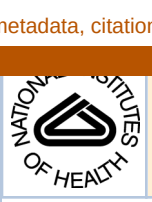

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Exhaustive glycosylation, PEGylation, and glutathionylation of a [G4]-ene$_{48}$ dendrimer via photoinduced thiol-ene coupling

Mauro Lo Conte[1], **Maxwell J. Robb**[2], **Yvonne Hed**[3], **Alberto Marra**[1], **Michael Malkoch**[3], **Craig J. Hawker**[2], and **Alessandro Dondoni**[1]

[1]Dipartimento di Chimica, Laboratorio di Chimica Organica, Università di Ferrara, Via L. Borsari 46, 44100 Ferrara, Italy.

[2]Department of Chemistry and Biochemistry, Materials Department, and Materials Research Laboratory (MRL), University of California, Santa Barbara, California 93106, USA.

[3]The Royal Institute of Technology, School of Chemical Science and Engineering, Division of Coating Technology, Teknikringen 56-58, SE-10044 Stockholm, Sweden.

Abstract

We report in this paper the use of free-radical thiol-ene coupling (TEC) for the introduction of carbohydrate, poly(ethylene glycol), and peptide fragments at the periphery of an alkene functional dendrimer. Four different sugar thiols including glucose, mannose, lactose and sialic acid, two PEGylated thiols and the natural tripeptide glutathione were reacted with a fourth generation alkene functional dendrimer [G4]-ene$_{48}$ upon irradiation at λ_{max} 365 nm. In all cases, the ^{1}H NMR spectra of the crude reaction mixture revealed the complete disappearance of alkene proton signals indicating the quantitative conversion of all 48 alkene groups of the dendrimer. With one exception only, all dendrimer conjugates were isolated in high yields (70–94%), validating the high efficiency of multiple TEC reactions on a single substrate. All isolated and purified compounds were analyzed by MALDI-TOF spectrometry and gave spectra consistent with the assigned structure.

Keywords

Carbohydrates; Click Chemistry; Dendrimers; Irradiation; MALDI

Introduction

The post-synthetic modification of dendrimers by conjugation to biologically relevant molecules is a key operation toward biomedical applications.[1] Given the versatility and robustness of the Cu-catalyzed alkyne-azide cycloaddition (CuAAC),[2,3] this prototypical click process is an attractive ligation tool for reaching this goal.[4–10] This versatility has been previously demonstrated by the preparation of an unsymmetrical dendrimer which was functionalized with sixteen mannose residues on one dendron and two coumarin fluorescent units attached to the chain ends of the smaller G-1 dendron through CuAAC chemistry.[11] Despite the unquestionable synthetic value of CuAAC as documented by an array of applications within the fields of material and life sciences,[12] a limitation is the intrinsic toxicity of the copper catalyst and associated difficulty in its removal. Consequently, this often results in the inability to translate this synthetic approach to a wide variety of biological applications. Furthermore, the difficulty in completely removing metallic

Correspondence to: Michael Malkoch; Craig J. Hawker; Alessandro Dondoni.

impurities from polymeric materials can also lead to detrimental effects in optical and electronic properties.[13] Substantial copper contamination of PEGylated poly(amide)-based dendrons and dendrimers prepared via CuAAC chemistry was recently reported by Weck and co-workers.[14] This drawback was circumvented by using the copper-free strain-promoted alkyne-azide cycloaddition (SPAAC) approach developed by Bertozzi[15,16] and Boons[17,18] that exploits the reactivity of cyclooctyne derivatives toward azides or through the development of new ligands with increased catalytic activity. Despite these advances, the introduction of rigid cyclooctatriazole units derived from SPAAC can alter the topology of the scaffold, thus reducing the ability of conjugated groups to interact with complementary biomolecules. The presence of residual ligands may also lead to unwanted side reactions.[19,20]

In order to address these concerns and to expand the portfolio of viable conjugation chemistries, it is convenient to examine the toolbox of click reactions[21–23] to find an alternative synthetic process that can be complementary to both the CuAAC and SPAAC-based approaches. In this context, we herein report on the validation of the free-radical thiol-ene coupling (TEC) for the introduction of carbohydrate, poly(ethylene glycol), and peptide fragments at the periphery of an alkene functional dendrimer. While the power of TEC for poly(thioether) dendrimer formation and functionalization has been recently highlighted,[24] its wide scope and synthetic utility are amply documented by the wide range of applications in polymer and material chemistry and, to a lesser extent, in organic synthesis and bioorganic chemistry.[25–39] This reaction is referred to as a click process for its high chemo- and regioselectivity as well as complete atom economy and unique tolerance to oxygen. Significantly, TEC does not require the use of a metal catalyst as it can be initiated by irradiation at a wavelength close to visible light and occurs at room temperature in aqueous solvents under neutral conditions. TEC has even been shown to proceed upon exposure to unfocused sunlight,[29] demonstrating the success of TEC under mild and benign conditions while requiring minimal energy. These characteristics aptly facilitate the conjugation of chemically sensitive biomolecules such as carbohydrates, peptides and proteins that could easily become altered under more harsh reaction conditions. Finally, TEC establishes a robust thioether linkage, which in addition to being flexible and sterically non-demanding, is stable toward chemical and enzymatic hydrolysis.[40]

Results and Discussion

We selected a fourth generation alkene functional dendrimer [G4]-ene$_{48}$ **1** (Figure 1) as it represents a versatile dendritic platform that is readily obtained through combining both CuAAC and TEC reactions via an accelerated AB_2+CD_2 growth approach.[41] However, the decoration of higher generation dendrimers via TEC with complex biomolecules including carbohydrates and peptides has not been investigated primarily because of the low availability of many biomolecules of interest and difficulties in purification of the highly functionalized products. In order to eliminate any trace of copper contamination derived from the CuAAC employed in the second and fourth steps of the dendrimer synthesis, a solution of the crude product **1** in CH_2Cl_2 was washed with a saturated aqueous solution of EDTA disodium salt and the product was analyzed by atomic absorption spectroscopy (AAS). This analysis showed the presence of copper below 60 ppm (w/w), a value that is consistent with other bioconjugation studies using CuAAC.[42] Thus, the purified [G4]-ene$_{48}$ **1** was employed as a scaffold for TECs with a library of thiol functional biomolecules shown in Figure 1.

Initial studies were directed towards the introduction of sugar residues to the dendrimer **1**. Glycodendrimers are promising candidates for a number of applications, including their use in enantioselective catalysis,[43,44] as glycoclusters for multivalent interaction with proteins

such as lectins,[45] and as hyperbranched glycomimetics of bioactive carbohydrates found as a part of the glycocalix surrounding cells.[46] Suitable reaction conditions were investigated by studying the photoinduced reaction of **1** with a simple sugar thiol such as 1-thio-β-D-glucopyranose **2a**. A mixture of MeOH:H_2O (1:1) or pure DMF were found to be the most appropriate solvents for conducting the TEC reaction. Coupling of **1** with 2–4 equiv of **2a**/ene were carried out in these solvents under previously established standard conditions for multiple TECs on calixarene scaffold,[30] i.e. irradiation for 1 h at λ_{max} 365 nm in the presence of 2,2-dimethoxy-2-phenylacetophenone (DPAP) as the initiator. Under these conditions and using 0.5–0.7 mM solutions of **1** (Table 1, entries 1 and 2) only partial addition took place as evidenced by the presence of residual alkene proton signals in the 5–6 ppm region of the NMR spectra (CD_3OD) of the crude reaction mixtures. However, quantitative conjugation of alkene functional groups could be achieved as evidenced by ^1H NMR analysis by increasing the concentration of the reaction mixture.

The resulting glycodendrimer **5a** (Figure 2) was successfully synthesized using a 9.2 mM solution of **1** in DMF and 4 equiv of **2a**/ene (Table 1, entry 3) and isolated by chromatography over Sephadex LH-20 in excellent yield (94%). Complete *S*-glycosylation in **5a** was demonstrated by GPC analysis (see Figure S1 in Supporting Information). In order to probe the effect of bulk and differing configuration in the thiols, the *S*-glycosylation of **1** with 1-thio-β-D-mannopyranose **2b** and the disaccharide 1-thio-β-D-lactopyranose **2c** was carried out. Under the same optimized reaction conditions described above, the photoinduced reaction of **1** with **2b** proceeded to completion to give the product **5b** in 77% isolated yield (Figure 2). For the reaction of **1** with the sterically demanding disaccharide **2c**, a further increase in concentration to 28 mM solution of dendrimer **1** in DMF and 5 equiv of **2c**/ene was necessary while an irradiation time of 2 h was found sufficient for the reaction to reach completion (Table 1, entry 6). As with previous results, the corresponding disaccharide glycoconjugate **5c** (Figure 2) was obtained in high isolated yield (93%) and purity.

Given the importance of assembling sialoclusters tethered to dendritic scaffolds,[47] and the efficiency of sialoclusters to inhibit viral replication,[48] we set out to introduce the most common sialic acid, 5-*N*-acetyl-neuraminic acid (Neu5Ac) at the chain ends of dendrimer **1**. To this end we prepared the new sialic acid thiol **2d** (see Supporting Information) featuring an alkyl bridge between the sulfhydryl group and the carbohydrate moiety. This alkyl chain was introduced in order to avoid encumbering interactions in the TEC due to the bulky sialyl fragment. Significantly, the photoinduced coupling of **1** with excess of **2d** in DMF resulted in complete consumption of all 48 ene groups of the dendrimer as revealed by NMR analysis of the crude reaction mixture with the sialodendrimer **5d** being isolated in 70% yield after purification (Figure 2).

Another important dendrimer modification entails the introduction of hydrophilic groups such as poly(ethylene glycol) (PEG) chains to induce water solubility and biocompatibility. This is especially useful in applications such as drug delivery as the ‘stealthy’ character imparted by PEG chains allows the scaffold to evade the reticuloendothelial system (RES) response and improves circulation lifetimes, favoring delivery of the bioactive agent to the target site.[49–53] In this context, the PEGylation of dendrimer **1** via TEC using the thiol **3a** containing a short methyl ether terminated PEG chain was examined. While concentrated solutions of **1** (from 17 to 46 mM in DMF) (entries 8 and 9) gave incomplete thiol-ene coupling as shown by NMR analysis, further investigation revealed that dendrimer **1** and thiol **3a** formed a homogeneous and transparent solution and therefore the photoreaction could be carried out without the use of any solvent (entry 10). The successful PEGylation of **1** was achieved as illustrated by the complete disappearance of the alkene proton signals in the NMR spectrum of the crude reaction mixture with the dendrimer **6a** (Figure 2) being

isolated in good yield (73%). PEGylation of dendrimer **1** was carried out under the same neat conditions by using the thiol **3b** bearing a PEG chain with a terminal hydroxyl group. In this case the yield of the modified dendrimer **6b** (Figure 2) isolated by chromatography was 93% with both PEGylated dendrimers **6a** and **6b** being fully water soluble.

As a final demonstration of the robustness of the thiol-ene coupling, the introduction of multiple peptide units to the chain ends of dendrimer **1** was performed using glutathione (GSH, **4**) as a model peptide (Figure 1). Initial conditions for the photoinduced reaction of **1** with **4**, employed a 2:2:1 mixture of H_2O-MeOH-DMF in order to dissolve the dendrimer and peptide (Table 1, entry 12). Under these conditions, after1 h irradiation a precipitate was formed that turned out to be insoluble in a wide range of organic solvents. Moreover a significant amount of **1** was recovered. In order to overcome solubility problems the reaction was carried out using the hydrochloride salt of **4**. Following irradiation of **1** and **4˙HCl** in a 4:1 mixture of DMF-H_2O (Table 1, run 15) for 1 h, the ^{1}H NMR spectrum of the isolated material showed the absence of signals in the region of olefinic protons, indicating that all 48 ene groups of **1** had reacted with the cysteine residue of **4˙HCl**. Unfortunately, the amphiphilic conjugate product **7˙HCl** (Figure 2) was only isolated with a yield of 36% as a result of significant column interaction with Sephadex LH-20. Nevertheless, the dendrimer-peptide conjugate **7** displaying the free amino group of glutamic acid may have the advantage of being able to be conjugated to other molecules via an amide linkage, which is one of the most fundamental and widespread chemical bonds in nature.

A variety of techniques were employed to fully characterize these highly functionalized dendritic macromolecules. ^{1}H and ^{13}C provided evidence for the successful incorporation of the functional building blocks to the dendritic chain ends and for the quantitative functionalization of the terminal alkene groups. Gel permeation chromatography (GPC) was then used to confirm the monodisperse nature of the products and in each case, the GPC chromatograms showed low polydispersity peaks, consistent with the dendritic starting materials and minimal chain-end coupling reactions. Further structural confirmation was obtained from MALDI-TOF mass spectrometry (Figure 3). In the case of dendrimer **1**, MALDI-TOF analysis revealed isotopically defined peaks that corresponded to the expected molecular weight of the monodisperse structure. Similarly, the dendrimer **7**, with 48 copies of peptides, displayed molecular weights in agreement with calculated values. For the glycodendrimers and to a lower extent, the PEG-substituted dendrimers, isotopic resolution of compounds **5–6** was unattainable. This was likely due to a high degree of fragmentations coupled with the inherent difficulties of identifying the most preferable sample preparation for optimal ionization of these multifunctional materials.[54,55]

These findings further strengthen the inherent complications of employing MALDI-TOF technique as a universal method to analyze highly complex and high molecular weight dendrimers. Inconsistent ionization performance into the gas phase leads to limited degree of accuracy in terms of peak maximum, shape, and dispersity. One such implication is the different ionization behavior found for dendrimers with sugars and peptide chain ends. However the combination of MALDI-TOF, gel permeation chromatography (GPC) and NMR spectroscopy did provide insight into the structural fidelity of the dendrimer conjugates and the efficient nature of the coupling chemistry.

Experimental

All moisture-sensitive reactions were performed under a nitrogen atmosphere using oven-dried glassware. Anhydrous solvents were dried over standard drying agents[56] 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[57] was performed on silica gel

60 (40–63 μm). Optical rotations were measured at 20 ± 2 °C in the stated solvent; $[\alpha]_D$ values are given in deg·mL·g^{-1}·dm^{-1}. ^{1}H NMR (300 and 400 MHz) and ^{13}C NMR spectra (75 MHz) were recorded in the stated solvent 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 MS analyses were conducted on a Bruker UltraFlex MALDI-TOF MS with SCOUT-MTP Ion Source (Bruker Daltonics, Bremen) equipped with a nitrogen laser (337 nm), a gridless ion source, and reflector design. This instrument was operated in both linear and reflector modes. All spectra, without MLC 710, were obtained in linear mode. The laser intensity was set to the lowest value possible. The obtained spectra were analyzed with FlexAnalysis Bruker Daltonics, Bremen, version 2.2. The matrixes solutions (10 g L^{-1}) used were 2-mercaptobenzothiazole (MTB), 2-(4-hydroxyphenylazo)-benzoic acid (HABA) or 1,8,9-antracenotriol (dithranol) and the salts (1 g L^{-1} in THF) were either trifluoroacetic acid (TFA) sodium salt or TFA silver salt. The former matrix was dissolved in 50:50:0.3 of water-acetonitrile-TFA, while HABA and dithranol was dissolved in THF. The samples were dissolved in chloroform, in 1:1 methanol-water or water (3 g L^{-1} to 5 g L^{-1}). From these mixtures 0.5 mL was added to the MALDI target plate. When the sample was dissolved in chloroform the mixture was allowed to crystallize at room temperature while the samples dissolved in methanol-water or water were placed in the vacuum oven for 15 min at 50 °C.

Gel permeation chromatography (GPC) was performed in DMF or $CHCl_3$ on a Waters 2690 separation module equipped with a Waters 2414 refractive index detector and Waters 2996 photodiode array detector. Molecular weights were calculated relative to linear PS or PEG standards.

The photoinduced thiol-ene reactions were carried out in a glass vial located 2.5 cm away from the household UVA lamp apparatus equipped with four 15 W tubes (1.5 × 27 cm each).

2-[2-(2-Methoxyethoxy)ethoxy]-1-ethanethiol (3a)

The thiol **3a** was prepared from commercially available triethylene glycol monomethyl ether by tosylation and nucleophilic substitution by potassium thioacetate as described.[58]

2-[2-(2-Hydroxyethoxy)ethoxy]-1-ethanethiol (3b)

A solution of commercially available di(ethylene glycol) vinyl ether (1.06 g, 8.00 mmol), thioacetic acid (0.63 mL, 8.80 mmol), and 2,2-dimethoxy-2-phenylacetophenone (DPAP, 45 mg, 0.17 mmol) in anhydrous THF (5 mL) was irradiated at r.t. for 45 min under magnetic stirring and then concentrated. The residue was filtered through a short column of silica gel (2 × 10 cm) with AcOEt to give the corresponding thioacetyl derivative (1.63 g, 98%). A solution of this product in a 0.2 M solution of MeONa in MeOH (20 mL) was kept at r.t. for 14 h in a nitrogen atmosphere, then neutralized with Amberlite IR-120 resin (H^+ form, activated immediately before the use), filtered through a sintered glass filter and concentrated to give pure **3b** (1.26 g, 97%). The physical and spectral data of **3b** were in agreement with those reported in the literature.[59,60]

Glycodendrimer 5a

A solution of dendrimer **1** (10 mg, 0.69 μmol), glucosyl thiol **2a** (26 mg, 0.13 mmol), and DPAP (3.4 mg, 13.3 μmol) in DMF (70 μL) was irradiated at r.t. for 1 h under magnetic stirring and then concentrated. The residue was eluted from a column of Sephadex LH-20 (1 × 30 cm) with 1:1 H_2O-MeOH to give **5a** (15.5 mg, 94%) as a white foam; $[\alpha]_D = -6.2$ (*c*

1.0, H_2O). ^1H NMR (300 MHz, D_2O) selected data: δ 7.76 (s, 30H, 30 H-5 Tr.), 4.36 36 (d, 48H, $J_{1,2}$ = 10.0 Hz, 48 H-1), 3.75 (dd, 48H, $J_{5,6a}$ = 1.5, $J_{6a,6b}$ = 11.8 Hz, 48 H-6a), 3.56 (dd, 48H, $J_{5,6b}$ = 4.5 Hz, 48 H-6b), 1.10 (s, 45H, 15 Me), 1.04 (s, 90H, 30 Me). ^{13}C NMR (75 MHz, D_2O): δ 176.6 (C), 174.4 (C), 173.8 (C), 145.0 (C), 123.9 (CH), 85.7 (CH), 80.1 (CH), 77.6 (CH), 73.7 (CH), 72.6 (CH), 70.1 (CH), 69.8 (CH_2), 65.8 (C), 60.8 (CH_2), 50.1 (CH), 49.1 (CH), 48.1 (CH), 46.6 (C), 34.8 (CH_2), 33.3 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 26.8 (CH_2), 21.6 (CH_2), 17.7 (CH_3), 17.6 (CH_3). MALDI-TOF MS: *m/z* calcd for $C_{975}H_{1642}N_{123}O_{423}S_{63}$ $(M+H)^+$ 23876.13, found 23876.77.

Glycodendrimer 5b

The photoinduced addition of mannosyl thiol **2b** to dendrimer **1** was performed as described for the preparation of **5a** to give, after chromatography on Sephadex LH-20 column (1:1 H_2O-MeOH), **5b** (77%) as a white foam; $[\alpha]_D$ = +1.3 (*c* 0.7, H_2O). ^1H NMR (300 MHz, D_2O) selected data: δ 7.76 (bs, 30H, 30 H-5 Tr.), 1.01 (bs, 135H, 45 Me). ^{13}C NMR (75 MHz, D_2O) selected data: δ 176.5 (C), 174.1 (C), 145.2 (C), 123.9 (CH), 84.8 (CH), 80.5 (CH), 74.2 (CH), 73.5 (C), 72.5 (CH), 71.5 (CH), 70.0 (CH_2), 66.8 (CH), 61.3 (CH_2), 29.5 (CH_2), 27.9 (CH_2), 21.6 (CH_2), 17.7 (CH_3). MALDI-TOF MS: *m/z* calcd for $C_{975}H_{1642}N_{123}O_{423}S_{63}$ $(M+H)^+$ 23876.13, found 21805.62.

Glycodendrimer 5c

A solution of dendrimer **1** (20 mg, 1.38 μmol), lactosyl thiol **2c** (119 mg, 0.33 mmol), and DPAP (8.5 mg, 33.2 μmol) in DMF (50 μL) was irradiated at r. t. for 2 h under magnetic stirring and then concentrated. The residue was eluted from a column of Sephadex LH-20 (1 × 30 cm) with 3:1 H_2O-MeOH to give **5c** (40.7 mg, 93%) as a white foam; $[\alpha]_D$ = −10.3 (*c* 0.3, H_2O). ^1H NMR (300 MHz, DMSO-d_6 + D_2O, 120 °C): δ 7.70 (s, 30H, 30 H-5 Tr.), 4.35-4.27 (m, 190H), 4.19 (t, *J* = 6.5 Hz, 30H), 4.16 (bs, 48H), 2.32 (t, *J* = 7.2 Hz, 60H), 1.90-1.70 (m, 142H), 1.60-1.48 (m, 60H), 1.18 (s, 45H, 15 Me), 1.01 (s, 90H, 30 Me). ^{13}C NMR (75 MHz, D_2O): δ 176.6 (C), 145.0 (C), 123.9 (CH), 103.1 (CH), 85.5 (CH), 78.9 (CH), 78.5 (CH), 76.1 (CH), 75.6 (CH), 73.6 (CH_2), 72.8 (CH), 72.3 (CH), 71.2 (CH), 70.1 (CH_2), 68.8 (CH), 61.2 (CH_2), 60.5 (CH_2), 50.2 (CH), 48.1 (CH), 33.3 (CH_2), 29.5 (CH_2), 26.8 (CH_2), 21.7 (CH_2), 17.7 (CH_3). MALDI-TOF MS: *m/z* calcd for $C_{1263}H_{2122}N_{123}O_{663}S_{63}$ $(M+H)^+$ 31658.88, found 30547.86.

Glycodendrimer 5d

The photoinduced addition of sialyl thiol **2d** to dendrimer **1** was performed as described for the preparation of **5a** to give, after chromatography on Sephadex LH-20 column (1:1 H_2O-MeOH), **5d** (79%) as a white foam; $[\alpha]_D$ = −4.8 (*c* 0.9, H_2O). ^1H NMR (300 MHz, D_2O) selected data: δ 7.70 (bs, 30H, 30 H-5 Tr.), 1.92 (s, 144H, 48 Ac), 1.02 (bs, 135H, 45 Me). ^{13}C NMR (75 MHz, D_2O) selected data: δ 172.9 (C), 171.8 (C), 170.0 (C), 168.4 (C), 141.7 (C), 120.0 (CH), 96.3 (C), 70.1 (CH), 67.7 (CH), 66.7 (C), 64.4 (CH), 63.9 (CH), 59.7 (CH_2), 59.3 (CH_2), 48.6 (CH), 44.5 (C), 35.2 (CH_2), 26.1 (CH_2), 25.7 (CH_2), 24.6 (CH_2), 18.9 (CH_3), 18.2 (CH_3), 14.4 (CH_3). MALDI-TOF MS: *m/z* calcd for $C_{1359}H_{2266}N_{171}O_{615}S_{63}$ $(M+H)^+$ 32861.40, found 31965.45.

Dendrimer 6a

A solution of dendrimer **1** (20 mg, 1.38 μmol), DPAP (6.8 mg, 26.6 μmol), and thiol **3a** (48 mg, 0.26 mmol) was irradiated at r. t. for 1 h under magnetic stirring and then concentrated. The residue was eluted from a column of Sephadex LH-20 (1 × 30 cm) with 1:1 H_2O-MeOH to give **6a** (23.5 mg, 73%) as a colorless liquid. ^1H NMR (300 MHz, CD_3OD): δ 8.02 (t, 30H, *J* = 5.5 Hz, 30 NH), 7.81 (s, 30H, 30 H-5 Tr.), 4.50-4.41 (m, 136H), 4.30-4.20 (m, 100H), 3.68-3.59 (m, 396H), 3.58-3.50 (m, 334H), 3.36 (s, 144H, 48 OMe), 2.84-2.74 (m,

28H), 2.70 (t, *J* = 6.8 Hz, 92H), 2.61 (t, *J* = 7.2 Hz, 112H), 2.43 (t, *J* = 7.0 Hz, 64H), 2.00-1.90 (m, 56H), 1.87-1.78 (m, 112H), 1.68-1.57 (m, 64H), 1.28 (s, 45H, 15 Me), 1.20 (s, 90H, 30 Me). ^{13}C NMR (100 MHz, $CDCl_3$): δ 207.0 (C), 175.1 (C), 172.3 (C), 145.1 (C), 122.1 (CH), 77.3 (CH), 73.7 (CH_2), 71.9 (CH_2), 70.9 (CH_2), 70.6 (CH_2), 70.3 (CH_2), 69.9 (CH_2), 65.0 (CH_2), 63.8 (C), 59.1 (CH), 53.4 (CH_2), 49.8 (CH_2), 47.4 (C), 35.0 (CH_2), 33.1 (CH_2), 31.4 (CH_2), 31.0 (CH), 30.2 (CH_2), 29.6 (CH_2), 29.1 (CH_2), 21.7 (CH_2), 18.3 (CH_3), 17.9 (CH_3). MALDI-TOF MS: *m/z* calcd for $C_{1023}H_{1834}N_{123}O_{327}S_{63}$ $(M+H)^+$ 23110.22, found 23194.97.

Dendrimer 6b

The photoinduced addition of thiol **3b** (88 mg, 0.53 mmol) to dendrimer **1** (20 mg, 1.38 μmol) in the presence of DPAP (13.5 mg, 53.2 μmol) was performed as described for the preparation of **6a** to give, after chromatography on Sephadex LH-20 column (1:1 H_2O-MeOH), **6b** (29 mg, 93%) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.61 (t, 30H, *J* = 5.3 Hz, 30 NH), 7.57 (s, 30H, 30 H-5 Tr.), 4.49 (d, *J* = 5.5 Hz, 74H), 4.36 (t, *J* = 7.0 Hz, 64H), 4.30-4.19 (m, 58H), 3.78-3.72 (m, 96H), 3.71-3.61 (m, 358H), 3.56-3.50 (m, 276H), 3.00 (bs, 48 OH), 2.78-2.70 (m, 120H), 2.61 (t, *J* = 7.2 Hz, 112H), 2.38 (t, *J* = 7.2 Hz, 64H), 2.00-1.80 (m, 120H), 1.72-1.59 (m, 112H), 1.27 (s, 45H, 15 Me), 1.16 (s, 90H, 30 Me). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.3 (C), 73.8 (CH_2), 72.6 (CH_2), 70.4 (CH_2), 65.1 (CH_2), 61.7 (CH_2), 33.2 (CH_2), 29.5 (CH_2), 17.9 (CH_3). MALDI-TOF MS: *m/z* calcd for $C_{975}H_{1738}N_{123}O_{327}S_{63}$ $(M+H)^+$ 22436.95, found 22760.27.

Peptidodendrimer 7·HCl

A solution of dendrimer **1** (10 mg, 0.69 μmol), glutathione chloridrate **4·HCl** (45 mg, 0.13 mmol, prepared by freeze-drying a solution of **4** in aqueous HCl), and 2,2-dimethoxy-2-phenylacetophenone (DPAP, 3.4 mg, 13.3 μmol) in 4:1 DMF-H_2O (70 μL) was irradiated at r. t. for 1 h under magnetic stirring and then concentrated. The residue was eluted from a column of Sephadex LH-20 (1 × 30 cm) with 1:1 H_2O-MeOH to give **7·HCl** (7.6 mg, 36%) as a white foam; $[\alpha]_D = -12.0$ (*c* 0.8, H_2O). ^{1}H NMR (300 MHz, D_2O) selected data: δ 7.75 (bs, 30H, 30 H-5 Tr.), 3.88 (s, 96H), 2.08-1.87 (m, 96H), 1.02 (bs, 135H, 45 Me). ^{13}C NMR (75 MHz, D_2O): δ 176.5 (C), 174.8 (C), 172.4 (C), 73.7 (CH_2), 70.0 (CH_2), 53.4 (CH), 52.9 (CH), 50.1 (CH_2), 41.4 (CH_2), 33.3 (CH_2), 31.6 (CH_2), 28.9 (CH_2), 28.5 (CH_2), 26.4 (CH_2), 21.6 (CH_2), 17.5 (CH_3). MALDI-TOF MS: *m/z* calcd for $C_{1167}H_{1881}KN_{267}O_{471}S_{63}$ $(M+H)^+$ 29246.99, found 29249.32.

Conclusion

In conclusion, based on the efficiency and fidelity of thiol-ene chemistry, we have presented the conjugation of a wide array of functional building blocks such as carbohydrates, a peptide, and short PEG chains onto the periphery of an ene-functional dendrimer without the need for protecting groups. Under optimized conditions the various thiol-ene couplings afforded the corresponding conjugated products in high yields with no observable side reactions. This work demonstrates the robust, efficient and orthogonal nature of thiol-ene chemistry and its versatility as a click ligation process for complex and multifunctional systems. Applications of these molecules as globular multivalent tools can be foreseen. In this respect, the sialoconjugate **5d** is particularly attractive in view of the participation of neuraminic acid in a variety of biomolecular recognition processes such as in viral adhesion to cells.[61] This potential role of **5d** is suggested by our recent finding on the calix[4]arene-based sialocluster inhibitor activity of influenza A virus and BK virus replication.[48] Studies in this direction are the objective of future work in our laboratories.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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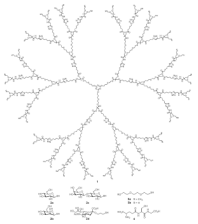

Figure 1.
Dendrimer [G4]ene$_{48}$ **1** and thiols **2–4** employed in photoinduced thiol-ene coupling reactions – note that no protecting groups were required.

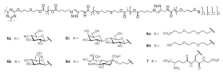

Figure 2.
Functionalized dendrimers **5–7** with carbohydrate, PEG, and peptide fragments at the chain ends.

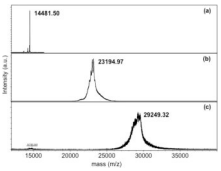

Figure 3.
MALDI-TOF spectrum of a) dendrimer **1** obtained in reflector mode using 2-(4-hydroxyphenylazo)-benzoic acid (HABA) as matrix; b) dendrimer **6a** obtained in linear mode using HABA as matrix; c) peptidodendrimer **7** obtained in linear mode using 2-mercaptobenzothiazole (MTB) as matrix.

Table 1

Photoinduced addition (λ_{max} 365 nm, r.t., 1 h) of thiols **2–4** to dendrimer **1** in the presence of DPAP (10 mol%).

Entry	Thiol (eq.)[a]	Solvent	Conc. (mM)	Product	Yield[b]
1	**2a** (2)	1:1 H_2O/MeOH	0.5	5a	_[c]
2	**2a** (4)	DMF	0.7	5a	_[c]
3	**2a** (4)	DMF	9.2	5a	94
4	**2b** (4)	DMF	10.6	5b	77
5	**2c** (4)	DMF	11.5	5c	_[c]
6	**2c** (5)	DMF	28	5c	93
7	**2d** (4)	DMF	12	5d	70
8	**3a** (4)	DMF	17	6a	_[c]
9	**3a** (4)	DMF	46	6a	_[c]
10	**3a** (4)	neat	-	6a	73
11	**3b** (8)	neat	-	6b	93
12	**4** (4)	2:2:1 H_2O/MeOH/DMF	3	7	_[c]
13	4·HCl (4)	1:1 H_2O/MeOH	1.7	7·HCl	_[c]
14	4·HCl (4)	DMF	11	7·HCl	_[c]
15	4·HCl (4)	4:1 DMF/H_2O	7	7·HCl	36

[a] Equivalents of thiol per alkene function.

[b] Isolated yield after column chromatography on Sephadex LH20.

[c] Incomplete reaction (^{1}H NMR analysis).