Synthesis of sialoclusters appended to calix[4]arene platforms *via* multiple azide-alkyne cycloaddition. New inhibitors of hemagglutination and cytopathic effect mediated by BK and influenza A viruses

Alberto Marra,*[a] Lisa Moni,[a] Daniele Pazzi,[a] Alfredo Corallini,*[b] Deborah Bridi[b] and Alessandro Dondoni*[a]

Received 14th January 2008, Accepted 7th February 2008
First published as an Advance Article on the web 28th February 2008
DOI: 10.1039/b800598b

Tetra- and octavalent sialoside clusters were prepared in good yields exploiting for the first time the multiple copper-catalyzed cycloaddition of a propargyl thiosialoside with calix[4]arene polyazides. The cycloadducts featured the hydrolytically stable carbon-sulfur bond at the anomeric position and the 1,4-disubstituted triazole ring as the spacer between the sialic acid moieties and the platform. It was demonstrated that these unnatural motifs did not hamper the desired biological activity of the sialoclusters. In fact, they were able to inhibit, at submillimolar concentrations, the hemagglutination and the viral infectivity mediated both by BK and influenza A viruses.

Introduction

Naturally occurring sialic acids constitute a family of more than 50 structurally distinct nine-carbon 3-deoxy-ulosonic acids,[1] the most widespread derivative being 5-*N*-acetyl-neuraminic acid (Neu5Ac, **1**) (Fig. 1). These monosaccharides are found at the non-reducing end of glycoconjugates, where they are invariably α-D-linked to a hexopyranose, usually D-galactose or D-galactosamine, or other sialic acid fragments. Due to their peculiar position within cell-surface glycoproteins and glycolipids, sialic acids are exposed to the external environment and involved in numerous physiological and pathological recognition phenomena, including the adhesion of bacteria and viruses to human cells.[2]

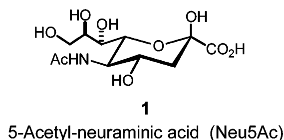


Fig. 1 The most common sialic acid found in Nature.

Examples of the latter pathogens are the BK and influenza A viruses. BK virus (BKV) is a human polyomavirus known to be the etiological agent of a severe form of nephropathy, a complication observed in 5–8% of kidney transplants, which often leads to the organ loss.[3] The extensive immunosuppressive treatment used to prevent kidney rejection enables a dramatic enhancement of the BKV replication resulting in the polyomavirus associated nephropathy (PVN). However, primary infection by BKV is usually asymptomatic and only occasionally may be accompanied by mild respiratory illness or urinary tract disease. During primary infection, viremia occurs and the virus spreads to several organs of the infected individual where it remains in a latent state. Virus isolation and Southern blot hybridization analysis established that the kidney is the main site of BKV latency in healthy individuals. By these technical approaches, BKV sequences were also detected in other organs, such as liver, stomach, lungs, parathyroid glands, and lymph nodes.[4] The detection of BKV DNA in tonsils suggests that the oropharynx may be the initial site of BKV infection.[5] Besides the above mentioned PVN disease, other inflammatory syndromes affecting several organs were described after BKV infection or reactivation in both immunocompetent[6] and immunosuppressed[6,7] individuals. Moreover, an association between hemorrhagics cystitis and BKV was shown in immunosuppressed bone marrow transplant recipients[8] as well as in immunocompetent people.[9] It has been recently demonstrated by one of us (A. C.) that whole BKV, its complete DNA, and also subgenomic DNA fragments containing the early region, are able to transform embryonic fibroblasts and cells cultured from kidney and brain of hamster, mouse, rat, rabbit and monkey.[10] BKV is highly oncogenic in rodents[10] since young or newborn hamsters, mice and rats, inoculated with BKV through different routes, developed tumors.[11] However, the role of BKV in human neoplasia is still uncertain.

Since no reliable drugs against BKV are available to date,[3b] there is a great need to develop new, non-conventional antiviral molecules based on recent discoveries of the actual receptors of BKV onto the host cell. Early experiments showed that BKV does not bind to sialic acid-depleted erythrocytes[12] and Vero cells.[13] Then, further evidence[14] of the Neu5Ac-mediated attachment of the virus to the cell has proved the key role exerted by 5-*N*-acetyl-neuraminic acid, linked to *N*-glycoproteines or gangliosides, as ligand of the viral capsid proteins.[15,16] Therefore, artificial Neu5Ac-decorated structures able to interfere with this sugar-protein recognition process may act as anti-BKV agents. It is worth noting that the potential of sialic acid-based unnatural ligands of BKV, as inhibitors of its binding to the host cell, has never been explored.[17]

A similar approach can be envisaged to design new antiviral drugs against other pathogens such as the influenza A viruses, which include the human, avian, swine, and equine influenza

[a]*Dipartimento di Chimica, Laboratorio di Chimica Organica, Università di Ferrara, Via Borsari 46, I-44100, Ferrara, Italy*
[b]*Dipartimento di Medicina Sperimentale e Diagnostica, Sezione di Microbiologia, Università di Ferrara, Via Borsari 46, I-44100, Ferrara, Italy*

 This journal is © The Royal Society of Chemistry 2008

viruses. Although both influenza A and B type viruses are responsible for annual flu epidemics in humans, only the A virus can cause sporadic pandemics, characterized by excess mortality and morbidity, since there is no animal reservoir of the influenza B virus.[18] Aquatic birds provide the natural reservoir for the influenza A viruses, although avian flu is, in general, asymptomatic in wild birds. Occasionally, highly pathogenic strains of the influenza A viruses (*e.g.* H5N1) cause serious systemic infections in domestic poultry and even cross the species barrier infecting humans.[19] These single-stranded RNA viruses carry two surface glycoproteins, a trimeric lectin (hemagglutinin, HA)[20] and a tetrameric glycosidase (neuraminidase, NA),[18c] and are subtyped according to the reactivity of HA (15 subtypes) and NA (9 subtypes). Both proteins are present in multiple copies on the virus surface (*ca.* 500 units of HA and 100 units of NA per virion) and recognize the same host cell sugar, 5-*N*-acetyl-neuraminic acid. The hemagglutinin mediates the adhesion of the virus to the cell, followed by the fusion of viral and cell membranes, whereas the neuraminidase cleaves the terminal sialic acid linked to the glycoconjugate receptors to allow progeny virus release and to promote the spread of the infection facilitating the movement of the viral particles through mucus.[21,22] Therefore, designed molecules that bind stronger than the natural ligand to either HA or NA would be, in principle, good anti-flu drugs. During the past two decades both issues have been actively addressed, but only some neuraminidase inhibitors,[23] zanamivir (**2**) and oseltamivir (**3**), have reached the market (Fig. 2), whereas compounds targeted against hemagglutinin[24] failed to become drugs.[25] This was mainly due to the weak HA-binding properties shown by monomeric sialic acid derivatives. In fact, compound **6**, one of the best monovalent inhibitors of the bromelain-released hemagglutinin (a soluble form of HA that lacks the C-terminal anchoring peptide), has a dissociation constant of 3.7 μM,[26] only three orders of magnitude lower than that found for methyl α-sialoside **5** ($K_D = 2.8$ mM), the prototypal HA ligand (Fig. 3). On the other hand, good monomeric neuraminidase inhibitors,[27] including the commercially available ones, have dissociation constants in the picomolar range.[28] However, NA inhibitors are effective only after the onset of infection in the host and are not suitable for prophylactic purposes. To this end, a great deal of work has been devoted to the synthesis of multivalent HA ligands,[29] non-natural compounds displaying multiple copies of sialic acid moieties, for which it was anticipated a strong HA affinity due to simultaneous binding events.

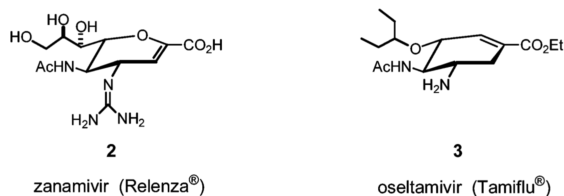


Fig. 2 Commercially available neuraminidase inhibitors.

Results and discussion

Synthesis of multivalent sialosides

Many recognition processes take place through the attachment of multiple binding sites on one molecule (*e.g.* a protein) or organism (*e.g.* a virus) to multiple receptor sites on another one (*e.g.* a cell).[30] The resulting affinity enhancement is orders of magnitude greater than that expected on the basis of a mere multiplication factor of valency. Indeed, multivalency is a means Nature has found to increase weak interactions to biologically relevant levels. This finding is especially important in carbohydrate-protein recognition events, in which monovalent affinities are usually quite low. In the case where a protein, which is bearing clustered binding sites, links to a multivalent ligand displaying sugar units with proper orientation and spacing, the phenomenon is termed[31] glycoside cluster effect.[30b]

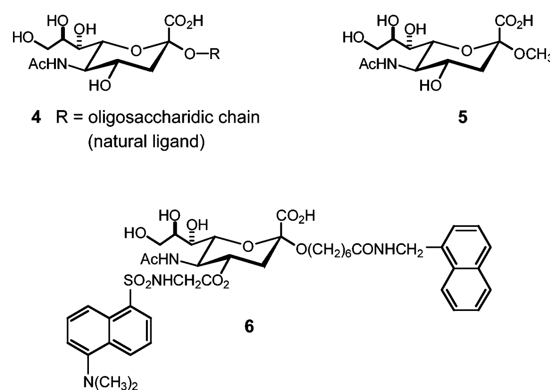


Fig. 3 Natural and unnatural ligands of influenza A virus hemagglutinin.

Non-natural multivalent HA ligands that present α-sialoside units tethered to proteins,[32] homopeptides,[33] polysaccharides,[34] polymers,[35] liposomes,[36] nanostructures,[37] dendrimers,[38] and low molecular weight scaffolds (to form clusters)[39] have been prepared and, in most cases, tested as inhibitors of the hemagglutination and the cytopathic effects mediated by influenza A viruses.[40] High molecular weight multivalent sialosides[32–37] showed a potent HA inhibitory activity, in some cases[35h] higher than that observed for the most effective natural inhibitor (equine α_2-macroglobulin), since the minimum concentrations[41] required to inhibit hemagglutination by the virus were in the nano- or picomolar range. However, serious concerns[34,35l,37b,42] were raised about the toxicity of polymeric sialosides having a polyacrylamide backbone.[35a–35i,35m,35n,35p] In addition, the polydisperse nature of macromolecular materials, which prevents rigorous purification and characterization, together with their potential immunogenicity, may constitute an insurmountable obstacle to the approval as antiviral therapeutics by national regulatory agencies. Therefore, structurally well-defined multivalent sialosides, such as dendrimer[38] and cluster[39] derivatives, are valuable lead compound candidates.

Our interest in sugar clusters dates back to the mid-1990s, when we reported the first synthesis[43] of glycosylated calixarenes (calixsugars) such as the water-soluble tetra-*O*-galactosyl-calix[4]arene **7** (Fig. 4). Then, various calixsugars were prepared by us[44] and others,[39e,45] while the synthesis of hydrolytically stable, carbon-linked glycosyl-calixarenes turned out to be a quite difficult task. In fact, the Wittig olefination of calixarene aldehydes by anomeric sugar phosphoranes gave poor results, since only a bis-*C*-glycosylated calix[4]arene was obtained in modest yield.[44c] Also the approach based on the Pd(0)-mediated Sonogashira-Heck-Cassar cross-coupling between poly-iodoarenes and ethynyl *C*-glycosides (*C*-glycosyl acetylenes), was inefficient for the

synthesis of *C*-calixsugars, while it was successfully applied to the preparation of a different class of *C*-glycoside clusters.[46] Finally, taking advantage of the potential of the Cu(I)-mediated azide-alkyne cycloaddition,[47,48] ethynyl *C*-glycosides were coupled with various scaffolds bearing multiple azide functions to give in high yields *C*-glycoclusters, including the *C*-calixsugar **8** (Fig. 4).[49] These molecules displayed a 1,4-disubstituted 1,2,3-triazole ring as the linker, a formidable keystone,[48e] connecting the sugar ligand to the scaffold. Recently, following a two-step procedure developed by Sharpless and Demko,[50] the hitherto unreported 1,5-disubstituted tetrazole-linked glycoclusters were made available as well.[51] Specifically, the facile cycloaddition between azidomethyl *C*-glycosides and tosyl cyanide gave the corresponding 1-glycosyl-5-sulfonyl-tetrazole derivatives, which were submitted to multiple nucleophilic substitution by calixarene tetrols to afford tetravalent *C*-glycoclusters,[51] *e.g.* **9** (Fig. 4).

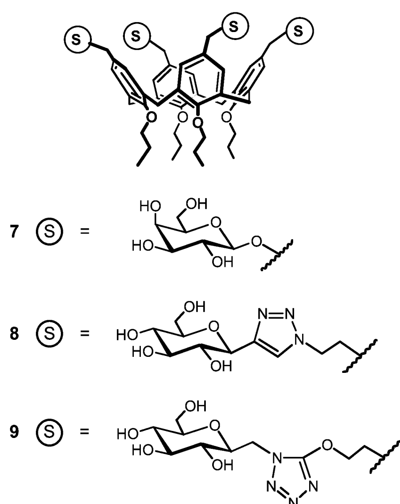


Fig. 4 Calixarene-based glycoside clusters prepared in the Authors' laboratory.

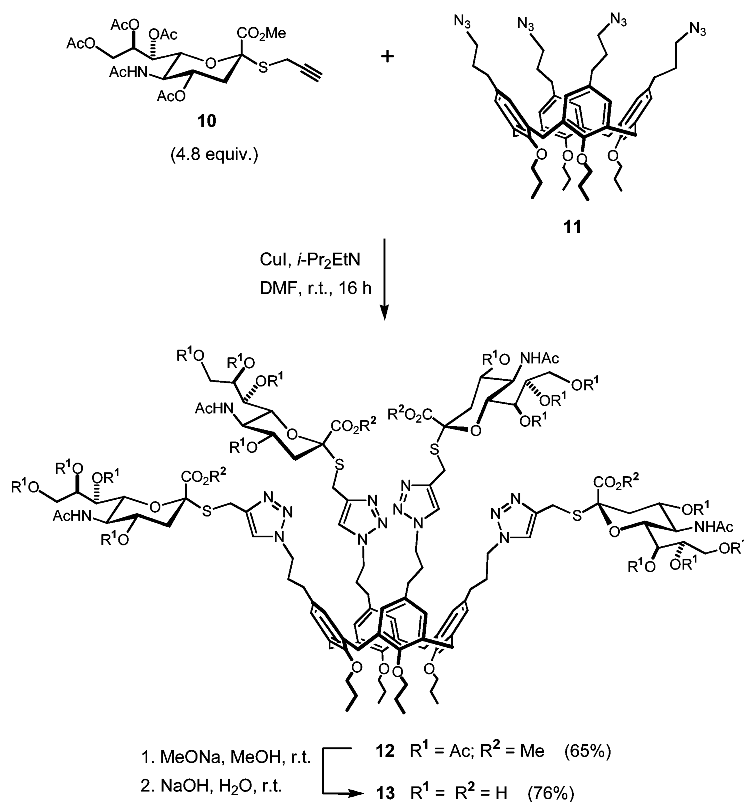
Unfortunately, the latter methodology cannot be exploited for the synthesis of sialoside clusters, because the acyl functions of the Neu5Ac units do not tolerate the conditions of the nucleophilic substitution step. Hence, we envisaged the copper-catalyzed azide-alkyne cycloaddition to be a convenient entry to multivalent sialosides. Calix[4]arenes, the most versatile members of this class of cyclic oligophenols,[52] were chosen as scaffolds due to the easy derivatization at both the upper (wide) and lower (narrow) rims and their three-dimensional preorganized architecture. In particular, among the four possible conformations (cone, partial cone, 1,2-alternate, 1,3-alternate) adopted by calix[4]arenes, the cone conformer allows a spatially close arrangement of up to four sugar ligands at either sides of the hydrophobic cavity. In order to prepare glycoclusters resistant to enzymatic hydrolysis for potential *in vivo* applications, the native anomeric oxygen atom of the sialic acid moieties had to be replaced by a carbon or sulfur[53] atom. The resulting isosteres would retain the hemagglutinin binding affinity because the functional groups involved in this sugar-protein recognition,[54] *i.e.* axial carboxylate, amide function, and glycerol side chain, are not altered.

Since α-D-linked *C*-sialosides bearing a terminal triple bond in their aglycon chain appeared to us to be difficult access, the known[39g] propargyl thiosialoside **10** was chosen as dipolarophile in the planned Cu-assisted cycloaddition reactions (Scheme 1). As the 1,3-dipole counterpart, we used the calixarene tetra-azide **11**, a stable compound adopting the fixed cone conformation that has been recently synthesized in our laboratory.[49] Hence, the tetra-azide **11** was allowed to react at room temperature overnight with the sugar alkyne **10** (1.2 equiv. per azide group) in the presence of freshly distilled *N*,*N*-diisopropylethylamine (Hünig's base) and 25 mol% of commercially available copper(I) iodide. To achieve complete solubility of the cycloaddition partners, anhydrous DMF, instead of toluene, was used as the reaction solvent. As partially deacetylated sialoclusters were formed due to the presence of adventitious water, the crude reaction mixture was acetylated (Ac_2O, Py, r.t., 3 h). The tetravalent sialocluster **12** was isolated in 65% yield by column chromatography on silica gel. The trivalent cycloadduct was recovered in 20% yield, although contaminated by **12** and other byproducts. The MS and ^{1}H NMR analyses readily confirmed the C_4-symmetric structure of **12**, while the 1,4-disubstitution pattern of the 1,2,3-triazole spacers was firmly established by ^{13}C NMR spectroscopy. In fact, a large positive $\Delta(\delta_{C4} - \delta_{C5})$ value (20.7 ppm) was found in the ^{13}C spectrum of **12**, as expected for 1,4-disubstituted triazole derivatives,[55] including sugar clusters.[49] On the other hand, it has been demonstrated that 1,5-disubstituted triazole rings display small negative values (ca. −5 ppm) for the chemical shift difference of the same carbon atoms.[49,55] To provide suitable material for biological studies, the sialocluster **12** was first deacetylated by transesterification (MeONa, MeOH, r.t., 3 h) to give the corresponding methyl ester derivative. Then, the saponification (aqueous NaOH, r.t., 18 h) of the crude product afforded the water soluble tetra-acid **13** in 76% overall yield after filtration through a short column of C18 silica gel.

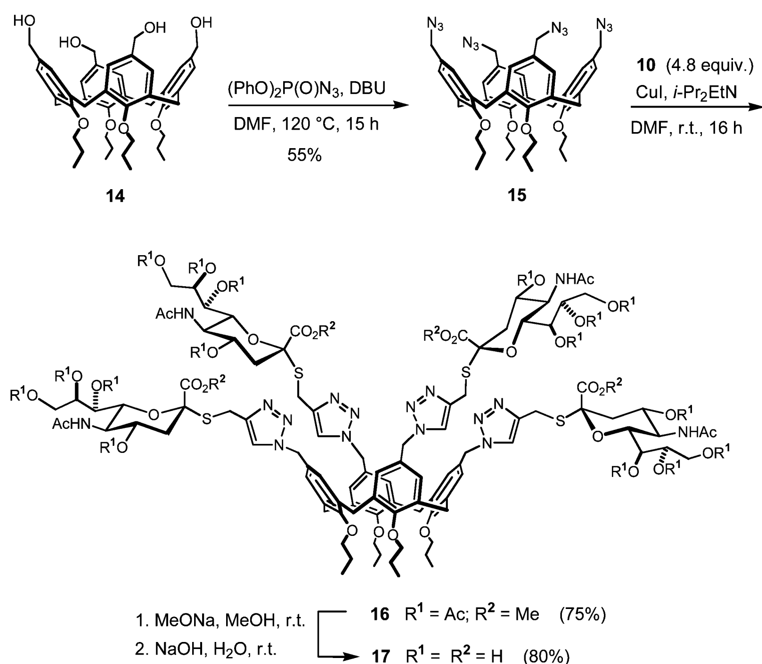
Aiming at investigating the effect of the chain length on the molecular recognition properties of the sialoside clusters, we targeted the calix[4]arene **15** in which the azide functions are linked to the aromatic ring through single methylene spacers (Scheme 2). Hence, the known[44b] upper rim calix[4]arene tetrol **14** was converted into **15** upon treatment with diphenyl phosphoryl azide (DPPA, 1.5 equiv. per hydroxy group) and 1,8-diazabicyclo[5.4.0.]undec-7-ene (DBU) at 120 °C for 15 h. After column chromatography on silica gel, the short-arm calix[4]arene tetra-azide **15** was isolated in 55% yield as a white crystalline solid. The cycloaddition between **15** and the propargyl thiosialoside **10** was carried out as described above to afford the peracetylated tetravalent sialocluster **16** in 75% isolated yield. Also in this case, the 1,4-regiochemistry of the triazole ring was established by ^{13}C-NMR analyses ($\Delta(\delta_{C4} - \delta_{C5}) = 20.8$ ppm). Then, the removal of the acetyl protecting groups and the hydrolysis of the methyl esters, followed by C18 silica gel purification, gave the tetra-acid **17** in 80% yield.

The approach to sialoclusters displaying the sugar moieties close to each other was carried out using a cone-configured calix[4]arene azidated at the lower rim. This side of the macrocycle is significantly narrower than the upper side. Therefore, the known[56] tetra-allyl-calixarene **18** (Scheme 3) was transformed into the tetrol **19** by one-pot multiple hydroboration and oxidation (73%). Then, compound **19** was submitted to the azidation using DPPA and DBU (120 °C, 18 h) to give, after column chromatography on silica gel, the lower rim tetra-azide **20** in 67% yield as a white crystalline

 This journal is © The Royal Society of Chemistry 2008

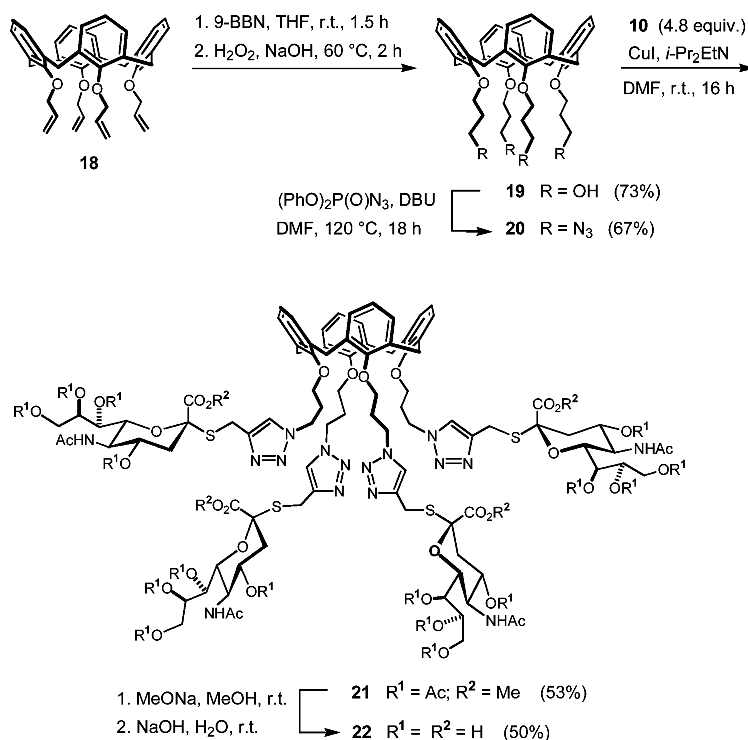


Scheme 1



Scheme 2

solid. The Cu-catalyzed cycloaddition of **20** with thiosialoside **10** (4.8 equiv.) under standard conditions afforded crystalline **21** in 53% isolated yield. The main reason for the moderate yield of isolated **21** was due to the loss of the product in the course of the chromatographic purification because of the low solubility of the cycloadduct in the eluent employed. Unfortunately, direct crystallization of the crude mixture, as well as size-exclusion chromatography were not suitable means for the purification of **21**. The C_4-symmetric structure and the regioisomeric assignment of the disubstituted triazole residues were confirmed by MS and NMR analyses (see Experimental Section). Moreover, the fixed cone conformation of the calix[4]arene scaffold was substantiated



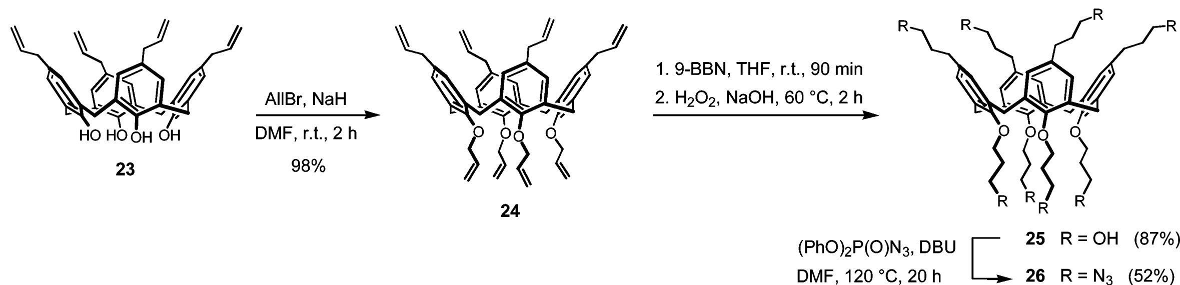
Scheme 3

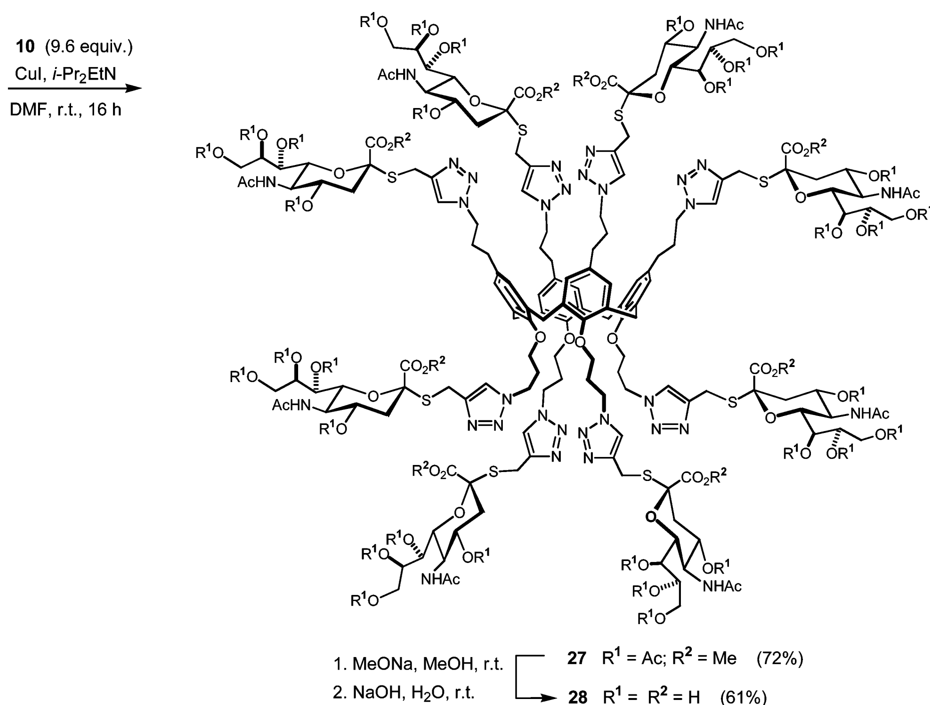
by the presence of signals for the equatorial and axial protons of the methylene bridges between the phenyl rings as large doublets at 3.15 and 4.30 ppm, respectively.[57] Finally, the acetyl and methyl protecting groups were removed under basic conditions as described for the above sialoclusters at the upper rim to give the tetra-acid **22** in 50% yield as a white crystalline solid.

Since sialylation at either the upper or lower rim of calix[4]arene scaffolds was quite efficient, we planned to prepare a cluster constituted of sialic acid residues at both sides of the calixarene cavity. In principle, such a densely sialylated molecule can bind simultaneously to a couple of hemagglutinin trimers located onto a single virion or two distinct viral particles. In both cases the HA inhibitory activity was expected to be stronger than that displayed by the tetravalent sialoclusters **13**, **17**, and **22**. To this end, the tetra-phenol **23** (Scheme 4) was further allylated at the lower rim to give the octa-allyl derivative **24** in nearly quantitative yield. The fixed cone conformation of this product was proved[57] by the presence in its ^{1}H NMR spectrum of two doublets at 3.10 and 4.38 ppm (J = 13.0 Hz) corresponding to the methylene bridges protons. Then **24** was submitted to the hydroboration-oxidation one-pot sequence to afford the octaol **25** (87%), which in turn was azidated using DPPA and DBU at high temperature. The syrupy calix[4]arene octa-azide **26** was isolated in pure form (52%) by column chromatography on Sephadex LH-20. The purification of this polyazide on a silica gel column was unsuccessful very likely because of the strong interactions with this stationary phase. The cycloaddition of **26** with propargyl thiosialoside **10** (1.2 equiv. per azide group) was performed using our standard procedure to give the octavalent cluster **27** in 72% yield after Sephadex LH-20 column chromatography. By means of this technique the sialocluster **27** was recovered as a brown solid, probably due to a complex with copper salts. A colorless, analytical sample of **27** was obtained by deacetylation, chromatography on C18 silica gel column, and acetylation (see Experimental Section). This compound showed very broadened signals in its ^{1}H NMR spectrum recorded at room temperature, probably due to a slow equilibrium between the conformations adopted by the glycosylated chains linked to the metacyclophane core. Fortunately, the sharp spectrum acquired at 120 °C (in DMSO-d$_6$) allowed to assign the C_4-symmetric structure and the cone conformation to **27**, while the formation of 1,4-disubstituted triazole rings was confirmed by ^{13}C NMR analysis ($\Delta(\delta_{C4} - \delta_{C5}) = \sim 19$ ppm). Although slightly contaminated by copper salts, compound **27** was transformed into the target octavalent sialocluster **28** by standard transesterification of the acetate functions and methyl ester saponification, followed by column chromatography on C18 silica gel. By this procedure, **28** was isolated in a pure form and 61% yield. Also the octavalent sialocluster **28** showed a complex ^{1}H NMR spectrum at 25 °C, while raising the temperature to 120 °C caused the coalescence of the signals.

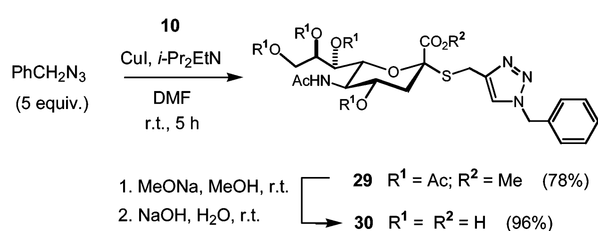
A rigorous evaluation of the enhancement of hemagglutinin inhibitory activity shown by glycoclusters with respect to a monovalent ligand, *i.e.* the glycoside cluster effect, required a monomeric sugar featuring the same aglycon structure.[58] Hence, the thiosialoside **30** (Scheme 5) appeared to be more appropriate than methyl *O*-sialoside **5** (Fig. 3) as the reference monovalent ligand. Actually, compound **30** embodied all the functional groups present in the above multivalent sialosides, such as the equatorial thioglycoside linkage, the 1,4-disubstituted triazole moiety, and the phenyl ring. Its protected derivative, the acetylated methyl ester **29**, was readily prepared in 78% yield by Cu-promoted cycloaddition of **10** with crude benzyl azide (ca. 5 equiv.) prepared

 This journal is © The Royal Society of Chemistry 2008





Scheme 4



Scheme 5

in situ immediately before use (Scheme 5). Then, standard deprotection reactions allowed the conversion of **29** into water soluble **30** in almost quantitative yield.

Biological assays

The capability of the synthesized multivalent sialosides to bind BKV particles was tested using the hemagglutination inhibition (HI) assay (Table 1). All compounds were able to inactivate BKV at submillimolar concentrations, the lower rim tetrasialoside **22** being the most potent of this set of inhibitors (0.11 mM, entry 4). Therefore, the use of the non-natural calixarene scaffold and triazole linker did not prevent the molecular recognition properties of the sugar ligand. Although each multivalent sialoside was more active than the monovalent derivative **30** (relative potencies: from 1.12 to 2.55), the same was no longer true when the actual concentration of sialic acid moieties was taken into consideration (relative potencies: from 0.17 to 0.64). Indeed, the installation of multiple copies of neuraminic acid onto a platform led to a decrease of the inhibition activity per sugar unit, this being in contrast to the occurrence of the glycoside cluster effect. These disappointing results may be ascribed to a low surface concentration of viral hemagglutinin, which hampers multiple

Table 1 HI of BKV by multivalent sialosides

Entry	Compound	HI activity[a] (mM)	Relative potency[b]
1	**30**	0.28	1
2	**13**	0.17 (0.68)[c]	1.65 (0.41)[d]
3	**17**	0.25 (1.00)[c]	1.12 (0.28)[d]
4	**22**	0.11 (0.44)[c]	2.55 (0.64)[d]
5	**28**	0.20 (1.60)[c]	1.40 (0.17)[d]

[a] Minimum concentration required for complete HI. [b] All potencies normalized to that of monovalent sialoside **30**. [c] Actual concentration of sialic acid units. [d] Based on the sialic acid contents.

concomitant interactions to take place. Unfortunately, no data are available in the literature about the number and spatial arrangement of hemagglutinin on the BKV surface.

Preliminary tests demonstrated that compounds **13**, **17**, **22**, **28**, and **30** did not manifest cytotoxicity against Vero cells. Hence, to further assess the ability of these sialosides to bind BKV virions, experiments of neutralisation of infection were carried out. Eight to ten days after infection in Vero cells, an evident cytopathic effect (CPE) was present in cultures infected with the control positive, whereas all other cultures, infected with BKV but pretreated with the mono- and multivalent sialosides (at the concentrations indicated in Table 1) or anti-BKV antibodies, did not show CPE. In order to confirm these results, all media obtained from the infected cultures were frozen and thawed three times, then submitted to the hemagglutination assay. Only the medium derived from cultures infected with the control positive gave hemagglutination, indicating the formation of new viral particles due to a cycle of BKV infection. Finally, the presence of structural antigens was analyzed by immunofluorescence (IF) in mock-infected cells, infected cells, and in cells infected with the artificial inhibitor-preincubated BKV (Fig. 5). When BKV was preincubated with the mono- and multivalent sialosides, the adsorption step was inhibited and the nuclei of the cells did not show the presence of viral proteins (Fig. 5c–g). The same result was obtained when the virus was preincubated with specific antibodies (Fig. 5h), whereas in cells infected with BKV, not pretreated, viral proteins were expressed and the nuclei of the cells appeared fluorescent (Fig. 5b).

The biological activity of the four sialoside clusters prepared was also tested against the influenza A virus. The standard HI assay (Table 2) demonstrated that **13**, **22**, and **28** were able to inhibit the virus-induced hemagglutination, whereas the upper rim tetrasialoside **17** failed to show HI activity at concentrations up to 50 mM (entry 3). In striking contrast to that observed with BKV, the monovalent sialoside **30** was a very weak hemagglutination inhibitor (0.28 *vs.* 100 mM, entry 1 in Table 1 and 2). On the other hand, the upper and lower rim polysialosides **13**, **22**, and **28** were active at submillimolar concentrations showing similar potencies (0.25–0.37 mM). These results indicate that a moderate glycoside cluster effect was operative since, considering the inhibition activity per sialic acid unit, the above multivalent sialosides were 50–83 times more active than the monovalent derivative **30** (Table 2). Moreover, it appears from the HI assay that the reduced length and flexibility of the methylene spacers at the upper rim of **17** did not allow an efficient sugar-protein recognition. Finally, the HI activity of the octasialoside **28** (Table 2, entry 5) was close to that of the tetrasialosides **13** and **22**, indicating that only one of the two sets of sialic acid units linked at both calixarene rims was involved in the interaction with the viral hemagglutinin. We suspect that this behaviour is due to the unsuitable distance between the two sets of sugar ligands, very likely too short to allow the simultaneous binding to two distinct virions.

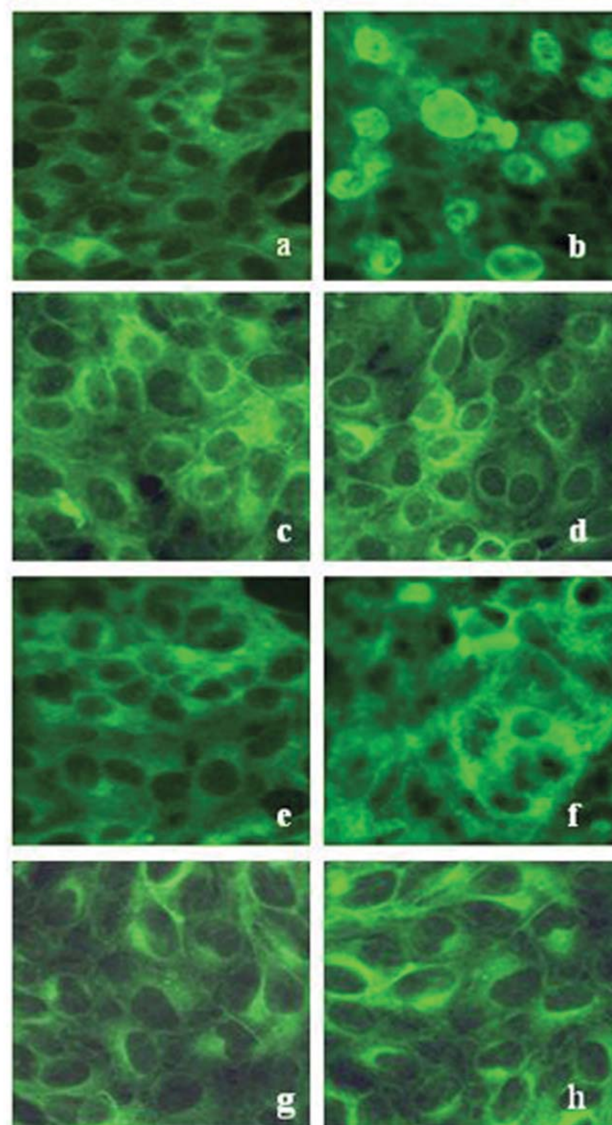


Fig. 5 Inhibition of the cytopathic effect in Vero cell cultures infected by BKV. The fluorescent nuclei indicate the expression of viral proteins. a) Mock-infected cells. b) Cells infected with BKV. c-g) Cells infected with BKV pretreated with monovalent sialoside **30**, upper rim tetrasialoside **13**, upper rim tetrasialoside **17**, lower rim tetrasialoside **22**, octasialoside **28**, respectively. h) Cells infected with BKV, pretreated with specific antibodies.

Table 2 HI of influenza A virus by multivalent sialosides

Entry	Compound	HI activity[a] (mM)	Relative potency[b]
1	**30**	100	1
2	**13**	0.37 (1.48)[c]	270 (68)[d]
3	**17**	>50 (>200)[c]	—
4	**22**	0.30 (1.20)[c]	333 (83)[d]
5	**28**	0.25 (2.00)[c]	400 (50)[d]

[a] Minimum concentration required for complete HI. [b] All potencies normalized to that of monovalent sialoside **30**. [c] Actual concentration of sialic acid units. [d] Based on the sialic acid contents.

The multivalent sialosides **13**, **22**, and **28** were also submitted to a neutralisation assay of influenza A virus (H3N2 strain) infectivity performed as described for the BKV (see Experimental Section). Ten days after infection of MDCK cells, the cytopathic effect (CPE) was present in cells infected with the virus, not pretreated, whereas the same cultures, infected with the virus and pretreated with the above inhibitors, did not show CPE.

 This journal is © The Royal Society of Chemistry 2008

Conclusions

While non-polymeric multivalent sialosides have been already described[38,39] and, in some cases, submitted to biological assays, our results pave the way for the synthesis of viral hemagglutinin inhibitors *via* multiple azide-alkyne cycloaddition. This powerful ligation method allowed us to prepare both inhibitors of the influenza A virus showing a moderate glycoside cluster effect and the first artificial inhibitors of the BKV.

Experimental section

All moisture-sensitive reactions were performed under a nitrogen atmosphere using oven-dried glassware. Anhydrous solvents were dried over standard drying agents[59] and freshly distilled prior to use. Reactions were monitored by TLC on silica gel 60 F_{254} with detection by charring with sulfuric acid. Flash column chromatography[60] was performed on silica gel 60 (230–400 mesh). Melting points were determined with a capillary apparatus. Optical rotations were measured at 20 ± 2 °C in the stated solvent; $[\alpha]_D$ values are given in deg $cm^3 \cdot g^{-1} \cdot dm^{-1}$. ^{1}H NMR (300 and 400 MHz) and ^{13}C NMR spectra (75 and 100 MHz) were recorded for $CDCl_3$ solutions at room temperature unless otherwise specified. Peak assignments were aided by ^{1}H-^{1}H COSY and gradient-HMQC experiments. In the ^{1}H NMR spectra reported below, the *n* and *m* values quoted in geminal or vicinal proton-proton coupling constants $J_{n,m}$ refer to the number of the corresponding sugar protons. MALDI-TOF mass spectra were acquired using 2,5-dihydroxy-benzoic acid as the matrix. ESI mass spectra were recorded for 6:4 CH_3CN-H_2O solutions containing 0.1% of trifluoroacetic acid.

5,11,17,23-Tetrakis{{3-{4-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)methyl]-1*H*-1,2,3-triazol-1-yl}propyl}}-25,26,27,28-tetrapropoxy-calix[4]arene (12)

A mixture of calix[4]arene tetra-azide **11** (92 mg, 0.10 mmol), propargyl thiosialoside **10** (262 mg, 0.48 mmol), freshly distilled *N*,*N*-diisopropylethylamine (418 µL, 2.40 mmol), CuI (23 mg, 0.12 mmol), and anhydrous DMF (5 cm^3) was sonicated in an ultrasound cleaning bath for 1 min, then magnetically stirred in the dark at room temperature for 16 h, and concentrated. A solution of the residue in pyridine (2 cm^3) and acetic anhydride (2 cm^3) was kept at room temperature for 3 h, then concentrated, diluted with AcOEt (100 cm^3), washed with phosphate buffer at pH 7 (2 × 10 cm^3), dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel with acetone to give first a tris-adduct (51 mg, 20%) slightly contaminated by the tetra-adduct **12**. ^{1}H NMR (400 MHz) selected data: δ 7.53 (s, 1H, H-5 Tr.), 7.52 (s, 2H, 2 H-5 Tr.), 6.44 (d, 4H, J = 3.3 Hz, Ar), 6.39 (s, 4H, Ar), 4.87 (ddd, 3H, $J_{3ax,4} = J_{4,5}$ = 10.9, $J_{3eq,4}$ = 4.7 Hz, 3 H-4), 4.38 and 3.04 (2d, 8H, J = 13.0 Hz, 4 ArCH_2Ar), 3.69, 3.68, and 3.66 (3 s, 9H, 3 OMe), 2.73 (dd, 3H, $J_{3ax,3eq}$ = 12.8 Hz, 3 H-3eq). MALDI-TOF MS (2561.88): 2585.9 (M^+ + H + Na), 2600.9 (M^+ + K). Eluted second was **12** (202 mg, 65%) as a syrup; $[\alpha]_D$ = +40.0 (*c* 0.4, $CHCl_3$). ^{1}H NMR (300 MHz): δ 7.56 (s, 4H, 4 H-5 Tr.), 6.44 (s, 8H, Ar), 5.52 (d, 4H, $J_{5,NH}$ = 10.0 Hz, 4 NH), 5.48 (ddd, 4H, $J_{7,8}$ = 8.5, $J_{8,9a}$ = 2.7, $J_{8,9b}$ = 5.5 Hz, 4 H-8), 5.36 (dd, 4H, $J_{6,7}$ = 2.1 Hz, 4 H-7), 4.90 (ddd, 4H, $J_{3ax,4}$ = 11.4, $J_{3eq,4}$ = 4.6, $J_{4,5}$ = 10.5 Hz, 4 H-4), 4.41 and 3.07 (2d, 8H, J = 13.3 Hz, 4 ArCH_2Ar), 4.34 (dd, 4H, $J_{9a,9b}$ = 12.5 Hz, 4 H-9a), 4.23 (t, 8H, J = 7.0 Hz, 4 $ArCH_2CH_2CH_2$), 4.10 (dd, 4H, 4 H-9b), 4.09 (ddd, 4H, $J_{5,6}$ = 10.6 Hz, 4 H-5), 4.03 and 3.98 (2d, 8H, J = 14.0 Hz, 4 SCH_2), 3.91 (dd, 4H, 4 H-6), 3.83 (t, 8H, J = 7.5 Hz, 4 $CH_3CH_2CH_2O$), 3.68 (s, 12H, 4 OMe), 2.76 (dd, 4H, $J_{3ax,3eq}$ = 12.5 Hz, 4 H-3eq), 2.32 (t, 8H, J = 7.2 Hz, 4 $ArCH_2CH_2CH_2$), 2.20, 2.19, 2.16, 2.05, and 1.90 (5 s, 60H, 20 Ac), 2.04–1.91 (m, 20H, 4 H-3ax, 4 $ArCH_2CH_2CH_2$, 4 $CH_3CH_2CH_2O$), 1.00 (t, 12H, J = 7.5 Hz, 4 $CH_3CH_2CH_2O$). ^{13}C NMR (100 MHz): δ 170.8, 170.2, 170.1, and 168.2 (CO), 155.0 (C Ar), 143.2 (C-4 Tr.), 134.9 (C Ar), 133.2 (C Ar), 127.9 (CH Ar), 122.5 (C-5 Tr.), 82.8 (C-2), 76.6 ($CH_3CH_2CH_2O$), 74.1 (C-6), 69.5 (C-4), 68.1 (C-8), 67.2 (C-7), 62.4 (C-9), 53.0 (OMe), 49.6 ($ArCH_2CH_2CH_2$), 49.3 (C-5), 37.6 (C-3), 32.0 ($ArCH_2CH_2CH_2$, $ArCH_2CH_2CH_2$), 31.0 ($ArCH_2Ar$), 23.3 (SCH_2, $CH_3CH_2CH_2O$), 23.2, 21.3, and 20.9 (CH_3CO), 10.3 ($CH_3CH_2CH_2O$); MALDI-TOF MS (3107.41): 3130.3 (M^+ + Na), 3146.1 (M^+ + K). Anal. Calcd. for $C_{144}H_{192}N_{16}O_{52}S_4$: C, 55.66; H, 6.23; N, 7.21. Found: C, 55.91; H, 6.35; N, 7.40.

5,11,17,23-Tetrakis{{3-{4-[(5-acetamido-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)methyl]-1*H*-1,2,3-triazol-1-yl}propyl}}-25,26,27,28-tetrapropoxy-calix[4]arene (13)

A solution of **12** (62 mg, 0.02 mmol) in a 0.2 M solution of NaOMe in MeOH (4 cm^3, prepared from Na and MeOH immediately before use) was stirred at room temperature for 3 h in a nitrogen atmosphere, then neutralized with Dowex 50 × 2–400 resin (H^+ form, activated and washed with H_2O and MeOH immediately before the use), and filtered through a sintered glass filter. The resin was washed with MeOH, and the solution was concentrated to give crude 5,11,17,23-tetrakis{{3-{4-[(methyl 5-acetamido-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)methyl]-1*H*-1,2,3-triazol-1-yl}-propyl}}-25,26,27,28-tetrapropoxy-calix[4]arene. ^{1}H NMR (300 MHz, CD_3OD) selected data: δ 7.92 (s, 4H, 4 H-5 Tr.), 6.56 (s, 8H, Ar), 4.44 and 3.11 (2d, 8H, J = 12.8 Hz, 4 $ArCH_2Ar$), 4.21 (t, 8H, J = 6.8 Hz, 4 $ArCH_2CH_2CH_2$), 4.13 and 4.01 (2d, 8H, J = 14.1 Hz, 4 SCH_2), 3.79 (s, 12H, 4 OMe), 2.79 (dd, 4H, $J_{3eq,4}$ = 4.5, $J_{3ax,3eq}$ = 12.7 Hz, 4 H-3eq), 2.02 (s, 12H, 4 Ac), 1.84 (dd, 4H, $J_{3ax,4}$ = 11.3 Hz, 4 H-3ax), 1.04 (t, 12H, J = 7.4 Hz, 4 $CH_3CH_2CH_2O$). A solution of the methyl ester tetra-adduct in 0.2 M aqueous NaOH (2 cm^3) was kept at room temperature for 18 h in a nitrogen atmosphere, then neutralized with Dowex 50 × 2–400 resin, and filtered through a sintered glass filter. The resin was washed with H_2O, and the solution was concentrated. The residue was eluted from a C18 silica gel cartridge with 1:1 H_2O-MeOH, then MeOH, and dried under high vacuum to give **13** (36 mg, 76%) as an amorphous solid; $[\alpha]_D$ = +24.7 (*c* 0.7, MeOH). ^{1}H NMR (300 MHz, CD_3OD) selected data: δ 7.90 (s, 4H, 4 H-5 Tr.), 6.55 (s, 8H, Ar), 4.44 and 3.11 (2d, 8H, J = 12.8 Hz, 4 $ArCH_2Ar$), 4.20 (t, 8H, J = 7.0 Hz, 4 $ArCH_2CH_2CH_2$), 4.15 and 4.02 (2d, 8H, J = 14.0 Hz, 4 SCH_2), 2.82 (dd, 4H, $J_{3eq,4}$ = 3.6, $J_{3ax,3eq}$ = 12.5 Hz, 4 H-3eq), 2.03 (s, 12H, 4 Ac), 1.84 (dd, 4H, $J_{3ax,4}$ = 10.5 Hz, 4 H-3ax), 0.94 (t, 12H, J = 7.5 Hz, 4 $CH_3CH_2CH_2O$). ^{13}C NMR (75 MHz, CD_3OD): δ 175.3 (C), 173.2 (C), 156.1 (C), 145.4 (C), 136.1 (C), 135.0 (C), 129.5 (CH), 124.9 (CH), 84.4 (C), 78.0 (CH_2), 77.2 (CH), 72.7 (CH), 70.2 (CH), 69.2

(CH), 64.5 (CH_2), 53.7 (CH), 50.7 (CH_2), 42.1 (CH_2), 33.0 (CH_2), 32.8 (CH_2), 31.8 (CH_2), 24.5 (CH_2), 22.7 (CH_3), 10.9 (CH_3). ESI MS (2378.71): 1190.5 (M + 2)/2, 793.9 (M + 3)/3. Anal. Calcd. for $C_{108}H_{152}N_{16}O_{36}S_4$: C, 54.53; H, 6.44; N, 9.42. Found: C, 54.38; H, 6.51; N, 9.33.

5,11,17,23-Tetrakis(3-azidomethyl)-25,26,27,28-tetrapropoxy-calix[4]arene (15)

A mixture of calixarene tetrol **14** (285 mg, 0.40 mmol), sodium azide (208 mg, 3.20 mmol), diphenyl phosphoryl azide (520 µL, 2.40 mmol), 1,8-diazabicyclo[5.4.0.]undec-7-ene (240 µL, 1.60 mmol), and anhydrous DMF (4 cm^3) was stirred at 120 °C for 15 h then partially concentrated, diluted with Et_2O (100 cm^3), washed with H_2O (2 × 10 cm^3), dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel with 15:1 cyclohexane-AcOEt to give **15** (179 mg, 55%) as a white solid. Mp 149–150 °C (cyclohexane). ^{1}H NMR (300 MHz): δ 6.64 (s, 8H, Ar), 4.48 and 3.18 (2d, 8H, $J = 13.4$ Hz, 4 ArCH_2Ar), 3.98 (s, 8H, 4 CH_2N_3), 3.88 (t, 8H, $J = 7.5$ Hz, 4 $CH_3CH_2CH_2O$), 1.96 (tq, 8H, $J = 7.5$, 7.5 Hz, 4 $CH_3CH_2CH_2O$), 1.02 (t, 12H, $J = 7.5$ Hz, 4 $CH_3CH_2CH_2O$). ^{13}C NMR (75 MHz): δ 156.5 (C), 135.2 (C), 128.6 (C), 128.5 (CH), 76.9 (CH_2), 54.2 (CH_2), 30.8 (CH_2), 23.2 (CH_2), 10.2 (CH_3); MALDI-TOF MS (812.96): 835.8 (M^+ + Na), 851.8 (M^+ + K). Anal. Calcd. for $C_{44}H_{52}N_{12}O_4$: C, 65.01; H, 6.45; N, 20.68. Found: C, 65.12; H, 6.52; N, 20.81.

5,11,17,23-Tetrakis{{4-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyrano-sylonate)methyl]-1*H*-1,2,3-triazol-1-yl}methyl}-25,26,27,28-tetra-propoxy-calix[4]arene (16)

The cycloaddition between the tetra-azide **15** (24 mg, 0.03 mmol) and propargyl thiosialoside **10** (79 mg, 0.14 mmol) was carried out as described for the preparation of **12** to give, after acetylation of the crude mixture and column chromatography on silica gel (acetone), **16** (68 mg, 75%) as a syrup; $[\alpha]_D = +45.5$ (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz): δ 7.40 (s, 4H, 4 H-5 Tr.), 6.58 (bs, 8H, Ar), 5.45 (ddd, 4H, $J_{7,8} = 8.5$, $J_{8,9a} = 2.6$, $J_{8,9b} = 5.3$ Hz, 4 H-8), 5.33 (dd, 4H, $J_{6,7} = 2.3$ Hz, 4 H-7), 5.32 (d, 4H, $J_{5,NH} = 10.0$ Hz, 4 NH), 5.23 and 5.18 (2d, 8H, $J = 14.6$ Hz, 4 ArCH_2N), 4.89 (ddd, 4H, $J_{3ax,4} = 11.2$, $J_{3eq,4} = 4.7$, $J_{4,5} = 10.5$ Hz, 4 H-4), 4.40 and 3.09 (2d, 8H, $J = 13.6$ Hz, 4 ArCH_2Ar), 4.29 (dd, 4H, $J_{9a,9b} = 12.5$ Hz, 4 H-9a), 4.08 (dd, 4H, 4 H-9b), 4.06 (ddd, 4H, $J_{5,6} = 10.7$ Hz, 4 H-5), 4.00 and 3.94 (2d, 8H, $J = 14.2$ Hz, 4 SCH_2), 3.88 (dd, 4H, 4 H-6), 3.81 (t, 8H, $J = 7.5$ Hz, 4 $CH_3CH_2CH_2O$), 3.57 (s, 12H, 4 OMe), 2.70 (dd, 4H, $J_{3ax,3eq} = 12.6$ Hz, 4 H-3eq), 2.16, 2.14, 2.02, 2.01, and 1.88 (5 s, 60H, 20 Ac), 1.96 (dd, 4H, 4 H-3ax), 1.89 (tq, 8H, $J = 7.4$, 7.5 Hz, 4 $CH_3CH_2CH_2O$), 0.98 (t, 12H, $J = 7.4$ Hz, 4 $CH_3CH_2CH_2O$). ^{13}C NMR (75 MHz): δ 170.8, 170.7, 170.2, 170.1, and 168.2 (CO), 156.9 (C Ar), 143.2 (C-4 Tr.), 135.4 (C Ar), 128.3 (CH Ar), 128.2 (C Ar), 122.4 (C-5 Tr.), 82.9 (C-2), 77.0 ($CH_3CH_2CH_2O$), 74.0 (C-6), 69.4 (C-4), 68.2 (C-8), 67.2 (C-7), 62.3 (C-9), 53.8 (ArCH_2N), 52.9 (OMe), 49.3 (C-5), 37.6 (C-3), 30.8 (ArCH_2Ar), 23.4 (SCH_2), 23.15, 21.3, 20.80, and 20.76 (CH_3CO), 23.10 ($CH_3CH_2CH_2O$), 10.2 ($CH_3CH_2CH_2O$); MALDI-TOF MS (2995.19): 3018.7 (M^+ + Na), 3034.7 (M^+ + K). Anal. Calcd. for $C_{136}H_{176}N_{16}O_{52}S_4$: C, 54.54; H, 5.92; N, 7.48. Found: C, 54.73; H, 6.01; N, 7.62.

5,11,17,23-Tetrakis{{4-[(5-acetamido-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)methyl]-1*H*-1,2,3-triazol-1-yl}methyl}-25,26,27,28-tetrapropoxy-calix[4]arene (17)

The sialocluster **16** (30 mg, 0.01 mmol) was deacetylated and demethylated as described for the preparation of **13**. The crude product was eluted from a C18 silica gel cartridge with MeOH, concentrated, and dried under high vacuum to give **17** (18 mg, 80%) as an amorphous solid; $[\alpha]_D = +16.0$ (*c* 0.5, MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ 7.86 (s, 4H, 4 H-5 Tr.), 6.69 (s, 8H, Ar), 5.32 (s, 8H, 4 ArCH_2N), 4.45 and 3.20 (2d, 8H, $J = 13.3$ Hz, 4 ArCH_2Ar), 4.15 and 4.05 (2d, 8H, $J = 14.5$ Hz, 4 SCH_2), 3.87 (t, 8H, $J = 7.5$ Hz, 4 $CH_3CH_2CH_2O$), 3.81–3.72 (m, 16H), 3.67–3.59 (m, 4H), 3.58–3.50 (m, 8H), 2.86 (bd, 4H, $J_{3ax,3eq} = 12.0$ Hz, 4 H-3eq), 2.03 (s, 12H, 4 Ac), 1.95 (tq, 8H, $J = 7.5$, 7.5 Hz, 4 $CH_3CH_2CH_2O$), 1.76–1.68 (m, 4H, 4 H-3ax), 1.02 (t, 12H, $J = 7.5$ Hz, 4 $CH_3CH_2CH_2O$). ^{13}C NMR (75 MHz, CD_3OD): δ 175.4 (C), 174.3 (C), 158.0 (C), 146.4 (C), 136.7 (C), 130.2 (C), 129.5 (CH), 124.8 (CH), 86.1 (C), 78.1 (CH_2), 76.9 (CH), 70.3 (CH), 69.5 (CH), 64.5 (CH_2), 54.9 (CH_2), 53.9 (CH), 42.5 (CH_2), 31.7 (CH_2), 24.6 (CH_2), 24.4 (CH_2), 22.7 (CH_3), 10.7 (CH_3). ESI MS (2266.50): 1134.4 (M + 2)/2, 756.7 (M + 3)/3. Anal. Calcd. for $C_{100}H_{136}N_{16}O_{36}S_4$: C, 52.99; H, 6.05; N, 9.89. Found: C, 52.88; H, 6.11; N, 9.80.

25,26,27,28-Tetrakis(3-hydroxypropoxy)-calix[4]arene (19)

To a cooled (0 °C), stirred solution of **18** (1.17 g, 2.0 mmol) in anhydrous THF (10 cm^3) was added dropwise 9-boracyclo[3.3.1]nonane (48 cm^3, 24.0 mmol, of a 0.5 M solution in hexane). The solution was allowed to reach room temperature in 1.5 h, then cooled to 0 °C and slowly diluted with 10 M NaOH (1.5 cm^3) and 35% H_2O_2 (3.8 cm^3). The mixture was stirred at room temperature for 30 min and then warmed to 60 °C. Stirring was continued for an additional 1.5 h, then the mixture was cooled to room temperature, diluted with 1 M phosphate buffer at pH 7 (50 cm^3), concentrated to remove the organic solvents, and extracted with CH_2Cl_2 (2 × 100 cm^3). The combined organic phases were dried (Na_2SO_4) and concentrated. The residue was eluted from a short column (5 × 5 cm) of silica gel with AcOEt, then 1:1 AcOEt-acetone, acetone, and 9:1 acetone-MeOH to give **19** (0.96 g, 73%) as a white solid. Mp 256–257 °C (Acetone). ^{1}H NMR (300 MHz, $CDCl_3$ + D_2O): δ 6.67–6.58 (m, 12H, Ar), 4.42 and 3.23 (2d, 8H, $J = 13.5$ Hz, 4 ArCH_2Ar), 4.08 (t, 8H, $J = 7.0$ Hz, 4 $HOCH_2CH_2CH_2O$), 3.90 (t, 8H, $J = 6.3$ Hz, 4 $HOCH_2CH_2CH_2O$), 2.20 (s, 3H, 0.5 $(CH_3)_2CO$), 2.18 (tt, 8H, $J = 6.3$, 7.0 Hz, 4 $HOCH_2CH_2CH_2O$). ^{13}C NMR (75 MHz): δ 155.8 (C Ar), 134.9 (C Ar), 128.3 (CH Ar), 122.3 (CH Ar), 72.4 ($HOCH_2CH_2CH_2O$), 60.2 ($HOCH_2CH_2CH_2O$), 32.9 ($HOCH_2CH_2CH_2O$), 30.9 (ArCH_2Ar); MALDI-TOF MS (656.80): 679.8 (M^+ + Na), 695.8 (M^+ + K). Anal. Calcd. for $C_{40}H_{48}O_8 \cdot 0.5(CH_3)_2CO$ C, 72.68; H, 7.49. Found: C, 72.76; H, 7.54.

25,26,27,28-Tetrakis(3-azidopropoxy)-calix[4]arene (20)

The calixarene tetrol **19** (328 mg, 0.50 mmol) was azidated as described for the preparation of **15** to give, after column chromatography on silica gel (3:1 CH_2Cl_2-cyclohexane), **20** (253 mg, 67%) as a white solid. Mp 108–110 °C (MeOH). ^{1}H NMR (300 MHz): δ 6.63 (s, 12H, Ar), 4.36 and 3.24 (2d, 8H, $J = 13.5$ Hz,

4 ArCH_2Ar), 4.00 (t, 8H, $J = 7.0$ Hz, 4 $N_3CH_2CH_2CH_2O$), 3.55 (t, 8H, $J = 6.7$ Hz, 4 $N_3CH_2CH_2CH_2O$), 2.18 (tt, 8H, $J = 6.7$, 7.0 Hz, 4 $N_3CH_2CH_2CH_2O$). ^{13}C NMR (75 MHz): δ 155.8 (C), 134.7 (C), 128.4 (CH), 122.6 (CH), 71.8 (CH_2), 48.7 (CH_2), 30.9 (CH_2), 29.5 (CH_2); MALDI-TOF MS (756.86): 780.0 (M^+ + Na). Anal. Calcd. for $C_{40}H_{44}N_{12}O_4$: C, 63.48; H, 5.86; N, 22.21. Found: C, 63.61; H, 5.94; N, 22.30.

25,26,27,28-Tetrakis{3-{4-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)methyl]-1*H*-1,2,3-triazol-1-yl}propoxy}-calix[4]arene (21)

The cycloaddition between the tetra-azide **20** (76 mg, 0.10 mmol) and propargyl thiosialoside **10** (262 mg, 0.48 mmol) was carried out as described for the preparation of **12** to give, after acetylation of the crude mixture and column chromatography on silica gel (2:1 acetone-AcOEt, then acetone), **21** (156 mg, 53%) as a white solid. Mp 144–146 (dec.) (AcOEt-cyclohexane); $[\alpha]_D = +41.0$ (*c* 1.5, $CHCl_3$). ^{1}H NMR (400 MHz): δ 7.55 (s, 4H, 4 H-5 Tr.), 6.58 (s, 12H, Ar), 5.44 (ddd, 4H, $J_{7,8} = 8.7$, $J_{8,9a} = 2.9$, $J_{8,9b} = 5.3$ Hz, 4 H-8), 5.35 (d, 4H, $J_{5,NH} = 10.0$ Hz, 4 NH), 5.34 (dd, 4H, $J_{6,7} = 2.3$ Hz, 4 H-7), 4.87 (ddd, 4H, $J_{3ax,4} = 11.5$, $J_{3eq,4} = 4.5$, $J_{4,5} = 10.6$ Hz, 4 H-4), 4.50 (t, 8H, $J = 6.8$ Hz, 4 $CH_2CH_2CH_2O$), 4.30 (dd, 4H, $J_{9a,9b} =$ 12.5 Hz, 4 H-9a), 4.30 and 3.15 (2d, 8H, $J = 13.6$ Hz, 4 ArCH_2Ar), 4.09 (dd, 4H, 4 H-9b), 4.07 (ddd, 4H, $J_{5,6} = 10.8$ Hz, 4 H-5), 4.02 and 3.94 (2d, 8H, $J = 14.2$ Hz, 4 SCH_2), 3.93 (t, 8H, $J = 7.0$ Hz, 4 $CH_2CH_2CH_2O$), 3.86 (dd, 4H, 4 H-6), 3.68 (s, 12H, 4 OMe), 2.72 (dd, 4H, $J_{3ax,3eq} = 12.8$ Hz, 4 H-3eq), 2.42 (tt, 8H, $J = 6.8$, 7.0 Hz, 4 $CH_2CH_2CH_2O$), 2.16, 2.14, 2.02, 2.01, and 1.88 (5 s, 60H, 20 Ac), 1.99 (dd, 4H, 4 H-3ax). ^{13}C NMR (100 MHz): δ 170.8, 170.3, 170.1, and 168.2 (CO), 155.7 (C Ar), 143.2 (C-4 Tr.), 134.6 (C Ar), 128.5 (CH Ar), 122.9 (CH Ar), 122.6 (C-5 Tr.), 82.9 (C-2), 74.0 (C-6), 71.3 ($CH_2CH_2CH_2O$), 69.5 (C-4), 68.2 (C-8), 67.2 (C-7), 62.3 (C-9), 53.1 (OMe), 49.2 (C-5), 47.4 ($CH_2CH_2CH_2O$), 37.6 (C-3), 30.9 (ArCH_2Ar, $CH_2CH_2CH_2O$), 23.3 (SCH_2, $CH_3CH_2CH_2O$), 23.2, 21.3, and 20.8 (CH_3CO); MALDI-TOF MS (2939.09): 2961.1 (M^+ + Na), 2977.1 (M^+ + K). Anal. Calcd. for $C_{132}H_{168}N_{16}O_{52}S_4$: C, 53.94; H, 5.76; N, 7.63. Found: C, 54.08; H, 5.82; N, 7.80.

25,26,27,28-Tetrakis{3-{4-[(5-acetamido-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)methyl]-1*H*-1,2,3-triazol-1-yl}propoxy}-calix[4]arene (22)

The sialocluster **21** (59 mg, 0.02 mmol) was deacetylated and demethylated as described for the preparation of **13**. The crude product was eluted from a C18 silica gel cartridge with 1:1 H_2O-MeOH, then MeOH, concentrated, and dried under high vacuum to give **22** (22 mg, 50%) as a white solid. Mp 192–194 (dec.) (AcOEt); $[\alpha]_D = +28.9$ (*c* 0.9, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 7.95 (s, 4H, 4 H-5 Tr.), 6.63–6.55 (m, 12H, Ar), 4.58 (t, 8H, $J = 6.7$ Hz, 4 $CH_2CH_2CH_2O$), 4.36 and 3.15 (2d, 8H, $J = 13.6$ Hz, 4 ArCH_2Ar), 4.12 and 4.01 (2d, 8H, $J = 14.5$ Hz, 4 SCH_2), 3.92 (t, 8H, $J = 6.6$ Hz, 4 $CH_2CH_2CH_2O$), 3.84–3.71 (m, 16H), 3.61 (dd, 4H, $J_{8,9b} = 5.4$, $J_{9a,9b} = 11.3$ Hz, 4 H-9b), 3.54–3.48 (m, 8H), 2.79 (dd, 4H, $J_{3eq,4} = 4.5$, $J_{3ax,3eq} = 12.8$ Hz, 4 H-3eq), 2.47 (tt, 8H, $J = 6.6$, 6.7 Hz, 4 $CH_2CH_2CH_2O$), 2.00 (s, 12H, 4 Ac), 1.79 (dd, 4H, $J_{3ax,4} = 10.8$, 4 H-3ax). ^{13}C NMR (75 MHz, CD_3OD): δ 175.2 (C), 173.0 (C), 157.1 (C), 145.6 (C), 136.1 (C), 129.6 (CH), 125.2 (CH), 123.6 (CH), 84.2 (C), 77.2 (CH), 72.7 (CH), 72.7 (CH_2), 70.2 (CH), 69.1 (CH), 64.5 (CH_2), 53.7 (CH), 48.9 (CH_2), 42.0 (CH_2), 32.0 (CH_2), 24.5 (CH_2), 22.7 (CH_3). ESI MS (2210.39): 1106.2 (M + 2)/2, 738.0 (M + 3)/3. Anal. Calcd. for $C_{96}H_{128}N_{16}O_{36}S_4$: C, 52.16; H, 5.84; N, 10.14. Found: C, 52.10; H, 5.89; N, 10.03.

5,11,17,23-Tetra-allyl-25,26,27,28-allyloxy-calix[4]arene (24)

To a cooled (0 °C), stirred solution of tetrol **23** (1.05 g, 1.8 mmol) in DMF (18 cm^3) was added NaH (0.43 g, 10.8 mmol, of a 60% dispersion in oil) and, after 15 min, allyl bromide (0.75 cm^3, 8.6 mmol). The mixture was stirred at room temperature for 2 h, then diluted with CH_3OH (1 cm^3) and, after 10 min, diluted with 1 M phosphate buffer at pH 7 (50 cm^3) and extracted with Et_2O (2 × 100 cm^3). The combined organic phases were dried (Na_2SO_4) and concentrated. The residue was eluted from a column of silica gel with 2:1 cyclohexane-CH_2Cl_2 to give **24** (1.31 g, 98%) as a syrup. ^{1}H NMR (300 MHz): δ 6.50 (s, 8H, Ar), 6.38 (ddt, 4H, $J = 6.4$, 10.3, 17.2 Hz, 4 $CH_2{=}CHCH_2O$), 5.82 (ddt, 4H, $J =$ 6.5, 10.2, 17.0 Hz, 4 $ArCH_2CH{=}CH_2$), 5.27 (ddt, 4H, $J = 1.5$, 1.5, 17.2 Hz, H*cis* of 4 $CH_2{=}CHCH_2O$), 5.19 (ddt, 4H, $J = 1.1$, 1.5, 10.3 Hz, H*trans* of 4 $CH_2{=}CHCH_2O$), 4.98 (ddt, 4H, $J =$ 1.5, 1.8, 10.2 Hz, H*trans* of 4 $ArCH_2CH{=}CH_2$), 4.90 (ddt, 4H, $J = 1.8$, 1.8, 17.0 Hz, H*cis* of 4 $ArCH_2CH{=}CH_2$), 4.46 (ddd, 8H, $J = 1.1$, 1.5, 6.4 Hz, 4 $CH_2{=}CHCH_2O$), 4.38 and 3.10 (2d, 8H, $J = 13.0$ Hz, 4 ArCH_2Ar), 3.09 (ddd, 8H, $J = 1.5$, 1.8, 6.5 Hz, 4 $ArCH_2CH{=}CH_2$). ^{13}C NMR (75 MHz): δ 154.1 (C), 138.2 (CH), 135.9 (CH), 134.9 (C), 133.2 (C), 128.3 (CH), 116.7 (CH_2), 114.9 (CH_2), 75.9 (CH_2), 39.4 (CH_2), 31.3 (CH_2), 26.9 (CH_2). MALDI-TOF MS (745.00): 767.9 (M^+ + Na), 783.9 (M^+ + K). Anal. Calcd. for $C_{52}H_{56}O_4$: C, 83.83; H, 7.58. Found: C, 84.02; H, 7.70.

5,11,17,23-Tetrakis(3-hydroxypropyl)-25,26,27,28-tetrakis(3-hydroxypropoxy)-calix[4]arene (25)

The octa-allyl-calixarene **24** (372 mg, 0.50 mmol) was submitted to the hydroboration-oxidation as described for the preparation of **19**. The crude mixture was triturated with cyclohexane, then Et_2O, and finally with AcOEt to give **25** (386 mg, 87%) as a white solid. Mp. 213–215 °C (Acetone). ^{1}H NMR (300 MHz, DMSO-d_6 + D_2O): δ 6.47 (s, 8H, Ar), 4.28 and 3.05 (2d, 8H, $J = 12.7$ Hz, 4 ArCH_2Ar), 3.86 (t, 8H, $J = 7.3$ Hz, 4 $HOCH_2CH_2CH_2O$), 3.57 (t, 8H, $J = 6.3$ Hz, 4 $HOCH_2CH_2CH_2O$), 3.30 (t, 8H, $J = 6.3$ Hz, 4 $ArCH_2CH_2CH_2OH$), 2.24 (t, 8H, $J =$ 7.5 Hz, 4 $ArCH_2CH_2CH_2OH$), 2.03 (tt, 8H, $J = 6.3$, 7.3 Hz, 4 $HOCH_2CH_2CH_2O$), 1.48 (tt, 8H, $J = 6.3$, 7.5 Hz, 4 $ArCH_2CH_2CH_2OH$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 154.0 (C), 134.9 (C), 134.0 (C), 127.7 (CH), 72.3 (CH_2), 60.0 (CH_2), 58.1 (CH_2), 34.3 (CH_2), 33.0 (CH_2), 31.0 (CH_2). MALDI-TOF MS (889.12): 912.1 (M^+ + Na). Anal. Calcd. for $C_{52}H_{72}O_{12}$: C, 70.24; H, 8.16. Found: C, 70.40; H, 8.27.

5,11,17,23-Tetrakis(3-azidopropyl)-25,26,27,28-tetrakis(3-azidopropoxy)-calix[4]arene (26)

The calixarene octaol **25** (178 mg, 0.20 mmol) was azidated as described for the preparation of **15** to give, after column chromatography on Sephadex LH-20 (CH_2Cl_2), **26** (113 mg, 52%) as a dark yellow syrup. An analytical sample was obtained by

chromatography on preparative TLC (silica gel 60, 0.5 mm, 3.5:1 CH_2Cl_2-cyclohexane). ^1H NMR (300 MHz): δ 6.48 (s, 8H, Ar), 4.30 and 3.15 (2d, 8H, J = 13.4 Hz, 4 ArCH_2Ar), 3.97 (t, 8H, J = 7.2 Hz, 4 $CH_2CH_2CH_2O$), 3.54 (t, 8H, J = 6.7 Hz, 4 $ArCH_2CH_2CH_2$), 3.19 (t, 8H, J = 6.8 Hz, 4 $CH_2CH_2CH_2O$), 2.40 (t, 8H, J = 7.5 Hz, 4 $ArCH_2CH_2CH_2$), 2.19 (tt, 8H, J = 6.8, 7.2 Hz, 4 $CH_2CH_2CH_2O$), 1.73 (tt, 8H, J = 6.7, 7.5 Hz, 4 $ArCH_2CH_2CH_2$). ^{13}C NMR (75 MHz): δ 154.0 (C Ar), 134.6 (C Ar), 134.4 (C Ar), 128.3 (CH Ar), 72.0 ($CH_2CH_2CH_2O$), 50.5 ($CH_2CH_2CH_2O$), 48.7 ($ArCH_2CH_2CH_2$), 32.0 ($ArCH_2CH_2CH_2$), 30.9 ($ArCH_2Ar$), 30.5 ($ArCH_2CH_2CH_2$), 29.5 ($CH_2CH_2CH_2O$). MALDI-TOF MS (1089.22): 1112.4 (M^+ + Na). Anal. Calcd. for $C_{52}H_{64}N_{24}O_4$: C, 57.34; H, 5.92; N, 30.86. Found: C, 57.56; H, 6.08; N, 31.01.

5,11,17,23-Tetrakis{3-{4-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)methyl]-1*H*-1,2,3-triazol-1-yl}propyl}-25,26,27,28-tetrakis{3-{4-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)methyl]-1*H*-1,2,3-triazol-1-yl}propoxy}-calix[4]arene (27)

The cycloaddition between the octa-azide **26** (44 mg, 0.04 mmol) and propargyl thiosialoside **10** (209 mg, 0.38 mmol) was carried out as described for the preparation of **12**. The crude mixture was acetylated (1:1 pyridine-acetic anhydride) at room temperature for 3 h and concentrated. A solution of the residue in AcOEt (40 cm^3) was washed with phosphate buffer at pH 7 (10 cm^3) and water (10 cm^3), then concentrated. The residue was eluted from a column of Sephadex LH-20 with 1:1 CH_2Cl_2-MeOH to give **27** (157 mg, 72%) as a light brown solid. In order to obtain an analytical sample, compound **27** was deacetylated under basic conditions (MeOH, Dowex 1 × 8 resin, r.t., 48 h), eluted from a C18 silica gel cartridge with MeOH, and acetylated (1:1 pyridine-Ac_2O, r.t., 14 h). A solution of the residue in CH_2Cl_2 was washed with water and concentrated to give **27** as a colorless amorphous solid; $[\alpha]_D$ = +43.8 (c 0.5, $CHCl_3$). ^1H NMR (300 MHz, DMSO-d_6, 120 °C) selected data: δ 7.84 (s, 4H, 4 H-5 Tr.), 7.79 (s, 4H, 4 H-5 Tr.), 7.38 (bd, 8H, $J_{5,NH}$ = 9.0 Hz, 8 NH), 6.53 (s, 8H, Ar), 5.37–5.30 (m, 8H, 8 H-8), 5.21 (bd, 8H, $J_{7,8}$ = 7.0 Hz, 8 H-7), 4.82 (ddd, 8H, $J_{3ax,4}$ = 11.0, $J_{3eq,4}$ = 4.5, $J_{4,5}$ = 10.0 Hz, 8 H-4), 4.50 (t, 8H, J = 6.8 Hz, 4 $CH_2CH_2CH_2O$), 4.20 (t, 8H, J = 6.6 Hz, 4 $ArCH_2CH_2CH_2$), 3.77 (s, 12H, 4 OMe), 3.75 (s, 12H, 4 OMe), 3.12 (d, 4H, J = 13.0 Hz, 4 Heq of 4 $ArCH_2Ar$), 2.71 (dd, 8H, $J_{3ax,3eq}$ = 12.8 Hz, 8 H-3eq), 2.08 and 2.07 (2 s, 24H, 8 Ac), 2.04 (s, 24H, 8 Ac), 1.96 and 1.95 (2 s, 24H, 8 Ac), 1.94 (s, 24H, 8 Ac), 1.84 (dd, 8H, 8 H-3ax), 1.72 (s, 24H, 8 Ac). ^{13}C NMR (75 MHz, DMSO-d_6) selected data: δ 170.0, 169.6, 169.4, 169.2, 169.0, and 168.0 (CO), 153.7 (C Ar), 141.8 (C-4 Tr.), 141.7 (C-4 Tr.), 134.0 (C Ar), 128.0 (CH Ar), 123.2 (C-5 Tr.), 123.0 (C-5 Tr.), 82.6 (C-2), 73.5 (C-6), 69.4 (C-4), 67.5 (C-8), 66.9 (C-7), 61.8 (C-9), 53.0 (OMe), 47.6 (C-5), 37.2 (C-3), 22.5, 20.9, and 20.5 (CH_3CO); MALDI-TOF MS (5453.69): 5477.8 (M^+ + H + Na), 5492.7 (M^+ + K). Anal. Calcd. for $C_{236}H_{312}N_{32}O_{100}S_8$: C, 51.97; H, 5.77; N, 8.22. Found: C, 52.26; H, 5.89; N, 8.40.

5,11,17,23-Tetrakis{3-{4-[(5-acetamido-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)methyl]-1*H*-1,2,3-triazol-1-yl}propyl}-25,26,27,28-tetrakis{3-{4-[(5-acetamido-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)methyl]-1*H*-1,2,3-triazol-1-yl}propoxy}-calix[4]arene (28)

A solution of **27** (54 mg, 0.01 mmol) in a 0.2 M solution of NaOMe in MeOH (3 cm^3, prepared from Na and MeOH immediately before the use) was stirred at room temperature in a nitrogen atmosphere. After 1.5 h the solution turned turbid, therefore was diluted with H_2O (ca. 1 cm^3) and stirred for an additional 2 h, then neutralized with Dowex 50 × 2–400 resin (H^+ form, activated and washed with H_2O and MeOH immediately before the use), and filtered through a sintered glass filter. The resin was washed with H_2O and MeOH, and the solution was concentrated. A solution of the methyl ester octa-adduct in 0.2 M aqueous NaOH (3 cm^3) was kept at room temperature for 24 h in a nitrogen atmosphere, then neutralized with Dowex 50 × 2–400 resin, and filtered through a sintered glass filter. The resin was washed with H_2O, and the solution was concentrated. The residue was eluted from a C18 silica gel cartridge with H_2O-MeOH (from 2:1 to 1:1), then MeOH, and dried under high vacuum to give **28** (24 mg, 61%) an amorphous solid; $[\alpha]_D$ = +6.7 (c 0.2, H_2O). ^1H NMR (300 MHz, DMSO-d_6 + D_2O, 120 °C) selected data: δ 7.88 (bs, 4H, 4 H-5 Tr.), 7.81 (bs, 4H, 4 H-5 Tr.), 6.45 (s, 8H, Ar), 1.90 (s, 24H, 8 Ac). ^{13}C NMR (75 MHz, DMSO-d_6 + D_2O) selected data: δ 173.5 (C), 172.0 (C), 144.9 (C), 134.8 (C), 128.7 (CH), 124.0 (CH), 85.8 (C), 75.5 (CH), 71.9 (CH), 69.3 (CH), 68.1 (CH), 63.4 (CH_2), 53.1 (CH), 49.5 (CH_2), 47.5 (CH_2), 42.2 (CH_2), 31.7 (CH_2), 30.5 (CH_2), 23.9 (CH_2), 23.0 (CH_3). ESI MS (3996.30): 1332.9 (M + 3)/3, 1000.6 (M + 4)/4. Anal. Calcd. for $C_{164}H_{232}N_{32}O_{68}S_8$: C, 49.29; H, 5.85; N, 11.22. Found: C, 49.15; H, 5.92; N, 11.13.

1-Benzyl-4-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)methyl]-1*H*-1,2,3-triazole (29)

A mixture of benzyl bromide (150 μL, 1.26 mmol), sodium azide (246 mg, 3.78 mmol), and MeOH (2.5 cm^3) was stirred at room temperature for 16 h, then partially concentrated using a stream of nitrogen, diluted with H_2O (10 cm^3), and extracted with Et_2O (2 × 20 cm^3). The combined organic phases were dried (Na_2SO_4), the solvent was partially removed under vacuum, and the solution concentrated to dryness using a stream of nitrogen to give benzyl azide as a colorless oil, which was used in the next step without further purification. A mixture of crude benzyl azide (ca. 1.25 mmol), propargyl thiosialoside **10** (138 mg, 0.25 mmol), freshly distilled *N*,*N*-diisopropylethylamine (220 μL, 1.26 mmol), CuI (12 mg, 0.06 mmol), and anhydrous DMF (2.5 cm^3) was sonicated in an ultrasound cleaning bath for 1 min, then magnetically stirred in the dark at room temperature for 6 h, and concentrated. A solution of the residue in pyridine (1 cm^3) and acetic anhydride (1 cm^3) was kept at room temperature for 14 h, then concentrated. The residue was eluted from a column of silica gel with AcOEt to give **29** (134 mg, 78%) as a colorless syrup; $[\alpha]_D$ = +37.0 (c 1.3, $CHCl_3$). ^1H NMR (300 MHz): δ 7.48 (s, 1H, H-5 Tr.), 7.42–7.28 (m, 5H, Ar), 5.56 and 5.47 (2d, 2H, J = 14.8 Hz, PhCH_2), 5.45 (ddd, 1H, $J_{7,8}$ = 8.9, $J_{8,9a}$ = 2.7, $J_{8,9b}$ = 5.5 Hz, H-8), 5.32 (dd, 1H, $J_{6,7}$ = 2.1 Hz, H-7), 5.17 (d, 1H, $J_{5,NH}$ = 10.0 Hz, NH), 4.87 (ddd, 1H, $J_{3ax,4}$ = 11.6, $J_{3eq,4}$ = 4.5, $J_{4,5}$ = 10.6 Hz, H-4), 4.30 (dd, 1H, $J_{9a,9b}$ = 12.5 Hz, H-9a), 4.07 (dd, 1H, H-9b), 4.06 (ddd, 1H, $J_{5,6}$ = 10.8 Hz, H-5), 4.01 and 3.97 (2d, 2H,

This journal is © The Royal Society of Chemistry 2008

J = 13.5 Hz, SCH_2), 3.85 (dd, 1H, H-6), 3.58 (s, 3H, OMe), 2.72 (dd, 1H, $J_{3ax,3eq}$ = 12.7 Hz, H-3eq), 2.16, 2.14, 2.04, 2.02, and 1.89 (5 s, 15H, 5 Ac), 1.99 (dd, 1H, H-3ax). ^{13}C NMR (75 MHz): δ 170.8, 170.7, 170.2, 170.1, and 168.1 (CO), 143.9 (C-4 Tr.), 134.7 (C Ar), 129.0 (CH Ar), 128.7 (CH Ar), 128.1 (CH Ar), 122.4 (C-5 Tr.), 82.7 (C-2), 73.9 (C-6), 69.4 (C-4), 68.1 (C-8), 67.2 (C-7), 62.3 (C-9), 54.0 (PhCH_2), 52.8 (OMe), 49.3 (C-5), 37.6 (C-3), 23.14 (SCH_2), 23.10, 21.1, 20.8, and 20.7 (CH_3CO); MALDI-TOF MS (678.71): 701.2 (M^+ + Na), 717.8 (M^+ + K). Anal. Calcd. for $C_{30}H_{38}N_4O_{12}S$: C, 53.09; H, 5.64; N, 8.25. Found: C, 53.26; H, 5.77; N, 8.40.

1-Benzyl-4-[(5-acetamido-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)methyl]-1*H*-1,2,3-triazole (30)

The thioglycoside **29** (68 mg, 0.10 mmol) was deacetylated as described for the preparation of **13** to give crude 1-benzyl-4-[(methyl 5-acetamido-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)methyl]-1*H*-1,2,3-triazole (52 mg). ^{1}H NMR (300 MHz, D_2O) selected data: δ 7.78 (s, 1H, H-5 Tr.), 7.33–7.22 (m, 5H, Ar), 5.43 (s, 2H, PhCH_2), 3.95 and 3.80 (2d, 2H, J = 11.0 Hz, SCH_2), 3.24 (s, 3H, OMe), 2.62 (dd, 1H, $J_{3eq,4}$ = 4.6, $J_{3ax,3eq}$ = 12.9 Hz, H-3eq), 1.88 (s, 3H, Ac), 1.70 (dd, 1H, $J_{3ax,4}$ = 11.6 Hz, H-3ax). The crude methyl ester derivative was hydrolyzed as described for the preparation of **13** to give, after chromatography (C18 silica gel cartridge, 2:1 MeOH-H_2O) **30** (48 mg, 96%) as an amorphous solid; $[\alpha]_D$ = +1.8 (c 0.2, MeOH). ^{1}H NMR (300 MHz, D_2O) selected data: δ 7.78 (s, 1H, H-5 Tr.), 7.32–7.26 (m, 3H, Ar), 7.24–7.18 (m, 2H, Ar), 5.46 (s, 2H, PhCH_2), 3.88 and 3.78 (2d, 2H, J = 15.0 Hz, SCH_2), 3.66 (dd, 1H, $J_{4,5}$ = $J_{5,6}$ = 10.3 Hz, H-5), 3.52 (ddd, 1H, $J_{3ax,4}$ = 11.2, $J_{3eq,4}$ = 4.8 Hz, H-4), 2.64 (dd, 1H, $J_{3ax,3eq}$ = 12.8 Hz, H-3eq), 1.88 (s, 3H, Ac), 1.68 (dd, 1H, H-3ax). ^{13}C NMR (75 MHz, D_2O): δ 175.2 (C), 174.0 (C), 145.6 (C), 135.0 (C), 129.3 (CH), 128.9 (CH), 128.2 (CH), 124.4 (CH), 86.4 (C), 74.9 (CH), 71.9 (CH), 68.7 (CH), 68.1 (CH), 62.5 (CH_2), 54.0 (CH_2), 51.8 (CH), 40.8 (CH_2), 23.7 (CH_2), 22.2 (CH_3). Anal. Calcd. for $C_{21}H_{28}N_4O_8S$: C, 50.80; H, 5.68; N, 11.28. Found: C, 50.68; H, 5.75; N, 11.20.

Cells and viruses

Vero and MDCK cells were grown and propagated in Dulbecco's modified Eagle's minimal essential medium (DMEM) supplemented with 10% foetal calf serum (FCS) at 37 °C in a humidified atmosphere with 5% CO_2. Prototype BKV was grown in Vero cells as described previously.[61] BKV was titrated by hemagglutination of type 0 human erythrocytes[62] and by the fluorescent antibody (FA) focus assay in Vero cells.[63] Influenza A virus (H3N2 strain) was grown in MDCK cell monolayers and viral hemagglutination units (HAU) were determined as described previously.[64]

Hemagglutination and hemagglutination-inhibition assays

Hemagglutination titration was carried out in a plastic 96-well microplate. 25 μL of virus were diluted serially with 25 μL of phosphate-buffered saline at pH 7.4 (PBS) and to each dilution was added 50 μL of a 1% suspension of group 0 human red cells. After the addition of erythrocytes the microplate was kept at 4 °C for 5 h. The hemagglutination titer was calculated on the basis of the highest virus dilution that gave a complete hemagglutination.

For HI titrations, the compounds were diluted serially with 25 μL of PBS on a microtiter plate. A 25 μL of viral suspension containing eight hemagglutination doses were added to each well and the mixture was kept at 37 °C for 1 h. Then 50 μL of a 1% suspension of red cells in PBS were added to each well. The results were read after 5 h of incubation at 4 °C. The HI titer was defined as the maximum dilution of each compound that caused complete inhibition of viral hemagglutination.

Neutralisation assay of viral infectivity

Neutralisation of viral infectivity was carried out by incubating different amounts of each compound with 10–50 $TCID_{50}$ (50% tissue-culture infectious dose) of BKV or influenza A virus at 37 °C for 1 h. Then, the suspensions were added to Vero or MDCK monolayers and, after 2 h at 37 °C, the inoculum was removed, the monolayers were washed three times with DMEM, and then incubated in the medium containing 2% FCS. The cultures were observed by light microscope for the presence of viral cytopathic effects (CPE) for 8–10 days. As a positive control, two cultures were infected with the virus alone and as a negative control, two monolayers were inoculated with the virus pre-treated with specific antibodies. Then, when the positive controls developed CPE, the medium of each well was frozen awaiting determination of the presence of virions by hemagglutination.

Immunofluorescence

Viral coat protein antigens were detected by indirect immunofluorescence. Subconfluent cell monolayers, cultured on cover slips, were infected with BK or influenza A viruses, pre-treated or not with the sugar derivatives under examination. Four to six days after infection, cultures were fixed for 10 min in cold acetone, incubated at 37 °C for 30 min with specific antibodies anti-coat antigens, washed three times in PBS, and incubated at 37 °C for 30 min with fluorescein-conjugated goat anti-rabbit IgGs (Antibodies Incorporated, Davis, CA). After extensive washing in PBS, the preparations were mounted in buffered glycerol and observed with a fluorescence microscope.

Acknowledgements

The influenza A virus was kindly provided by Dr. R. Cusinato (Dipartimento di Microbiologia e Virologia, Azienda Ospedaliera, University of Padova, Italy). We are grateful to Codexis Inc. (USA) for supplying 5-*N*-acetyl-neuraminic acid. This work was financially supported by the University of Ferrara.

References

1 (*a*) For recent reviews, see: T. Angataa and A. Varki, *Chem. Rev.*, 2002, **102**, 439–469; (*b*) M. J. Kiefel and M. von Itzstein, *Chem. Rev.*, 2002, **102**, 471–490; (*c*) I. Hemeon and A. J. Bennet, *Synthesis*, 2007, 1899–1926.
2 (*a*) K. J. Yarema and C. R. Bertozzi, *Curr. Opin. Chem. Biol.*, 1998, **2**, 49–61; (*b*) C. A. Lingwood, *Curr. Opin. Chem. Biol.*, 1998, **2**, 695–700; (*c*) P. M. Lanctot, F. H. Gage and A. P. Varki, *Curr. Opin. Chem. Biol.*, 2007, **11**, 373–380.

3 (*a*) For reviews, see: A. S. Dugan, S. Eash and W. J. Atwood, *Transpl. Infect. Dis.*, 2006, **8**, 62–67; (*b*) P. Randhawa and D. C. Brennan, *Am. J. Transplant.*, 2006, **6**, 2000–2005.
4 (*a*) M. A. Israel, M. A. Martin, K. K. Takemoto, P. M. Howley, S. A. Aaronson, D. Solomon and G. Khoury, *Virology*, 1978, **90**, 187–196; (*b*) M. M. Pater, A. Pater, M. Fiori, J. Slota, and G. Di Mayorca, in *Viruses in Naturally Occurring Cancers*, eds. M. Essex, G. Todaro, and H. zur Hausen, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1980, pp 329-341.
5 J. Goudsmit, P. Wetheim-van Dillen, A. van Strien and J. von der Noordaa, *J. Med. Virol.*, 1982, **10**, 91–99.
6 (*a*) G. Bratt, A. L. Hammarin, M. Grandien, B. G. Hedquist, I. Nennesmo, B. Sundelin and S. Seregard, *AIDS*, 1999, **13**, 1071–1075; (*b*) O. Cubukcu-Dimopulo, A. Greco, A. Kumar, D. Karluk, K. Mittal and J. Jagirdar, *Am. J. Surg. Pathol.*, 2000, **24**, 145–149.
7 (*a*) V. Nickeleit, T. Klimkait, I. F. Binet, P. Dalquen, V. Del Zenero, G. Thiel, M. J. Mihatsch and H. H. Hirsch, *N. Engl. J. Med.*, 2000, **342**, 1309–1315; (*b*) M. D. Reploeg, G. A. Storch and D. B. Clifford, *Clin. Infect. Dis.*, 2001, **33**, 191–202.
8 A. Azzi, R. Fanci, A. Bosi, S. Ciappi, K. Zakrzewska, R. de Santis, D. Laszlo, S. Guidi, R. Saccardi and A. M. Vannucchi, *Bone Marrow Transplant.*, 1994, **14**, 235–240.
9 G. L. Stoner, R. Alappan, D. V. Jobes, C. F. Ryschkewitsch and M. L. Landry, *Am. J. Kidney Dis.*, 2002, **39**, 1102–1112.
10 A. Corallini, M. Tognon, M. Negrini, and G. Barbanti-Brodano, in *Human Polyomaviruses: Molecular and Clinical Perspectives*, eds. K. Khalili, and G. L. Stoner, Wiley-Liss Inc, New York, 2001, pp. 431-460.
11 A. Corallini, G. Altavilla, M. G. Cecchetti, G. Fabris, M. P. Grossi, P. G. Balboni, G. Lanza and G. Barbanti-Brodano, *J. Natl. Cancer Inst.*, 1978, **61**, 875–883.
12 L. Seganti, P. Mastromarino, F. Superti, L. Sinibaldi and N. Orsi, *Acta Virol.*, 1981, **25**, 177–181.
13 L. Sinibaldi, P. Goldoni, V. Pietropaolo, C. Longhi and N. Orsi, *Arch. Virol.*, 1990, **113**, 291–296.
14 (*a*) J. Gilbert, J. Dahl, C. Riney, J. You, C. Cui, R. Holmes, W. Lencer and T. Benjamin, *J. Virol.*, 2005, **79**, 615–618; (*b*) A. S. Dugan, S. Eash and W. J. Atwood, *J. Virol.*, 2005, **79**, 14442–14445; (*c*) J. A. Low, B. Magnuson, B. Tsai and M. J. Imperiale, *J. Virol.*, 2006, **80**, 1361–1366.
15 A. D. Hale, D. Bartkeviciute, A. Dargeviciute, L. Jin, W. Knowles, J. Staniulis, D. W. G. Brown and K. Sasnauskas, *J. Virol. Methods*, 2002, **104**, 93–98.
16 For a recent review, see: G. V. Gee, A. S. Dugan, N. Tsomaia, D. F. Mierke and W. J. Atwood, *Glycoconj. J.*, 2006, **23**, 19–26.
17 *N*-Modified sialic acids have been biosynthesized and directly incorporated into glycoconjugates of cultivated cells. In the presence of 5-*N*-propanoyl- and *N*-butanoyl-neuraminic acids the Vero cells were infected by BK virus more efficiently; on the other hand, when the 5-*N*-pentanoyl derivative was incorporated, the same cells became resistant to infection by BKV. See: O. T. Keppler, P. Stehling, M. Herrmann, H. Kayser, D. Grunow, W. Reutter and M. Pawlita, *J. Biol. Chem.*, 1995, **270**, 1308–1314.
18 (*a*) Reviews: M. C. Zambon, *Rev. Med. Virol.*, 2001, **11**, 227–241; (*b*) D. M. Morens and A. S. Fauci, *J. Infect. Dis.*, 2007, **195**, 1018–1028; (*c*) J. Gong, W. Xu and J. Zhang, *Curr. Med. Chem.*, 2007, **14**, 113–122.
19 (*a*) Reviews: G. A. Poland, R. M. Jacobson and P. V. Targonski, *Vaccine*, 2007, **25**, 3057–3061; (*b*) M. Gauthier-Clerc, C. Lebarbenchon and F. Thomas, *Ibis*, 2007, **149**, 202–214; (*c*) J. S. M. Peiris, M. D. de Jong and Y. Guan, *Clin. Microbiol. Rev.*, 2007, **20**, 243–267.
20 R. J. Russell, D. J. Stevens, L. F. Haire, S. J. Gamblin and J. J. Skehel, *Glycoconj. J.*, 2006, **23**, 85–92.
21 R. Wagner, M. Matrosovich and H.-D. Klenk, *Rev. Med. Virol.*, 2002, **12**, 159–166.
22 (*a*) Recent studies have revealed the involvement of additional, non-sialic, carbohydrate lectin recognition in both A and B influenza viruses. See: S. J. Stray, R. D. Cummings and G. M. Air, *Glycobiology*, 2000, **10**, 649–658; (*b*) E. M. Rapoport, L. V. Mochalova, H.-J. Gabius, J. Romanova and N. V. Bovin, *Glycoconj. J.*, 2006, **23**, 115–125.
23 (*a*) Reviews: E. De Clercq, *Nature Rev. Drug. Discov.*, 2006, **5**, 1015–1025; (*b*) Y. Liu, J. Zhang and W. Xu, *Curr. Med. Chem.*, 2007, **14**, 2872–2891.
24 (*a*) M. Matrosovich and H.-D. Klenk, *Rev. Med. Virol.*, 2003, **13**, 85–97; (*b*) J. Stevens and R. O. Donis, *Infect. Disord. Drug Targets*, 2007, **7**, 329–335.
25 For a recent review on anti-influenza agents other than NA and HA inhibitors, see: H.-P. Hsieh and J. T.-A. Hsu, *Curr. Pharm. Design*, 2007, **13**, 3531–3542.
26 E. G. Weinhold and J. R. Knowles, *J. Am. Chem. Soc.*, 1992, **114**, 9270–9275.
27 (*a*) A few multivalent zanamivir analogues have been synthesized. See: M. Chandler, R. Conroy, A. W. J. Cooper, R. B. Lamont, J. J. Scicinski, J. E. Smart, R. Storer, N. G. Weir, R. D. Wilson and P. G. Wyatt, *J. Chem. Soc., Perkin Trans. 1*, 1995, 1189–1197; (*b*) T. Honda, S. Yoshida, M. Arai, T. Masuda and M. Yamashita, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 1929–1932; (*c*) T. Masuda, S. Yoshida, M. Arai, S. Kaneko, M. Yamashita and T. Honda, *Chem. Pharm. Bull.*, 2003, **51**, 1386–1398; (*d*) K. G. Watson, R. Cameron, R. J. Fenton, D. Gower, S. Hamilton, B. Jin, G. Y. Krippner, A. Luttick, D. McConnell, S. J. F. MacDonald, A. M. Mason, V. Nguyen, S. P. Tucker and W.-Y. Wu, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 1589–1592; (*e*) Y. Lu and J. Gervay-Hague, *Carbohydr. Res.*, 2007, **342**, 1636–1650.
28 W. M. Kati, D. Montgomery, R. Carrick, L. Gubareva, C. Maring, K. McDaniel, K. Stefly, A. Molla, F. Hayden, D. Kempf and W. Kohlbrenner, *Antimicrob. Agents Chemother.*, 2002, **46**, 1014–1021.
29 For a recent review, see: X.-L. Sun, *Curr. Med. Chem.*, 2007, **14**, 2304–2313.
30 (*a*) For reviews dealing with biological and thermodynamic implications of multivalent interactions, see: M. Mammen, S.-K. Choi and G. M. Whitesides, *Angew. Chem., Int. Ed.*, 1998, **37**, 2754–2794; (*b*) J. J. Lundquist and E. J. Toone, *Chem. Rev.*, 2002, **102**, 555–578; (*c*) A. Mulder, J. Huskens and D. N. Reinhoudt, *Org. Biomol. Chem.*, 2004, **2**, 3409–3424; (*d*) J. D. Badji,'c, A. Nelson, S. J. Cantrill, W. B. Turnbull and J. F. Stoddart, *Acc. Chem. Res.*, 2005, **38**, 723–732; (*e*) M. Ambrosi, N. R. Cameron and B. G. Davis, *Org. Biomol. Chem.*, 2005, **3**, 1593–1608; (*f*) L. L. Kiessling, J. E. Gestwicki and L. E. Strong, *Angew. Chem., Int. Ed*, 2006, **45**, 2348–2368.
31 Y. C. Lee and R. T. Lee, *Acc. Chem. Res.*, 1995, **28**, 321–327.
32 (*a*) R. Roy, C. A. Laferrière, A. Gamian and H. J. Jennings, *J. Carbohydr. Chem.*, 1987, **6**, 161–167; (*b*) R. Roy and C. A. Laferrière, *Can. J. Chem.*, 1990, **68**, 2045–2054; (*c*) R. Roy and C. A. Laferrière, *J. Chem. Soc., Chem. Commun.*, 1990, 1709–1711.
33 (*a*) H. Kamitakahara, T. Suzuki, N. Nishigori, Y. Suzuki, O. Kanie and C.-H. Wong, *Angew. Chem., Int. Ed.*, 1998, **37**, 1524–1528; (*b*) K. Totani, T. Kubota, T. Kuroda, T. Murata, K. I. P. J. Hidari, T. Suzuki, Y. Suzuki, K. Kobayashi, H. Ashida, K. Yamamoto and T. Usui, *Glycobiology*, 2003, **13**, 315–326; (*c*) M. Ogata, T. Murata, K. Murakami, T. Suzuki, K. I. P. J. Hidari, Y. Suzuki and T. Usui, *Bioorg. Med. Chem.*, 2007, **15**, 1383–1393.
34 Y. Makimura, S. Watanabe, T. Suzuki, Y. Suzuki, H. Ishida, M. Kiso, T. Katayama, H. Kumagai and K. Yamamoto, *Carbohydr. Res.*, 2006, **341**, 1803–1808.
35 (*a*) R. Roy and C. A. Laferrière, *Carbohydr. Res.*, 1988, **177**, C1–C4; (*b*) M. N. Matrosovich, L. V. Mochalova, V. P. Marinina, N. E. Byramova and N. V. Bovin, *FEBS Lett.*, 1990, **272**, 209–212; (*c*) A. Spaltenstein and G. M. Whitesides, *J. Am. Chem. Soc.*, 1991, **113**, 686–687; (*d*) R. Roy, F. O. Andersson, G. Harms, S. Kelm and R. Schauer, *Angew. Chem., Int. Ed.*, 1992, **31**, 1478–1481; (*e*) M. A. Sparks, K. W. Williams and G. M. Whitesides, *J. Med. Chem.*, 1993, **36**, 778–783; (*f*) N. V. Bovin, E. Y. Korchagina, T. V. Zemlyanukhina, N. E. Byramova, O. E. Galanina, A. E. Zemlyakov, A. E. Ivanov, V. P. Zubov and L. V. Mochalova, *Glycoconj. J.*, 1993, **10**, 142–151; (*g*) W. J. Lees, A. Spaltenstein, J. E. Kingery-Wood and G. M. Whitesides, *J. Med. Chem.*, 1994, **37**, 3419–3433; (*h*) M. Mammen, G. Dahmann and G. M. Whitesides, *J. Med. Chem.*, 1995, **38**, 4179–4190; (*i*) G. B. Sigal, M. Mammen, G. Dahmann and G. M. Whitesides, *J. Am. Chem. Soc.*, 1996, **118**, 3789–3800; (*j*) S.-K. Choi, M. Mammen and G. M. Whitesides, *J. Am. Chem. Soc.*, 1997, **119**, 4103–4111; (*k*) A. Tsuchida, K. Kobayashi, N. Matsubara, T. Muramatsu, T. Suzuki and Y. Suzuki, *Glycoconj. J.*, 1998, **15**, 1047–1054; (*l*) J. D. Reuter, A. Myc, M. M. Hayes, Z. Gan, R. Roy, D. Qin, R. Yin, L. T. Piehler, R. Esfand, D. A. Tomalia and J. R. Baker, *Bioconjugate Chem*, 1999, **10**, 271–278; (*m*) W.-Y. Wu, B. Jin, G. Y. Krippner and K. G. Watson, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 341–343; (*n*) T. Furuike, S. Aiba, T. Suzuki, T. Takahashi, Y. Suzuki, K. Yamada and S.-I. Nishimura, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3000–3005; (*o*) Z.-Q. Yang, E. B. Puffer, J. K. Pontrello and L. L. Kiessling, *Carbohydr. Res.*, 2002, **337**, 1605–1613; (*p*) A. S. Gambaryan, E. Y. Boravleva, T. Y. Matrosovich, M. N. Matrosovich, H.-D. Klenk, E. V. Moiseeva, A. B. Tuzikov, A. A.

 This journal is © The Royal Society of Chemistry 2008

Chinarev, G. V. Pazynina and N. V. Bovin, *Antiviral Res.*, 2005, **68**, 116–123.
36 (*a*) J. E. Kingery-Wood, K. W. Williams, G. B. Sigal and G. M. Whitesides, *J. Am. Chem. Soc.*, 1992, **114**, 7303–7305; (*b*) W. Spevak, J. O. Nagy, D. H. Charych, M. E. Schaefer, J. H. Gilbert and M. D. Bednarski, *J. Am. Chem. Soc.*, 1993, **115**, 1146–1147; (*c*) A. Reichert, J. O. Nagy, W. Spevak and D. H. Charych, *J. Am. Chem. Soc.*, 1995, **117**, 829–830; (*d*) C.-T. Guo, X.-L. Sun, O. Kanie, K. F. Shortridge, T. Suzuki, D. Miyamoto, K. I. P. J. Hidari, C.-H. Wong and Y. Suzuki, *Glycobiology*, 2002, **12**, 183–190.
37 (*a*) A. B. Tuzikov, A. A. Chinarev, A. S. Gambaryan, V. A. Oleinkov, D. V. Kinov, N. B. Matsko, V. A. Kadykov, M. A. Ermishov, I. V. Demin, V. V. Demin, P. D. Rye and N. V. Bovin, *ChemBioChem*, 2003, **4**, 147–154; (*b*) N. V. Bovin, A. B. Tuzikov, A. A. Chinarev and A. S. Gambaryan, *Glycoconj. J*, 2004, **21**, 471–478.
38 (*a*) R. Roy, D. Zanini, S. J. Meunier and A. Romanowska, *J. Chem. Soc., Chem. Commun.*, 1993, 1869–1872; (*b*) R. Roy and D. Zanini, *J. Org. Chem.*, 1996, **61**, 7348–7354; (*c*) M. Llinares and R. Roy, *Chem. Commun.*, 1997, 2119–2120; (*d*) S. J. Meunier, Q. Wu, S.-N. Wang and R. Roy, *Can. J. Chem.*, 1997, **75**, 1472–1482; (*e*) K. Matsuoka, H. Oka, T. Koyama, Y. Esumi and D. Terunuma, *Tetrahedron Lett.*, 2001, **42**, 3327–3330; (*f*) J.-I. Sakamoto, T. Koyama, D. Miyamoto, S. Yingsakmongkon, K. I. P. J. Hidari, W. Jampangern, T. Suzuki, Y. Suzuki, Y. Esumi, K. Hatano, D. Terunuma and K. Matsuoka, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 717–721.
39 (*a*) G. D. Glick and J. R. Knowles, *J. Am. Chem. Soc.*, 1991, **113**, 4701–4703; (*b*) G. D. Glick, P. L. Toogood, D. C. Wiley, J. J. Skehel and J. R. Knowles, *J. Biol. Chem.*, 1991, **266**, 23660–23669; (*c*) S. Sabesan, J. Duus, P. Domaille, S. Kelm and J. C. Paulson, *J. Am. Chem. Soc.*, 1991, **113**, 5865–5866; (*d*) S. Sabesan, J. Duus, S. Neira, P. Domaille, S. Kelm, J. C. Paulson and K. Bock, *J. Am. Chem. Soc.*, 1992, **114**, 8363–8375; (*e*) S. J. Meunier and R. Roy, *Tetrahedron Lett.*, 1996, **37**, 5469–5472; (*f*) K. Fujimoto, O. Hayashida, Y. Aoyama, C.-T. Guo, K. I. P. J. Hidari and Y. Suzuki, *Chem. Lett.*, 1999, 1259; (*g*) Z. Gan and R. Roy, *Can. J. Chem.*, 2002, **80**, 908–916; (*h*) T. Ohta, N. Miura, N. Fujitani, F. Nakajima, K. Niikura, R. Sadamoto, C.-T. Guo, T. Suzuki, Y. Suzuki, K. Monde and S.-I. Nishimura, *Angew. Chem., Int. Ed.*, 2003, **42**, 5186–5189; (*i*) S. A. Kalovidouris, O. Blixt, A. Nelson, S. Vidal, W. B. Turnbull, J. C. Paulson and J. F. Stoddart, *J. Org. Chem.*, 2003, **68**, 8485–8493.
40 The inhibitory activity of these products toward BK virus was not evaluated.
41 Expressed as the total concentration of sialic acid units in the molecule, not on the basis of the concentration of the polymeric material.
42 A. S. Gambaryan, A. B. Tuzikov, A. A. Chinarev, L. R. Juneja, N. V. Bovin and M. N. Matrosovich, *Antiviral Res.*, 2002, **55**, 201–205.
43 A. Marra, M.-C. Scherrmann, A. Dondoni, A. Casnati, P. Minari and R. Ungaro, *Angew. Chem., Int. Ed.*, 1994, **33**, 2479–2481.
44 (*a*) A. Marra, A. Dondoni and F. Sansone, *J. Org. Chem.*, 1996, **61**, 5155–5158; (*b*) A. Dondoni, A. Marra, M.-C. Scherrmann, A. Casnati, F. Sansone and R. Ungaro, *Chem.–Eur. J.*, 1997, **3**, 1774–1782; (*c*) A. Dondoni, M. Kleban, X. Hu, A. Marra and H. D. Banks, *J. Org. Chem.*, 2002, **67**, 4722–4733.
45 (*a*) C. Félix, H. Parrot-Lopez, V. Kalchenko and A. W. Coleman, *Tetrahedron Lett.*, 1998, **39**, 9171–9174; (*b*) R. Roy and J. M. Kim, *Angew. Chem., Int. Ed.*, 1999, **38**, 369–372; (*c*) C. Saitz-Barria, A. Torres-Pinedo and F. Santoyo-González, *Synlett*, 1999, 1891–1894; (*d*) F. Calvo-Flores, J. Isac-García, F. Hernández-Mateo, F. Pérez-Balderas, J. A. Calvo-Asin, E. Sanchéz-Vaquero and F. Santoyo-González, *Org. Lett.*, 2000, **2**, 2499–2502; (*e*) F. Pérez-Balderas and F. Santoyo-González, *Synlett*, 2001, 1699–1702; (*f*) F. Sansone, E. Chierici, A. Casnati and R. Ungaro, *Org. Biomol. Chem.*, 2003, **1**, 1802–1809; (*g*) G. M. L. Consoli, F. Cunsolo, C. Geraci and V. Sgarletta, *Org. Lett.*, 2004, **6**, 4163–4166; (*h*) M. G. ten Cate, M. Omerović, G. V. Oshorsky, M. Crego-Calama and D. N. Reinhoudt, *Org. Biomol. Chem.*, 2005, **3**, 3727–3733; (*i*) U. Schädel, F. Sansone, A. Casnati and R. Ungaro, *Tetrahedron*, 2005, **61**, 1149–1154; (*j*) K. Krenek, M. Kuldová, K. Hulíková, I. Stibor, P. Lhoták, M. Dudic, J. Budka, H. Pelantová, K. Bezouska, A. Fiserova and V. Kren, *Carbohydr. Res.*, 2007, **342**, 1781–1792; (*k*) S. P. Bew, R. A. Brimage, N. L'Hermite and S. V. Sharma, *Org. Lett.*, 2007, **9**, 3713–3716.
46 A. Dondoni, A. Marra and M. G. Zampolli, *Synlett*, 2002, 1850–1854.
47 (*a*) C. W. Tornфe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057–3064; (*b*) V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596–2599.
48 (*a*) For reviews, see:V. D. Bock, H. Hiemstra and J. H. van Maarseven, *Eur. J. Org. Chem.*, 2006, 51–68; (*b*) P. Wu and V. V. Fokin, *Aldrichim. Acta*, 2007, **40**, 7–17; (*c*) J.-F. Lutz, *Angew. Chem., Int. Ed.*, 2007, **46**, 1018–1025; (*d*) S. Dedola, S. A. Nepogodiev and R. A. Field, *Org. Biomol. Chem.*, 2007, **5**, 1006–1017; (*e*) A. Dondoni, *Chem. Asian J.*, 2007, **2**, 700–708; (*f*) J. E. Moses and A. D. Moorhouse, *Chem. Soc. Rev.*, 2007, **36**, 1249–1262.
49 A. Dondoni and A. Marra, *J. Org. Chem.*, 2006, **71**, 7546–7557.
50 Z. P. Demko and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2110–2113.
51 A. Dondoni and A. Marra, *Tetrahedron*, 2007, **63**, 6339–6345.
52 (*a*) For reviews, see:C. D. Gutsche, *Aldrichim. Acta*, 1995, **28**, 3–9; (*b*) V. Böhmer, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 713–745; (*c*) V. Böhmer, *Liebigs Ann./Recueil*, 1997, 2019–2030; (*d*) A. Ikeda and S. Shinkai, *Chem. Rev.*, 1997, **97**, 1713–1734.
53 For a recent work on the stability of thiosialoside toward neuraminidases, see: J. C. Wilson, M. J. Kiefel, D. I. Angus and M. von Itzstein, *Org. Lett.*, 1999, **1**, 443–446.
54 N. K. Sauter, J. E. Hanson, G. D. Glick, J. H. Brown, R. L. Crowther, S.-J. Park, J. J. Skehel and D. C. Wiley, *Biochemistry*, 1992, **31**, 9609–9621.
55 N. A. Rodios, *J. Heterocycl. Chem.*, 1984, **21**, 1169–1173.
56 C. D. Gutsche, B. Dhawan, J. A. Levine, K. H. No and L. J. Bauer, *Tetrahedron*, 1983, **39**, 409–426.
57 M. Iqbal, T. Mangiafico and C. D. Gutsche, *Tetrahedron*, 1987, **43**, 4917–4930.
58 J. Tejler, E. Tullberg, T. Frejd, H. Leffler and U. J. Nilsson, *Carbohydr. Res.*, 2006, **341**, 1353–1362.
59 W. L. F. Armarego, and C. L. L. Chai, *Purification of Laboratory Chemicals*, 5th ed., Butterworth-Heinemann, Amsterdam, 2003.
60 W. C. Still, M. Kahn and A. Mitra, *J. Org. Chem.*, 1978, **43**, 2923–2925.
61 N. M. Maraldi, G. Barbanti-Brodano, M. Portolani and M. La Placa, *J. Gen. Virol.*, 1975, **27**, 71–80.
62 M. Portolani, G. Barbanti-Brodano and M. La Placa, *J. Virol.*, 1974, **15**, 420–422.
63 S. A. Aaronson and G. J. Todaro, *Proc. Soc. Exp. Biol. Med.*, 1970, **134**, 103–106.
64 Y. Suzuki, T. Suzuki, M. Matsunaga and M. Matsumoto, *J. Biochem. (Tokyo)*, 1985, **97**, 1189–1199.