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A highly versatile convergent/divergent "onion peel" synthetic strategy toward potent multivalent glycodendrimers†

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Both convergent and divergent strategies for the synthesis of "onion peel" glycodendrimers are reported which resulted in one of the best multivalent ligands known against the virulent factor from a bacterial lectin isolated from *Pseudomonas aeruginosa*.

Dendrimers are well defined, hyperbranched tree like macromolecules which have shown great potential for applications in diverse areas ranging from nanoengineering to medicine.[1] Their striking architecture leads to excellent properties, but unfortunately brings in many synthetic challenges as well. Traditionally, their iterative construction emanates from a central core in a layer-by-layer fashion using repetitive moieties *via* most popular divergent[2] and convergent[3] methods. Both strategies have their own drawbacks and often require tedious repetitive synthetic steps, with classically only a slow enhancement in the number of peripheral functionalities at each generation. To meet the increasing demand of dendrimers for advanced applications, the scientific focus has been shifted towards their efficient and rapid construction involving a minimum number of reactions and with access to a large number of surface active functionalities. Notably, the introduction of orthogonal building blocks and the use of hyperfunctionalized synthons combined with robust and highly efficient chemical reactions have recently fulfilled these specifications.[4–6]

Glycodendrimers in particular, with their widespread applications[7] as microbial antiadhesins, biosensors, vaccines, drug delivery vectors, and gene transfection agents, do not depart from this efficacy pursuit. In this context, we recently reported a novel divergent "onion peel" approach to construct glycodendrimers using distinct and orthogonal building blocks at each generation growth.[8] Using this strategy, we demonstrated that structural diversities could be efficiently and rapidly harnessed at low generations. Notably, distinct hydrophobic/hydrophilic and rigidity/flexibility balances together with presentation of different epitopes clearly influenced their potencies as protein ligands. In complement to this rationally programmed arrangement of branching units, we wish to report herein an inverted strategy with multivalent presentation of different types of ligands around a fixed "onion peel" dendritic scaffold. Chemically heterogeneous layers were assembled at each generation by both convergent and divergent strategies using a combination of orthogonal building blocks and highly efficient chemical reactions such as radical initiated photochemical thiol–ene coupling (TEC) reactions,[5,9] amidation,[10] and copper-catalyzed azide–alkyne cycloaddition (CuAAc) reactions.[11]

The divergent construction of these novel dendrimers was initiated with inexpensive commercially available dipentaerythritol **1** serving as a dense A_6 core. Per-*O*-allylation with allyl bromide in the presence of NaH in DMF provided *hexakis*allylated G(0) derivative **2** in 80% yield (Scheme 1). Complete allylation was clearly confirmed by ^{1}H NMR analysis, which showed the characteristic allylic signals at δ 5.90 and 5.34–5.08 ppm and the disappearance of OH signals together with its predicted HRMS. Core structure **2** was next subjected to a radical TEC reaction with excess of cysteamine hydrochloride in the presence of photoinitiator 2,2-dimethoxy-2-phenylacetophenone (DMPAP, 10 mol%) under UV irradiation at 365 nm in DMF. Water soluble hydrochloride **3** was uneventfully isolated in 75% yield after dialysis and fully characterized by ^{1}H- and ^{13}C-NMR spectroscopy that showed the absence of olefinic signals, and by HRMS. Polyamine **3** was then treated with tripropargylated gallic acid derivative **4**[12] by amidation under classical carbodiimide coupling (72%). Notably, the use of AB_3 monomer **4**, when combined with our A_6 core **2**, readily provided G(1) hypercore **5** already possessing eighteen surface functional groups. For comparison purposes, PAMAM dendrimers and the like, built around AB_2 monomers, only reach these values at the G(2) level. Dendrimer **5** was next treated with peracetylated β-D-galactopyranosyl azide **6**[13] under classical click reaction conditions ($CuSO_4 \cdot 5H_2O$, Na-ascorbate in THF/H_2O) to afford octadecavalent galactodendrimer **7**. The ^{1}H-NMR spectrum showed the complete disappearance of the propargylic C≡C*H* signals at δ 2.50 ppm and the expected appearance of two distinct

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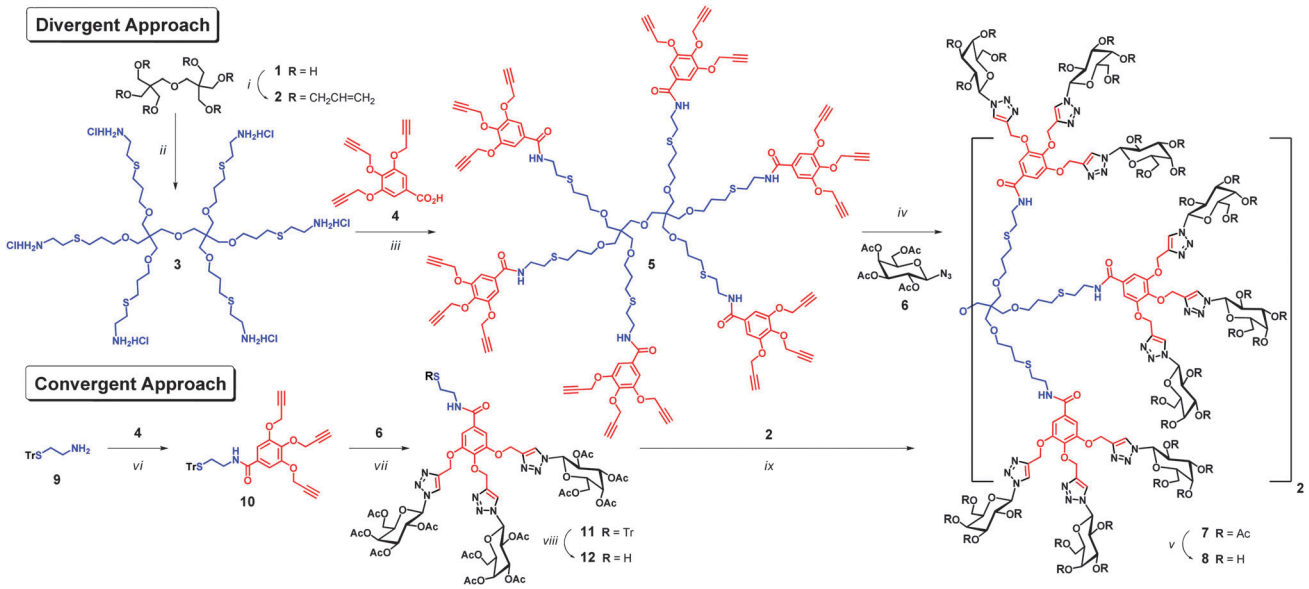
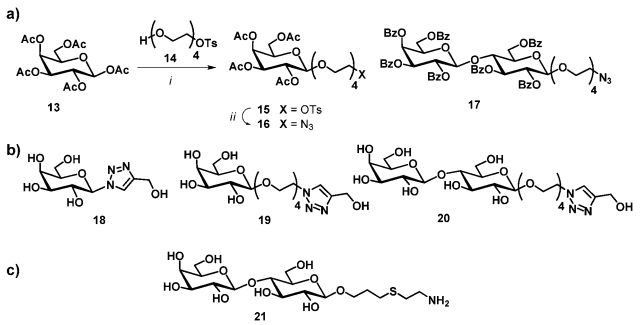


Scheme 1 Divergent and convergent synthesis of octadecavalent galactodendrimer **8**. *Reagents and conditions*: (i) NaH, Allyl bromide, DMF, 0 °C to rt, 5 h, 80%; (ii) cysteamine·HCl, DMPAP, DMF, 365 nm, 3 h, 75%; (iii) EDC, DMPAP, DIPEA, DMF, 60 °C, o.n., 72%; (iv) $CuSO_4{\cdot}5H_2O$, Na ascorbate, THF/H_2O (1 : 1), 40 °C, 12 h, 81%; (v) MeONa/MeOH, rt, o.n., 88%; (vi) EDC, DMPAP, DMF, rt, o.n., 78%; (vii) $CuSO_4{\cdot}5H_2O$, Na ascorbate, THF/H_2O (1 : 1), 40 °C, 5 h, 84%; (viii) Et_3SiH, TFA, 0 °C, 3 h, DCM, 85%; (ix) AIBN, dioxane, 75 °C, 5 h, 53%.

triazole signals integrating in a 2 : 1 ratio at δ 8.09 and 8.16 ppm. Another evidence of the monodispersity of the dendritic structure was further confirmed by gel permeation chromatography (GPC) which showed a narrow and symmetrical Gaussian pattern with a PDI of 1.03. Subsequently, de-*O*-acetylation of **7** under Zemplén conditions (NaOMe, MeOH) provided the final glycodendrimer **8** having 18 deprotected galactopyranoside moieties in quantitative yield (a molecule having 72-OH groups)!

In order to illustrate the full versatility of this "onion peel" strategy for the rapid access to structurally diversified dendrimers, we also envisaged the construction of dendrimer **7** by a convergent approach. This alternative was initiated with *S*-trityl cysteamine **9** prepared by a slight modification (ESI,† Scheme S1) of the literature procedure.[14] Under classical amidation conditions, **4** (EDC, DMPAP, DMF) provided intermediate **10** in 78% yield. A Cu-catalyzed click reaction was then performed in the presence of galactosyl azide **6** to afford wedged glycodendron **11** in 84% yield. Once again, the apparition of two discrete triazole singlets in the ^{1}H NMR spectrum with suitable integration (δ 8.04 and 8.15 ppm; 2 : 1 ratio), coupled with the disappearance of propargylic signals confirmed the triple grafting of the sugar ligand. Chemoselective deprotection of the thiol group using 5% TFA in the presence of Et_3SiH as a cation scavenger afforded dendron **12** in excellent yield (85%), without any trace of disulfide side-products. The aromatic protons corresponding to the trityl group at δ 7.46–7.17 ppm completely disappeared. Notably, the triplet corresponding to CH_2 in the α-position of tritylated thiol **11** at δ 2.50 ppm shifted down-field at δ 2.75 ppm. Final ligation of thiol **12** with *hexakis*allylated core **2** was achieved through a thiol–ene coupling reaction (AIBN, dioxane, 75 °C, 5 h) to provide pure glycodendrimer **7** in a 53% yield (30% yield under UV/DMPAP). Hence, we clearly demonstrated that the convergent sequence could be applied toward the construction of functionalized "onion peel" glycodendrimers without substantial loss of efficiency (5 steps and 24% overall yield from **1** *vs.* 4 steps and 35% using the divergent method).

It is well established that the key factors for improving the overall avidity of glycodendritic architectures against bacterial and leguminous lectins through multivalent binding processes originate from: (1) the relative accessibility of the sugar ligands at the dendritic surfaces[8] and (2) the inner scaffold structures/valency themselves.[7c,15,16] In order to further our understanding and for rationalization of these features using the above unique flexible "onion peel" template from which galactopyranoside ligands with different aglycones emanated, glycodendrimers with longer penultimate spacers were next constructed. Hence, for lectin's better accessibility toward the sugar ligands, longer branching residues and the choice of the peripheral sugars should constitute an improved design. Toward this goal, we synthesised both galactopyranoside and lactoside dendrimers using tetraethylene glycol (TEG) spacers (Scheme 2).



Scheme 2 (a) Syntheses of monomeric azido precursors **16** and **17**, (b) reference compounds **18** and **19** and (c) a lactoside derivative immobilized on the chip for SPR studies. *Reagents and conditions*: (i) $BF_3{\cdot}Et_2O$, DCM, 0 °C to rt, 4 h, 55%; (ii) NaN_3, DMF, 90 °C, o.n., 82%.

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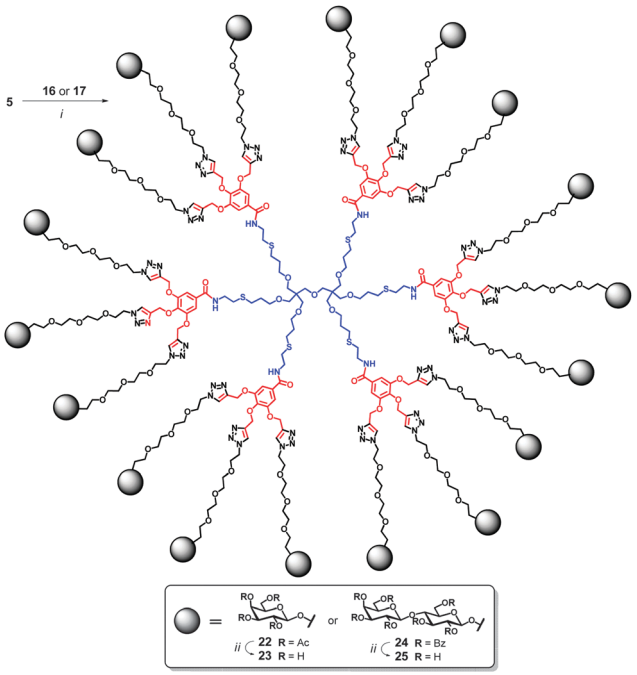

Scheme 3 Synthesis of glycodendrimers **23** and **25**. *Reagents and conditions*: (i) $CuSO_4{\cdot}5H_2O$, Na ascorbate, THF/H_2O, 40 °C, 12 h, 76% **22**, 77% **24**; (ii) NaOMe, DCM, MeOH, pH 9–10, rt, o.n., 90% **23**, 86% **25**.

Treatment of galactopyranose pentaacetate **13** with monotosylated tetraethylene glycol **14**[17] under Lewis acid-catalyzed conditions ($BF_3{\cdot}Et_2O$ in DCM) afforded compound **15** in 55% yield. Substitution of the tosylate in **15** by a terminal azide functionality was readily accomplished using NaN_3 in DMF to give **16** in 82% yield. Analogously, coupling of tosylated TEG derivative **14** onto its peracetylated lactose homolog, followed by substitution with azide was performed as previously described,[17] but better results were ultimately obtained through the *per*-benzoylated derivative **17**, which allowed easier purification and increased yields (see the ESI† for the protocol). Both azido-terminated sugar ligands **16** and **17** were coupled onto scaffold **5** *via* CuAAc to afford glycodendrimers **22** and **24** in 76–77% yields, which correspond to nearly quantitative individual coupling (Scheme 3).

Unequivocally, both ^1H and ^{13}C NMR spectra indicated complete disappearance of propargylic signals and sugar incorporation with calculated relative integration. HRMS together with the presence of molecular ions and fragmentations corresponding to regular losses of carbohydrate moieties gave convincing proofs of the structural integrity. Zemplén transesterification (NaOMe and MeOH) furnished two additional water soluble glycodendritic candidates **23** (90%) and **25** (86%) for comparative inhibition experiments with a bacterial lectin from *Pseudomonas aeruginosa*. Note that dendrimer **25** possesses 126 peripheral OH groups and thus, can serve on its own as an interesting precursor for further functionalization and applications.

In this context, the relative binding affinities of three novel glycodendrimers **8**, **23**, and **25** were evaluated by competitive surface plasmon resonance (SPR) using a galactoside specific bacterial lectin from the Gram-negative bacteria *P. aeruginosa*.[6,18]

Table 1 IC_{50} values of glycodendrimers and their monomeric analogs derived from competitive inhibition SPR studies

Entry	Cpd	IC_{50} (μM)	R.p.[a]	R.p./sugar[b]	β^b
Galactoside					
1	**18**	43 ± 1.5	1	1	11
2	**8**	0.22 ± 0.02	195	11	
TEG-Galactoside					
3	**19**	21 ± 1.5[c]	2	2	**32**
4	**23**	**0.037 ± 0.005**	**1162**	**65**	
TEG-Lactoside					
5	**20**	958 ± 34	0.05	0.05	11
6	**25**	4.2 ± 0.4	10	0.6	

[a] Relative potency. [b] Potency enhancement of individual sugars throughout the same family. [c] This value is consistent with the one previously described for the tri(ethylene)glycol congener.[21]

This protein constitutes a virulence factor and is involved in the pathogenesis of the bacteria in cystic fibrosis patients. To suitably evaluate the beneficial presentation of the multivalent sugar ligands, monomeric standards **18**,[19] **19** and **20** corresponding to mimetics of the peripheral saccharidic belt of each conjugate were synthesized. To this end, CuAAc conditions were applied to glycosyl azides **6** and **16**, and peracetylated derivative of **17**, respectively, in the presence of propargylic alcohol, followed by classical de-*O*-acetylation under the Zemplén conditions (see the ESI† for protocols).

For the competitive inhibition studies, the lactoside derivative **21**[20] was immobilized onto a commercial SPR sensor chip (CM5) following the manufacturer's procedure. IC_{50} values (Table 1) were determined using the pre-incubated mixtures of the PA-IL lectin (1.5 μM) with increasing concentrations of monomers or glycodendrimers used as analytes over the surface of CM5-bound **21**.

The SPR experiments clearly demonstrated that glycodendrimers **8**, **23**, and **25** exhibited much higher binding affinity compared to their corresponding monovalent derivatives **18**, **19**, and **20** due to the "multivalent or glycoside cluster effect".[22] As expected, monomeric lactoside **20** represented a weaker ligand for PA-IL[18] while the addition of a TEG linker to the galactoside moiety (**19** *vs.* **18**) allowed a 2-fold enhancement of the affinity for the lectin. Thus, the additional glucoside residues in lactosides have a detrimental effect which therefore cannot be simply accounted for by a longer linker. Interestingly, galactosylated dendrimer **8** exhibited low micromolar IC_{50} values (0.22 μM), while most notably galactodendrimer **23** afforded one of the best ligands known to date with an IC_{50} value of 37 nM that compared well with the results obtained with multivalent conjugates built around flexible or rigid scaffolds.[23] These results unambiguously highlight the key role of linkers in the interactions with lectins, with a counter-balanced entropic cost due to their flexibility. Additionally, tri-dimensional distribution of terminal and optimized galactosides crucially contributed to high potencies since a substantial improvement (32-fold) was observed for each ligand in **23**, when compared to monomeric reference **19**, while weaker individual enhancements were obtained with congested (**8** *vs.* **18**) or unoptimized (**20** *vs.* **25**) conjugates (11-fold).

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In summary, we demonstrated that the structural diversity in the construction of ''onion peel'' dendrimers, accessible *via* both convergent and divergent routes, represents an additional strategy for the build-up of dense surface groups at low dendrimer generations. It also represents clear advantages over existing approaches by providing versatile hypercore building blocks. Moreover, by not restricting layer-by-layer syntheses using identical subunits, one can programme the physical/biophysical properties of the dendrimers, as exemplified here using TEG residues. Of particular interest in this instance, is the use of underexploited dipentaerythritol as an A_6 core molecule. In fact, work is now in progress for further application of this useful building block as an AB_5 moiety. The work presented herein will undoubtedly be useful to generate efficient and programmable multivalent antiadhesive agents against bacterial infections.[7a,24] Rationalization of the preferential binding mode(s) together with determination of the precise role of each structural parameter leading to high avidity ligands such as in compound **23** are under investigation. Multivalent ''onion peel'' inhibitors harbouring optimized sugar epitopes, notably containing aromatic residues, are also presently under study. Further applications as antiadhesins towards galectins,[17] or as vectors for vaccines or drug targeting nanomaterials[25] are also under investigation.

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