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Solution-phase synthesis of linear and cyclic β- and α,β-peptoids was coupled to photo-induced thiol-ene coupling reaction to readily access multivalent thioglycoclusters. A tetrameric cyclic β-peptoid scaffold displaying 1-thio-β-D-galactose or 1-thio-α-D-mannose has revealed by ITC experiments efficient binding potency for bacterial lectins LecA and BC2L-A, respectively.

The design of multivalent glycoconjugates with high-affinity for proteins that possess multiple glycoside binding sites such as lectins and antibodies has been widely developed during the last decades.[1] Lectins are carbohydrate-specific proteins that mediate numerous cellular recognition events: development, differentiation, morphogenesis, fertilisation, immune response, implantation, cell migration and cancer metastasis.[2] Lectins contain two or more specific sugar-combining sites and as a consequence display strong avidity towards clustered sugars compared to monomeric ones. The lectin-binding efficiency and specificity of glycoclusters have been found to be dependent not only on the epitope density but also on the nature of the backbone and on the geometrical characteristics of the multivalent assembly.[3]

In the field of multivalent glycoside recognition, although poorly studied, *S*-glycoclusters present notable benefits over their *O*-glycoside counterparts, especially in terms of chemical stability and low susceptibility to enzymatic degradation.[4] Furthermore due to similar spatial arrangement of *S*- and *O*-glycosides, many lectins tolerate the O to S replacement, and some have even stronger affinity toward *S*-glycosides than the corresponding *O*-glycosides.[5] Thioglycoclusters can be prepared by photochemical thiol-ene coupling (TEC reactions) between 1-thiosugars and alkene-functionalized scaffolds (Fig. 1).[6] This type of ligation has been widely exploited in the field of polymer science[7] and this century-old reaction[8] has ignited growing interest in the past few years for preparing multivalent carbohydrate-based constructs due to its "click chemistry profile".[9] Well-defined thioglycoclusters were first efficiently prepared by the group of Stoddart from β-cyclodextrin scaffolds using protected 1-thiosugars.[10] The method was further extended to other scaffolds including peptides, calixarenes and dendrimers. However the reaction conditions, notably an excess of carbohydrate partners, the use of organic solvents, protected carbohydrates and purifications by chromatography were sometimes quite far from click chemistry criteria.[11] By adapting continuous flow chemistry to photochemical TEC, Hartmann's group demonstrated the power of this reaction to access sequence-defined carbohydrate-functionalised oligo(amidoamines).[12] The TEC reaction is a particularly appealing ligation method for the preparation of heteroglycoclusters when combined with the Copper(I)-catalysed Alkyne-Azide Cycloaddition (CuAAC)[13] or with the thiol-chloroacetyl coupling (TCC).[14]

α-Peptoids (N-substituted glycine oligomers) and β-peptoids (N-substituted β-alanine oligomers) are peptidomimetics characterised by resistance to proteases, rapid cellular uptake, and straightforward synthesis with a great potential for diversity.[15] Glycopeptoids have been developed as proteolytically stable glycopeptide mimics and the use of peptoid to access multivalent glycocluster constructs has proven particularly interesting.[16] The CuAAC reaction has been widely used for functionalizing peptoid scaffolds and particularly for accessing glycoclusters.[17] By contrast, the TEC reaction still remains nearly unexploited in the peptoid field.[18] The only access to peptoid-type thioglycoclusters was achieved by a solid-phase submonomer approach using acetylated sugars bearing at the anomeric position a thioethyl amino linker.[19] Obviously, the multivalent TEC ligation on already build peptoid scaffolds represents the most convergent way to multivalent thioglycopeptoids. The present study combines an efficient synthetic process for the preparation of alkene-functionalized linear

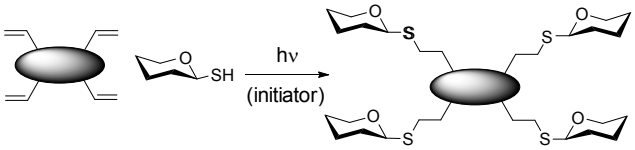

Fig. 1 Multivalent photochemical thiol-ene reaction

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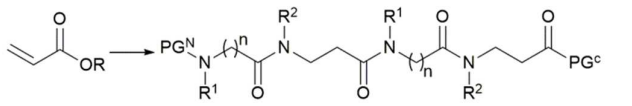
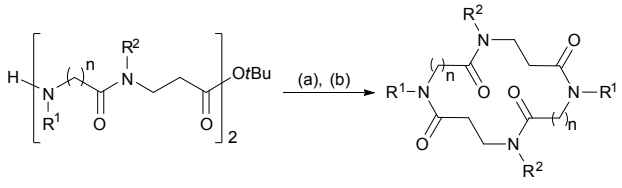
and cyclic peptoids with multivalent thiol-ene coupling to access thioglycoclusters ready for biological evaluation towards bacterial lectins. To reach this objective, gram-scale syntheses of linear β- and alternated α,β-peptoids carrying allyl side chains were efficiently performed thanks to a solution-phase methodology using volatile amines developed previously in our lab and avoiding intermediate purification steps.[18a] Briefly, the peptoid residues are created in two steps by acylation of the N-terminus of the peptoid, followed by reaction of the acylated intermediate (a bromoacetamide for an α residue or an acrylamide for a β residue) with the desired volatile amine (allylamine or isopropylamine in the present study). Linear peptoid scaffolds were thus obtained from tertbutyl or methyl acrylate, in an iterative manner with single final column chromatography purification, the intermediates being merely purified by filtration and/or evaporation (Table 1). The cyclic peptoid scaffolds **8** and **9** were efficiently formed by cyclisation of their linear precursors **4** and **7** under EDCI/HOBt conditions after TFA deprotection of the C-terminus (Scheme 1).[20] Following this approach, ten linear or cyclic β- or α,β-peptoids with 2 or 4 ligation sites and different capping groups have been synthesised to highlight the scope of photoinduced thiol-ene coupling with 1-thiosugars. The TEC process was optimized following as much as possible click reaction criteria.[11] First of all, free 1-thiosugars were used,[12,18b,21] thus eliminating the need of any deprotection step before biological evaluation. Another major concern was to drive the thiol-ene coupling reactions to 100% conversion using only stoichiometric amounts of thiol partners relative to the alkene pendant side chains, in order to avoid complex product isolation. A careful optimization of the TEC process using 1-thio-β-D-glucose (βGlcSNa) as model thiosugar (see ESI for details) led to the following conditions: βGlcSNa / 1M HCl (1.1 eq. per alkene moiety), 20 mol% of 2,2-dimethoxy-2-phenylacetophenone (DPAP) as photoinitiator,[22] overnight irradiation in water using a pyrex filter. After irradiation, the photoinitiator was removed from the crude by extraction with dichloromethane. A simple purification of the glycocluster was then performed using a C18 SPE cartridge. While the glycopeptoid was retained on the stationary phase, washing with one volume of water eliminated the remaining thioglucose and NaCl resulting from thiolate neutralization. Then elution with one volume of methanol and successive vaccum evaporation provided the multivalent glycopeptoid in pure form. The photoclick-optimized conditions were then applied to various linear and cyclic scaffolds using 1-thio-β-D-glucose and two other thiosugars of interest for lectin recognition: 1-thio-β-D-galactose and 1-thio-α/β-D-mannose. The dimeric and tetrameric glycoclusters **10**-**18** were thus obtained in good to excellent yields (Table 2).

Table 1 Structures of linear peptoid scaffolds



scaffold	n	PG^C	PG^N	R^1	R^2	Global yield (%)[a]
1	1	O*t*Bu	H	allyl	allyl	42
2	1	O*t*Bu	Ac	allyl	allyl	29
3	1	OMe	Ac	allyl	allyl	34
4	1	O*t*Bu	H	allyl	*i*Pr	36
5	1	OMe	Ac	allyl	*i*Pr	25
6	2	OMe	Ac	allyl	allyl	32
7	2	O*t*Bu	H	allyl	allyl	40

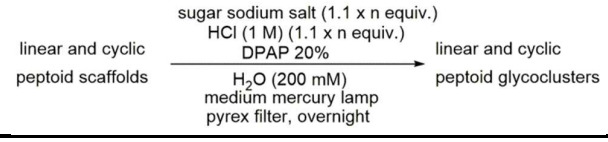
[a] Yield calculated for 7 to 8 steps from *tert*butyl acrylate or methyl acrylate. See SI for experimental conditions



4 n = 1, R^1 = allyl, R^2 = *i*Pr
7 n = 2, R^1,R^2 = allyl
8 n = 1, R^1 = allyl, R^2 = *i*Pr, **80%**
9 n = 2, R^1,R^2 = allyl, **50%**

Scheme 1 Cyclisation. Key: (a) TFA/CH_2Cl_2 then (b) EDCI (3.0), HOBt (3.0), Et_3N (6.0), CH_2Cl_2, r.t.

Table 2 Linear and cyclic peptoid glycoclusters



Entry	Scaffold	Sugar[a]	Glycocluster	n[b]	Yield (%)
1	**3**	1-thio-β-D-Glc	**10**	4	90
2	**5**	1-thio-β-D-Glc	**11**	2	90
3	**6**	1-thio-β-D-Glc	**12**	4	81
4	**8**	1-thio-β-D-Glc	**13**	2	68
5	**9**	1-thio-β-D-Glc	**14**	4	73
6	**3**	1-thio-β-D-Gal	**15**	4	76
7	**9**	1-thio-β-D-Gal	**16**	4	66
8	**9**	1-thio-α-D-Man	**17**	4	66
9	**9**	1-thio-β-D-Man	**18**	4	67

[a] Sodium salt. [b] Valency.

The binding affinities of selected thioglycoclusters were evaluated towards two soluble lectins produced by *Pseudomonas aeruginosa* and *Burkholderia cenocepacia* which are pathogenic bacteria involved in opportunistic infection in patients with immunosuppression or in life-threatening lung infection in cystic fibrosis patients.[23] LecA (also named PA-IL) is a cytotoxic galactose-specific lectin from *P. aeruginosa* involved in bacterial adhesion and biofilm formation.[24] The X-ray structure of the lectin/galactose complex reveals a tetrameric quaternary structure with the presence of a bridging calcium ion in the binding site (Fig. 2).[25] BC2L-A is one of the four soluble lectins identified in *B. cenocepacia*.[26] This mannose specific lectin shows a dimeric arrangement with a binding site involving two calcium ions.

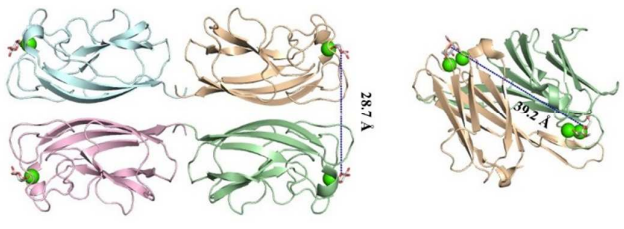


Fig. 2 Crystal structure of LecA/galactose complex (PDB code 1OKO)[25] and BC2L-A/mannoside complex (PDB code 2VNV)[26a]. Dashed lines represent the distances between sugar ring oxygen atoms.

The study of the interaction between glycoclusters **15**-**18** (Fig. 3) and these two lectins was performed by isothermal titration microcalorimetry (ITC) (Table 3). This bioanalytical technique is particularly suited since it furnishes complete thermodynamics providing a general overview of the binding process involved.[27]

Linear and cyclic tetrameric thiogalactoside glycoclusters **15** and **16** were evaluated towards LecA (Fig. 2). First, the binding ability of LecA to the model ethyl 1-thio-β-D-galactopyranoside was evaluated in order to assess the influence of anomeric oxygen replacement by sulfur. Indeed, previous studies showed a tenfold higher affinity of aromatic thiogalactosides (Kd 6.3 μM) compared to α- or β-D-GalOMe (Kd 70 μM).[5d] The monovalent ligand β-D-GalSEt gave ITC values similar to those of β-D-GalOMe (Kd 41 μM)[28] showing that replacement of oxygen by sulfur has no influence on the binding affinity (Table 3). Both linear and cyclic tetrameric glycoclusters **15** and **16** displayed greatly improved affinity compared to the monovalent β-D-GalSEt. The binding stoichiometry (n) for both compounds demonstrates a 1:4 glycocluster/lectin ratio, i.e. the implication of the four thiosugars in the binding event as previously obtained with a cyclic β-tetrapeptoid as scaffold.[28] The cyclic thioglycopeptoid **16** with a Kd value of 97 nM is in the range of the most potent LecA ligand identified to date.[29] As expected, the more rigid cyclic peptoid **16** presents an entropy barrier lower than those of the linear ones, and the 2 KJ/mol difference in the TΔS entropy contribution explains the higher affinity for the cyclic molecules, since both compounds display the same enthalpy of binding.

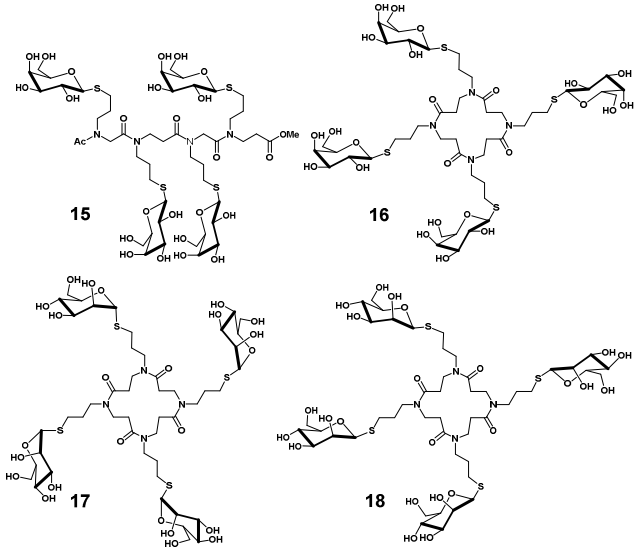


Fig. 3 Structures of thiogalactoside ligands **15** and **16** evaluated towards LecA and thiomannoside ligands **17** and **18** evaluated towards BC2L-A

For the dimeric BC2L-A lectin, the ITC values of the monovalent ligand α-D-ManSEt were shown to be almost identical to those measured for α-D-ManOMe (Table 3).[26a] The β-thiomannoside bound with almost equivalent affinity (Kd 9.6 μM). For both compounds, analysis of the thermodynamic contribution indicates a favourable entropy term, which is unusual in protein-carbohydrate interaction, but previously observed in this family of lectin and attributed to the presence of two calcium atoms in the binding site.[30] The cyclic tetrameric glycopeptoids **17** and **18**, differing only by the α/β anomeric configuration, gave improved avidity for BC2L-A compared to their monovalent counterparts (see ESI, Fig. S1). Particularly, the 1-thio-α-D-mannoside cluster **17** displayed a 204 nM dissociation constant similar to that of a rigid dimannoside compound recently published but with different binding thermodynamics.[26b] The tetrameric cluster **17** gave a higher enthalpy but together with a higher entropic cost. The stoichiometry and enthalpy values indicate that 3 to 4 BCL2-A monomers are bound to each cluster.

The high efficiency of glycolusters for binding to multivalent lectin is generally observed when the geometry is well suited for chelating two neighbouring binding sites.[28,29d,f] In the present case, the tetravalent mannosylated clusters cannot chelate the two binding sites of BC2L-A that are 40 Å apart (Fig. 2). Indeed, building peptoid models using the crystal structure of the scaffold[31] and extended conformation of the mannoyslated arms do not give extension larger than 20 Å (see ESI, Fig. S2). Therefore only aggregation can occur for BC2L-A interacting with **17** and **18**. Interestingly, the same conclusion was reached for LecA, since the binding sites are 30 Å away, and ligands **15** and **16** could not extend more than 25 Å. It is of interest to observe that very strong affinities can be obtained, even when aggregation, and not chelation dominates the binding.

Compounds able to aggregate lectins may also play a role on the agglutination of bacteria that are presenting such lectins. A systematic study on the in vitro and in vivo effect of galactosylated clusters on *P. aeruginosa* demonstrated that inhibition of bacterial aggregation may be one mode of action of such compounds.[32] These results open the way to further studies aiming to better understand binding properties of this type of glycoclusters with BC2L-A, a lectin of interest still under-studied.

Table 3 Isothermal titration microcalorimetry (ITC) measurements for the binding of ligands β-D-GalSEt, **15** and **16** to LecA and ligands α-D-ManSEt, β-D-ManSEt, **17** and **18** to BC2L-A. Standard deviations are indicated on experimentally derived values (at least two experiments)

Ligand	Valency	n	ΔH [kJ mol^{-1}]	ΔG [kJ mol^{-1}]	$T\Delta S$ [kJ mol^{-1}]	K_d [μM]	Relative potency [β]
β-D-GalSEt	1	1 (fixed)	-31 ± 7	-25	-6	41.6 ± 1.5	1
15	4	0.23 ± 0.01	-124 ± 6	-38	-86	0.214 ± 0.05	194
16	4	0.18 ± 0.01	-124 ± 10	-40	-84	0.097 ± 0.01	429
α-D-ManSEt	1	1.06 ± 0.1	-23.0 ± 1	-31.3	+ 8.3	3.3 ± 0.5	1
β-D-ManSEt	1	1.06 ± 0.02	-21.6 ± 0.1	-28.7	+7.1	9.57 ± 0.05	0.3 (vs α -ManSMe)
17	4	0.33 ± 0.01	-108.5 ± 2.6	-38.2	-70.3	0.204 ± 0.001	16 (vs α -ManSMe)
18	4	0.34 ± 0.01	-76.2 ± 2.5	-33.3	-42.9	1.45 ± 0.3	6.6 (vs β -ManSMe)

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