Single and dual glycoside clustering around calix[4]arene scaffolds *via* click thiol–ene coupling and azide–alkyne cycloaddition†

Michele Fiore,[a] Angela Chambery,[b] Alberto Marra*[a] and Alessandro Dondoni*[a]

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We present the first synthesis of calix[4]arene-based *S*-glycoclusters *via* photoinduced multiple thiol–ene coupling of tetra- and octa-allyl calix[4]arenes with peracetylated glucosyl thiol (67–88% yields). Moreover we describe the dual clustering at the upper and lower rim of a calix[4]arene with two different sugars (galactose and glucose) *via* sequential copper(I)-catalyzed azide–alkyne cycloaddition and photoinduced thiol–ene coupling.

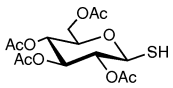
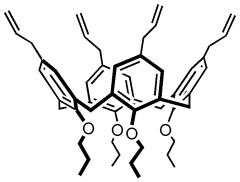
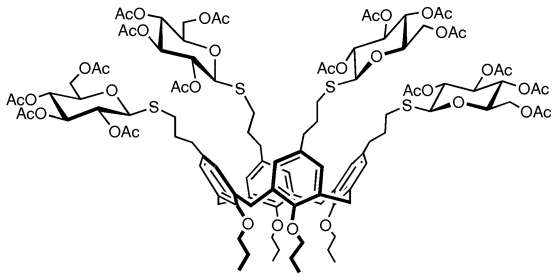
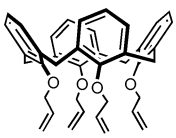
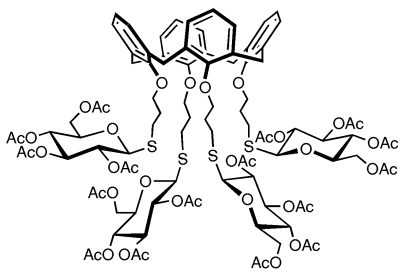
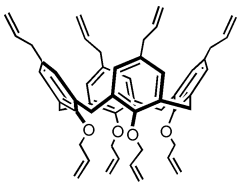
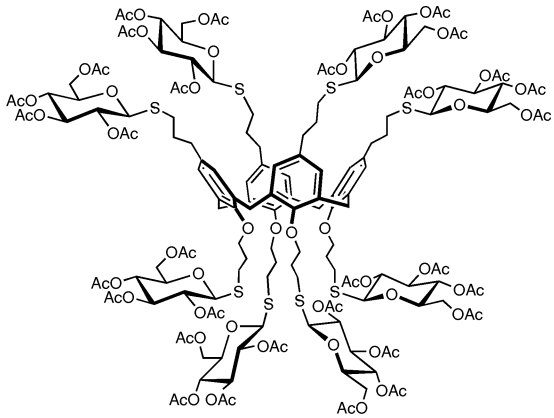
Ever since we reported in the mid-1990s on the first synthesis of calix[4]arene *O*-glycosides,[1] increasing interest has been focused by us[2] and others[3,4] on multiple glycosylation of calix[4]arene platforms to give glycoclusters. The final goal of those synthetic efforts was the preparation of structurally well-defined clusters to be used as probes in carbohydrate–lectin molecular recognition processes. Thus, in view of the fact that non-natural *C*-glycosides are impervious toward hydrolytic degradation,[5] subsequent research in our laboratory was carried out on the assembly of calix[4]arene-based *C*-glycoclusters. Accordingly, we first synthesized compounds in which the carbohydrate fragments were grafted to the macrocycle through a carbon chain formed by Wittig reaction of calix[4]arene aldehydes and sugar phosphoranes.[2c] In a second instance we prepared *C*-glycoclusters *via* *N*-heterocycle ring formation, namely tetrazole from thermal azide–nitrile cycloaddition (ANC)[6] and triazole from Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC).[7] Recently, we took advantage of the efficiency of the latter reaction, the quintessential click process,[8] for anchoring up to eight sialyl residues to calix[4]arene scaffolds in a one-pot process.[3] In this case *S*-sialoclusters were formed because all sialyl residues featured an anomeric thioether linkage. *S*-Linked glycoconjugates are known to display low susceptibility to enzymatic hydrolysis because the rate of hydrolysis of the thioglycosidic bond by glycohydrolases is several order of magnitudes slower than that of the corresponding *O*-glycosides.[9] The calix[4]arene-based *S*-sialoclusters proved to be active at submillimolar concentrations against BK (etiological agent of nephropathies) and influenza A viruses. Hence, giving the need to prepare calix[4]arene-based glycoclusters with robust tethers by the use of simple and efficient ligation reactions, we would like to report here on calixarene glycoclustering through sulfide bridges formed *via* photoinduced addition of glycosyl thiols to alkene functionalized calix[4]arenes. The century old radical addition of thiols to terminal alkenes,[10] the so called thiol-ene coupling (TEC), has been recently highlighted by one of us[11] as an exemplar case of click process by virtue of some special features. These include high efficiency, total atom economy, orthogonality to a broad range of reagents, compatibility with water and oxygen, use of a green catalyst such as irradiation close to visible light. The high efficiency of multiple TEC reactions on functionalized scaffolds has been recently demonstrated by Hawker with the synthesis of dendrimers,[12] by Lindhorst with the synthesis of glycoclusters,[13] and by Waldmann with the immobilization of proteins on solid surfaces.[14] In addition to this encouraging scenario on the potential of TEC, we were motivated to prepare calix[4]arene-based *S*-glycoclusters because we considered these compounds as the simplest and stable mimics of *O*-glycoclusters. Indeed, the substitution of oxygen by sulfur linked to the sugar anomeric carbon represents the smallest step away in terms of bond length and angle.[15]

Taking advantage of optimized TEC established for *S*-disaccharide synthesis,[16] we decided to perform reactions of sugar thiols with alkene-functionalized calix[4]arenes under similar conditions, *i.e.* irradiation at λ_{max} 365 nm in CH_2Cl_2 at rt and in the presence of air using 2,2-dimethoxy-2-phenylacetophenone (DPAP) as the sensitizer. Accordingly, the coupling of per-*O*-acetylated β-D-glucosyl thiol **1**[17] with the known[2c] upper-rim tetra-*C*-allyl calix[4]arene **2** afforded after 1 h the target tetravalent *S*-glycocluster **3** (Table 1) in which each glucosyl fragment was anchored to the calix[4]arene platform through a sulfide bridge. While using a slight excess of thiol **1** the isolated yield of pure **3** was low (28%), the yield increased substantially to 78% using 3 equiv. of thiol per allyl group. This indicated that under the latter conditions each hydrothiolation reaction occurred with very high efficiency to give the corresponding sulfide in an average yield of 94%. Due to the large amount of residual **1**, transesterification was likely to take place to some extent during the work-up and purification steps leading to variable amounts of partially deacetylated cycloadducts. Therefore, the reaction mixture was treated with Ac_2O and pyridine to transform **1** into the corresponding thioacetate derivative, thus avoiding transesterification reactions. It is noteworthy that the calix[4]arene scaffold did not deteriorate under the reaction conditions as the only side products were partially glycosylated calix[4]arenes. Moreover, in the ^{1}H NMR spectrum of **3**, the presence of signals for the equatorial and axial protons of the methylene bridges between the phenyl rings as large doublets at *ca.* 3.0 and 4.4 ppm, respectively, clearly indicated that the macrocycle was in its original cone conformation.

[a]*Dipartimento di Chimica, Laboratorio di Chimica Organica, Università di Ferrara, Via L. Borsari 46, 44100, Ferrara, Italy. E-mail: adn@unife.it; Fax: 39 0532 455167; Tel: 39 0532 455176*
[b]*Dipartimento di Scienze della Vita, II Università di Napoli, Via Vivaldi 43, 81100, Caserta, Italy*

† Electronic supplementary information (ESI) available: Experimental procedures, analytical data, copies of the ^{1}H and ^{13}C NMR spectra of all new compounds. See DOI: 10.1039/b912686d

Table 1 Photoinduced glycosylation of calixarenes **2**, **4**, and **6** by thiol **1** to give *S*-linked glycoclusters. All reactions were performed in CH_2Cl_2 (0.05 M) with irradiation at λ_{max} 365 nm in the presence of DPAP (10 mol%)

Entry	Thiol	Alkene	Product (Yield)
1	 **1**	 **2**	 **3** (78%)
2	**1**	 **4**	 **5** (88%)
3	**1**	 **6**	 **7** (67%)

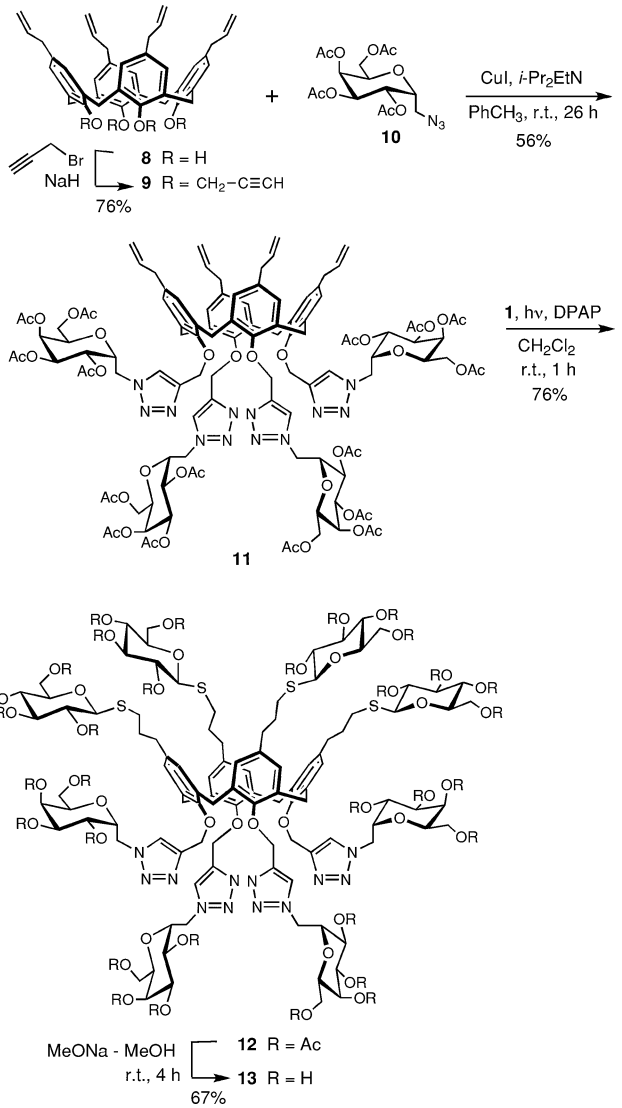
In a second instance we considered the coupling of **1** with tetra-*O*-allyl calix[4]arene **4**[18] (Table 1, entry 2) with the aim of forming a glycocluster at the lower and narrower rim of the macrocycle. Although *O*-allyl bearing substrates were used in photoinduced thiol–ene reactions at λ_{max} 360–365 nm,[19] we were concerned about the stability of **4** under the same conditions. Fortunately enough, it was demonstrated that **4** in a solution of CH_2Cl_2 remained unaltered under irradiation at λ_{max} 365 in the presence of DPAP. Thus, we were pleased to observe that the multi-TEC of excess **1** with **4** under the above photocatalyzed conditions afforded the product **5** in excellent isolated yield after chromatography (88%).

Encouraged by these results we decided to install tetravalent *S*-galactoside clusters at both calix[4]arene upper and lower rims using the octa-allyl derivative[3] **6** as a substrate reacting with thiol **1** (Table 1, entry 3). Thus, a solution of calix[4]arene **6** and 3 equiv. per allyl group of **1** in CH_2Cl_2 was irradiated at λ_{max} 365 in the presence of DPAP for 1 h. After this time, the ^{1}H NMR spectrum of the reaction mixture indicated that **6** was completely consumed as shown by the absence of the signals at *ca.* 5.8 and 6.4 ppm corresponding to the alkene protons. The acetylation of the crude mixture followed by column chromatography gave the octavalent *S*-glycoside cluster **7** in 67% yield corresponding to a 95% average yield for each thiol–ene coupling.

Because some bacteria exhibit various lectins recognizing different carbohydrate residues and a single lectin can interact with various types of carbohydrate moieties,[20] we focused on a dual scaffolding round the same calix[4]arene platform by two different glycoside residues. To this aim we considered sequential CuAAC and TEC as a dual ligation strategy.

According to this plan, the tetra-*C*-allyl and tetra-*O*-propargyl functionalized calix[4]arene **9**, readily prepared from the known[21]

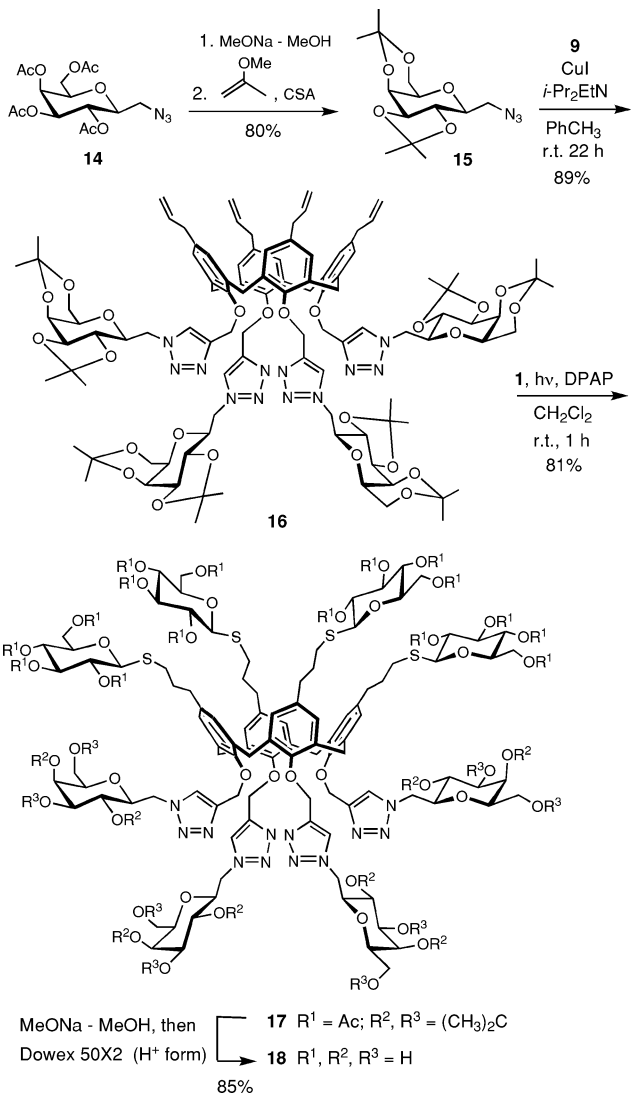
tetrol **8**, was allowed to react with a sugar azide first and then with a sugar thiol (Scheme 1).



Scheme 1

The choice of this reaction sequence was quite logical based on the known photoinduced reactivity of thiols with double and triple bonds[22] whereas CuAAC is highly specific. Thus, a mixture of calix[4]arene **9**, peracetylated α-D-linked galactosylmethyl azide[23] **10** (1.1 equiv. per propargyl group), catalytic CuI and *N,N*-diisopropylethylamine in toluene was allowed to react at rt for 26 h. After that time the reaction product **11** featuring a tetravalent glycocluster anchored to the calix[4]arene platform through triazole-spacers was isolated by column chromatography in 56% yield (Scheme 1). Then, compound **11** was mixed with glucosyl thiol **1** (3 equiv. per allyl group) and catalytic DPAP in CH_2Cl_2 and the solution was irradiated at λ_{max} 365 for 1 h. The crude reaction mixture was acetylated with Ac_2O/Py and the glycosylated calix[4]arene **12** was isolated in 76% yield. Compound **12** featured two different tetravalent glycoclusters attached to the calix[4]arene scaffold, one being constituted of galactose residues and the other of glucose ones. Deacetylation of **12** by transesterification afforded **13**. As galactose and glucose are known to be recognized by different lectins,[24] compound **13** can play a double role in molecular recognition processes.

Aiming at broadening the scope of the above dual clustering on calix[4]arene scaffold, we set out to use the known[7a] β-linked galactosylmethyl azide **14** as the reaction partner in the initial CuAAC process with **9**. Quite surprisingly, this reaction afforded the expected calix[4]arene galactoside **19** (see ESI†) in only 35% yield after 72 h at rt. The low yield of isolated **19** was unacceptable for the development of an efficient dual glycosylation protocol.[25] Therefore, we decided to remove the *O*-acetyl protective groups of **14** and set out to use the diacetonide protected[26] sugar azide **15** as a new reagent (Scheme 2). Much to our delight the CuI catalyzed cycloaddition of **15** with **9** (1.1 equiv. per propargyl group) in toluene at rt proceeded smoothly to give after 22 h the calix[4]arene *C*-galactoside **16** in 89% isolated yield. The reaction of **16** with **1** under the standard photoinduced conditions described above for **11** resulted quite efficient as the dually glycosylated calix[4]arene **17** was isolated by column chromatography in 81% yield. The acetyl and isopropylidene protecting groups of the carbohydrate residues



Scheme 2

of **17** were removed by one-pot treatment with MeONa in MeOH and strongly acidic ion-exchange resin to give the fully deprotected product **18** in 85% yield. Also this compound exhibited tetravalent glucose and galactose clusters at the upper and lower rim of the calix[4]arene scaffold and therefore can play a double role in lectin recognition processes.

In conclusion, we believe we have demonstrated the great efficiency of TEC as a ligation tool that enables the installation of carbohydrate residues at the lower and upper rims of calix[4]arene scaffolds through a robust anomeric thioether linkage. Thus, the definition of TEC as a *photoclick* reaction appears to be appropriate. Quite remarkably, it has been shown for the first time that TEC can be combined with the click Cu(I)-catalyzed azide–alkyne cycloaddition for the introduction of two different carbohydrate fragments at each rim of the scaffold.

Experimental section

Glycocluster **3**. The reaction was carried out in a glass vial (diameter: 1 cm; wall thickness: 0.65 mm), sealed with a natural rubber septum, located 2.5 cm away from the UVA lamp. To a solution of **1** (218 mg, 0.60 mmol) and **2** (38 mg, 0.05 mmol) in dry CH_2Cl_2 (1 mL) was added DPAP (15.4 mg, 0.06 mmol). The solution was irradiated at rt for 1 h under magnetic stirring, then concentrated. The residue was treated with Ac_2O (1 mL) and pyridine (1 mL) at rt for 2 h and then concentrated. The pale yellow residue was eluted from a column of silica gel with cyclohexane–AcOEt (from 1:1 to 1:2) to give **3** (86 mg, 78%) as a white foam; $[\alpha]_D = -40.9$ (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.40 (s, 8H, Ar), 5.25 (dd, 4H, $J_{2,3} = J_{3,4} = 9.3$ Hz, 4 H-3), 5.11 (dd, 4H, $J_{4,5} = 10.0$ Hz, 4 H-4), 5.04 (dd, 4H, $J_{1,2} = 10.0$ Hz, 4 H-2), 4.53 (d, 4H, 4 H-1), 4.39 and 3.04 (2d, 8H, $J = 13.2$ Hz, 4 ArCH_2Ar), 4.28 (dd, 4H, $J_{5,6a} = 4.8$ Hz, $J_{6a,6b} = 12.4$ Hz, 4 H-6a), 4.14 (dd, 4H, $J_{5,6b} = 2.2$ Hz, 4 H-6b), 3.81 (t, 8H, $J = 7.5$ Hz, 4 $CH_3CH_2CH_2O$), 3.73 (ddd, 4H, 4 H-5), 2.70–2.56 (m, 8H, 4 $CH_2CH_2CH_2S$), 2.37 (t, 8H, $J = 7.5$ Hz, 4 $CH_2CH_2CH_2S$), 2.08, 2.06, 2.05, and 2.04 (4 s, 48H, 16 Ac), 1.98–1.86 and 1.80–1.70 (2 m, 16H, 4 $CH_3CH_2CH_2O$, 4 $CH_2CH_2CH_2S$), 0.99 (t, 12H, $J = 7.5$ Hz, 4 $CH_3CH_2CH_2O$). ^{13}C NMR (75 MHz): δ 170.6 (C), 170.1 (C), 169.4 (C), 169.3 (C), 154.8 (C), 134.6 (C), 134.1 (C), 127.9 (CH), 83.8 (CH), 76.9 (CH_2), 75.7 (CH), 73.9 (CH), 70.0 (CH), 68.3 (CH), 62.1 (CH_2), 34.1 (CH_2), 31.6 (CH_2), 30.9 (CH_2), 29.9 (CH_2), 23.1 (CH_2), 20.7 (CH_3), 20.6 (CH_3), 10.3 (CH_3). HRMS (ESI/Q-TOF) *m*/*z* calcd for $(C_{108}H_{152}N_2O_{40}S_4)/2$ $(M + 2NH_4)^{2+}$ 1122.4402, found 1122.4423.

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