 (OCH$_3$), 31.0 (C2, C8, C14, C20). ESI-MS: M+Na$^+$, found 1211.8. $C_{56}H_{76}N_4O_{24}Na$ requires 1211.47.

*4.1.3. 5,11,17,23-Tetraamino-25,26,27,28-tetrakis[2-(2-(2-methoxyethoxy)ethoxy)ethoxy]-calix[4]arene (**5**).* A solution of compound **4** (966 mg, 0.812 mmol) in AcOEt–MeOH (9:1, 100 mL) was vigorously stirred with Pd/C catalyst under H_2 atmosphere for 17 h at 50 °C. The catalyst was removed by filtration and the filtrate was concentrated under vaccum to give tetra-amine **5** as a colourless amorphous solid (823 mg, 95%). ^{1}H NMR ($CDCl_3$, 297 K): δ=6.05 (s, 8H, Ar), 4.31 (d, 4H, J=13.2 Hz, ArCH_2Ar), 3.99 (t, 8H, J=5.8 Hz, ArOCH_2), 3.82 (t, 8H, J=5.8 Hz, ArOCH$_2$CH_2O), 3.60–3.66 (m, 24H, OCH_2CH_2OCH_2CH$_2$OCH$_3$), 3.55–3.50 (m, 8H, CH_2OCH$_3$), 3.38 (s, 12H, OCH$_3$), 2.90 (d, 4H, J=13.2 Hz, ArCH_2Ar), 2.81 (s, 8H, NH$_2$). ^{13}C NMR ($CDCl_3$, 297 K): δ=149.6 (C5, C11, C17, C23), 140.6 (C25, C26, C27, C28), 135.5 (C1, C3, C7, C9, C13, C15, C19, C21), 115.7 (C4, C6, C10, C12, C16, C18, C22, C24), 72.8 (ArOCH$_2$), 71.9 (CH$_2$OCH$_3$), 70.6, 70.5, 70.3, 70.2 (CH$_2$OCH$_2$CH$_2$OCH$_2$-CH$_2$OCH$_3$), 58.9 (OCH$_3$), 30.9 (C2, C8, C14, C20). ESI-MS: M+Na$^+$, found 1091.7. $C_{56}H_{84}N_4O_{16}Na$ requires 1091.58.

*4.1.4. 5,11,17,23-Tetrakis-(6-azidohexanamido)-25,26,27,28-tetrakis-[2-(2-(2-methoxyethoxy)ethoxy)ethoxy]-calix[4]arene (**7**).* A mixture of tetra-amine **5** (300 mg, 0.28 mmol) and activated ester **6**[18] (535 mg, 2.10 mmol) in dry CH_2Cl_2 (5 mL) was stirred for 17 h at 20 °C. The mixture was poured into 1 N HCl (150 mL) and the product was extracted with CH_2Cl_2 and the solution was dried and concentrated. Purification by column chromatography (EtOAc–MeOH 90:10) gave compound **7** as a colourless syrup (387 mg, 85%). ^{1}H NMR ($CDCl_3$, 297 K): δ=6.91 (s, 8H, Ar), 4.54 (d, J=13.2 Hz, 4H, ArCH_2Ar), 4.11 (t, J=5.2 Hz, 8H, ArOCH_2), 3.91 (t, J=5.1 Hz, 8H, ArOCH$_2$CH_2), 3.57–3.65 (m, 24H, OCH_2CH_2OCH_2CH$_2$OCH$_3$), 3.53–3.42 (m, 8H, CH_2OCH$_3$), 3.33 (s, 12H, OCH$_3$), 3.25–3.31 (m, 8H, CH$_2$N$_3$), 3.09 (d, J=13.2 Hz, 4H, ArCH_2Ar), 2.24 (t, J=7.5 Hz, 8H, NHCOCH_2), 1.61 (m, 16H, CH_2CH$_2$CH_2CH$_2$N$_3$), 1.46–1.39 (m, 8H, CH_2CH$_2$CH$_2$N$_3$). ^{13}C NMR ($CDCl_3$, 297 K): δ=173.6 (CO), 154.1 (C5, C11, C17, C23), 136.1 (C25, C26, C27, C28), 133.7 (C1, C3, C7, C9, C13, C15, C19, C21), 121.6 (C4, C6, C10, C12, C16, C18, C22, C24), 74.6 (ArOCH$_2$), 72.7 (CH$_2$OCH$_3$), 71.4, 71.2, 71.1 (CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$OCH$_3$), 58.8 (OCH$_3$), 52.0 (CH$_2$N$_3$), 37.3 (NHCOCH_2), 31.9 (C2, C8, C14, C20), 29.3 (CH$_2$CH$_2$N$_3$), 27.1 (NHCOCH$_2$CH$_2$), 26.0 (CH$_2$CH$_2$CH$_2$N$_3$). ESI-MS: M+Na$^+$, found 1648.0. $C_{80}H_{120}N_{16}O_{20}Na$ requires 1647.88.

*4.1.5. 2-(Prop-2-ynylcarbamoyl)ethyl 2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-1-thio-β-D-glucopyranoside (**9**).* Compound **8**[19] (200 mg, 0.24 mmol) was treated with propargylamine (17 μl, 0.24 mmol) in dry CH_2Cl_2 (4.0 mL), the mixture was stirred for 17 h at 20 °C, diluted with CH_2Cl_2 (20 mL), filtered and the filtrate was concentrated under vacuum. The resulting residue was purified by column chromatography (hexane–EtOAc 1:1) to afford amide **9** as a colourless amorphous solid (115 mg, 62%), $[\alpha]_D^{20}$ −2.0 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 330 K): δ 6.40 (s, 1H, NH), 5.36 (d, J=3.3 Hz, 1H, H-4 Gal), 5.21 (t, J=9.7 Hz, 1H, H-3 Glc), 5.12 (dd, J=7.9, 10.4 Hz, 1H, H-2 Gal), 4.98 (dd, J=3.3, 10.4 Hz, 1H, H-3 Gal), 4.93 (t, J=9.7 Hz, 1H, H-2 Glc), 4.70 (br d, J=11.1 Hz, 1H, H-6a Glc), 4.54 (br d, J ~9 Hz, 2H, H-1 Gal, H-1 Glc), 4.19–4.01 (m, 5H, H-6b Glc, H-6a and H-6b Gal, NHCH_2), 3.89 (t, J=6.7 Hz, 1H, H-5 Gal), 3.79 (t, J=9.7 Hz, 1H, H-4 Glc), 3.65–3.59 (m, 1H, H-5 Glc), 3.08–3.00 (m, 1H, CH$_2$S), 2.89–2.77 (m, 1H, CH$_2$S), 2.54 (t, J=7.2 Hz, 2H, CH_2CONH), 2.24 (t, J=2.6 Hz, 1H, C≡CH), 2.11 (s, 6H, 2× Ac), 2.02 (s, 3H, Ac), 2.01 (s, 12H, 4× Ac), 1.97 (s, 3H, Ac). ^{13}C NMR ($CDCl_3$, 330 K): δ=171.2 (NHCO), 170.7, 170.3, 170.1, 170.0, 169.7, 169.6, 168.9 (CH$_3$CO), 100.1 (C-1 Gal), 84.5 (C-1 Glc), 79.5 (C≡CH), 76.7 (C-5 Glc), 75.8 (C-4 Gal), 73.6 (C-3 Glc), 71.5 (C≡CH), 70.9 (C-3 Gal), 70.7 (C-5 Gal), 69.9 (C-2 Glc), 69.1 (C-2 Gal), 66.6 (C-3 Glc), 61.5 (C-6 Glc), 60.7 (C-6 Gal), 37.2 (CH$_2$CONH), 29.1 (NHCH$_2$), 27.0 (SCH$_2$) 20.9, 20.8, 20.7, 20.5, 20.4 (CH$_3$CO). ESI-MS: M+Na$^+$, found 784.3. $C_{32}H_{43}NO_{18}SNa$ requires 784.21.

*4.1.6. 2-(2-(2-(Prop-2-ynyloxy)ethoxy)ethoxy)ethyl 2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**12**).* To a mixture of trichloroacetimidate **10**[20] (500 mg, 0.64 mmol), 2-(2-(2(prop-2-inyloxy)ethoxy)ethoxy)ethanol (**11**)[21] (165 mg, 0.77 mmol) and 4 Å molecular sieves (100 mg) in dry CH_2Cl_2 (3.0 mL) was added TMSOTf (36 μl, 0.13 μmol). The mixture was stirred for 1 h at −30 °C and then for 17 h at 20 °C, when it was neutralized with Et_3N (100 μl) and filtered. The filtrate was diluted with CH_2Cl_2 (20 mL) and extracted with H_2O (3×10 mL). The organic layer was dried, the solvent was removed under vacuum and the residue was purified by column chromatography (hexane–EtOAc 75:25) to afford compound **12** as a colourless amorphous solid (242 mg, 50%), $[\alpha]_D^{20}$ +22 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 330 K) δ 5.24 (d, J=2.6 Hz, 1H, H-4 Gal), 5.09 (t, J=9.3 Hz, 1H, H-3 Glc), 5.00 (dd, J=7.9 10.4 Hz, 1H, H-2 Gal), 4.86 (dd, J=3.4, 10.4 Hz, 1H, H-3 Gal), 4.79 (dd, J=7.9, 9.3 Hz, 1H, H-2 Glc), 4.48 (d, J=7.9 Hz, 1H, H-1 Glc), 4.43–4.36 (m, 2H, H-6a Glc, H-1 Gal), 4.11 (d, J=2.4 Hz, 2H, NHCH_2), 4.05–3.95 (m, 3H, H-6b Glc, H-6a and 6-b Gal), 3.85–3.75 (m, 2H, H-5 Gal, GlcOCH_aH$_b$), 3.70 (t, J=9.3 Hz, 1H, H-4 Glc) 3.66–3.48 (m, 12H, H-5 Glc, OCH$_2$), 2.34 (t, J=2.4 Hz, 1H, C≡CH), 2.06 (s, 3H, Ac), 2.03 (s, 3H, Ac), 1.96 (s, 3H, Ac), 1.95 (s, 3H, Ac), 1.94 (s, 6H, 2× Ac), 1.87 (s, 3H, Ac). ^{13}C NMR ($CDCl_3$, 330 K): δ=170.6, 170.5, 170.3, 170.2, 169.9, 169.8, 169.2 (CO), 101.2 (C-1 Gal), 100.7 (C-1 Glc), 79.8 (CH$_2$CCH), 76.4 (C-4 Glc, C-5 Glc), 74.8 (CCH), 72.9 (C-3 Glc), 72.7 (OCH$_2$), 71.7(C-2 Glc), 71.1 (C-3 Gal), 70.73, 70.7, 70.5, 70.4 (OCH$_2$), 69.2 (C-5 Gal), 69.1 (C-2 Gal), 66.7 (C-4 Gal), 62.2 (C-6 Glc), 60.9 (C-6 Gal), 58.1 (OCH$_2$CCH), 20.8, 20.5, 20.3, 20.2 (CH$_3$CO). ESI-MS: M+Na$^+$, found 829.5. $C_{35}H_{50}O_{21}Na$ requires 829.27.

4.2. General procedure for the Cu(I) catalysed 1,3-dipolar cycloaddition

A solution of the calix[4]arene-azide or tetra-*O*-propargyl-calix[4]arene (1 equiv), a lactose derivative (alkyne or azide, respectively (5 equiv)), 1 M aq $CuSO_4 \cdot 5H_2O$ (0.2 equiv) and 1 M aq sodium ascorbate (0.4 equiv) in DMF (1 mL per 20 mg of the calix[4]arene derivative) was heated at 70 °C in a microwave reactor for 20 min. The solvent was evaporated, the residue was taken up in CH_2Cl_2 (1 mL) and purified by filtration through a pad of silica gel (CH_2Cl_2–MeOH 95:5).

4.3. General deacetylation procedure

A solution of an acetylated compound in methanolic 0.1 M $NaOCH_3$ was stirred at room temperature for 2 h. The mixture was neutralized with Amberlite IR-120H (H$^+$) resin, the resin was filtered off and the solvent was removed under reduced pressure to give unprotected calixsugar, which was characterised without further purification.

*4.3.1. 5,11,17,23-Tetrakis(5-(4-(2-[O-(2,3,4,6-tetra-O-acetyl-β-D-galatopyranosyl)-(1→4)-(2,3,6-tri-O-acetyl-1-thio-β-D-glucopyranosyl) thio]ethylcarbamoyl)-1H-1,2,3-triazol-1-yl)pentylcarbamoyl)-25,26,27,28-tetrakis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-calix[4] arene (**13**).* Compounds **7** (20 mg, 0.012 mmol) and **9** (47 mg, 0.062 mmol) were reacted following the general cycloaddition procedure to give conjugate **13** as a white amorphous solid (46 mg, 80%). ^{1}H NMR (CD_3OD–$CDCl_3$ 4:1, 330 K): δ 7.69 (s, 4H, triazole), 6.94 (s, 8H, Ar), 5.38 (d, J=2.6 Hz, 4H, H-4 Gal), 5.21 (t, J=9.1 Hz, 4H, H-3 Glc), 5.08 (dd, J=9.1, 9.6 Hz, 4H, H-2 Gal), 5.01 (dd, J=2.6, 10.4 Hz, 4H, H-3 Gal), 4.90 (t, J=9.1 Hz, 4H, H-2 Glc), 4.65–4.50 (m, 12H, H-1 Glc, H-1 Gal, H-6a Glc), 4.45 (d, J=13.1 Hz, 4H, ArCH_2Ar), 4.35 (t, J=7.1 Hz, 8H, CH_2CH_2–triazole), 4.15–4.00 (m, 20H, H-6b Glc, H-6a and 6b Gal, ArOCH_2), 3.99–3.95 (m, 4H, H-5 Gal), 3.92–3.86 (m, 16H, $ArOCH_2CH_2$, $NHCH_2$–triazole), 3.82 (t, J=9.1 Hz, 4H, H-4 Glc), 3.73–3.58 (m, 28H, H-5 Glc, $CH_2CH_2OCH_2CH_2OCH_3$) 3.54 (t, J=5.2 Hz, 8H, CH_2OCH_3), 3.38 (s, 12H, OCH_3), 3.12 (d, J=13.1 Hz, 4H, ArCH_2Ar), 3.06–2.83 (m, 8H, CH_2S), 2.56 (t, J=7.2 Hz, 8H, SCH_2CH_2), 2.26 (br t, 8H, $NHCOCH_2CH_2CH_2$), 2.17 (s, 12H, Ac), 2.11 (s, 12H, Ac), 2.08 (s, 12H, Ac), 2.07 (s, 12H, Ac), 2.06 (s, 12H, Ac), 2.03 (s, 12H, Ac), 1.98 (s, 12H, Ac), 1.94 (br t, 8H, CH_2CH_2–triazole), 1.70 (br t, 8H, $NHCOCH_2CH_2CH_2$), 1.40 (br t, 8H, $NHCOCH_2CH_2CH_2$). ^{13}C NMR (CD_3OD–$CDCl_3$ 4:1, 297 K): δ 171.3 (CONH), 170.6, 170.4, 170.2, 170.1, 169.7, 169.1(CH_3CO), 152.7 (C5, C11, C17, C23), 144.8 (C-4 triazole), 135.1 (C25, C26, C27, C28), 132.6 (C1, C3, C7, C9, C13, C15, C19, C21), 122.8 (C-5 triazole), 120.9 (C4, C6, C10, C12, C16, C18, C22, C24), 100.9 (C-1 Gal), 84.1 (C-1 Glc), 76.9 (C-5 Glc), 75.9 (C-4 Glc), 73.7 (C-3 Glc), 73.2 (ArOCH_2), 71.9 (CH_2OCH_3), 71.0 (C-3 Gal), 70.7, 70.6, 70.5, 70.4, 70.3, 70.2 (C-2 Glc, C-5 Gal, $ArOCH_2CH_2OCH_2$-$CH_2OCH_2CH_2OCH_3$, $NHCH_2$–triazole), 70.3 ($ArOCH_2CH_2$), 70.1 (C-2 Glc), 69.1 (C-2 Gal), 66.6 (C-4 Gal), 61.8 (C-6 Glc), 60.7 (C-6 Gal), 58.9 (OCH_3), 50.7 (CH_2CH_2–triazole), 36.6 (SCH_2), 36.3 ($NHCOCH_2$), 31.1 (C2, C8, C14, C20), 29.5 (CH_2CH_2–triazole), 26.8 (SCH_2CH_2), 25.3 ($NHCOCH_2CH_2CH_2$), 24.4 ($NHCOCH_2CH_2CH_2$), 20.9, 20.8, 20.7, 20.6, 20.5 (CH_3CO). MALDI-TOF MS: M+Na^+, found 4695.60. $C_{208}H_{292}N_{20}O_{92}S_4Na$ requires 4694.76.

*4.3.2. 5,11,17,23-Tetrakis(5-(4-(2-[O-(β-D-galatopyranosyl)-(1→4)-(β-D-glucopyranosyl)thio]ethylcarbamoyl)-1H-1,2,3-triazol-1-yl)pentylcarbamoyl)-25,26,27,28-tetrakis(2-(2-(2-methoxyethoxy)ethoxy) ethoxy)-calix[4]arene (**14**).* Compound **13** (44 mg, 0.094 mmol) was deacetylated following the general procedure to give calixsugar **14** as a white amorphous solid (27 mg, 81%). ^{1}H NMR (CD_3OD–$CDCl_3$ 4:1, 330 K): δ 7.87 (s, 4H, triazole), 6.85 (s, 8H, Ar), 4.50–4.30 (m, 28H, ArCH_2Ar, CH_2CH_2–triazole, $NHCH_2$–triazole, H-1 Gal, H-1 Glc), 4.12–4.04 (m, 8H, ArOCH_2), 3.90–3.38 (m, 84H, $ArOCH_2CH_2$, $OCH_2CH_2OCH_2CH_2OCH_3$, H-2, H-3, H-4, H-5 and H-6 Glc, H-3, H-4, H-5 and H-6 Gal), 3.28–3.33 (m, 16H, H-2 Gal, OCH_3), 3.11 (d, J=13.3 Hz, 4H, ArCH_2Ar), 3.08–2.88 (m, 8H, CH_2S), 2.61 (s, 8H, CH_2CH_2S), 2.26 (s, 8H, $NHCOCH_2$), 1.94 (s, 8H, CH_2CH_2–triazole), 1.68 (s, 8H, $NHCOCH_2CH_2$), 1.38 (s, 8H, $NHCOCH_2CH_2CH_2$). ^{13}C NMR (CD_3OD–$CDCl_3$ 4:1, 297 K): δ 172.8, 172.2 (CO), 152.7 (C5, C11, C17, C23), 144.6 (C-4 triazole), 134.9 (C25, C26, C27, C28), 132.4 (C1, C3, C7, C9, C13, C15, C19, C21), 123.6 (C-5 triazole), 120.7 (C4, C6, C10, C12, C16, C18, C22, C24), 103.7 (C-1 Gal), 85.8 (C-1 Glc), 79.6, 79.0, 76.3, 75.6, 73.4, 73.2, 72.4, 72.4, 71.8, 71.7, 70.5, 70.3, 70.2, 68,6 (C2–C5 Glc, C2–C5 Gal, OCH_2CH_2O), 61.3, 61.0 (C-6 Glc, C-6 Gal), 58.5 (OCH_3), 50.4 (CH_2CH_2–triazole), 36.9 (SCH_2CH_2), 36.5 ($NHCOCH_2CH_2CH_2$), 34.5 ($NHCH_2$–triazole), 30.9 (C2, C8, C14, C20), 29.5 (CH_2CH_2–triazole), 26.9 (SCH_2), 25.4, 24.6 ($NHCOCH_2CH_2CH_2$). MALDI-TOF MS: M+Na^+, found 3518.2. $C_{152}H_{236}N_{20}O_{64}S_4Na$ requires 3517.46.

*4.3.3. 5,11,17,23-Tetrakis[5-(4-(2-(2-(2-[(2,3,4,6-tetra-O-acetyl-β-D-galatopyranosyl)-(1→4)-(2,3,6-tri-O-acetyl-β-D-glucopyranosyloxy)] ethoxy)ethoxy)ethoxymethyl)1H-1,2,3-triazol-1-yl) pentylcarbamoyl]-25,26,27,28-tetrakis(2-(2-(2-methoxyethoxy)ethoxy)-calix[4]arene (**15**).* Azide **7** (20 mg, 0.012 mmol) and alkyne **12** (50 mg, 0.062 mmol) were reacted following the general procedure to give title compound **15** as a white amorphous solid (47 mg, 80%). ^{1}H NMR ($CDCl_3$–CD_3OD 4:1, 330 K) δ 7.82 (s, 4H, H-triazole), 6.90 (s, 8H, Ar), 5.35 (d, J=2.6 Hz, 4H, H-4 Gal), 5.15 (t, J=9.4 Hz, 4H, H-3 Glc), 5.07–5.01 (m, 8H, H-2 Gal, H-3 Gal), 4.85 (dd J=9.4, 10.4 Hz, 4H, H-2 Glc), 4.64–4.60 (m, 12H, OCH_2–triazole, H-1 Gal, H-1 Glc), 4.50 (m, 8H, $ArCH_2Ar$, H-6a Glc), 4.38 (t, J=7.1 Hz, 8H, CH_2CH_2–triazole), 4.32 (s, 4H, NH), 4.15–4.10 (m, 20H, $ArOCH_2$, H-6b Glc, H-6a and H-6b Gal), 4.04–4.01 (m, 4H, H-5 Gal), 3.94–3.88 (m, 12H, GlcOCHa, $ArOCH_2CH_2$), 3.84 (t, J=9.4 Hz, 4H, H-4 Glc), 3.81–3.61 (m, 72H, 8× OCH_2, GlcOCHb, H-5 Glc), 3.55–3.52 (m, 8H, CH_2OCH_3), 3.75 (s, 12H, OCH_3), 3.12 (d, J=13.1 Hz, 4H, ArCH_2Ar), 2.24 (t, J=7.8 Hz, 8H, $NHCOCH_2$), 2.14 (s, 12H, Ac), 2.11 (s, 12H, Ac), 2.06 (s, 12H, Ac), 2.05 (s, 12H, Ac), 2.04 (s, 12H, Ac), 2.02 (s, 12H, Ac), 1.96 (s, 12H, Ac), 1.95–1.90 (m, 8H, CH_2CH_2–triazole), 1.71–1.65 (m, 8H, $NHCOCH_2CH_2$), 1.40–1.32 (m, 8H, $NHCOCH_2CH_2CH_2$). ^{13}C NMR (CD_3OD–$CDCl_3$ 4:1, 297 K): δ 172.1 (CONH), 171.0, 170.7, 170.6, 170.4, 170.3, 170.1, 169.7 ($COCH_3$), 152.7 (C5, C11, C17, C23), 144.6 (C-4 triazole), 134.9 (C25, C26, C27, C28), 132.4 (C1, C3, C7, C9, C13, C15, C19, C21), 123.5 (C-5 triazole), 120.7 (C4, C6, C10, C12, C16, C18, C22, C24), 100.7 (C-1 Gal), 100.4 (C-1 Glc), 76.2 (C-4 Glc), 73.2 ($ArOCH_2$), 73.1 (C-3 Glc), 72.5 (C-5 Glc), 71.7 (C-2 Glc, CH_2OCH_3), 71.1 (C-3 Gal), 70.5, 70.4, 70.36, 70.3, 70.1, 69.5, 69.3 (OCH_2), 70.2 (C-5 Gal), 68.9 (C-2 Gal), 66.9 (C-4 Gal), 63.9 (OCH_2–triazole), 62.2 (C-6 Glc), 60.9 (C-6 Gal), 58.4 (OCH_3), 50.0 (CH_2CH_2–triazole), 36.3 ($NHCOCH_2$), 30.9 (C2, C8, C14, C20), 29.8 (CH_2CH_2–triazole), 25.8 ($NHCOCH_2CH_2CH_2$), 24.8 ($NHCOCH_2CH_2$), 20.4, 20.2, 20.1, 20.0, 19.9, 19.8 (CH_3CO). MALDI-TOF MS: M+Na^+, found 4875.2. $C_{220}H_{320}N_{16}O_{104}Na$ requires 4875.02.

*4.3.4. 5,11,17,23-Tetrakis(5-(4-(2-(2-(2-([O-β-D-galatopyranosyl-(1→4)-β-D-glucopyranosyloxy)ethoxy)ethoxy)]ethoxymethyl)1H-1,2,3-triazol-1-yl)pentylcarbamoyl)-25,26,27,28-tetrakis-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-calix[4]arene (**16**).* Calixsugar **15** (45 mg, 0.0093 mmol) was deacetylated following the general procedure to give title compound **16** as a white amorphous solid (27 mg, 79%). ^{1}H NMR ($CDCl_3$–CD_3OD 4:1, 297 K): δ 8.10 (s, 4H, H-triazole), 6.99 (s, 8H, Ar), 4.65 (s, 8H, OCH_2–triazole), 4.55 (d, J=13.0 Hz, 4H, $ArCH_2Ar$), 4.47 (t, J=7.1 Hz, 8H, CH_2CH_2–triazole), 4.37 (d, J=7.7 Hz, 4H, H-1 Gal), 4.35 (d, J=7.9 Hz, 4H, H-1 Glc), 4.17–13 (m, 8H, $ArOCH_2$), 4.02–3.97 (m, 4H, GlcOCHa), 3.94 (br t, J=4.9 Hz, 8H, $ArOCH_2CH_2$), 3.90 (dd, J=2.1, 11.9 Hz, 4H, H-6a Gal), 3.86 (dd, J=3.6, 11.9 Hz, 4H, H-6b Gal), 3.84 (d, J=3.2 Hz, 4H, H-4 Gal), 3.78 (dd, J=6.0, 11.4 Hz, 4H, H-6a Glc), 3.75–3.58 (m, 78H, 9× OCH_2, H-6b Glc), 3.58–3.48 (m, 20H, H-2 and H-5 Gal, H-3, H-4 and H-5 Glc), 3.44–3.40 (m, 4H, H-3 Gal), 3.39 (s, 12H, OCH_3), 3.28 (t, J=9.6 Hz, 4H, H-2 Gal), 3.15 (d, J=13.0 Hz, 4H, $ArCH_2Ar$), 2.26 (br t, J=7.1 Hz, 8H, $NHCOCH_2CH_2CH_2$), 1.97–1.91 (m, 8H, CH_2CH_2–triazole), 1.70–1.65 (m, 8H, $NHCOCH_2CH_2CH_2$), 1.39–1.32 (m, 8H, $NHCOCH_2CH_2CH_2$). ^{13}C NMR (CD_3OD–$CDCl_3$ 4:1, 297 K): δ 172.2 (CONH), 152.8 (C5, C11, C17, C23), 144.0 (C-4 triazole), 134.9 (C25, C26, C27, C28), 132.5 (C1, C3, C7, C9, C13, C15, C19, C21), 124.9 (C-5 triazole), 120.6 (C4, C6, C10, C12, C16, C18, C22, C24), 103.8 (C-1 Gal), 102.8 (C-1 Glc), 79.6 (C-4 Glc), 75.7 (C-5 Glc), 75.0 (C-3 Gal), 74.9, 71.2 (C-2 Gal, C-3 Glc), 73.4 (C-5 Gal), 73.3 (C-2 Glc), 73.2 ($ArOCH_2$), 71.7 (CH_2OCH_3), 70.4, 70.3, 70.2, 70.15, 70.13, 70.1, 70.08, 69.5, 68.4 (OCH_2), 68.8 (C-4 Gal), 63.4 (OCH_2–triazole), 61.2 (C-6 Glc), 60.7 (C-6 Gal), 58.1 (OCH_3), 50.4 (CH_2–triazole), 36.2 ($COCH_2$), 30.9 (C2, C8, C14, C20), 29.6 (CH_2CH_2–triazole), 25.7 ($NHCOCH_2CH_2CH_2$), 24.7 ($NHCOCH_2CH_2CH_2$). MALDI-TOF MS: M+Na^+, found 3697.5. $C_{164}H_{264}N_{16}O_{76}Na$ requires 3697.72.

*4.3.5. 5,11,17,23-Tetrakis(1,1-dimethylethyl)-25,26,27,28-tetrakis-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)-calix[4]arene (**18**).* To a stirred

solution of *p-tert*-butylcalix[4]arene (100 mg, 0.15 mmol) in dry DMF (2.5 mL) were added NaH (60% suspension, 185 mg, 4.6 mmol) followed after 10 min by tosylate **17**[21] (355 mg, 1.08 mmol). The mixture was heated at 75 °C for 17 h, cooled to room temperature and then poured into 1 N HCl (150 mL). The crude product was extracted with CH_2Cl_2, combined organic extracts were washed with water, dried and solvents were removed under vacuum. The residue was purified by column chromatography (EtOAc–MeOH 95:5) to give compound **18** as a pale yellow oil (98 mg, 50%). ^{1}H NMR ($CDCl_3$, 297 K) δ 6.77 (s, 8H, Ar), 4.43 (d, J=12.6 Hz, 4H, ArCH_2Ar), 4.12 (t, J=5.9 Hz, 8H, ArOCH_2), 3.96 (t, J=5.9 Hz, 8H, ArOCH$_2$CH_2), 3.77–3.58 (m, 24H, OCH_2CH_2OCH_2CH_2N$_3$), 3.36 (d, J=9.3 Hz, 8H, CH_2N$_3$), 3.11 (d, J=12.6 Hz, 4H, ArCH_2Ar), 1.07 (s, 36H, C(CH_3)$_3$). ^{13}C NMR ($CDCl_3$, 297 K): δ 153.1 (C5, C11, C17, C23), 144.5 (C25, C26, C27, C28), 133.6 (C1, C3, C7, C9, C13, C15, C19, C21), 124.8 (C4, C6, C10, C12, C16, C18, C22, C24), 72.7 (ArOCH_2), 71.8, 70.6, 70.3, 69.9 (ArOCH$_2$CH_2OCH_2CH_2OCH_2CH_2N$_3$), 50.9 (CH_2N$_3$), 33.7 (*C*(CH$_3$)$_3$), 31.3 (C(CH_3)$_3$), 30.9 (C2, C8, C14, C20). ESI-MS: M+Na$^+$, found 1299.70. $C_{68}H_{100}N_{12}O_{12}Na$ requires 1299.75.

*4.3.6. 5,11,17,23-Tetrakis(1,1-dimethylethyl)-25,26,27,28-tetrakis(2-(2-(2-(4-(2-(2-(2-[O-(2,3,4,6-tetra-O-acetyl-β-D-galatopyranosyl)-(1→4)-(2,3,6-tri-O-acetyl-β-D-glucopyranosyloxy)]ethoxy)ethoxy)ethoxymethyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)-calix[4]arene (**19**).* Reaction of tetra-azide **18** (15 mg, 0.012 mmol) with alkyne **12** (45 mg, 0.060 mmol) according to general procedure for cycloaddition gave title compound **19** as a white amorphous solid (28 mg, 52%). ^{1}H NMR ($CDCl_3$–CD_3OD 4:1, 330 K) δ 8.22 (s, 4H, H-triazole), 6.77 (s, 8H, Ar), 5.35 (d, J=3.4 Hz, 4H, H-4 Gal), 5.20 (t, 4H, J=9.3 Hz, H-3 Glc), 5.08 (dd, J=8.0, 10.4 Hz, 4H, H-2 Gal), 5.02 (dd, J=3.4, 10.4 Hz, 4H, H-3 Gal), 4.90 (dd, J=9.3, 8.4 Hz, 4H, H-2 Glc), 4.61 (d, J=8.4 Hz, 4H, H-1 Glc), 4.60 (d, J=8.0 Hz, 4H, H-1 Gal,), 4.55–4.49 (m, 12H, H-6a Glc, OCH$_2$–triazole), 4.46 (d, J=12.6 Hz, 4H, ArCH_2Ar), 4.16–4.10 (m, 20H, H-6a and H-6b Gal, H-6b Glc, ArOCH_2), 4.02–3.98 (m, 4H, H-5 Gal), 3.96–3.89 (m, 20H ArOCH$_2$CH_2, OCH$_2$, GlcOC*H*a), 3.85 (t, J=9.3 Hz, 4H, H-4 Glc) 3.75–3.60 (m, 72H, H-5 Glc, 7× OCH$_2$, OCH$_2$CH_2–triazole, GlcOC*H*b), 3.18 (d, J=12.6 Hz, 4H, ArCH_2Ar), 2.13 (s, 12H, Ac), 2.11 (s, 12H, Ac), 2.04 (s, 12H, Ac), 2.03 (s, 12H, Ac), 2.02 (s, 12H, Ac), 2.01 (s, 12H, Ac), 1.94 (s, 12H, Ac), 1.04 (s, 36H, C(CH_3)$_3$). ^{13}C NMR (CD_3OD–$CDCl_3$ 4:1, 297 K): δ 170.9, 170.8, 170.6, 170.4, 170.3, 170.1, 169.6 (CO), 163.2 (C5, C11, C17, C23), 153.2 (C25, C26, C27, C28), 144.9 (C-4 triazole), 133.7 (C1, C3, C7, C9, C13, C15, C19, C21), 125.1 (C-5 triazole, C4, C6, C10, C12, C16, C18, C22, C24), 100.8 (C-1 Gal), 100.5 (C-1 Glc), 76.4 (C-4 Glc), 72.8 (C-3 Glc, ArOCH_2), 72.6 (C-5 Glc), 71.7 (C-2 Glc), 71.1 (C-3 Gal), 70.3 (C-5 Gal), 70.5, 70.43, 70.4, 70.2, 69.2, 69.1 (OCH$_2$), 69.0 (C-2 Gal), 66.9 (C-4 Gal), 64.2 (OCH$_2$–triazole), 62.1 (C-6 Glc), 60.8 (C-6 Gal), 31.2 ((*C*H$_3$)$_3$C), 30.9 ((CH$_3$)$_3$*C*), 29.3 (C2, C8, C14, C20), 20.5, 20.47, 20.4, 20.36, 20.3, 20.2, 20.1 (*C*H$_3$CO). MALDI-TOF MS: M+Na$^+$, found 4525.6. $C_{208}H_{300}N_{12}O_{96}Na$ requires 4526.89.

*4.3.7. 5,11,17,23-Tetrakis(1,1-dimethylethyl)-25,26,27,28-tetrakis(2-(2-(2-(4-(2-(2-(2-[O-(β-D-galatopyranosyl)-(1→4)-(β-D-glucopyranosyloxy)]ethoxy)ethoxy)ethoxymethyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)-calix[4]arene (**20**).* Compound **19** (28 mg, 0.062 mmol) was deacetylated following the general procedure to give the title compound **20** as a white amorphous solid (19 mg, 92%). ^{1}H NMR (CD_3OD–$CDCl_3$ 4:1, 297 K): 7.73 (s, 8H, Ar), 4.67–4.58 (m, 16H, OCH$_2$–triazole, OCH$_2$CH_2–triazole), 4.49 (d, J=13.0 Hz, 4H, ArCH_2Ar), 4.33 (d, J=9.7 Hz, 4H, H-1 Gal), 4.32 (d, J=9.7 Hz, 4H, H-1 Glc), 4.09 (t, J=5.4 Hz, 8H, ArOCH_2), 4.03 (m, 4H, GlcOC*H*a), 3.97–3.93 (m, 8H, ArOCH$_2$CH_2), 3.93–3.90 (m, 8H, OCH_2CH$_2$–-triazole), 3.88 (br d, 4H, H-4 Gal) 3.87–3.75 (m 12H, H-6a Glc, H-6a and H-6b Gal), 3.73–3.63 (m, 44H, 5× OCH$_2$, H-6b Glc), 3.63–3.58 (m, 4H, H-5 Glc) 3.57–3.55 (m, 12H, H-3 and H-4 Glc, H-2 Gal), 3.50 (dd, 4H, J=3.0, 10.2 Hz, H-5 Gal), 3.44–3.39 (m, 4H, H-3 Gal), 3.28 (t, J=9.7 Hz, 4H, H-2 Glc), 3.22 (d, J=13.0 Hz, 4H, ArCH_2Ar), 1.1 (s, 36H, C(CH$_3$)$_3$). ^{13}C NMR (CD_3OD–$CDCl_3$ 4:1, 297 K): δ 153.3 (C5, C11, C17, C23), 144.7 (C-4 triazole), 137.5 (C25, C26, C27, C28), 133.7 (C1, C3, C7, C9, C13, C15, C19, C21), 128.1 (C4, C6, C10, C12, C16, C18, C22, C24), 124.9 (C-5 triazole), 103.8 (C-1 Gal), 102.8 (C-1 Glc), 79.6 (C-4 Glc), 75.7 (C-5 Glc), 75.0 (C-3 Gal), 74.8, 71.1 (C-2 Gal, C-3 Glc), 73.4 (C-5 Gal), 73.2 (C-2 Glc), 73.0 (ArOCH_2), 70.4, 70.2, 70.17, 70.1, 70.0 (OCH$_2$), 69.7 (ArOCH$_2$CH$_2$) 68.9 (C-4 Glc), 68.8 (OCH$_2$CH$_2$–triazole), 68.4 (OCH$_2$), 61.2 (C-6 Glc), 60.7 (C-6 Gal), 30.9 ((CH$_3$)$_3$*C*, C2, C8, C14, C20), 29.4 ((*C*H$_3$)$_3$C). MALDI-TOF MS: M+Na$^+$, found 3349.3. $C_{152}H_{244}N_{12}O_{68}Na$ requires 3349.59.

*4.3.8. 5,11,17,23-Tetrakis(1,1-dimethylethyl)-25,26,27,28-tetrakis(1-(2-(2-(2-[O-(2,3,4,6-tetra-O-acetyl-β-D-galatopyranosyl)-(1→4)-(2,3,6-tri-O-acetyl-β-D-glucopyranosyloxy)]ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-ylmethoxy)-calix[4]arene (**24**).* Reaction of calixarene derivative **21**[24,26] (20 mg, 0.025 mmol) and azide **23**[27] (99 mg, 0.012 mmol) was carried out following the general cycloaddition procedure affording the title compound **24** as a white amorphous solid (55 mg, 55%). ^{1}H NMR (CD_3OD–$CDCl_3$ 4:1, 330 K) δ 7.80 (s, 4H, H-triazole), 6.98 (s, 8H, Ar), 5.30 (d, J=3.3 Hz, 4H, H-4 Gal), 5.15 (t, J=9.6 Hz, 4H, H-3 Glc), 5.11 (dd, J=7.9, 10.3 Hz, 4H, H-2 Gal), 4.97–4.94 (m, 12H, H-3 Gal, ArOCH_2), 4.85 (dd, J=9.6, 7.8 Hz, 4H, H-2 Glc), 4.58 (t, J=7.5 Hz, 8H, CH$_2$CH_2–triazole), 4.52 (d, J=7.8 Hz, 4H, H-1 Glc) 4.51 (d, J=7.9 Hz, 4H, H-1 Gal), 4.46 (dd, J=12.0, 1.8 Hz, 4H, H-6a Glc), 4.11–4.01 (m, 16H, ArCH_2Ar, H-6b Glc, H-6a and H-6b Gal), 3.95–3.85 (m, 16H, CH_2CH$_2$–triazole, GlcOC*H*a, H-5 Gal,), 3.76 (t, J=9.6 Hz, 4H, H-4 Glc), 3.66–3.58 (m, 8H, GlcOC*H*b, H-5 Glc), 3.58–3.52 (m, 24H, 3× OCH$_2$), 3.05 (d, J=12.2 Hz, 4H, ArCH_2Ar), 2.30 (s, 12H, Ac), 2.20 (s, 12H, Ac), 2.15 (s, 12H, Ac), 2.04 (s, 12H, Ac), 2.00 (s, 12H, Ac), 1.99 (s, 12H, Ac), 1.91 (s, 12H, Ac), 1.1 (s, 36H, C(CH$_3$)$_3$). ^{13}C NMR (CD_3OD–$CDCl_3$ 4:1, 297 K): δ 170.7, 170.6, 170.5, 170.3, 170.1, 169.9, 169.5 (CO), 149.6 (C5, C11, C17, C23), 147.9 (C25, C26, C27, C28), 142.6 (C-4 triazole), 134.5 (C1, C3, C7, C9, C13, C15, C19, C21), 125.7 (C-5 triazole), 124.9 (C4, C6, C10, C12, C16, C18, C22, C24), 100.9 (C-1 Gal), 100.5 (C-1 Glc), 76.1 (C-4 Glc), 72.9 (C-3 Glc), 72.6 (C-5 Glc), 71.6 (C-2 Glc), 70.9 (C-3 Gal), 70.5 (C-5 Gal), 70.4, 70.3, 70.0, 69.3, 69.2, (OCH$_2$), 69.0 (C-2 Gal), 68.5 (ArOCH$_2$) 66.7 (C-4 Gal), 61.9 (C-6 Glc), 60.7 (C-6 Gal), 50.2 (CH$_2$CH$_2$–triazole), 31.1 ((*C*H$_3$)$_3$C), 30.6 ((CH$_3$)$_3$*C*), 29.6 (C2, C8, C14, C20), 20.7, 20.6, 20.55, 20.5, 20.45, 20.4, 20.3 (*C*H$_3$CO). MALDI-TOF MS: M+Na$^+$, found 3996.9. $C_{184}H_{252}N_{12}O_{84}Na$ requires 3998.58.

*4.3.9. 5,11,17,23-Tetrakis(1,1-dimethylethyl)-25,26,27,28-tetrakis(1-(2-(2-(2-[O-(β-D-galatopyranosyl)-(1→4)-(β-D-glucopyranosyloxy)]ethoxy)ethoxy)ethyl)1H-1,2,3-triazol-4-ylmethoxy)-calix[4]arene (**25**).* Compound **24** (56 mg, 0.014 mmol) was deprotected following the general deacetylation procedure to give the title compound **25** as a white amorphous solid (38 mg, 96%). ^{1}H NMR (CD_3OD–$CDCl_3$ 4:1, 297 K): δ 8.02 (br s, 4H, H-triazole), 6.99 (s, 8H, Ar), 5.02 (s, 8H, OCH$_2$–triazole), 4.66 (t, J=4.8 Hz, 8H, OCH$_2$CH_2–-triazole), 4.37 (d, J=9.7 Hz, 4H, H-1 Gal), 4.33 (d, J=9.7 Hz, 4H, H-1 Glc), 4.15 (d, J=13.0 Hz, 4H, ArCH_2Ar), 4.00–3.93 (m, 12H, GlcOC*H*a, CH_2CH$_2$–triazole), 3.93–3.84 (br dd, 12H, H-4, H-6a and H-6b Gal), 3.80 (dd, J=7.8, 12.0 Hz, 4H, H-6a Glc), 3.74 (dd, J=4.2, 12.0 Hz, 4H, H-6b Glc), 3.73–3.68 (m, 4H, GlcOC*H*b), 3.66–3.58 (m, 28H, H-5 Glc, 3× OCH$_2$), 3.58–3.55 (m, 12H, H-3 and H-4 Glc, H-2 Gal), 3.52 (dd, J=2.5, 9.7 Hz, H-5 Gal), 3.44–3.41 (m, 4H, H-3 Gal), 3.20 (d, J=13.0 Hz, 4H, ArCH_2Ar), 1.12 (s, 36H, C(CH$_3$)$_3$). ^{13}C NMR (CD_3OD–$CDCl_3$ 4:1, 297 K): δ 150.7 (C5, C11, C17, C23), 146.7 (C25, C26, C27, C28), 143.3 (C-4 triazole), 134.5 (C1, C3, C7, C9, C13, C15, C19, C21), 125.3 (C4, C6, C10, C12, C16, C18, C22, C24), 124.9 (C-5 triazole), 103.8 (C-1 Gal), 102.7 (C-1 Glc), 79.6 (C-4 Glc), 75.7 (C-5 Glc), 75.0 (C-3 Gal), 74.8, 71.1 (C-3 Glc, C-2 Gal), 73.4 (C-5 Gal), 73.2 (C-2 Glc), 70.1, 70.0, 69.9, 69.2, 68.4 (OCH$_2$), 68.9 (C-4 Gal), 67.6

(OCH_2–triazole), 61.2 (C-6 Glc), 60.6 (C-6 Gal), 50.1 (OCH_2CH_2–triazole), 30.8 (($CH_3)_3C$, C2, C8, C14, C20), 29.4 (($CH_3)_3C$). MALDI-TOF MS: $M+Na^+$, found 2820.9. $C_{128}H_{196}N_{12}O_{56}Na$ requires 2821.28.

*4.3.10. 5,11,17,23-Tetrakis(1,1-dimethylethyl)-25,26,27,28-tetrakis(1-(2-(2-(2-[O-(2,3,4,6-tetra-O-acetyl-β-D-galactoopyranosyl)-(1→4)-(2,3,6-tri-O-acetyl-β-D-glucopyranosyloxy)]ethoxy)ethoxy)ethyl)1H-1,2,3-triazol-4-ylmethoxy)-calix[4]arene, partial cone (**26**).* Reaction of calixarene derivative **22**[25,26] (10 mg, 0.013 mmol) with azide **23**[27] (50 mg, 63 mmol) was carried out following the general cycloaddition procedure affording the title compound **26** as a white amorphous solid (33 mg, 66%). ^1H NMR (CD_3OD–$CDCl_3$ 4:1, 330 K): δ 8.05 (s, 2H, H-triazole), 7.85 (s, 1H, H-triazole), 7.83 (s, 1H, H-triazole), 7.05 (s, 2H, Ar), 6.85 (s, 2H, Ar), 6.81 (s, 2H, Ar), 6.36 (s, 2H, Ar), 5.38 (d, *J*=2.8 Hz, 4H, H-4 Gal), 5.22–5.17 (m, 4H, H-3 Glc), 5.10–5.07 (m, 4H, H-2 Gal), 5.00 (dd, *J*=2.8, 10.4 Hz, 4H, H-3 Gal), 4.91–4.82 (m, 10H, ArOCH_2, H-2 Glc), 4.70 (s, 4H, ArOCH_2), 4.60–4.48 (m, 20H, H-1 Glc, H-1 Gal, H-6a Glc, CH_2CH_2–triazole), 4.18–4.08 (m, 12H, H-6b Glc, H-6a and H-6b Gal), 3.98–3.85 (m, 16H, H-5 Gal, ArCH_2Ar, 1× OCH_2), 3.83 (m, 4H,), 3.80–3.48 (m, 40H, H-4 and H-5 Glc, 4× OCH_2), 2.81 (d, *J*=12.0 Hz, 4H, ArCH_2Ar), 2.20 (s, 12H, Ac), 2.05 (s, 12H, Ac), 1.99 (s, 12H, Ac), 1.92 (s, 12H, Ac), 1.90 (s, 12H, Ac), 1.89 (s, 12H, Ac), 1.87 (s, 12H, Ac), 1.21 (s, 18H, $C(CH_3)_3$), 0.92 (s, 9H, $C(CH_3)_3$), 0.86 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (CD_3OD–$CDCl_3$ 4:1, 297 K): δ 170.8, 170.7, 170.5, 170.4, 170.2, 169.9, 169.5 (CO), 154.4, 153.1, 150.6 (C5, C11, C17, C23), 144.3, 144.1, 143.9 (C-4 triazole), 136.1 (C25, C26, C27, C28), 132.7, 131.9, 131.8 (C1, C3, C7, C9, C13, C15, C19, C21), 128.5, 125.9, 125.6, 125.1 (C4, C6, C10, C12, C16, C18, C22, C24), 125.3, 125.2, 124.9 (C-5 triazole), 100.8 (C-1 Gal), 100.5 (C-1 Glc), 76.1 (C-4 Glc), 72.9 (C-3 Glc), 72.6 (C-5 Glc), 71.6 (C-2 Glc), 71.0 (C-3 Gal), 70.5 (C-5 Gal), 70.4, 70.3, 70.1, 70.0, 69.4, 69.3, 69.1, 69.08, 69.0 (OCH_2), 69.2 (C-2 Gal), 66.8 (C-4 Gal), 66.5, 64.9, 61.7 (ArOCH_2), 62.0 (C-6 Glc), 60.8 (C-6 Gal), 50.1, 50.0, 49.7 (CH_2CH_2–triazole), 33.8, 33.6, 33.5 (($CH_3)_3C$), 31.9, 31.5 (C2, C8, C14, C20), 31.1, 31.0 (($CH_3)_3C$), 20.6, 20.5, 20.4, 20.35, 20.3, 20.2 (CH_3CO). MALDI-TOF MS: $M+Na^+$, found 3997.3. $C_{184}H_{252}N_{12}O_{84}Na$ requires 3998.58.

*4.3.11. 5,11,17,23-Tetrakis(1,1-dimethylethyl)-25,26,27,28-tetrakis(1-(2-(2-(2-[O-(β-D-galatopyranosyl)-(1→4)-(β-D-glucopyranosyloxy)]ethoxy)ethoxy)ethyl)1H-1,2,3-triazol-4-ylmethoxy)-calix[4]arene, partial cone (**27**).* Compound **26** (31 mg, 0.008 mmol) was deprotected following the general deacetylation procedure to give the title compound **27** as a white amorphous solid (20 mg, 92%). ^1H NMR (CD_3OD–$CDCl_3$ 4:1, 297 K): δ 8.15 (s, 1H, H-triazole), 8.14 (s, 1H, H-triazole), 7.99 (s, 2H, H-triazole), 7.13 (s, 2H, Ar), 6.89 (s, 2H, Ar), 6.86 (s, 2H, Ar), 6.39 (s, 2H, Ar), 4.89 (s, 4H, OCH_2–triazole), 4.88 (s, 2H, OCH_2–triazole), 4.83 (s, 2H, OCH_2–triazole), 4.58–4.55 (m, 8H, OCH_2CH_2–triazole), 4.36 (d, *J*=9.7 Hz, 4H, H-1 Gal), 4.32 (d, *J*=9.7 Hz, 4H, H-1 Glc), 3.98–3.96 (m, 6H, ArCH_2Ar, GlcOC*H*a), 3.94–3.89 (m, 8H, OCH_2CH_2–triazole), 3.87–3.82 (m, 12H, H-6a and H-6b Gal, H-4 Gal), 3.82–3.79 (m, 4H, H-6a Glc), 3.75–3.69 (m, 4H, H-6b Glc), 3.69–3.60 (m, 8H, ArCH_2Ar, GlcOC*H*b), 3.60–3.55 (m, 40H, 3× OCH_2, H-2 Gal, H-3, H-4 and H-5 Glc), 3.53–3.48 (m, 4H, H-5 Gal), 3.43–3.38 (m, 4H, H-3 Gal), 3.30–3.33 (m, 4H, H-2 Glc), 2.95 (d, *J*=13.2 Hz, 2H, ArCH_2Ar) 1.25 (s, 18H, $C(CH_3)_3$), 0.97 (s, 9H, $C(CH_3)_3$), 0.92 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (CD_3OD–$CDCl_3$ 4:1, 297 K): δ 154.3, 153.6, 153.2 (C5, C11, C17, C23), 144.3, 144.1, 144.0 (C-4 triazole), 136.2 (C25, C26, C27, C28), 132.7, 131.9, 131.8 (C1, C3, C7, C9, C13, C15, C19, C21), 128.5, 126.0, 125.4, 125.1 (C4, C6, C10, C12, C16, C18, C22, C24), 125.8, 125.5, 125.1 (C-5 triazole), 103.7 (C-1 Gal), 102.87, 102.8, 102.78 (C-1 Glc), 79.7 (C-4 Glc), 75.5 (C-5 Glc), 74.9, 71.1 (C-2 Gal and C-3 Glc), 74.7 (C-3 Gal), 73.4 (C-5 Gal), 73.1 (C-2 Glc), 70.2, 70.1, 69.4, 69.1, 68.4 (OCH_2), 68.9 (C-4 Gal), 66.4, 64.7 (OCH_2–triazole), 61.2 (C-6 Glc), 60.8 (C-6 Gal), 50.1, 49.8 (OCH_2CH_2–triazole), 33.7, 33.5, 33.4 (($CH_3)_3C$) 31.9 (C2, C8, C14, C20), 31.2, 30.9 (($CH_3)_3C$). MALDI-TOF MS: $M+Na^+$, found 2821.0. $C_{128}H_{196}N_{12}O_{56}Na$ requires 2821.28.

4.4. Trypanocidal activity

Biological activities of the synthesised calixsugar compounds were evaluated against trypomastigote forms of *T. cruzi* Y strain obtained from culture with a fibroblast cell line (LLC-MK_2). Trypomastigote cultures were resuspended to 6.5×10^6 parasites/mL in RPMI (Roswell Park Memorial Institute), containing 10% foetal bovine serum and three parallel experiments were performed for each compound at six different concentrations (1000 μM, 750 μM, 500 μM, 250 μM, 100 μM and 50 μM) at 37 °C under 5% CO_2. Benznidazole **28** (*N*-benzyl-2-nitro-1-imidazolacetamide, Roche) was used as a reference trypanocidal drug (positive control). Results of parasite viability were determined by counting the number of motile forms according to Brener.[28] The activities of the compounds tested are represented as IC_{50try}, corresponding to concentrations that kill 50% of the parasites.[29] The IC_{50try} was determined with PRISM 5.0 software using Nonlinear Regression in based on a Sigmoidal dose-response.

Acknowledgements

This work was supported in part by the UK BBSRC and by the Italian National Research Council (CNR). E.G. is grateful to Consiglio Nazionale delle Ricerche, Istituto di Chimica Biomolecolare (Italy) for a travel grant. Shirley Fairhurst and Abdul Rashid at JIC are thanked for NMR spectroscopy and mass spectrometry support.

References and notes

1. Varki, A. *Glycobiology* **1993**, *3*, 97–130.
2. (a) Turnbull, J. E.; Field, R. A. *Nat. Chem. Biol.* **2007**, *3*, 74–77; (b) Kiessling, L. L.; Splain, R. A. *Annu. Rev. Biochem.* **2010**, *79*, 619–653; (c) Fais, M.; Karamanska, R.; Russell, D. A.; Field, R. A. *J. Cereal Sci.* **2009**, *50*, 306–311.
3. (a) Lee, R. T.; Lee, Y. C. *Glycoconjugate J.* **2000**, *17*, 543–551; (b) Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555–578.
4. (a) Chabre, Y. M.; Roy, R. *Adv. Carbohydr. Chem. Biochem.* **2010**, *63*, 165–393; (b) Lindhorst, T. K. *Top. Curr. Chem.* **2002**, *218*, 201–235; (c) Pieters, R. J. *Org. Biomol. Chem.* **2009**, *7*, 2013–2025.
5. (a) Gutsche, C. D. *Calixarenes*; Royal Society of Chemistry: Cambridge, 1992; (b) *Calixarenes 2001*; Asfari, Z., Böhmer, V., Harrowfield, J., Vicens, J., Eds.; Kluwer: Dordrecht, 2010.
6. (a) Dondoni, A.; Marra, A. *Chem. Rev.* **2010**, *110*, 4949–4977; (b) Fulton, D. A.; Stoddart, J. F. *Bioconjugate Chem.* **2001**, *12*, 655–672; (c) Casnati, A.; Sansone, F.; Ungaro, R. *Acc. Chem. Res.* **2003**, *36*, 246–254; (d) Baldini, L.; Casnati, A.; Sansone, F.; Ungaro, R. *Chem. Soc. Rev.* **2007**, *36*, 254–266.
7. (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599; (b) Meldal, M.; Tornoe, C. W. *Chem. Rev.* **2008**, *108*, 2952–3015; (c) Aragão-Leoneti, V.; Campo, V. L.; Gomes, A. S.; Field, R. A.; Carvalho, I. *Tetrahedron* **2010**, *66*, 9475–9492; (d) Dedola, S.; Nepogodiev, S. A.; Field, R. A. *Org. Biomol. Chem.* **2007**, *5*, 1006–1017; (e) Dondoni, A. *Chem.—Asian J.* **2007**, *2*, 700–708.
8. (a) Dondoni, A.; Marra, A.; Scherrmann, M. C.; Casnati, A.; Sansone, F.; Ungaro, R. *Chem.—Eur. J.* **1997**, *3*, 1774–1782; (b) Dondoni, A.; Kleban, M.; Hu, X. B.; Marra, A.; Banks, H. D. *J. Org. Chem.* **2002**, *67*, 4722–4733; (c) Perez-Balderas, F.; Santoyo-Gonzalez, F. *Synlett* **2001**, 1699–1702; (d) Sansone, F.; Chierici, E.; Casnati, A.; Ungaro, R. *Org. Biomol. Chem.* **2003**, *1*, 1802–1809; (e) Dondoni, A.; Marra, A. *J. Org. Chem.* **2006**, *71*, 7546–7557; (f) Bew, S. P.; Brimage, R. A.; L'Hermite, N.; Sharma, S. V. *Org. Lett.* **2007**, *9*, 3713–3716; (g) Meunier, S. J.; Roy, R. *Tetrahedron Lett.* **1996**, *37*, 5469–5472; (h) Roy, R.; Kim, J. M. *Angew. Chem., Int. Ed.* **1999**, *38*, 369–372; (i) Marra, A.; Moni, L.; Pazzi, D.; Corallini, A.; Bridi, D.; Dondoni, A. *Org. Biomol. Chem.* **2008**, *6*, 1396–1409; (j) Dondoni, A.; Marra, A. *Tetrahedron* **2007**, *63*, 6339–6345; (k) Geraci, C.; Consoli, G. M. L.; Galante, E.; Bousquet, E.; Pappalardo, M.; Spadaro, A. *Bioconjugate Chem.* **2008**, *19*, 751–758.
9. (a) Schadel, U.; Sansone, F.; Casnati, A.; Ungaro, R. *Tetrahedron* **2005**, *61*, 1149–1154; (b) Arosio, D.; Fontanella, M.; Baldini, L.; Mauri, L.; Bernardi, A.; Casnati, A.; Sansone, F.; Ungaro, R. *J. Am. Chem. Soc.* **2005**, *127*, 3660–3661.
10. Moni, L.; Pourceau, G.; Zhang, J.; Meyer, A.; Vidal, S.; Souteyrand, E.; Dondoni, A.; Morvan, F.; Chevolot, Y.; Vasseur, J. J.; Marra, A. *ChemBioChem* **2009**, *10*, 1369–1378.
11. (a) Giorgi, M. E.; Ratier, L.; Agusti, R.; Frasch, A. C. C.; de Lederkremer, R. M. *Glycoconjugate J.* **2010**, *27*, 549–559; (b) Agusti, R.; Paris, G.; Ratier, L.; Frasch, A. C. C.; de Lederkremer, R. M. *Glycobiology* **2004**, *14*, 659–670.

12. (a) Previato, J. O.; Andrade, A. F. B.; Pessolani, M. C. V.; Mendoncapreviato, L. *Mol. Biochem. Parasitol.* **1985**, *16*, 85–96; (b) Schenkman, S.; Jiang, M. S.; Hart, G. W.; Nussenzweig, V. *Cell* **1991**, *65*, 1117–1125; (c) de Lederkremer, R. M.; Agusti, R. *Adv. Carbohydr. Chem. Biochem.* **2009**, *62*, 311–366.
13. (a) Harrison, J. A.; Kartha, K. P. R.; Fournier, E. J. L.; Lowary, T. L.; Malet, C.; Nilsson, U. J.; Hindsgaul, O.; Schenkman, S.; Naismith, J. H.; Field, R. A. *Org. Biomol. Chem.* **2011**, *9*, 1653–1660; (b) Carvalho, I.; Andrade, P.; Campo, V. L.; Guedes, P. M. M.; Sesti-Costa, R.; Silva, J. S.; Schenkman, S.; Dedola, S.; Hill, L.; Rejzek, M.; Nepogodiev, S. A.; Field, R. A. *Bioorg. Med. Chem.* **2010**, *18*, 2412–2427; (c) Campo, V. L.; Carvalho, I.; Da Silva, C. H. T. P.; Schenkman, S.; Hill, L.; Nepogodiev, S. A.; Field, R. A. *Chem. Sci.* **2010**, *1*, 507–514; (d) van Well, R. M.; Collet, B. Y. M.; Field, R. A. *Synlett* **2008**, 2175–2177; (e) Campo, V. L.; Carvalho, I.; Allman, S.; Davis, B. G.; Field, R. A. *Org. Biomol. Chem.* **2007**, *5*, 2645–2657.
14. Neres, J.; Brewer, M. L.; Ratier, L.; Botti, H.; Buschiazzo, A.; Edwards, P. N.; Mortenson, P. N.; Charlton, M. H.; Alzari, P. M.; Frasch, A. C.; Bryce, R. A.; Douglas, K. T. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 589–596.
15. (a) Marra, A.; Dondoni, A.; Sansone, F. *J. Org. Chem.* **1996**, *61*, 5155–5158; (b) Felix, C.; Parrot-Lopez, H.; Kalchenko, V.; Coleman, A. W. *Tetrahedron Lett.* **1998**, *39*, 9171–9174; (c) Andre, S.; Sansone, F.; Kaltner, H.; Casnati, A.; Kopitz, J.; Gabius, H. J.; Ungaro, R. *ChemBioChem* **2008**, *9*, 1649–1661.
16. Kohmoto, S.; Mori, E.; Kishikawa, K. *J. Am. Chem. Soc.* **2007**, *129*, 13364–13365.
17. (a) Corbellini, F.; Mulder, A.; Sartori, A.; Ludden, M. J. W.; Casnati, A.; Ungaro, R.; Huskens, J.; Crego-Calama, M.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **2004**, *126*, 17050–17058; (b) Zhao, Y.; Ryu, E. H. *J. Org. Chem.* **2005**, *70*, 7585–7591.
18. Grandjean, C.; Boutonnier, A.; Guerreiro, C.; Fournier, J. M.; Mulard, L. A. *J. Org. Chem.* **2005**, *70*, 7123–7132.
19. Ashton, P. R.; Boyd, S. E.; Brown, C. L.; Nepogodiev, S. A.; Meijer, E. W.; Peerlings, H. W. I.; Stoddart, J. F. *Chem.—Eur. J.* **1997**, *3*, 974–984.
20. Zimmermann, P.; Bommer, R.; Bar, T.; Schmidt, R. R. *J. Carbohydr. Chem.* **1988**, *7*, 435–452.
21. Fernandez-Megia, E.; Correa, J.; Rodriguez-Meizoso, I.; Riguera, R. *Macromolecules* **2006**, *39*, 2113–2120.
22. Dedola, S.; Hughes, D. L.; Nepogodiev, S. A.; Rejzek, M.; Field, R. A. *Carbohydr. Res.* **2010**, *345*, 1123–1134.
23. Khanetskyy, B.; Dallinger, D.; Kappe, C. O. *J. Comb. Chem.* **2004**, *6*, 884–892.
24. Ryu, E. H.; Zhao, Y. *Org. Lett.* **2005**, *7*, 1035–1037.
25. Xu, W.; Vittal, J. J.; Puddephatt, R. J. *Can. J. Chem.* **1996**, *74*, 766–774.
26. Cecioni, S.; Lalor, R.; Blanchard, B.; Praly, J. P.; Imberty, A.; Matthews, S. E.; Vidal, S. *Chem.—Eur. J.* **2009**, *15*, 13232–13240.
27. Li, J.; Zacharek, S.; Chen, X.; Wang, J.; Zhang, W.; Janczuk, A.; Wang, P. G. *Bioorg. Med. Chem.* **1999**, *7*, 1549–1558.
28. Brener, Z. *Rev. Inst. Med. Trop. Sao Paulo* **1962**, *4*, 389–396.
29. (a) Silva, R. S. F.; Costa, E. M.; Trindade, U. L. T.; Teixeira, D. V.; Pinto, M. D. F. R.; Santos, G. L.; Malta, V. R. S.; De Simone, C. A.; Pinto, A. V.; de Castro, S. L. *Eur. J. Med. Chem.* **2006**, *41*, 526–530; (b) Guedes, P. M.; Oliveira, F. S.; Gutierrez, F. R.; da Silva, G. K.; Rodrigues, G. J.; Bendhack, L. M.; Franco, D. W.; Do Valle Matta, M. A.; Zamboni, D. S.; da Silva, R. S.; Silva, J. S. *Br. J. Pharmacol.* **2010**, *160*, 270–282.