CHEMISTRY
A EUROPEAN JOURNAL

FULL PAPER

DOI: 10.1002/chem.201300339

Controlled Click-Assembly of Well-Defined Hetero-Bifunctional Cubic Silsesquioxanes and Their Application in Targeted Bioimaging

M. Eugenia Pérez-Ojeda,[b] Beatriz Trastoy,[a, c] Álvaro Rol,[a] María D. Chiara,[d] Inmaculada García-Moreno,[b] and Jose Luis Chiara*[a]

Abstract: A general procedure for the assembly of hetero-bifunctional cubic silsesquioxanes with diverse functionality and a perfectly controlled distribution of functional groups on the inorganic framework has been developed. The method is based on a two-step sequence of mono- and hepta-functionalization through the ligand-accelerated copper(I)-catalyzed azide–alkyne cycloaddition of a readily available octaazido cubic silsesquioxane. The stoichiometry of the reactants and the law of binomial distribution essentially determine the selectivity of the key monofunctionalization reaction when a copper catalyst with strong donor ligands is used. The methodology has been applied to the preparation of a set of bifunctional nano-building-blocks with orthogonal reactivity for the controlled assembly of precisely defined hybrid nanomaterials and a fluorescent multivalent probe for application in targeted cell-imaging. The inorganic cage provides an improved photostability to the covalently attached dye as well as a convenient framework for the 3D multivalent display of the pendant epitopes. Thus, fluorescent bioprobes based on well-defined cubic silsesquioxanes offer interesting advantages over more conventional fully organic analogues and ill-defined hybrid nanoparticles and promise to become powerful tools for the study of cell biology and for biomedical applications.

Keywords: bioimaging · carbohydrates · click chemistry · hybrid materials · silsesquioxanes

Introduction

The "bottom-up" modular construction of 1D, 2D, and 3D nanomaterials with molecular precision at the nanometer scale is of paramount interest for the fine-tuned control of their macroscopic properties. For this endeavor, it is essential to have a ready and scalable access to a set of structurally well-defined, homogenous nano-building-blocks with diverse functionality as well as a set of efficient chemical reactions (ideally "click"-type)[1] for their functionalization. Within the last two decades, polyhedral oligosilsesquioxanes (POSS)[2] have emerged as an increasingly important group of 3D nano-building-blocks for the preparation of a variety of hybrid functional-materials.[3] POSS are cage-like molecules with a core-shell composition consisting of a siloxane inorganic scaffold decorated with organic substituents at the vertices. Due to their rigid inorganic core, these unique nanometer-sized hybrid molecules have superior mechanical and thermal stabilities that are partially transmitted to their derived materials. Applications in areas as diverse as polymers, composite materials, dendrimers, optical materials, coatings, liquid crystals, metal catalysts, drug carriers, and tissue engineering have been described, particularly in the patent literature.[3,4] POSS are usually synthesized by hydrolytic condensation of organosilicon monomers $RSiX_3$ (R = organic group; X = halogen or alkoxide group) and can be readily modified both on the inorganic cage (e.g., T_8, T_{10}, T_{12}, and so on)[5] and the peripheral organic functionality (mono-, multi-, homo- or heterofunctionalized).

The most promising POSS monomers are the highly symmetrical and topologically ideal cube-octameric frameworks (T_8), with the general formula type $(RSiO_{1.5})_8$ and a cage size of approximately 0.5–0.7 nm.[2b] Most known cubic POSS are homo-octafunctionalized. Among these, octahydridooctasilsesquioxane (**1**),[6] the simplest T_8 compound, together with octavinyloctasilsesquioxane (**2**),[7] octaphenyloctasilsesquioxane (**3**),[7f,8] octakis(3-chloropropyl)octasilsesquioxane (**4**),[5c,9] and octakis(3-aminopropyl)octasilsesquioxane (**5**)[10] (Scheme 1) have been the most useful precursors to a variety of different T_8 monomers and derived materials. However, the derivatization of the POSS cage

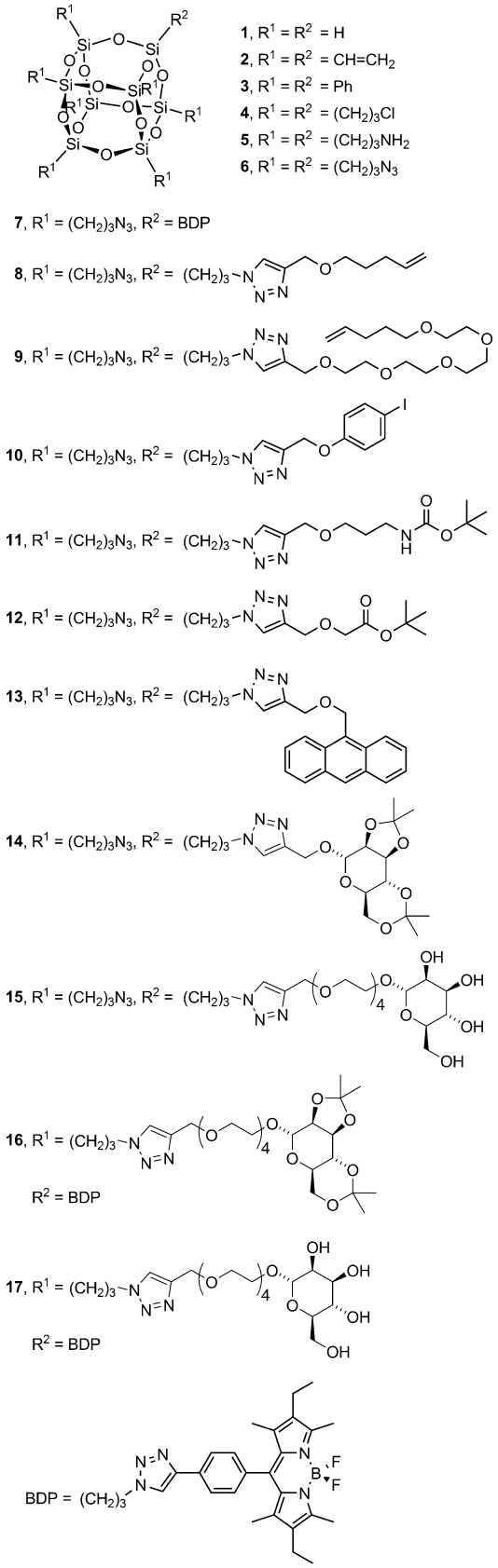
[a] Dr. B. Trastoy, Á. Rol, Dr. J. L. Chiara
Instituto de Química Orgánica General, IQOG-CSIC
Juan de la Cierva 3, 28006 Madrid (Spain)
E-mail: jl.chiara@csic.es

[b] M. E. Pérez-Ojeda, Prof. Dr. I. García-Moreno
Instituto de Química-Física "Rocasolano"
IQFR-CSIC, Serrano 119, 28006 Madrid (Spain)

[c] Dr. B. Trastoy
Present address: Institute of Human Virology
University of Maryland. School of Medicine
725 West Lombard Street, Baltimore, Maryland 21201 (USA)

[d] Dr. M. D. Chiara
Othorhinolaryngology Unit
Institute of Oncology of Principado de Asturias
Hospital Central de Asturias, Celestino Villamil s/n
33006 Oviedo (Spain)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201300339.



Scheme 1. Readily available homo-octafunctionalized POSS (**1**–**6**) used for the preparation of diverse hybrid materials and bifunctional POSS derivatives (**7**–**16**) prepared from **6** in this work.

with mixed sets of functional groups can provide still greater diversity, widely expanding their applications for polymerization,[3c,e] grafting,[3c] surface bonding,[3c] bioconjugation,[4c] and other useful transformations. POSS containing an assortment of two different organic groups (hetero-bifunctional POSS)[11] are the simplest class among these multifunctional derivatives and possibly the most useful, but those allowing independent derivatization of both functionalities have scarcely been reported. For example, the functionalization of the POSS cage with both a biorecognition element (e.g., a peptidic, nucleic acid, or carbohydrate epitope) and an "active" molecule, such as a fluorescent dye, a magnetic resonance imaging probe, a drug or other functional molecules, is of great interest for potential applications in bioimaging, vectorized drug-transport, or for the study of biological receptors.[4,12] The preparation of Janus-type POSS particles is also a very promising application that is being actively pursued.[13,14] Five different strategies have been described in the literature for the preparation of hetero-bifunctional POSS: 1) direct co-hydrolytic condensation of mixtures of different organosilicon monomers;[5d,15] 2) nucleophile-induced cage reshuffling of mixtures of different preformed POSS;[16] 3) cross-condensation of two cyclic tetrasiloxane tetraols ("half-cube" silsesquioxanes);[13b,17] 4) corner-capping of incompletely condensed silsesquioxanes;[12c,18,38] and 5) partial functionalization of homo-octafunctional POSS.[6d,9f,19] However, a majority of examples prepared by these routes contain simple alkyl or aryl groups as one of the two functionalities and, hence, are not readily amenable to further derivatization without compromising the integrity of the inorganic cage and/or the accompanying organic functionality, which considerably limits their potential applications. This is generally the case for the corner-capping route. In addition, only with the exception of this route and some peculiar cases of mono-[19c] and di-functionalized[19e,20] POSS, statistical mixtures of multi-functionalized cages and their various structural (and stereo-) isomers (Figure 1) are usually formed, which are difficult to separate.[15a,b,19h,n] Thus, the selective synthesis of bifunctional POSS with well-defined substitution patterns is still a complex and largely unsolved challenge, in spite of recent progress.

Our group has previously described the synthesis of octakis(3-azidopropyl)octasilsesquioxane (**6**, Scheme 1)[21] from readily available **5** by a diazo-transfer reaction[22] and its highly efficient octafunctionalization through copper(I)-catalyzed azide–alkyne 1,3-dipolar cycloaddition[23] (CuAAC, a paradigmatic example of "click" reaction[1]) with a variety of terminal alkynes.[21,24–26] Following this approach, several new octaglycosyl-POSS conjugates were readily assembled and their multivalent interaction with a complementary protein receptor, the model plant-lectin ConA, was thoroughly studied by using an array of biophysical techniques.[26] Armed with this simple and efficient methodology for the preparation of diverse homo-octafunctionalized POSS derivatives, we now describe that **6** is also an optimum scaffold for the controlled synthesis of structurally well-defined

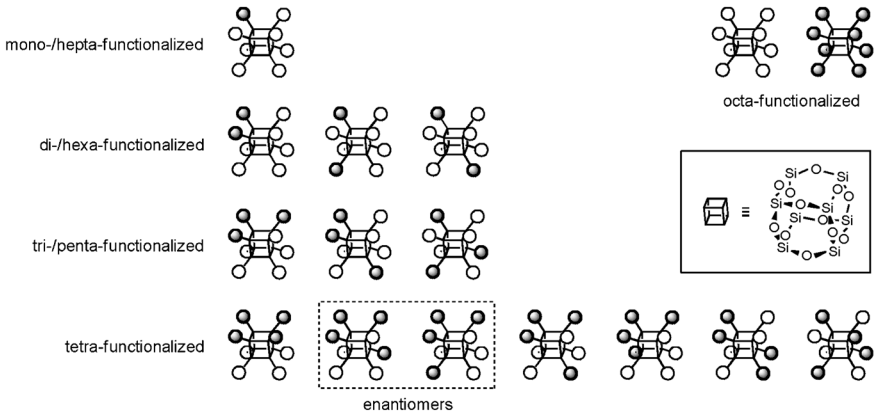
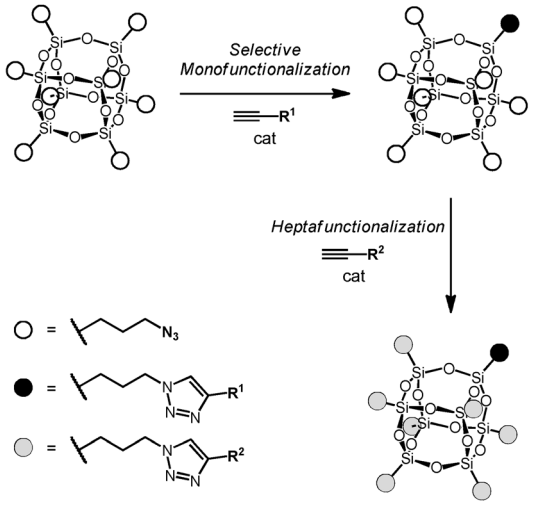


Figure 1. All possible POSS products and their different structural isomers (and stereoisomers) that can be generated in the reaction of a homo-octafunctional cubic POSS with a monofunctional reagent.

hetero-bifunctional POSS derivatives equipped with orthogonally reactive groups as versatile building blocks for the controlled assembly of diverse hybrid functional-materials. To this end, we have followed a simple two-step strategy (Scheme 2), which involves the highly selective monofunctionalization of **6** by a controlled CuAAC reaction with a



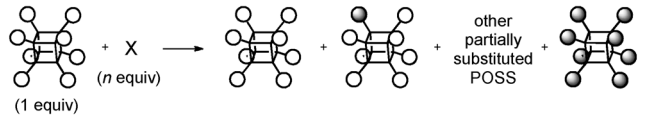
Scheme 2. Sequential click strategy for the preparation of structurally well-defined bifunctional POSS.

terminal functional alkyne followed by the subsequent CuAAC of the remaining seven azide groups with a second functional alkyne. This strategy, based on two sequential click reactions, is a powerful method for attaching virtually any pair of mutually compatible functionalities to the POSS framework. However, due to the octafunctional character and cubic symmetry of **6**, the key monofunctionalization step requires a careful optimization of the reaction conditions to avoid the formation of complex statistical mixtures of polytriazolyl-POSS derivatives (Figure 1) that would complicate the purification of the required monotriazolyl-POSS product. We have recently reported on the interesting photophysical and laser properties of a fluorescent derivative of **6** containing a single 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) chromophoric substituent (compound **7**).[24] We now disclose in detail the synthetic methodology and optimization studies that led to the hybrid dye **7** and the extension of the new methodology to the preparation of other bifunctional POSS (**8**–**17**, Scheme 1). As will be shown below, both the stoichiometry of the initial CuAAC monofunctionalization reaction as well as the nature of the employed copper(I) catalyst need to be carefully adjusted for optimum results. As a proof of concept, we have used this strategy for the preparation of a set of well-defined hetero-bifunctional POSS with a variety of orthogonally reactive functional groups as well as a POSS derivative that combines a cluster of sugar epitopes and a fluorescent dye, which has been shown to be a useful probe for bioimaging of cell surface receptors.

Results and Discussion

Synthetic methodology: Octaazide **6** is an ideal starting scaffold for the preparation of bifunctional POSS derivatives since the irreversible character, wide substrate-scope and outstanding efficiency of the CuACC reaction can enable the fine-tuned control of the extent of the derivatization with a diversity of functional terminal alkynes. However, with eight reactive and symmetry-equivalent vertex groups on cubic **6**, the formation of statistical mixtures of polyfunctionalized products (and their isomers; see Figure 1) is unavoidable under usual homogeneous reaction conditions. As for other cases of monoderivatization of a homo-multifunctional substrate, polysubstitution can in principle be minimized by a judicious selection of the appropriate stoichiometry of the reactants, depending on the number of equivalent reactive groups on the substrate.[27] Surprisingly, there is no consensus in the literature as to what stoichiometry should be preferably employed for the selective monoderivatization of a homo-octafunctional POSS, with reported reactant/POSS molar-ratios varying widely from 1:1 to 1:8. If one assumes that all functional groups on the starting octafunctional POSS as well as on its resultant partially substituted derivatives have all the same reactivity independently of the extent of substitution,[28] a statistical mixture of products will be formed that will ideally follow a binomial distribution.[27] Table 1 shows the theoretical binomial distribution of all possible products that can be obtained in the derivatization reaction of an octafunctional POSS (or any other molecule with 8 equivalent reactive groups) with a monofunctional reagent, ranging from unreacted (unsubstituted)

Table 1. Theoretical yields[a] of substituted POSS products formed in the reaction of a homo-octafunctional POSS with a monofunctional reagent (X) as a function of the reaction stoichiometry (n), predicted by the law of binomial distribution.[b]

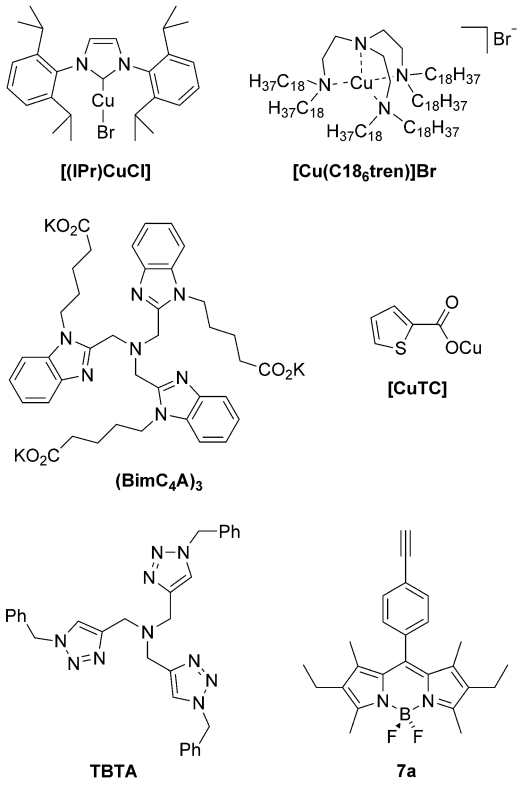


	Number of vertex substituents on POSS that have reacted								
n	0[c]	1	2	3	4	5	6	7	8
0.1	90.43	95.65	4.24	0.11	0.00	0.00	0.00	0.00	0.00
0.25	77.57	89.25	10.08	0.65	0.03	0.00	0.00	0.00	0.00
0.5	59.67	78.92	18.41	2.46	0.20	0.01	0.00	0.00	0.00
1.0	34.36	59.83	29.91	8.55	1.53	0.17	0.01	0.00	0.00
2.0	10.01	29.67	34.61	23.07	9.61	2.56	0.43	0.04	0.00
3.0	2.33	11.44	24.03	28.83	21.63	10.38	3.11	0.53	0.04
4.0	0.39	3.14	10.98	21.96	27.45	21.96	10.98	3.14	0.39
5.0	0.04	0.52	3.04	10.14	21.13	28.17	23.48	11.18	2.33
6.0	0.00	0.04	0.38	2.31	8.65	20.76	31.15	26.70	10.01
7.0	0.00	0.00	0.01	0.11	1.00	5.61	19.63	39.27	34.36
8.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00

[a] Calculated with respect to the limiting reagent. [b] For the statistical calculations, we have assumed that all functional groups on the different partially functionalized POSS derivatives have the same reactivity, independently of the extent of substitution. [c] Yield of unreacted POSS relative to total starting POSS.

starting substrate to fully (octa-) substituted product, as a function of the stoichiometry of the reaction. For an equimolar reaction mixture of POSS and derivatization reagent ($n=1$, Table 1, entry 4), the maximum yield of monosubstituted POSS theoretically expected is only 39.27%, accompanied by 34.36% unreacted POSS, and with the remaining substrate (26.37%) yielding a mixture of polysubstituted products (mainly di- and tri-substituted derivatives).[29] Thus, under these conditions, up to 60.73% of the derivatization reagent will be wasted in the production of undesired polysubstituted POSS cages, which would be an untenable situation for the introduction of expensive and highly elaborated substituents and/or for valuable POSS substrates. Recalculating the yields with respect to the total reacted POSS (i.e., subtracting the recovered starting material, see Table 1), the mixture of substituted products obtained in this case will consist mainly of 59.83% monosubstituted, 29.91% disubstituted, and 8.55% trisubstituted POSS, with negligible amounts (<2%) of higher-substituted derivatives. Lowering the reagent/POSS stoichiometry n (Table 1, entries 1–3) will improve the selectivity of monosubstitution at the expense of reducing overall POSS conversion. However, this is a simple and very effective strategy for the selective preparation of monosubstituted POSS compounds provided that excess unreacted POSS could be readily recovered from the reaction mixture and recycled.[30] Thus, by using a 1:10 reagent/POSS molar ratio ($n=0.1$; Table 1, entry 1) will produce the monosubstituted compound with very high (95.65%) selectivity with only 8.4% of derivatization reagent lost in producing higher substituted POSS derivatives, although with a low 9.16% yield of monosubstituted cage calculated with respect to total starting POSS. A 1:4 reagent/POSS molar ratio ($n=0.25$; Table 1, entry 2) gives about 90% selectivity for monosubstitution in 20% yield with respect to total starting POSS, with approximately 20% of derivatization reagent lost in producing polysubstituted derivatives, which could be a balanced compromise in the case of inexpensive reagents. One can also conclude from the analysis of Table 1 that no other partially substituted POSS derivative would be selectively accessible by simply modifying the stoichiometry of the functionalization reaction, since complex statistical mixtures of products will be always obtained for $1 \leq n < 8$ (Table 1, entries 5–10). Of course, this corollary does not necessarily hold true for the case of sterically hindered or polyfunctional derivatization reagents.

Although the CuAAC monofunctionalization of **6** could in principle be achieved by simply performing the reaction with a large excess of POSS over alkyne, as explained above, it is known that attempted CuAAC monofunctionalization of some polyazides can show unusual product distributions that deviate significantly from those expected on a purely statistical basis. Thus, 1,2- and conformationally constrained 1,3-diazides preferentially afford bis-triazoles at the expense of the expected monotriazole, even when an excess of diazide with respect to alkyne is employed.[31] This unusual result has been tentatively ascribed to direct participation of the initial Cu-triazolide intermediate in the subsequent click reaction of a vicinal-azide group.[31] Whereas **TBTA** (Scheme 3), a widely used accelerating copper(I)-ligand, did



Scheme 3. Copper catalysts, ligands, and model (fluorescent) alkyne used in the optimization studies of the CuAAC monofunctionalization of octaazide **6**.

not have a significant effect on the outcome of diazide reactions, Finn and co-workers have described that some (benzimidazolylmethyl)amine ligands (e.g., **(BimC$_4$A)$_3$**, Scheme 3) gave the monotriazole as the major product in the expected 2:1 mono-/bis-triazole statistical ratio in one example of conformationally rigid 1,3-diazide using a 1:1 diazide/alkyne reaction stoichiometry.[31b]

For the initial optimization study of the CuAAC monofunctionalization of octaazide **6**, we selected the fluorescent BODIPY dye **7a** (Scheme 3) as a model alkyne, which we have used previously for the preparation of an octa-BODIPY-POSS cluster.[21] The reaction could be easily followed by TLC by simple naked eye inspection, and affords a POSS derivative **7** (Scheme 1) labeled with a single fluorescent probe and seven additional reactive azide groups ready for further functionalization with any molecule of interest having a terminal alkynyl group. In addition, as we have already shown, compound **7** is an interesting hybrid dye for the preparation of solid-state laser materials with improved thermal and photochemical stability.[24] Based on the previous statistical calculations, we selected a rather conservative tenfold molar excess of **6** over alkyne **7a** to guarantee a very high statistical selectivity for the monofunctionalized product **7** (Table 1, entry 1). However, under the previously optimized conditions,[21] using $CuSO_4$/sodium ascorbate as catalyst in a biphasic CH_2Cl_2/H_2O (1:1)[32] solvent mixture, a very low yield (9%) of monotriazolyl-POSS **7** was obtained together with unreacted **6** and a complex mixture of uncharacterized polytriazolyl-POSS products (Table 2, entry 1). Thus, in the absence of added copper ligands, octaazide **6** showed the same strong preference for the formation of polytriazolyl products previously observed by Finn and co-workers[31] for apparently more conformationally constrained polyazides. By using the same **6**/**7a** = 10:1 molar ratio as above, we screened different copper catalysts (Scheme 3) and reaction conditions (e.g., aqueous or anhydrous solvents, with or without added base, at room temper-

Table 2. Optimization of the CuAAC monofunctionalization of octaazide POSS **6** with fluorescent alkyne **7a** to afford bifunctional POSS **7**.[a]

Entry	Catalyst	Base	Solvent (v/v ratio)	T [°C][b]	t [h]	Yield [%][c]
1	$CuSO_4$·5 H_2O, sodium ascorbate	–	CH_2Cl_2/H_2O (1:1)	25	20	9
2	[CuTC]	iPr$_2$NEt	THF	80 (MW)	2	25
3	[CuCl(IPr)]	iPr$_2$NEt	PhMe	80 (MW)	11	57
4	$CuSO_4$·5 H_2O, sodium ascorbate, (BimC$_4$A)$_3$	–	THF/H_2O (5:1)	25	15	48
5	[Cu(C18$_6$tren)]Br	iPr$_2$NEt	PhMe	80 (MW)	6	82

[a] A molar ratio **6**/**7a** = 10:1 was used in all cases. [b] MW indicates that the reaction was carried out under microwave heating. [c] Yields of the isolated product (column chromatography).

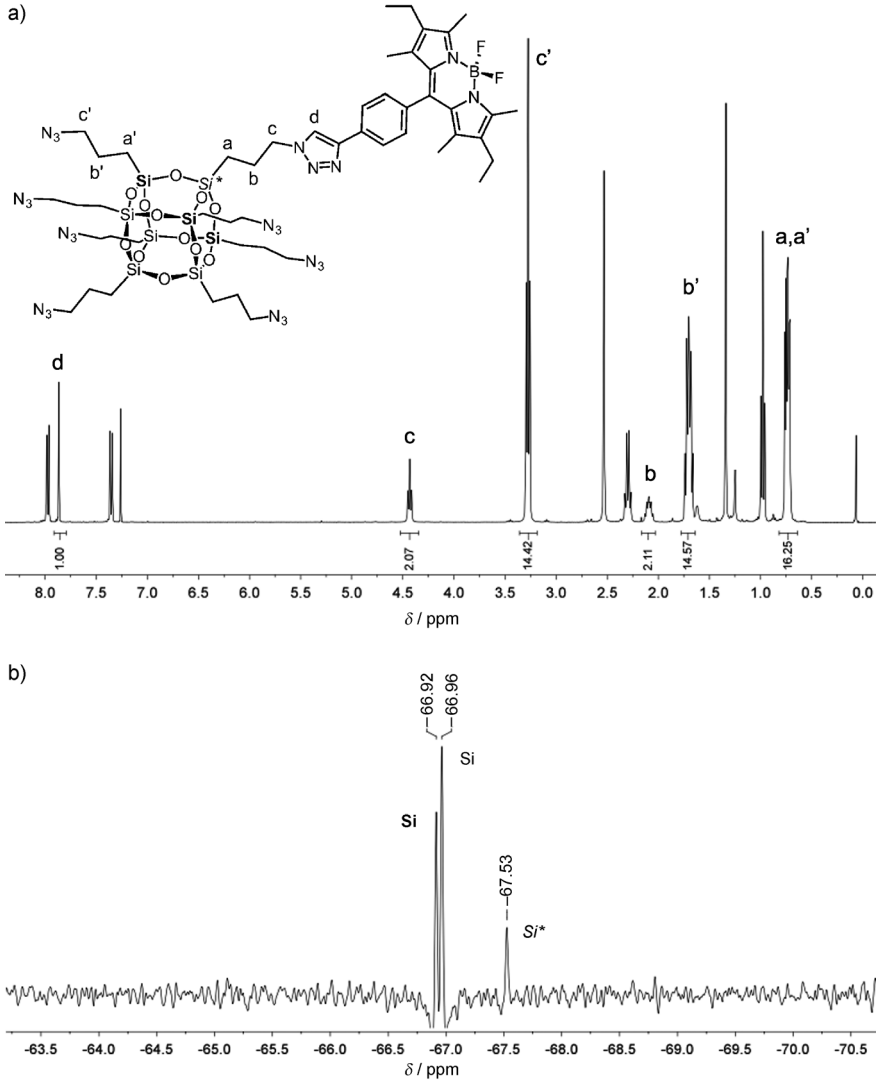
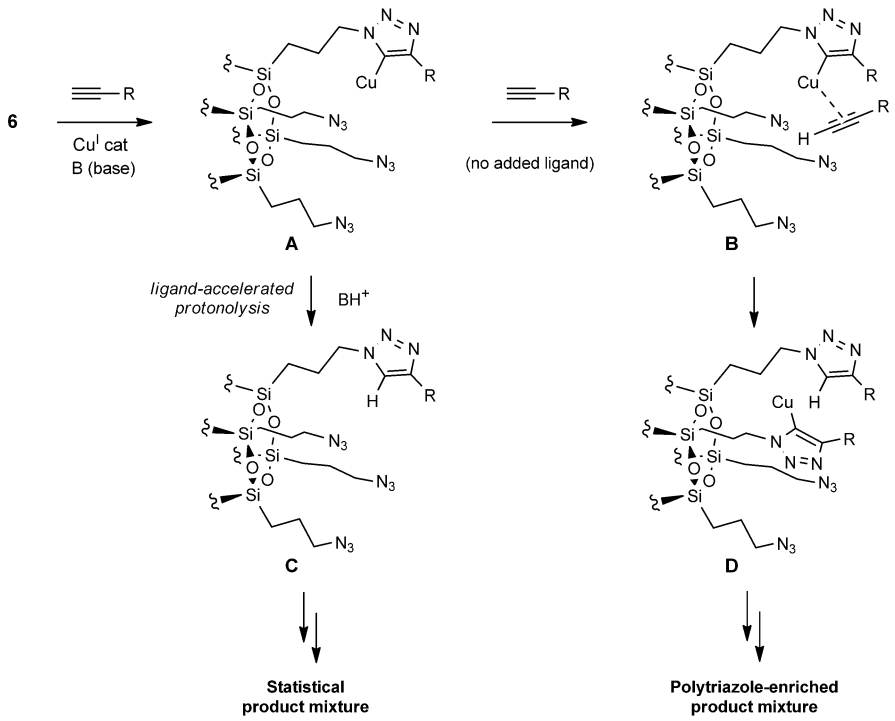


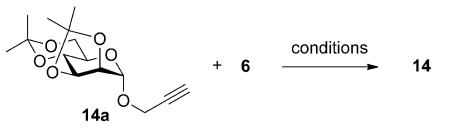
Figure 2. a) ^{1}H (400 MHz, $CDCl_3$) and b) ^{29}Si NMR (79.5 MHz, $CDCl_3$) spectra of compound **7** showing partial peak assignments. The numbers under the spectral line in (a) show relative integral regions for the corresponding peaks.

ature or under microwave heating) with the objective of minimizing the formation of polytriazolyl-POSS products to obtain **7** in the expected statistical yield. Copper(I) thiophene-2-carboxylate (**[CuTC]**, Scheme 3), which has been recently used in CuAAC reactions of sulfonyl azides,[33] gave a slightly better yield of **7** (25%) after heating for two hours under microwave irradiation in THF (Table 2, entry 2), but this outcome was still far from the expected statistical value. Better results were obtained with a copper(I) catalyst bearing an N-heterocyclic carbene[34] ligand (**[(IPr)CuCl]**, Scheme 3) or with Finn's tetradentate tripodal amino ligand **$(BimC_4A)_3$**[35] (Scheme 3; Table 2, entries 3 and 4, respectively). The tetramine **[Cu(C18$_6$tren)]Br** catalyst[36] (Scheme 3) with added Hünig's base in toluene as solvent and under microwave heating gave the highest yield of **7** (82%; Table 2, entry 5), close to the expected statistical value (95.65%). In all cases, the rest of alkyne **7a** was transformed into a complex mixture of polytriazolyl POSS products, as shown by ^{1}H NMR analysis of the remaining mixed fractions from column chromatography. The structure of **7** was unambiguously confirmed by high-resolution mass spectrometry and multinuclear (^{1}H, ^{13}C, ^{29}Si) NMR spectroscopy (see Figure 2 and the Supporting Information). The degree of substitution can be readily recognized by the 7(4+3):1 and 4:3:1 pattern distribution of ^{1}H (Figure 2a) and ^{29}Si NMR (Figure 2b) signals, respectively, as expected from molecular symmetry considerations.[15a] The ^{29}Si chemical shifts are within the expected region for an alkyl-substituted cubic POSS (ca. $\delta = -65$ to -70 ppm).[2b]

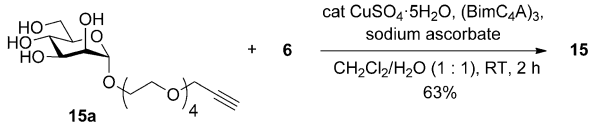
An informed speculation to explain the observed ligand effect on the selectivity of the CuAAC reaction is the following. According to previous mechanistic studies by Finn and co-workers,[31] the rate-limiting step of the CuAAC reaction apparently changes from the protonolysis of the Cu-triazolide intermediate to the alkyne deprotonation–cycloaddition sequence upon incorporation of chelating ligands on the copper catalyst. Therefore, under Sharpless conditions (no ligand) a build-up of Cu-triazolide **A** (Scheme 4) is expected to occur that kinetically favors its coordination to azide or alkyne (**B**) and the subsequent intramolecular formation of polytriazolyl products (through **D**). In contrast, chelating ligands hamper the further coordination of the metal in **A** and accelerate the protonolysis of **A** to **C**, diverting the copper catalyst towards another cycle of alkyne deprotonation and intermolecular cycloaddition to afford a statistically controlled mixture of triazole products with a final composition essentially determined by the initial stoichiometry of the reactants and the law of binomial distribution (Table 1). The apparent correspondence observed between the selectivity of the monofunctionalization reaction and the donor strength of the copper ligand, which approximately correlates with its proton affinity, is in agreement with this mechanistic view.[37]



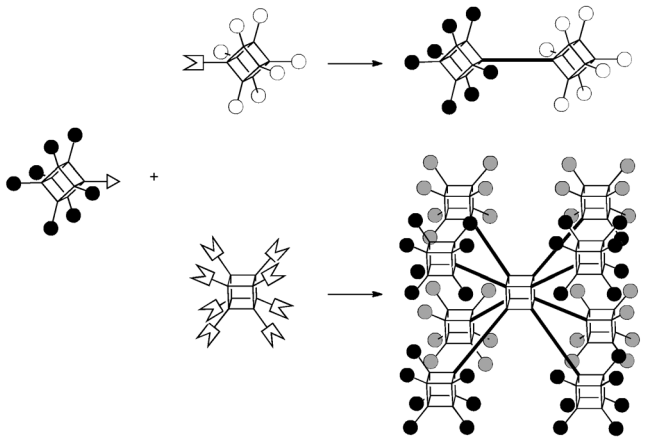
Scheme 4. Simplified mechanistic rationale for the observed ligand effect on the selectivity of the CuAAC monofuntionalization of octaazide **6**.



Conditions	Yield [%]
cat $CuSO_4 \cdot 5H_2O$, sodium ascorbate CH_2Cl_2/H_2O (1 : 1), RT, 3 h	10
cat [Cu(C18$_6$tren)]Br, *i*Pr$_2$NEt, toluene, 80 °C (MW), 6 h	79



Scheme 5. Synthesis of mono-glycosyl-POSS **14** and **15**. A 2:1 $(BimC_4A)_3$/copper molar-ratio was employed.

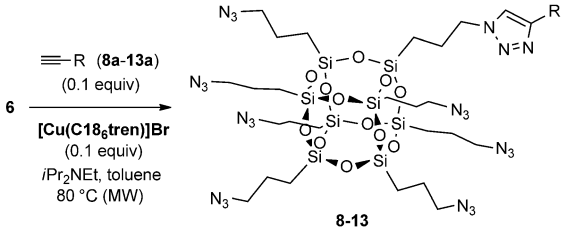


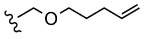
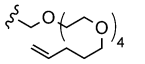
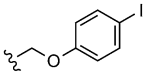
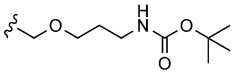
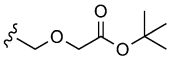
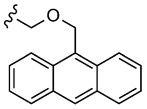
Scheme 6. Possible applications of complementary reactive mono-/heptafunctionalized POSS for nanoconstruction of dumbbell-shaped POSS dyads and complex 3D POSS assemblies.

A similar catalyst-effect on selectivity was observed for the CuAAC reaction of **6** with other alkynes (Scheme 5). Thus, the acetal-protected α-D-mannose glycoside **14a** gave also a low yield (10%) of monosubstituted POSS **14** under Sharpless conditions by using a biphasic aqueous solvent mixture and a tenfold molar excess of **6**, but the yield improved to 79% by using the **[Cu(C18$_6$tren)]Br** catalyst under the optimized reaction conditions with the same stoichiometry. However, these optimized conditions are not appropriate in the case of water-soluble alkynes such as **15a**, which is not readily soluble in apolar organic solvents. In this case, addition of the water-soluble ligand **(BimC$_4$A)$_3$** (in a 2:1 ligand/copper molar ratio) to a biphasic CH_2Cl_2/H_2O solvent reaction mixture containing a catalytic amount of $CuSO_4{\cdot}5H_2O$ and an excess of sodium ascorbate afforded the monosubstituted POSS **15** in a short reaction time and in a reasonably good yield.

To study the scope and versatility of the method, we synthesized a set of hetero-bifunctional POSS **8**–**13** with orthogonal reactivity by using a variety of alkynes functionalized with alkene (**8a** and **9a**), halobenzene (**10a**), protected amine (**11a**), protected carboxylic acid (**12a**), or anthracene (**13a**) end-groups (Table 3). The monotriazolyl-POSS products were obtained in good yield and with excellent selectivity by using the optimized conditions. The structures of the new POSS products were unambiguously confirmed by high-resolution mass spectrometry and multinuclear (^{1}H, ^{13}C, ^{29}Si) NMR spectroscopy (see the Supporting Information). Recovery of excess **6** from the crude reaction mixture was close to quantitative in all cases (see Table 3). This set of hetero-bifunctional POSS allows for the selective, stepwise modification of the cage functionality at either the azido groups or the other orthogonal functionality for the preparation of new bifunctional hybrid materials with well-defined structures. For example, the combination (in the appropriate order) of CuAAC heptafunctionalization with either alkene homo/cross-metathesis (**8**, **9**), transition-metal-catalyzed cross-coupling (**8**, **9**, **10**), deprotection followed by

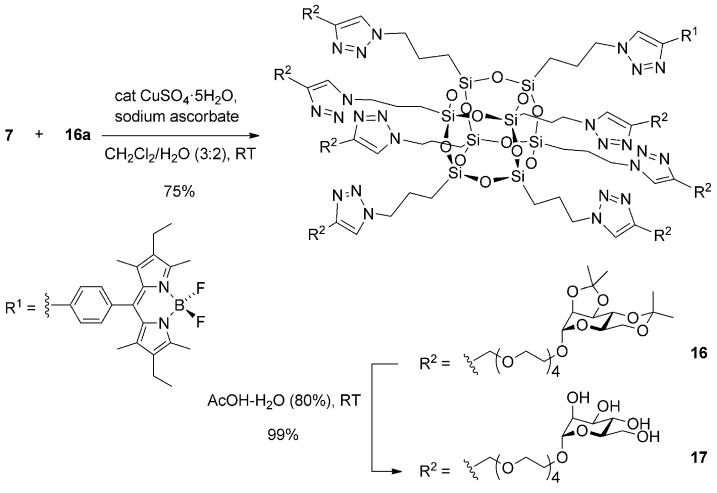
Table 3. Synthesis of hetero-bifunctional POSS **8**–**13** with orthogonal reactivity by selective CuAAC monofunctionalization of octaazide **6**.



Entry	R, POSS product		t [h]	Isolated product yield [%][a]
1		**8**	3	51 (87)
2		**9**	2	74 (99)
3		**10**	2	87 (89)
4		**11**	2	64 (96)
5		**12**	2	83 (94)
6		**13**	3	68 (85)

[a] Recovery yield of excess **6** is shown in parenthesis.

ester (**12**) or amide bond formation (**11**, **12**), amine deprotection followed by diazo-transfer and subsequent CuAAC reaction (**11**), or Diels–Alder cycloaddition (**13**) could provide access to a large variety of new hetero-bifunctional POSS. In addition, the obtained bifunctional POSS nanobuilding-blocks could be readily homo- or hetero-dimerized to afford, respectively, symmetric or asymmetric dumbbell-shaped POSS dyads,[13c,38] or they could be assembled with



Scheme 7. Synthesis of fluorescently labeled POSS glycoclusters **16** and **17**.

complementarily reactive homo-octafunctional-POSS monomers to produce even more complex 3D constructs (Scheme 6).

Confocal microscopy study: As a proof of concept to demonstrate the effectiveness of this synthetic strategy for the preparation of complex, well-defined bifunctional materials, we have prepared a multivalent fluorescent probe for application in targeted cell-imaging. To this end, we proceeded to react the seven azide groups of mono-BODIPY-POSS **7** with a terminal alkyne functionalized with a carbohydrate epitope (Scheme 7). The acetal-protected α-D-mannose derivative **16a**, equipped with a propargylated tetraethyleneglycol connector, was selected for this task. The hepta-click functionalization of **7** proceeded efficiently to afford the isolated **16** in 75% yield (=96% yield per azido group) by using the same optimized reaction conditions previously described by our group for the octafunctionalization of **6**.[21,26] Removal of the protecting acetal groups under mildly acidic conditions[21,26] produced the fluorescently labeled glyco-POSS cluster **17** in quantitative yield. The structures of **16** and **17** were unambiguously confirmed by MALDI-TOF and multi-nuclear (^{1}H, ^{13}C, ^{29}Si) NMR spectroscopy (see Figure 3 and the Supporting Information). As in the case of compound **7** (Figure 2), the degree of substitution could be readily recognized by the 4:3:1 pattern distribution of ^{1}H (Figure 3a) and ^{29}Si NMR (Figure 3b) signals, as expected from molecular symmetry considerations.[15a] The ^{29}Si chemical shifts are, again, within the expected region for an alkyl-substituted cubic POSS.[2b] As expected, the dispersion of ^{29}Si chemical shifts in these octatriazolyl-POSS derivatives (**16**, **17**) are narrower than those of the monotriazolyl-heptaazido-POSS derivatives **7**–**15**.

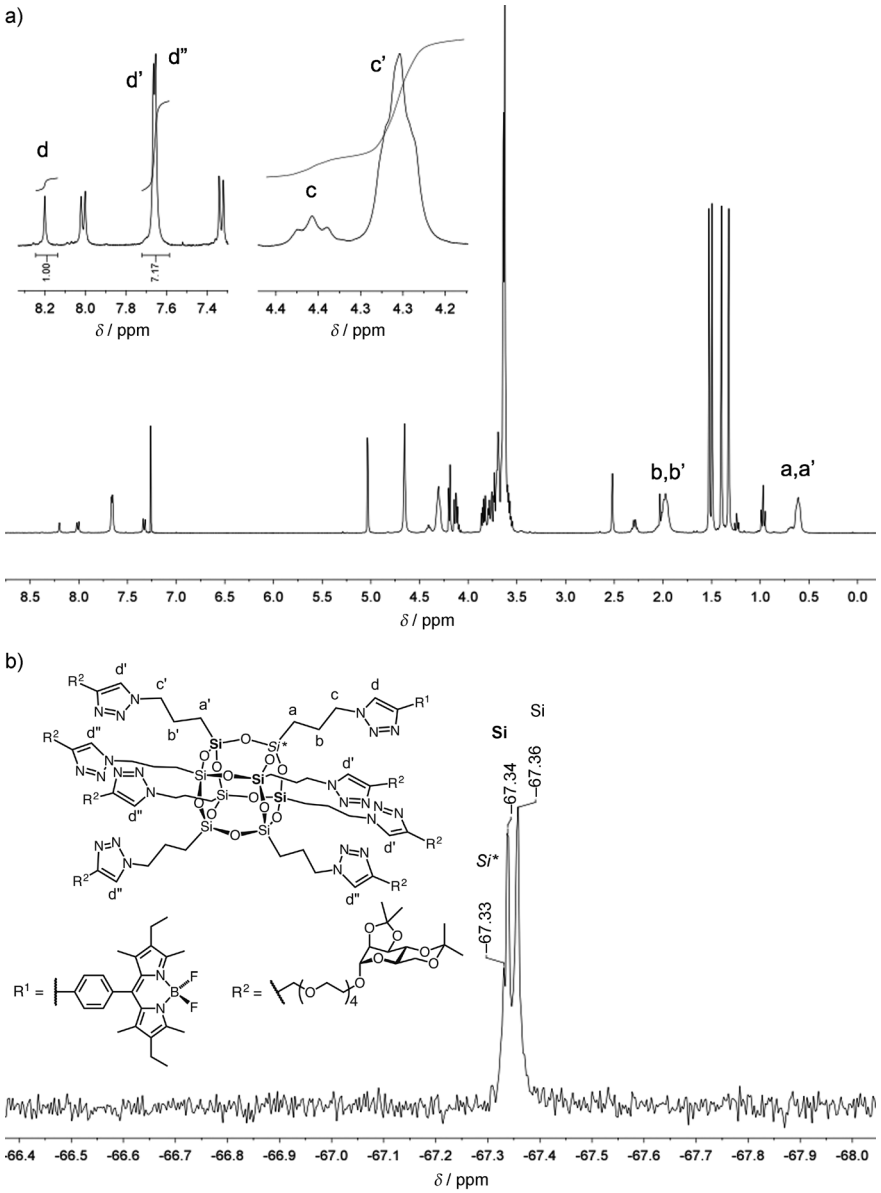


Figure 3. a) ^{1}H (400 MHz, $CDCl_3$) and b) ^{29}Si NMR (79.5 MHz, $CDCl_3$) spectra of compound **16** showing partial peak assignments. The numbers under the spectral line in the left inset of (a) show relative integral regions for the triazole protons.

Water-soluble glyco-POSS **17** was assayed as a fluorescent probe for the imaging of C-type lectin receptors (CLRs)[39] present on the surface of antigen-presenting cells by using fluorescence microscopy. An important group of CLRs recognize oligosaccharides containing mannose and/or fucose, including the mannose receptor (MR)[40] and DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin, also called CD209)[41] on dendritic cells (DC). DC-SIGN in particular has attracted much interest since its discovery in 2000,[42] because it binds to a large range of clinically relevant pathogens, including HIV, Ebola virus, Candida albicans and Leishmania, among others, facilitating their uptake for subsequent antigen presentation.[41] Both, MR and DC-SIGN bind to glycan patterns present on the surface of pathogens through multivalent carbohydrate–protein interactions. With its seven mannose epitopes, glyco-POSS **17** is properly equipped to sustain a multivalent binding to MR and DC-SIGN. In addition, the covalent attach-

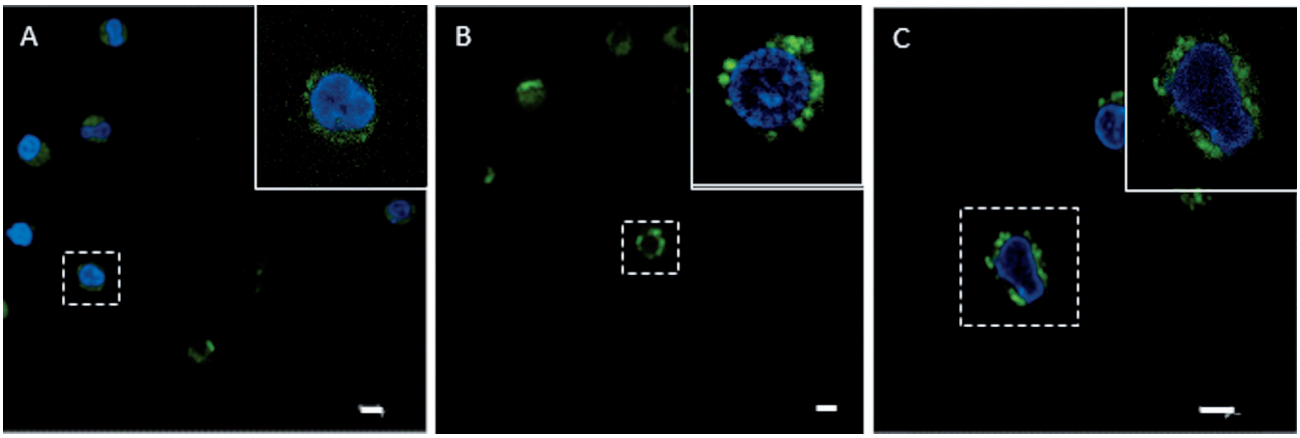


Figure 4. Live confocal microscopy analysis of the interaction of glyco-POSS **16** (panel A) and **17** (panels B and C) with stable K562 transfectant cells expressing DC-SIGN (K562-CD209). The cells were incubated with the glyco-POSS in phosphate-buffered saline for 10 min at 4 °C. The cell nuclei were subsequently stained with 4′,6-diamidino-2-phenylindole. Insets show large images for the indicated cell. Results are representatives of multiple cells in three independent experiments. Scale bar = 10 μm.

ment of the BODIPY chromophore to the inorganic cage of POSS confers an improved photostability to the multivalent construct as we have described recently,[24,43] which is particularly important for the new imaging techniques developed in optical microscopy that require high-laser-intensity irradiation.

We have used confocal laser-scanning microscopy to visualize the binding interaction of fluorescent glyco-POSS cluster **17** with cell surface receptors of human K562 cells stably transfected with DC-SIGN (K562-CD209),[44] which express a high level of endogenous DC-SIGN (Figure 4). Analogue **16**, which has all its key sugar hydroxyls blocked with acetal protecting groups and should therefore be unable to interact specifically with the sugar receptors, was used as control. The cells were incubated with the probe for a short time (10 min) and at low temperature (4 °C) to minimize the receptor-mediated endocytosis of the glyco-POSS. The slow off-rate of **17** from the receptors, as expected for a multivalent binding interaction, allowed imaging (at room temperature) to take place following washout of the unbound fluorescent probe. Live confocal images revealed the strong fluorescence of **17** located mainly at the cell membrane (Figure 4 b and c), although a minor internalization of the probe into the cytoplasm was unavoidable in spite of the precautions taken to avoid the receptor-mediated endocytosis. In comparison, only a very faint and diffuse (nonspecific) staining was observed for the control compound **16** (Figure 4 a).

Conclusion

We have shown that readily available octakis(3-azidopropyl)octasilsesquioxane (**6**) is an ideal starting material for the controlled assembly of well-defined hetero-bifunctional POSS. Successive grafting of two different groups on the POSS core has been efficiently achieved by using two sequential ligand-accelerated CuAAC functionalizations. The key initial monofunctionalization of **6** required a careful optimization of the reaction conditions to minimize formation of complex mixtures of polysubstituted POSS products. The selectivity of this reaction is not only dependent on the stoichiometry of the reactants, but also on the donor ability of the ligands on the copper catalyst. A simple statistical calculation proved to be essential for an informed decision on the appropriate reaction stoichiometry required for the selective monofunctionalization of homo-octafunctional POSS molecules. A screening of copper catalysts showed that **[Cu(C18$_6$tren)]Br** provided the reaction outcome closest to the expected statistically. This catalyst effect on selectivity can be ascribed to the ligand-accelerated protonolysis of the copper-triazolide intermediate of the CuAAC reaction in combination with the hampering of further coordination of the metal in this intermediate to alkyne or azide. The presence of a tethered carboxylic acid on the ligand does not seem to be really required for this accelerated hydrolysis.[31b]

The wide substrate scope and high efficiency of the click functionalization allow for the easy preparation of a large variety of unprecedented hetero-bifunctional POSS with a perfectly controlled distribution of functional groups on the cubic framework. To demonstrate the versatility of this methodology, we have prepared a set of bifunctional POSS with orthogonal reactivity, which can be used as nano-building-blocks for the controlled assembly of hybrid nanomaterials, and a multivalent fluorescent probe for application in targeted cell-imaging. The inorganic cage of POSS provides an improved photostability to the covalently attached dye,[24,43] as well as a convenient framework for the 3D multivalent display[26] of the pendant epitopes. In addition, the recently observed gradual disassembly of similar water-soluble octasilsesquioxanes under physiological conditions[26] and the expected low toxicity of the resulting monomeric organosilanes suggest that these hybrid multivalent constructs would be very attractive systems for in vivo applications. Accordingly, fluorescent bioprobes based on well-defined

POSS materials offer interesting advantages over more conventional, fully organic analogues and ill-defined hybrid nanoparticles and promise to become powerful tools for dissecting cellular interactions at the molecular level and for biomedical applications. We foresee that the described synthetic methodology can be easily transferred to similar "click"-type functionalization reactions, such as the highly efficient radical-mediated thiol-ene/yne reaction,[45,46] widely expanding the potential applications of this strategy in POSS chemistry.

Acknowledgements

This work was supported by the Spanish Ministerio de Ciencia e Innovación (projects MAT2010-20646-C04-01 and MAT2010-20646-C04-03), the European Social Fund and Comunidad de Madrid (project S2009/PPQ-1634 "AVANCAT"), Fondo de Investigación Sanitaria (FIS PI11/929), and Obra Social Cajastur-IUOPA. The Ministerio de Ciencia e Innovación is gratefully thanked for a FPU predoctoral contract to B.T. and CSIC for a JAEPRE contract to M.E.P.O. We also thank Prof. Angel Costela for a thoughtful critical review of the manuscript.

[1] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem.* **2001**, *113*, 2056–2075; *Angew. Chem. Int. Ed.* **2001**, *40*, 2004–2021.

[2] a) P. D. Lickiss, F. Rataboul, *Adv. Organomet. Chem.* **2008**, *57*, 1–116; b) D. B. Cordes, P. D. Lickiss, F. Rataboul, *Chem. Rev.* **2010**, *110*, 2081–2173.

[3] a) R. M. Laine, C. Zhang, A. Sellinger, L. Viculis, *Appl. Organomet. Chem.* **1998**, *12*, 715–723; b) R. M. Laine, *J. Mater. Chem.* **2005**, *15*, 3725–3744; c) S.-W. Kuo, F.-C. Chang, *Prog. Polym. Sci.* **2011**, *36*, 1649–1696; d) R. M. Laine, M. F. Roll, *Macromolecules* **2011**, *44*, 1073–1109; e) F. Wang, X. Lu, C. He, *J. Mater. Chem.* **2011**, *21*, 2775–2782.

[4] a) *Applications of Polyhedral Oligomeric Silsesquioxanes, in Advances in Silicon Science, Vol. 3*, (Ed.: C. Hartmann-Thompson), Springer, **2011**; b) K. Tanaka, Y. Chujo, *J. Mater. Chem.* **2012**, *22*, 1733–1746; c) S. Fabritz, S. Horner, O. Avrutina, H. Kolmar, *Org. Biomol. Chem.* **2013**, *11*, 2224–2236; d) K. Tanaka, Y. Chujo, *Polym. J.* **2013**, *45*, 247–254.

[5] a) E. Rikowski, H. C. Marsmann, *Polyhedron* **1997**, *16*, 3357–3361; b) F. J. Feher, R. Terroba, J. W. Ziller, *Chem. Commun.* **1999**, 2153–2154; c) V. Ervithayasuporn, X. Wang, Y. Kawakami, *Chem. Commun.* **2009**, 5130–5132; d) M. Z. Asuncion, R. M. Laine, *J. Am. Chem. Soc.* **2010**, *132*, 3723–3736; e) M. Ronchi, S. Sulaiman, N. R. Boston, R. M. Laine, *Appl. Organomet. Chem.* **2009**, *24*, 551–557; f) J. C. Furgal, J. H. Jung, T. Mizmuo, K. Chou, M. Schwartz, T. Goodson, III, R. M. Laine, *Polym. Prepr. Am. Chem. Soc. Div. Polym. Chem.* **2012**, *53*, 15–16; g) R. M. Laine, J. H. Jung, J. C. Furgal, K. Chou, M. Schwartz, T. Goodson, III, *Polym. Prepr. Am. Chem. Soc. Div. Polym. Chem.* **2012**, *53*, 543.

[6] a) R. Muller, C. Dathe, L. Heinrich, *J. Prakt. Chem.* **1959**, *9*, 71–74; b) C. L. Frye, W. T. Collins, *J. Am. Chem. Soc.* **1970**, *92*, 5586–5588; c) P. A. Agaskar, *Inorg. Chem.* **1991**, *30*, 2707–2708; d) G. Calzaferri, D. Herren, R. Imhof, *Helv. Chim. Acta* **1991**, *74*, 1278–1280.

[7] a) K. A. Andrianov, N. M. Petrovnina, T. V. Vasileva, V. E. Shklover, B. I. Dyachenko, *Zh. Obshch. Khim.* **1978**, *48*, 2692–2695; b) F. J. Feher, D. Soulivong, A. G. Eklund, K. D. Wyndham, *Chem. Commun.* **1997**, 1185–1186; c) P. G. Harrison, C. Hall, *Main Group Met. Chem.* **1997**, *20*, 515–529; d) B. W. Manson, J. J. Morrison, P. I. Coupar, P.-A. Jaffres, R. E. Morris, *J. Chem. Soc. Dalton Trans.* **2001**, 1123–1127; e) Y. Itami, B. Marciniec, M. Kubicki, *Chem. Eur. J.* **2004**, *10*, 1239–1248; f) E. O. Dare, L.-K. Liu, J. Peng, *Dalton Trans.* **2006**, 3668–3671; g) G. Cheng, N. R. Vautravers, R. E. Morris, D. J. Cole-Hamilton, *Org. Biomol. Chem.* **2008**, *6*, 4662–4667; h) S. Sulaiman, A. Bhaskar, J. Zhang, R. Guda, T. Goodson, III, R. M. Laine, *Chem. Mater.* **2008**, *20*, 5563–5573; i) P. Zak, B. Marciniec, M. Majchrzak, C. Pietraszuk, *J. Organomet. Chem.* **2011**, *696*, 887–891.

[8] a) K. Olsson, *Ark. Kemi* **1958**, *13*, 367–378; b) A. R. Bassindale, Z. Liu, I. A. MacKinnon, P. G. Taylor, Y. Yang, M. E. Light, P. N. Horton, M. B. Hursthouse, *Dalton Trans.* **2003**, 2945–2949; c) M. Kozelj, B. Orel, *Dalton Trans.* **2008**, 5072–5075.

[9] a) U. Dittmar, B. J. Hendan, U. Floerke, H. C. Marsmann, *J. Organomet. Chem.* **1995**, *489*, 185–194; b) S. Lucke, K. Stoppek-Langner, B. Krebs, M. Lage, *Z. Anorg. Allg. Chem.* **1997**, *623*, 1243–1246; c) A. Gultek, T. Seckin, H. I. Adiguzel, *Turk. J. Chem.* **2005**, *29*, 391–399; d) Y. Liu, X. Yang, W. Zhang, S. Zheng, *Polymer* **2006**, *47*, 6814–6825; e) B. Marciniec, M. Dutkiewicz, H. Maciejewski, M. Kubicki, *Organometallics* **2008**, *27*, 793–794; f) S. Fabritz, D. Heyl, V. Bagutski, M. Empting, E. Rikowski, H. Frauendorf, I. Balog, W.-D. Fessner, J. J. Schneider, O. Avrutina, H. Kolmar, *Org. Biomol. Chem.* **2010**, *8*, 2212–2218; g) D. Heyl, E. Rikowski, R. C. Hoffmann, J. J. Schneider, W.-D. Fessner, *Chem. Eur. J.* **2010**, *16*, 5509.

[10] a) M.-C. Gravel, R. M. Laine, *Polym. Prepr. Am. Chem. Soc. Div. Polym. Chem.* **1997**, *38*, 155–156; b) F. J. Feher, K. D. Wyndham, *Chem. Commun.* **1998**, 323–324; c) F. J. Feher, K. D. Wyndham, M. A. Scialdone, *Chem. Commun.* **1998**, 1469–1470; d) F. J. Feher, K. D. Wyndham, D. Soulivong, F. Nguyen, *J. Chem. Soc. Dalton Trans.* **1999**, 1491–1498.

[11] C. Marcolli, G. Calzaferri, *Appl. Organomet. Chem.* **1999**, *13*, 213–226, 3457.

[12] a) H. Ghanbari, B. G. Cousins, A. M. Seifalian, *Macromol. Rapid Commun.* **2011**, *32*, 1032–1046; b) K.-Y. Pu, B. Liu, *Adv. Funct. Mater.* **2011**, *21*, 3408–3423; c) F. Olivero, F. Reno, F. Carniato, M. Rizzi, M. Cannas, L. Marchese, *Dalton Trans.* **2012**, *41*, 7467–7473.

[13] a) R. M. Laine, M. Roll, M. Asuncion, S. Sulaiman, V. Popova, D. Bartz, D. J. Krug, P. H. Mutin, *J. Sol-Gel Sci. Technol.* **2008**, *46*, 335–347; b) M. Z. Asuncion, M. Ronchi, H. Abu-Seir, R. M. Laine, *C. R. Chim.* **2010**, *13*, 270–281; c) Y. Li, W.-B. Zhang, I. F. Hsieh, G. Zhang, Y. Cao, X. Li, C. Wesdemiotis, B. Lotz, H. Xiong, S. Z. D. Cheng, *J. Am. Chem. Soc.* **2011**, *133*, 10712–10715.

[14] Some Janus-type cubic silsesquioxanes are now commercially available from Mayaterials, Inc (http://www.mayaterials.com).

[15] a) B. J. Hendan, H. C. Marsmann, *J. Organomet. Chem.* **1994**, *483*, 33–38; b) S. Kraus-Ophir, I. Jerman, B. Orel, D. Mandler, *Soft Matter* **2011**, *7*, 8862–8869; c) C. Chen, S. Huang, M. Chen, Q. Lu, *High Perform. Polym.* **2012**, *24*, 119–124.

[16] a) Z. Li, Y. Kawakami, *Chem. Lett.* **2008**, *37*, 804–805; b) J. H. Jung, R. M. Laine, *Macromolecules* **2011**, *44*, 7263–7272.

[17] S. Tateyama, Y. Kakihana, Y. Kawakami, *J. Organomet. Chem.* **2010**, *695*, 898–902.

[18] a) F. J. Feher, D. A. Newman, J. F. Walzer, *J. Am. Chem. Soc.* **1989**, *111*, 1741–1748; b) S. T. Iacono, A. Vij, W. Grabow, D. W. Smith, Jr., J. M. Mabry, *Chem. Commun.* **2007**, 4992–4994; c) P. Żak, C. Pietraszuk, B. Marciniec, G. Spólnik, W. Danikiewicz, *Adv. Synth. Catal.* **2009**, *351*, 2675–2682; d) Y.-C. Lin, S.-W. Kuo, *J. Polym. Sci. Part A: Polym. Chem.* **2011**, *49*, 2127–2137; e) H. Liu, M. Puchberger, U. Schubert, *Chem. Eur. J.* **2011**, *17*, 5019–5023; f) B. M. Moore, S. M. Ramirez, G. R. Yandek, T. S. Haddad, J. M. Mabry, *J. Organomet. Chem.* **2011**, *696*, 2676–2680; g) W. Wang, Q. Shen, W. Zha, G. Zhu, *J. Polym. Res.* **2011**, *18*, 1119–1124; h) J. K. Hu, Q. C. Zhang, S. L. Gong, *Chin. Chem. Lett.* **2012**, *23*, 181–184; i) S. C. Kettwich, S. N. Pierson, A. J. Peloquin, J. M. Mabry, S. T. Iacono, *New J. Chem.* **2012**, *36*, 941–946; j) Y. Zheng, L. Wang, S. Zheng, *Eur. Polym. J.* **2012**, *48*, 945–955.

[19] a) C. Zhang, R. M. Laine, *J. Organomet. Chem.* **1996**, *521*, 199–201; b) A. Tsuchida, C. Bolln, F. G. Sernetz, H. Frey, R. Muelhaupt, *Macromolecules* **1997**, *30*, 2818–2824; c) F. J. Feher, K. D. Wyndham, R. K. Baldwin, D. Soulivong, J. D. Lichtenhan, J. W. Ziller, *Chem. Commun.* **1999**, 1289–1290; d) R. Knischka, F. Dietsche, R. Hanselmann, H. Frey, R. Mülhaupt, P. J. Lutz, *Langmuir* **1999**, *15*, 4752–4756; e) M. A. Said, H. W. Roesky, C. Rennekamp, M. Andruh, H.-G. Schmidt, M. Noltemeyer, *Angew. Chem.* **1999**, *111*, 702–705;

Angew. Chem. Int. Ed. **1999**, *38*, 661–664; f) N. Auner, J. W. Bats, D. E. Katsoulis, M. Suto, R. E. Tecklenburg, G. A. Zank, *Chem. Mater.* **2000**, *12*, 3402–3418; g) A. R. Bassindale, D. J. Parker, P. G. Taylor, A. C. Watt, *Can. J. Chem.* **2003**, *81*, 1341–1349; h) C. McCusker, J. B. Carroll, V. M. Rotello, *Chem. Commun.* **2005**, 996–998; i) K. Tanaka, K. Inafuku, Y. Chujo, *Bioorg. Med. Chem.* **2008**, *16*, 10029–10033; j) S.-y. Kuwahara, K. Yamamoto, J.-i. Kadokawa, *Chem. Lett.* **2010**, *39*, 1045–1047; k) X.-F. Yu, S. Zhong, X.-P. Li, Y.-F. Tu, S.-G. Yang, H. R. M. Van, C.-Y. Ni, D. J. Pochan, R. P. Quirk, C. Wesdemiotis, W.-B. Zhang, S. Z. D. Cheng, *J. Am. Chem. Soc.* **2010**, *132*, 16741–16744; l) W.-B. Zhang, Y. Li, X. Li, X. Dong, X. Yu, C.-L. Wang, C. Wesdemiotis, R. P. Quirk, S. Z. D. Cheng, *Macromolecules* **2011**, *44*, 2589–2596; m) Y. Li, X.-H. Dong, K. Guo, Z. Wang, Z. Chen, C. Wesdemiotis, R. P. Quirk, W.-B. Zhang, S. Z. D. Cheng, *ACS Macro Lett.* **2012**, *1*, 834–839; n) H. Lin, X. Wan, X. Jiang, Q. Wang, J. Yin, *J. Mater. Chem.* **2012**, *22*, 2616–2623; o) S. Hörner, S. Fabritz, H. D. Herce, O. Avrutina, C. Dietz, R. W. Stark, M. C. Cardoso, H. Kolmar, *Org. Biomol. Chem.* **2013**, *11*, 2258–2265.

[20] E. A. Rebrov, N. A. Tebeneva, A. M. Mouzafarov, Y. E. Ovchinnikov, Y. T. Struchkov, T. V. Strelkova, *Russian Chem. Bull.* **1995**, *44*, 1286–1292.

[21] B. Trastoy, M. E. Perez-Ojeda, R. Sastre, J. L. Chiara, *Chem. Eur. J.* **2010**, *16*, 3833–3841.

[22] J. R. Suárez, B. Trastoy, M. E. Perez-Ojeda, R. Marin-Barrios, J. L. Chiara, *Adv. Synth. Catal.* **2010**, *352*, 2515–2520.

[23] a) C. W. Tornøe, M. Meldal, "*Peptidotriazoles: Copper(I)-catalyzed 1,3-dipolar cycloadditions on solid-phase*", *Peptides* **2001**, *Proc. Am. Pept. Symp.*, American Peptide Society and Kluwer Academic Publishers, San Diego, **2001**, pp. 263- 264; b) C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057–3064; c) V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem.* **2002**, *114*, 2708–2711; *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599. For a comprehensive review, see: d) M. Meldal, C. W. Tornøe, *Chem. Rev.* **2008**, *108*, 2952–3015.

[24] M. E. Pérez-Ojeda, B. Trastoy, I. Lopez-Arbeloa, J. Banuelos, A. Costela, I. Garcia-Moreno, J. L. Chiara, *Chem. Eur. J.* **2011**, *17*, 13258–13268.

[25] For alternative syntheses of **6**, see ref. [5c, 9f and 9g]. For other examples of CuAAC functionalization of this substrate, see ref. [9f,g].

[26] B. Trastoy, D. A. Bonsor, M. E. Pérez-Ojeda, M. L. Jimeno, A. Méndez-Ardoy, J. M. García-Fernández, E. J. Sundberg, J. L. Chiara, *Adv. Funct. Mater.* **2012**, *22*, 3191–3201.

[27] In an excellent "teaching editorial", Donald M. Simons nicely explained the statistical relationship between chosen stoichiometry and reaction outcome for the derivatization of a homobifunctional spacer with a monofunctional reagent. Interestingly, this communication has been almost completely overlooked by the synthetic chemistry community (cited only once, according to Web of Knowledge): D. M. Simons, *Bioconjugate Chem.* **1999**, *10*, 3–8.

[28] This ideal case rarely occurs in real molecules, particularly for the most substituted derivatives due to increasing steric hindrance with increasing substitution; thus, ratios of multisubstituted products are somehow overestimated by this assumption.

[29] Not surprisingly, the examples of synthesis of monofunctional POSS by selective monofunctionalization of a homo-octafunctional POSS that has been described using a 1:1 stoichiometry rarely exceed a 30% isolated yield.

[30] The rather lipophilic octaazide **6** is an ideal substrate in this context too, since each azide to 1,2,3-triazole functional group transformation will produce a significant increase of the polarity of the molecule thus allowing for a facile chromatographic separation of unreacted POSS from the more polar mono-triazolyl-POSS product and also of the latter from all possible polytriazolyl-POSS derivatives that may have formed in the click reaction, which will all have an even higher polarity and, thus, a lower mobility in normal phase silica column chromatography.

[31] a) V. O. Rodionov, V. V. Fokin, M. G. Finn, *Angew. Chem.* **2005**, *117*, 2250–2255; *Angew. Chem. Int. Ed.* **2005**, *44*, 2210–2215; b) V. O. Rodionov, S. I. Presolski, D. Diaz Diaz, V. V. Fokin, M. G. Finn, *J. Am. Chem. Soc.* **2007**, *129*, 12705–12712.

[32] B.-Y. Lee, S. R. Park, H. B. Jeon, K. S. Kim, *Tetrahedron Lett.* **2006**, *47*, 5105–5109.

[33] J. Raushel, V. V. Fokin, *Org. Lett.* **2010**, *12*, 4952–4955.

[34] a) V. Jurkauskas, J. P. Sadighi, S. L. Buchwald, *Org. Lett.* **2003**, *5*, 2417–2420; b) S. Díez-González, A. Correa, L. Cavallo, S. P. Nolan, *Chem. Eur. J.* **2006**, *12*, 7558–7564.

[35] V. O. Rodionov, S. I. Presolski, S. Gardinier, Y.-H. Lim, M. G. Finn, *J. Am. Chem. Soc.* **2007**, *129*, 12696–12704.

[36] a) G. Barré, D. Taton, D. Lastecoueres, J.-M. Vincent, *J. Am. Chem. Soc.* **2004**, *126*, 7764–7765; b) N. Candelon, D. Lastecoueres, A. K. Diallo, J. Ruiz Aranzaes, D. Astruc, J.-M. Vincent, *Chem. Commun.* **2008**, 741–743.

[37] a) A linear relationship between the donor ability of the ligands (as determined by their proton affinity) and the activation barrier of the protonolytic cleavage of the metal–carbon bond has been found by DFT calculations for some transition-metal complexes, with better donors decreasing the barrier: H. M. Senn, D. V. Deubel, P. E. Blöchl, A. Togni, G. Frenking, *J. Mol. Struct.* **2000**, *506*, 233–242. The low donor ability of the **TBTA** ligand could thus explain its negligible effect on the outcome of diazide reactions. b) Likewise, the efficiency of **CuTC** in catalyzing the formation of 1-sulfonyl-1,2,3-triazoles over other competitive processes in the CuAAC reaction of alkynes with sulfonyl azides has been ascribed (see ref. [33]) to its ability to stabilize the Cu-triazolide intermediate and/or to promote the protonolytic cleavage of this intermediate, which could also explain its positive effect on the selectivity of the monofunctionalization of POSS polyazides.

[38] H. Araki, K. Naka, *Macromolecules* **2011**, *44*, 6039–6045.

[39] A. Cambi, M. Koopman, C. G. Figdor, *Cell. Microbiol.* **2005**, *7*, 481–488.

[40] L. East, C. M. Isacke, *Biochim. Biophys. Acta Gen. Subj.* **2002**, *1572*, 364–386.

[41] U. Svajger, M. Anderluh, M. Jeras, N. Obermajer, *Cell Signal* **2010**, *22*, 1397–1405.

[42] T. B. H. Geijtenbeek, R. Torensma, S. J. van Vliet, G. C. F. van Duijnhoven, G. J. Adema, Y. van Kooyk, C. G. Figdor, *Cell* **2000**, *100*, 575–585.

[43] See the Supporting Information for a laser and photostability study of compound **16**.

[44] E. Caparros, P. Munoz, E. Sierra-Filardi, D. Serrano-Gomez, A. Puig-Kroger, J. L. Rodriguez-Fernandez, M. Mellado, J. Sancho, M. Zubiaur, A. L. Corbi, *Blood* **2006**, *107*, 3950–3958.

[45] For recent reviews, see: a) C. E. Hoyle, C. N. Bowman, *Angew. Chem.* **2010**, *122*, 1584–1617; *Angew. Chem. Int. Ed.* **2010**, *49*, 1540–1573; b) A. Dondoni, A. Marra, *Chem. Soc. Rev.* **2012**, *41*, 573–586; c) A. Massi, D. Nanni, *Org. Biomol. Chem.* **2012**, *10*, 3791–3807.

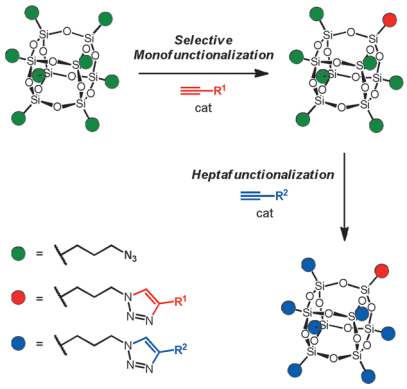
[46] However, the thiol-ene/yne reaction has narrower functional group compatibility than the CuAAC reaction. For some selected applications of the thiol-ene/yne reaction in POSS chemistry, see: a) Y. Gao, A. Eguchi, K. Kakehi, Y. C. Lee, *Org. Lett.* **2004**, *6*, 3457–3460; b) J. Xu, X. Li, C. M. Cho, C. L. Toh, L. Shen, K. Y. Mya, X. Lu, C. He, *J. Mater. Chem.* **2009**, *19*, 4740–4745; c) I. Nischang, O. Brueggemann, I. Teasdale, *Angew. Chem.* **2011**, *123*, 4688–4692; *Angew. Chem. Int. Ed.* **2011**, *50*, 4592–4596; d) M. Lo Conte, S. Staderini, A. Chambery, N. Berthet, P. Dumy, O. Renaudet, A. Marra, A. Dondoni, *Org. Biomol. Chem.* **2012**, *10*, 3269–3277; e) Z. Wang, Y. Li, X.-H. Dong, X. Yu, K. Guo, H. Su, K. Yue, C. Wesdemiotis, S. Z. D. Cheng, W.-B. Zhang, *Chem. Sci.* **2013**, *4*, 1345–1352; f) A. Marra, S. Staderini, N. Berthet, P. Dumy, O. Renaudet, A. Dondoni, *Eur. J. Org. Chem.* **2013**, 1144–1149. See also references [13c, 19l, and 19m].

Received: January 29, 2013
Published online: ■■ ■■, 2013

Click Chemistry

*M. E. Pérez-Ojeda, B. Trastoy, Á. Rol, M. D. Chiara, I. García-Moreno, J. L. Chiara** ..... ▮▮▮▮–▮▮▮▮

Controlled Click-Assembly of Well-Defined Hetero-Bifunctional Cubic Silsesquioxanes and Their Application in Targeted Bioimaging



Play the click dice game: The selectivity of the functionalization reaction of a symmetric polyfunctional substrate is statistically related to the stoichiometry of the reactants. For the copper(I)-catalyzed azide–alkyne cycloaddition monofunctionalization of the cubic octaazide shown, this statistical relationship holds only if a copper catalyst with strong donor ligands is employed for the click reaction, allowing the preparation of well-defined hetero-bifunctional hybrid nanoclusters.

