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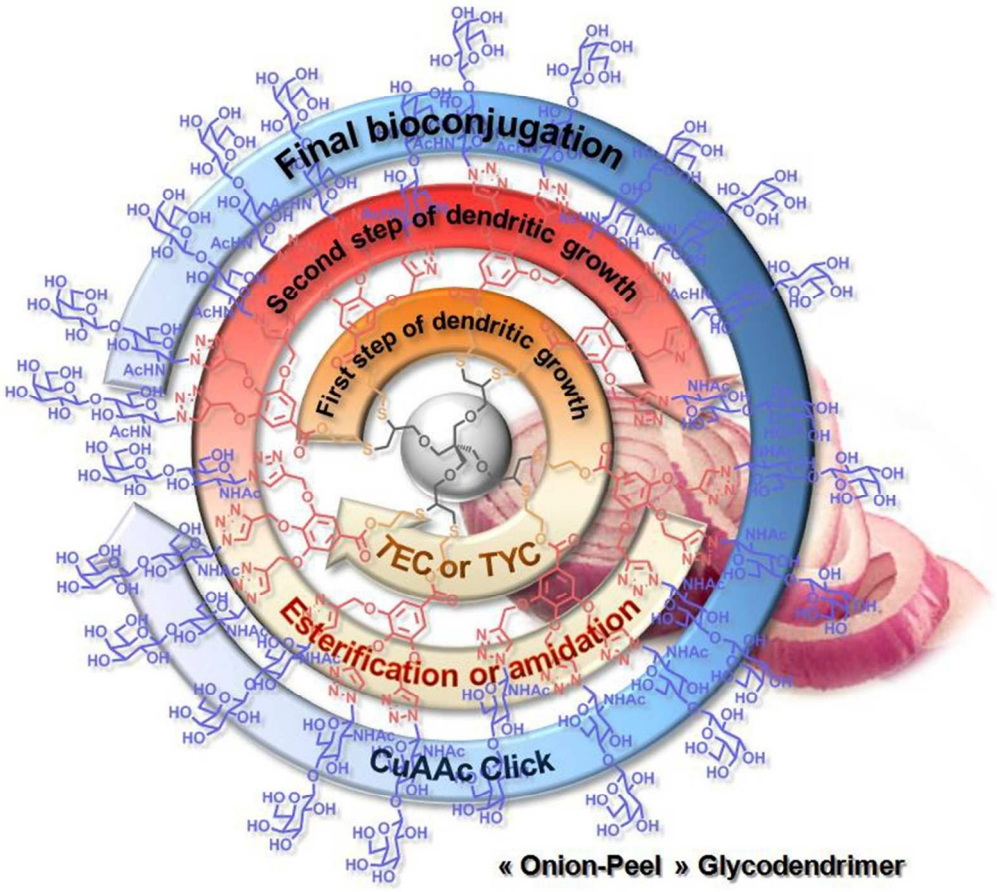

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ARTICLE TYPE

"Onion Peel" Dendrimers: A Straightforward Synthetic Approach Towards Highly Diversified Architectures

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We report herein a novel "onion peel strategy" for the divergent construction of glycodendrimers using different building blocks at each layer of the dendritic growth. A combination of successive highly efficient, versatile, and robust chemical reactions, namely thiol-ene or thiol-yne, esterification, and azide-alkyne click chemistry, generated dendrimers having chemically heterogeneous layers, some of which with UV-visible functions. The strategy is fundamentally different to conventional dendritic systems usually built from repetitive building nanosynthons of limited surface groups. The validity of this novel approach towards the construction of biologically active glycodendrimers having dense surface sugar residues within low dendrimer generations was fully demonstrated using *Erythrina Cristagalli*, a leguminous lectin known to bind natural killer cells through its galactoside recognition ability. The dendrimer's surface was decorated with an azido derivative of *N*-acetyllactosamine using click chemistry which led to new glycodendrimers having high affinities as compared to the corresponding monovalent analog. The ongoing quest for a better parameterization of critical carbohydrate-protein recognition factors urgently requires structures with tailored biophysical properties, sizes, and shapes together with optimized tri-dimensional architectures. The proposed methodology, for which entirely orthogonal building blocks can be applied, represents an additional contribution to the wide arsenal of existing strategies which can create higher structural diversity among dendritic structures of biological interests.

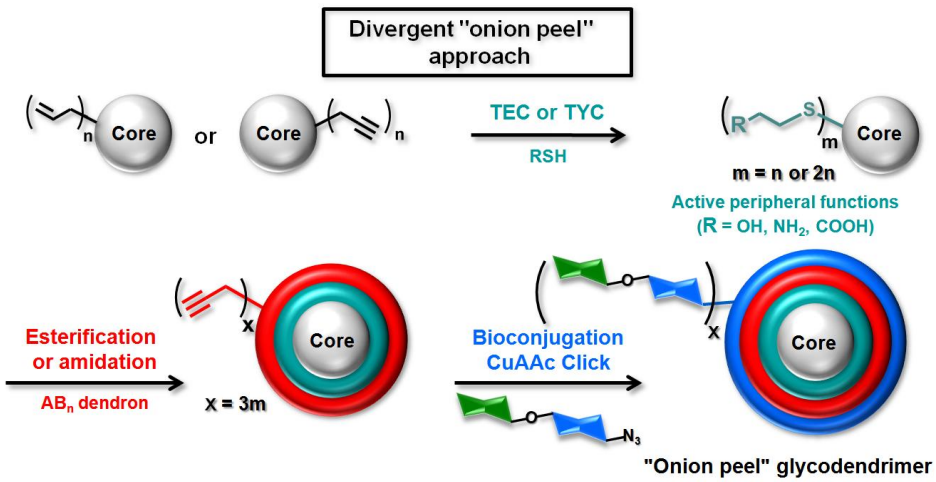
Introduction

Dendrimers are highly branched mono-disperse macromolecules with precise constitutions that have been explored in a wide variety of chemical, biological, and material studies.[1] A large number of synthetic strategies have been developed for their construction such as the most popular convergent, divergent and accelerated approaches.[1] However, despite these major achievements, their syntheses can still be tedious due to the inherent complexities associated with each repetitive methodology, most of which using narrow AB_2 monomer building blocks. Based on this observation, new strategies allowing easier preparation of homogenous and constitutionally diversified macromolecular structures are deemed necessary. Interestingly, besides classical strategies involving "hypercores"[2] and "hypermonomers",[3] one of the first breakthroughs towards accelerated dendritic syntheses has been carried out in the mid-90s[4] with the application of orthogonal coupling strategies[5] allowing the building of complex biomolecules.[6] The orthogonality concept dramatically reduced the number of required synthetic steps by using complementary bifunctional precursors that were coupled together by obviating deprotection or activation steps. Moreover, most common dendrimers are based on identical repeating units at each generation, thus greatly limiting the tailoring of biophysical properties that prevent structural diversity.

More particularly and despite their roles as well-defined artificial glycoconjugates, most glycodendrimers[7] do not depart from these constraints. Since the first report,[8] the ongoing quest toward more active hypervalent carbohydrate-loaded dendrimers exhibiting a range of activities has systematically grown.[9] The emergence of these mono-disperse glycomacromolecules has significantly contributed to our understanding of multivalent carbohydrate-protein interactions through the "cluster glycoside effect", according to which the binding affinities of multivalent carbohydrates are significantly higher than the sum of individual ligands.[10] In addition, glycodendrimers have received considerable attention for their use in biomedical applications, such as anti-adhesins, drug delivery, biosensors, gene transfections, and vaccines.[11] As the design of multivalent glycodendrimers strongly depends on the unique structural features of the protein receptors, the conception of tailored systems is highly desirable. It can thus be considered that the art of synthetic design of multivalent scaffolds remains open to alternative and improved strategies that will allow better

controlled structural diversity.

To address these issues, we propose a new type of "onion peel strategy" for the divergent construction of original dendritic architectures, involving the incorporation of different families of building blocks containing orthogonal functional groups at each layer (or generation) some of which having UV-visible moieties. The flexibility of the strategy will be demonstrated by choosing intentionally different but adapted building blocks that could differ in terms of constitution, valency, and peripheral functionalities. The layout diversity of each final biomolecule is thus programmed. Hence, the proposed approach leading to a controlled assembly of structural elements does not only rely on a "branching pattern" requirement but can extend the concept to smart multifunctional tools with tailored structures and properties. For example, once optimized, this approach may generate the desired hydrophobic/hydrophilic and rigidity/flexibility balances at each step of the dendritic growth. The general methodology for the sequential construction of our set of glycosylated architectures is proposed in Scheme 1. The application of a distinct mode of coupling at each layer generated an original heterogeneity in the internal functionalities and branched moieties, as opposed to conventional dendritic systems built from repetitive, or at least alternate, synthetic patterns. Thus, the proposed "onion peel" methodology, for which an entire orthogonality can be applied, could represent an alternative contribution to the wide arsenal of methodologies towards the rapid and sequential construction of dendritic architectures.



Scheme 1 Sequential construction of sugar decorated "onion peel" dendritic structures *via* an accelerated divergent strategy.

By successfully adapting the proposed onion peel strategy, we present herein the synthesis of a new family of model glycoclusters and glycodendrimers **1**-**5** decorated with *N*-acetyllactosamine (LacNAc) termini (Figure 1). The key-reactions involved in the elaboration of our set of glycosylated structures concerned the application of three different atom economical-"click" reactions that can provide high yields from simplified set-up and purification protocols, together with a highly desired tolerance toward a broad range of solvents and functional groups.[12] High chemo- and regioselectivities popularized some of these fast-growing orthogonal methodologies to ease the construction of sophisticated but well-defined (glyco)dendritic architectures.[13] Among the most efficient and orthogonal, the photolytic thiol-ene coupling (*TEC*)[14] will be advantageously applied to initiate the uniform growth of our dendritic scaffolds through the formation of internal robust thioether linkages. Higher degree of branching will be insured with the utilization of less-developed thiol-yne coupling (*TYC*)[15] involving a double hydrothiolation of terminal alkynes *via* a similar free-radical chain mechanism. EDC-mediated esterifications (or amidations)[16] will represent the last step of the dendritic growth with the introduction of polypropargylated dendrons equipped with the complementary focal function. The regioselective Cu(I)-catalyzed azide-alkyne [1,3]-dipolar Huisgen cycloaddition (CuAAc)[17] will be performed as an efficient ligation methodology for the peripheral glycoconjugation. In this context, LacNAc was chosen as a decorative sugar head group. It notably represents part of biologically active tumor associated carbohydrate antigens found in several natural glycoproteins and glycolipids presented by the blood groups, Lewis^X, and Lewis^Y.[18] Similarly important, LacNAc possess strong binding affinities toward a cancer associated family of proteins known as galectins.[19] In spite of its biological significance, the multivalent display of LacNAc residues onto dendritic scaffolds has been only reported in scarce occasions.[20] Consequently, the protein binding studies involving derivatives **1**-**5** have been assessed by using surface plasmon resonance (SPR) with a model leguminous lectin from *Erythrina cristagalli* agglutinin (ECA).[21] The main goal of this experiment is to establish the validity of the "onion peel" approach for the construction of biologically functional glycodendrimers. In addition, the influence of subtle structural parameters on the relative binding properties will be evaluated to extract fundamental trends towards optimized parameters.

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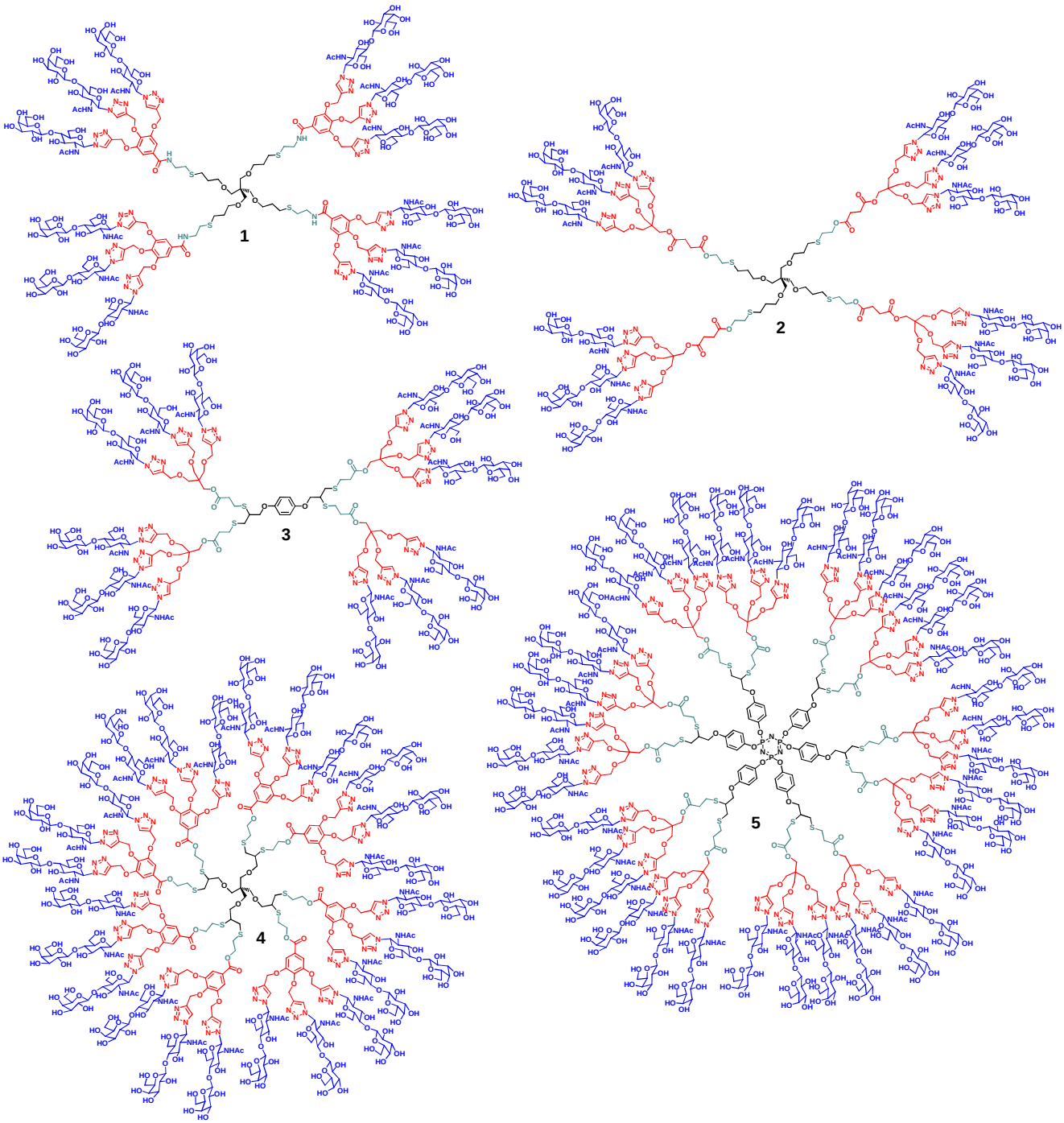
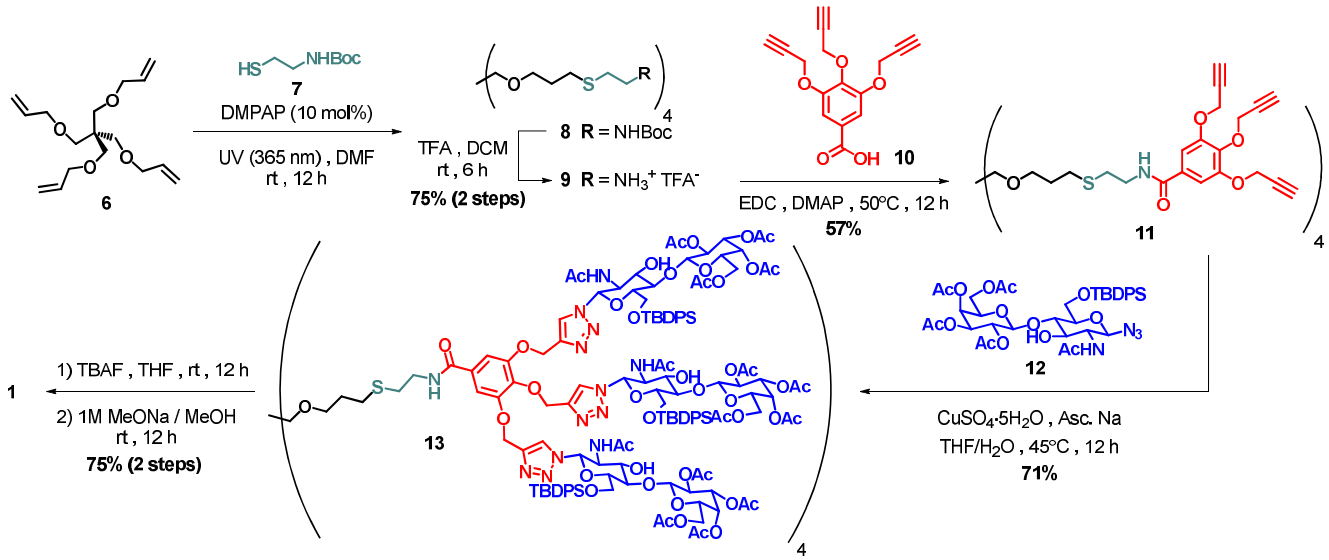


Figure 1. Molecular structures of targeted glycodendrimers **1**-**5**.

Results and discussion

Synthesis: The first step of our synthetic investigation dealt with the photolytic addition of *N*-Boc-cysteamine **7** on *tetrakis*-allylpentaerythritol **6** under standard *TEC* conditions (Scheme 2) to afford **8**. Similarly to well-documented quasi-exclusive *anti*-Markovnikov addition observed for this type of hydrothiolation,[14,22,23] we also noticed an analogous trend for some α-addition. (See *SI* for all tested conditions).



Scheme 2. Synthesis of glycocluster **1** through *TEC*-Amidation-*CuAAc* (4×1×3) sequence.

Subsequent removal of Boc-protecting groups in **8** using TFA in DCM furnished intermediate **9** after solvent evaporation in 75% yield over two steps. Amide coupling of **9** with bifunctional AB_3 derivative **10**[24] under basic conditions resulted in the formation of dodecapropargylated **11** in 57% yield (87% yield per amidation). The complete attachment of the protected β-azido LacNAc derivative **12**[25] under classical CuAAc conditions led to the multivalent derivative **13**. MALDI-TOF experiment furnished a unique signal in the expected region (11457.6 for a theoretical M.W. = 11448.9) while GPC indicated the uniformity of the structure (PDI (M_w/M_n) = 1.031) (*SI*). Finally, TBAF removal of TBDPS-protecting groups in the sugar residues, followed by de-*O*-acetylation under Zemplén conditions (NaOMe, MeOH) efficiently provided glycocluster **1** having twelve LacNAc moieties.

An alternative synthetic pathway was next explored to circumvent the above activation/deprotection steps in order to obtain congeners with equal or higher surface groups. As illustrated in Scheme 1, the optimized sequence was based on the integration of an orthogonal three steps-sequence consisting in hydrothiolation/esterification/click cycloaddition. Table 1 summarizes the structural elements that were assembled. Scheme 3 illustrates the critical steps towards the accelerated syntheses of glycoclusters **2**-**5** through an orthogonal and divergent dendritic growth.

The photoaddition of mercaptoethanol **14** on pentaerythritol derivative **6** afforded tetrahydroxylated core **15** in 85% yield which initiated the sequence towards the synthesis of glycocluster **2** (Scheme 3, sequence 1). Interestingly, the proportions of α-addition remained negligible in this case (≤ 5%), in agreement with previous works using hydroxylated thiol precursors.[14d,23]

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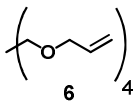
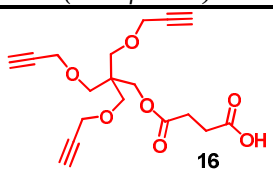
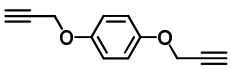
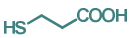
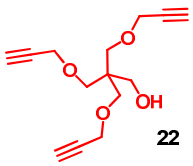
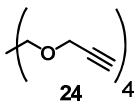
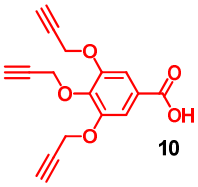
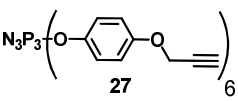
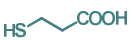
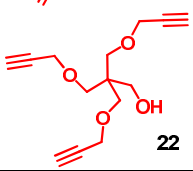
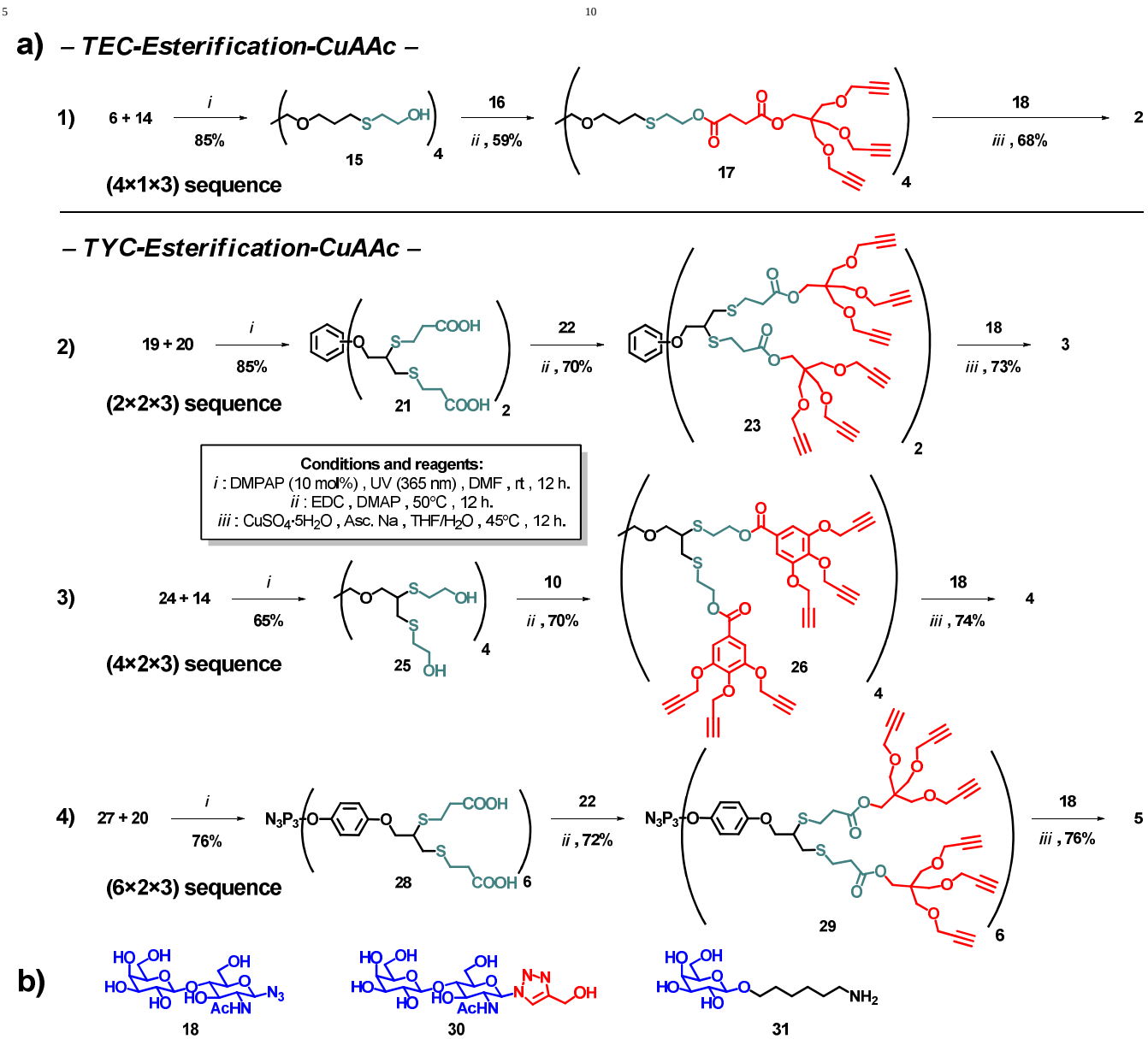
Table 1. Structural elements used to build polypropargylated scaffolds *via* an accelerated and orthogonal divergent strategy.

Entry	Core	Thiol *Reaction type*	Polypropargylated scaffolds *(Esterification)*	Dendritic growth	Valency	Polypropargylated scaffolds
1	 **6**	 **14** *TEC*	 **16**	4×1×3	12	**17**
2	 **19**	 **20** *TYC*	 **22**	2×2×3	12	**23**
3	 **24**	 **14** *TYC*	 **10**	4×2×3	24	**26**
4	 **27**	 **20** *TYC*	 **22**	6×2×3	36	**29**

The ^{1}H NMR spectra clearly illustrated completion of the multiple hydrothiolation process by the entire disappearance of signals belonging to the alkene function at δ 5.90 and 5.25 ppm together with the presence of the characteristic quintuplet signal at δ 1.80 ppm corresponding to the newly formed aliphatic -$OCH_2CH_2CH_2S$- motif (Figure 2). In addition, all the relative integrations of each proton presented in the external section of the core were in perfect agreement with those of the internal C_qCH_2O region. Esterification of tetraol **15** in the presence of TRIS-based AB_3 dendron **16**[26] further insured the efficient incorporation of surface active propargylic functionalities to provide **17**. Figure 2 illustrates completion of esterifications by the addition of characteristic signals of the succinate (δ 2.70 ppm), TRIS (δ 4.15 and 3.55 ppm), and propargylic signals (doublet at δ 4.10 and triplet at δ 2.45 ppm) showing the expected relative integrations.

Twelve deprotected LacNAc termini were subsequently grafted *via* standard CuAAc conditions. The presence of internal ester functions implicated the use of deprotected β-azido LacNAc **18**[25] which was successfully integrated to the polypropargylated scaffold to lead to dodecavalent cluster **2** in 68% yield. The direct coupling of hydroxylated

ligands advantageously avoided classical de-*O*-acetylation step and purifications by column chromatography when protected sugars are used.



Scheme 3. a) Accelerated divergent strategies for the syntheses of glycoclusters **2-5** harboring surface LacNAc residues; b) Structures of monomer used as references for SPR studies (see *SI* for the synthesis of **30**).

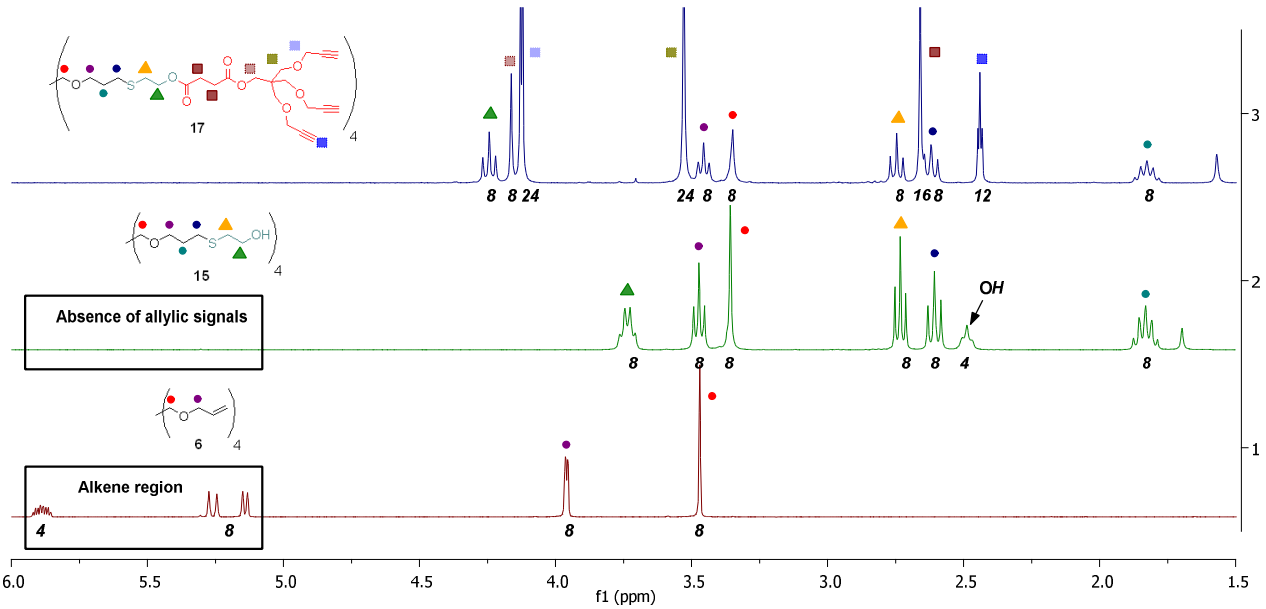


Figure 2. Comparison of ^{1}H NMR spectra ($CDCl_3$, 300 MHz) of **6**, **15** and **17** with the appearance/disappearence of characteristic signals towards the construction of dodecapropargylated scaffold **17** (observed proton integrations are indicated in italic below each signal).

In order to explore the flexibility of our global synthetic approach and to enhance the density of termini using a limited number of steps, poly-propargylated cores were used to perform TYC chemistry that enabled to double the number of attachments at each individual reactive terminal alkyne. Accordingly, a third dodecavalent homolog was synthesized, differing from the previous ones by the nature of the core from which emanated the clusters of epitopes, together with the mode of dendritic growth. To this end, a double hydrothiolation was first performed on dipropargylated hydroquinone **19**[27] by means of mercaptopropionic acid **20** to provide pure derivative **21** in 85% (Scheme 3, sequence 2). This scaffold, having four carboxylic acids, was subsequently treated with hydroxylated AB_3 dendron **22**[26] through the efficient formation of ester bonds *via* EDC/DMAP coupling. As described above, complete capping with LacNAc residues **18** was accomplished on the newly formed dodecavalent derivative **23** using the above CuAAc conditions to afford **3** in good yield. Thus, three linear orthogonal synthetic steps with an overall 43% yield allowed the straightforward formation of a dodecavalent LacNAc dendrimer. Interestingly, the application of similar three steps-sequences consisting in successive TEC/TYC-esterification-CuAAc allowed the addition of two dodecavalent "onion peel" glycoclusters to **1**, creating structural diversity in *1*) the dendritic growth with 4×1×3- (for **1** and **2**) or 2×2×3- (for **3**) patterns together with the possibility to integrate efficient orthogonality; *2*) the inner functionalities responsible for the stability of the constructs by the presence of thioether, ester and amide linkages; *3*) the compaction of the scaffolds; *4*) the aromatic/aliphatic character of the inner sections using gallic acid or pentaerythritol derivatives as secondary cores; *5*) the presentation of the peripheral sugar termini emanating from the main and secondary cores.

The generation of higher analogs containing more sugar residues has also been explored *via* the proposed orthogonal three steps sequence. Thus, the first hyperbranched glycosylated structure (**4**) emanated from the known **24**,[28] (*See SI for improved synthesis of* **24**) obtained in high yields according to optimized conditions on which was performed the TYC chemistry in the presence of mercaptoethanol **14** (Scheme 3, sequence 3). The resulting octa-hydroxylated scaffold **25** was further decorated with eight aromatic carboxylic acid precursor **10** to afford tetracosa-propargylated core **26** harboring 24 reactive propargyl functions in a 70% yield (96% yield per individual esterification sequence). Once again, complete derivatization was confirmed by mass spectrometry together with IR and NMR spectroscopy. In particularly, the ^{1}H NMR spectra clearly indicated the disappearance of propargylic signals for **25** (δ 4.20 and 2.40 ppm in precursor **24**) and the predicted relative integration of newly formed moiety in comparison to protons located in the core (Figure 3, middle section). In addition, esterification also led to distinctive addition of signals such as those corresponding to aromatic (δ 7.45 ppm) and terminal propargylic protons (δ 4.75 and δ 2.50 ppm) having the calculated integrations.

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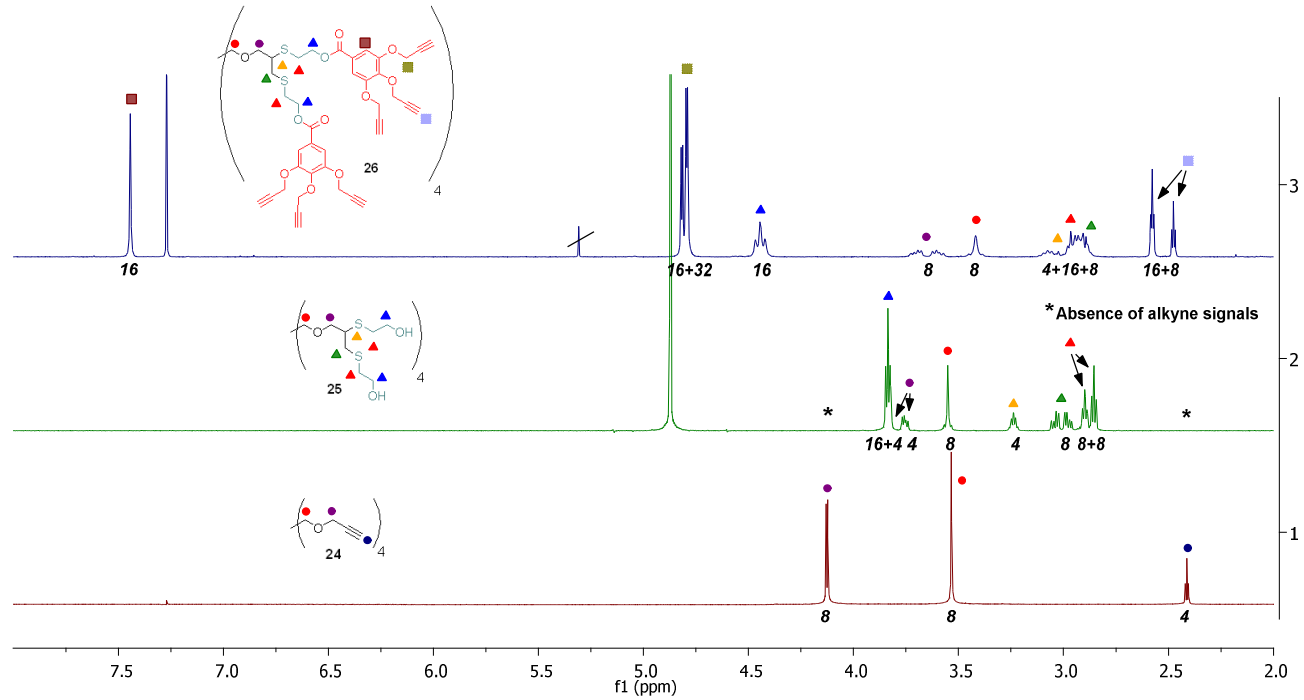

Figure 3. Direct comparison of ^{1}H NMR spectra (in $CDCl_3$ for top and bottom spectra and in D_2O for middle spectrum, 300 MHz) of **24**, **25** and **26** with the appearance/disappearence of characteristic signals towards the construction of tetracosavalent scaffold **26** (observed proton integrations are indicated in italic below each signal and stars in middle spectrum indicates the absence of propargylic signals).

As observed for previous analogs with exposed LacNAc residues, final bioconjugation proceeded efficiently using azido sugar **18** to afford the densely packed macromolecule **4** having 24 termini after dialysis.

A similar methodology was adapted with the same efficiency for the synthesis of higher analogue **5** containing 36 LacNAc appendages and an aliphatic backbone. The construction started from hexapropargylated cyclotriphosphazene **27**[29] known to afford 3-up/3-down wedges in both solid state and solution.[30,30b] Twelve-fold addition of mercaptopropionic acid **20** on **27** led to **28** in good yield (76%) after purification by silica gel chromatography (Scheme 3, sequence 4). Once again, high resolution mass spectrometry (ESI- technique) confirmed the formation of $[M\text{-}2H]^{2-}$ adducts, thus perfectly matching the expected theoretical pattern (Figure 4, see *SI* for full spectrum).

Tripropargylated AB_3 wedges **22** were subsequently anchored *via* carbodiimide–mediated esterification to achieve the construction of hypercore **29** in an excellent yield. The exhibited thirty-six propargylated peripheral functions of **29** were finally transformed into triazoles during the multiple CuAAc process. Complete bioconjugation of **29** in the presence of **18** provided glycodendrimer **5**, as seen from its ^{1}H NMR spectra showing the absence of propargylic signals using the above conditions.

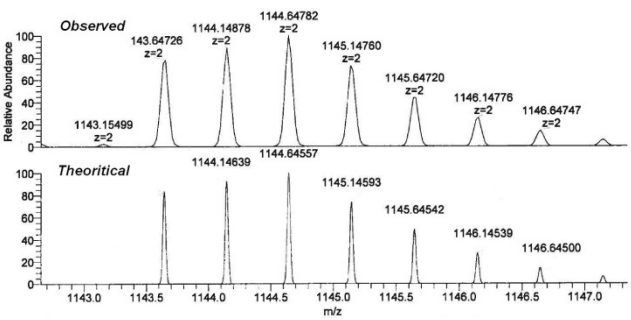

Figure 4. Specific region of negative HR-ESI observed (top) and theoretical (bottom) isotopic distributions for **28** exhibiting 12 carboxylic acid functions ($[M\text{-}2H]^{2-}$ signal).

Interestingly, low and high resolution Mass Spectrometry analyses furnished consistent results for the

hyperbranched macromolecules together with polypropargylated precursors as indicated in Table 2.

Table 2 Mass Spectrometry results obtained from MALDI-TOF, ESI, and APCI Techniques for hyperbranched derivatives.

Entry	Compound	M.W.[a]	Exp. Mass [adduct] (Technique)
		Polypropargylated scaffolds	
1	**11**	*1668.5501*	1691.5360 $[M+Na]^+$ HR-ESI^+
2	**17**	*1936.7585*	1937.7621 $[M+H]^+$ HR-$APCI^+$
3	**23**	*1538.5433*	1539.5506 $[M+H]^+$ HR-ESI^+
4	**26**	3043.4	3049.0 $[M+Li]^+$ LR-MALDI-TOF
5	**29**	5078.9	5077.5 LR-MALDI-TOF
		LacNAc-terminated dendrimers[b]	
6	**13**	11448.9	11457.9
7	**1**	6570.3	6597.9
8	**2**	6838.6	6862.3
9	**3**	6440.2	6464.0
10	**4**	12844.1	12735.6
11	**5**	19779.9	19774.8

[a] Exact mass values are indicated in italic when high resolution analyses were performed.[b] Low-resolution mass values were obtained by MALDI-TOF technique ($[M+Na]^+$ adducts).

Surface plasmon resonance studies: Subsequent to synthesis, surface plasmon resonance (SPR) studies have been conducted to assess the relative protein binding abilities of glycodendrimers **1**-**5** with the LacNAc-specific leguminous lectin (ECA) from *Erythrina Cristagalli*. In these studies, the lectin was immobilized onto CM5 sensor surface (Biacore) to a level of ~1200 RU, by using the manufacturer's amide coupling methodology. As a blank reference, ethanolamine was immobilized onto one of the flow cell of the sensor chip. Solutions with various concentrations of LacNAc-functionalized dendrimers have been flowed over surface-bound lectin and significant interactions were determined for each glycodendrimers and compared to monovalent standard **30**. A representative sensorgram was obtained for each ligand (see Figure 3 for glycodendrimer **4** and *SI* for the remaining compounds). Determination of the kinetic parameters relative to the glycodendrimer-lectin interactions were fitted by using a 1:1 Langmuir model available in BIAevaluation software.[31] The corresponding data (k_{on}, k_{off}, K_D and relative binding affinities) are given in Table 3.

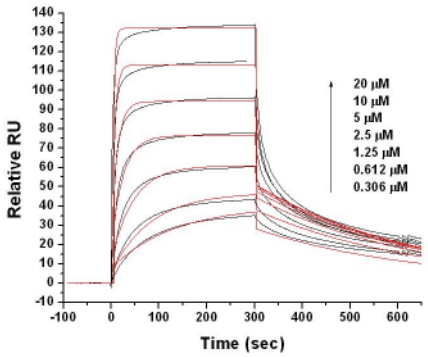

Figure 3. SPR sensorgrams for the interactions of glycodendrimer **4** (0.306 µM to 20 µM) with the surface bound ECA lectin. The binding data are overlaid with the fit (in red) of a 1:1 Langmuir interaction model.

Table 3. Kinetic parameters obtained for the interactions of glycodendrimers with the bound ECA. Data were fitted by using a 1:1 Langmuir model available in BIAevaluation software.

Cpd	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_D (nM)	r.p.[a]	r.p./sugar
30	375	7.26×10^{-3}	19400 ± 560	1	1
1	6.13×10^{3}	3.33×10^{-3}	543 ± 28	35	2.9
2	3.25×10^{4}	3.57×10^{-3}	109 ± 7	176	14.6
3	1.79×10^{4}	4.71×10^{-3}	263 ± 14	75	6.2
4	*3.08×10^{4}*	*2.82×10^{-3}*	*92* ± 4	***216***	***9***
5	3.05×10^{3}	1.00×10^{-3}	329 ± 20	58	4.8

[a] relative potency.

In each case, simple-exponential binding profiles were obtained with association phase free of mass transport phenomenon. Overall and as expected, the glycodendrimers exhibited higher k_{on} and lower k_{off} values than those of monovalent **30**. As a result, multivalent compounds exhibited high nanomolar affinities with the dimeric ECA. Although no K_D values were previously determined by SPR for monovalent LacNAc derivatives with ECA, the experimental value for **30** consistently stands in micromolar values as compared to similar references.[20d,32] The glycodendrimers exhibited interesting high relative potencies, with an up to 216-fold enhancement in global affinity for the best candidate **4**, while corresponding to a modest improvement for each peripheral LacNAc moieties of **4** compared to **30**. In fact, the meek glycocluster effects observed throughout the series is typical of divalent lectin interactions which usually reflect a predominance of kinetic (82-fold faster k_{on}) rather than thermodynamic improvement. In fact, the best recorded value was obtained with dodecavalent **2** for which each termini was only 14-fold more active than the reference monomer. With a noticeable exception,[33] this observation remains consistent with earlier investigations that ascertained the fact that ECA had a small

multivalency enhancement ability, as determined with LacNac-glycopolymers.[34]

Although no impressive thermodynamic trends can be extracted from the above data, the relative kinetic values can lead to interesting observations that pinpoint the influence of structural parameters toward relative affinity with ECA. First, glycodendrimer **5** harboring the largest number of peripheral sugars do not necessarily represent the best candidate, since its K_D value is worst than two of the three dodecavalent congeners **2** and **3**. Interestingly, although exhibiting similar lowest valencies, these clusters were built around distinct building blocks and displayed different kinetic values. Predominantly, distinct k_{on} values indicate that the rates of association were strongly dependent to the nature of the structural elements that dictate the tri-dimensional organization of the sugars. In this series, aromatic branching units as in **1** seemed to hamper the optimal display of the LacNAc residues while aliphatic homologs, and especially elongated **2** allowed a better recognition. On the other hand, a noticeable enhancement in affinity was obtained for **4** having UV-visible gallic acid moieties incorporated in the scaffold, in comparison to **1** (Figure 5).

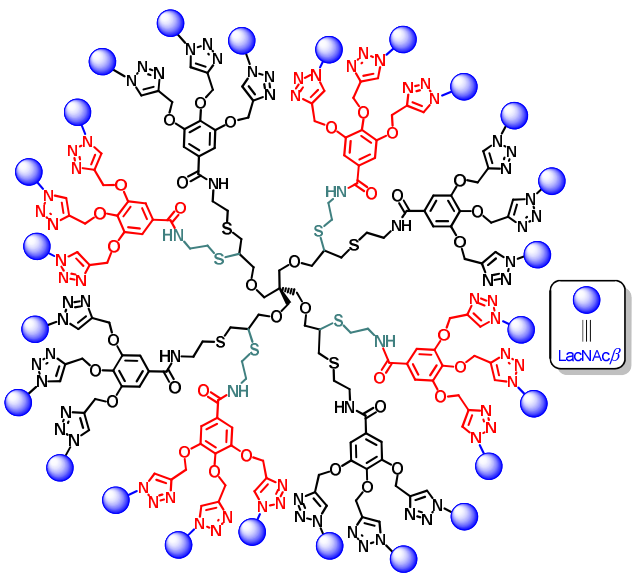

Figure 5. Superimposition of glycoclusters **1** (black, 12 sugars) and **4** (black + green + red, 24 sugars) to illustrate their similar composition but different epitopes' density.

This result further highlights the role of multivalency and more precisely the epitopes' density which led to an enhancement in activity from binding events. Noteworthy is the fact that this trend is not obvious when comparison between **3** and **5** is made. Although similar cluster of six epitopes emanating from the same dithiolated moieties are present at the periphery, their spatial presentation is insured from various cores and is responsible for the different binding behaviors against ECA. Thus, in this experiment, the focal branching emanating from the templates that direct the repeating units is also likely playing a critical role in the ligand-lectin recognition phenomenon. In addition, the stereoisomers created by the TYC reaction could not be accounted for the binding differences as the chiral centers are 14-15 atoms away from the anomeric carbon of the Glc residues bound to the external outer limit of the galactoside binding site (see S101).

In order to get more insight into the relative "multivalent effect" of the glycodendrimers, we have also performed another SPR-based assay involving a competitive inhibition studies. In this context, 6-amino hexyl β-D-galactopyranoside **31**[35] was immobilized onto the sensor surface to provide a more realistic mimetic system of the eukaryotic cell surface that can recognize the lectin. For the determination of IC_{50} values, equilibrium mixtures of ECA (5 µM) in contact with increasing concentrations of glycodendrimers **1**-**5** and monomers **18** and **30** have been used as analytes over the surface of galactoside **31**. Thus, the affinity of ECA towards the bound galactoside in the presence of different concentrations of glycodendrimers was measured (Table 4).

Table 4. IC_{50} values of the glycodendrimers **1**-**5** and monomers **18** and **30** derived from competitive inhibition SPR studies.

Cpd	IC_{50} (µM)	r.p.[a]	r.p./sugar
18	563 ± 34	-	-
30	362 ± 20	1	1
1	3.82 ± 0.23	95	8
2	3.07 ± 0.09	118	10
3	6.19 ± 0.52	58	5
4	0.61 ± 0.02	593	25
5	*0.31 ± 0.01*	*1168*	*32*

[a] relative potency.

A typical sensorgram profile and the corresponding inhibition curve derived from the sensorgrams are shown in Figure 6 for glycodendrimer **5,** the best ligand in this experiment (see *SI* for the remaining glycodendrimers **2**-**5** and for monomers **18** and **30**). Once again, consistent high micromolar IC_{50} values were obtained for monomers **18** and **30**, with a slightly better activity for the latter having a triazole group at the anomeric position which could be attributed to known "aglycon-assisted" binding events.[36]

Overall, similar tendencies obtained during the previous assays were observed, however with enhanced effects. Indeed, the improved affinity corresponded to an increased number of ligands with relative potencies exceeding 1000 for the best candidate **5**, resulting in a 32-

fold better affinity for each sugar in comparison to monomer **30**. Also, similar discrepancies were obtained throughout the dodecavalent glycoclusters **1**-**3**, reinforcing the importance of structural parameters' arrangement and the induced organization of dendronized moieties. In this series, elongated ligand **2** exhibited the best results with an interesting 3.1 µM value. As observed earlier, the addition of four trivalent dendrons (comparing between **1** and **4**, Figure 5) allowed favorable effects since the increase in density was responsible for an important drop in IC_{50} values. Notably, this corresponds to a relative potency enhancement of 2.5-fold for each epitope on tetra-cosavalent **4** compared to dodecavalent glycocluster **1**. Contrary to studies for K_D determination, this tendency is also effective with the multivalent contribution afforded by aliphatic scaffolds. While **3** constitutes the worst ligand overall, the multiplication of the hexavalent motif from dithiolation strategy afforded stunning enhancement in affinity with best results for **5**.

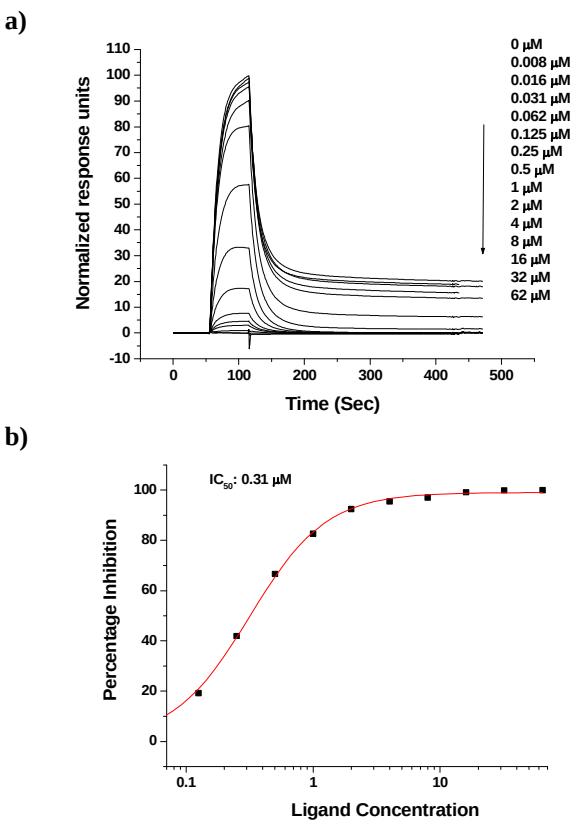

Figure 6. (a) Sensorgrams obtained by injection of ECA (5 µM) incubated with different concentrations of glycodendrimer **5** varying from 0.008 µM (top curve) to 62 µM (bottom curve) on the surface of immobilized galactoside **31**. (b) The inhibitory curve for the glycodendrimer **5**. IC_{50} value was extracted from the sigmoidal fit of the inhibition curve.

Though the relative affinities differ from both SPR studies, the proposed family of multivalent LacNAc-dendrimers **1**-**5** contains some of the best ECA ligands known to date, with high nanomolar affinities. The observed discrepancies throughout the assays may be explained from the fact that the kinetic data (Table 3) was obtained by assuming the simple 1:1 Langmuir model binding between the surface-bound dimeric ECA and the multivalent ligands, although attempts to avoid this situation were made by low density ligand immobilization. It is interesting to note that the relative potencies were found to be higher in competitive inhibition studies than in the surface-bound ECA. It may be partly attributable to the fact that in solution phase competitive studies, upon equilibration for 1 h, glycodendrimers may have enough time to bind almost irreversibly with ECA through multivalent cross-linking lattice interactions when compared to instantaneous binding in solid phase interactions.

Conclusions

In conclusion, a novel type of onion peel strategy was designed for the synthesis of glycodendrimers by using different families of building blocks containing orthogonal functional groups at each layer or generation of the dendritic growth. The synthesis was achieved by using highly efficient reactions, such as, thiol-ene or thiol-yne, esterification, and azide-alkyne click chemistry. The robustness and flexibility of this approach were translated by the efficiency of each coupling step, regardless of the nature of terminal reactive functionalities, as exemplified with the elaboration and use of polyamine, polyol, polyacid, polyalkene, and polyalkyne multivalent templates. The onion peel strategy presented herein may lead to new directions in dendrimer research for the synthesis of much richer family of functionalized dendritic structures and for creating higher structural diversity. To exemplify the influence of such structural diversity, two distinct SPR studies with the leguminous lectin *Erythrina Cristagalli* (ECA) as a model were conducted and led to interesting results towards the design of optimized lectin ligands. Most importantly, the proposed synthetic approach validates the concept according to which each structural element influences the recognition processes. Thus, this work brings a valuable complement to a recent study[37] that investigated the influence of different "click" ligation modes on glycodendrimers-induced lectin recognition. The present synthetic strategy allows a better rationally programmed arrangement of branching units towards biologically active multivalent constructs. This investigation is directed to the development and the application of this approach towards the construction of

potent ligands against human lectins. The conception of functionalized templates as promising candidates in vaccine immunotherapy[38] is also under the scope and will be reported in due course.

Notes and references

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