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PII: S0040-4039(18)31310-8
DOI: https://doi.org/10.1016/j.tetlet.2018.10.069
Reference: TETL 50379

To appear in: *Tetrahedron Letters*

Received Date: 5 September 2018
Revised Date: 24 October 2018
Accepted Date: 30 October 2018

Please cite this article as: Figueredo, A.S., Zamoner, L.O.B., Rejzek, M., Field, R.A., Carvalho, I., Cluster glycosides and heteroglycoclusters presented in alternative arrangements, *Tetrahedron Letters* (2018), doi: https://doi.org/10.1016/j.tetlet.2018.10.069

Cluster glycosides and heteroglycoclusters presented in alternative arrangements

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ABSTRACT

Multivalent carbohydrates, or glycoclusters, are useful tools to study glycan-lectin and glycan-enzyme recognition processes and have wide potential therapeutic applicability. Herein, we report the synthesis of novel glycoclusters presenting glucopyranose units in alternate arrangements by coupling through the C-1, C-2, C-3 or C-6 sugar positions and using tetra- and hexa-valent scaffolds for multivalent display. Coupling the appropriate azide-functionalised D-glucopyranose units with alkynlated penta- or dipenta-erythritol central cores was accomplished *via* copper-catalysed azide-alkyne cycloaddition (CuAAC), yielding a panel of eight tetra- and hexa-valent glycoclusters in good yields (52-83%). This click chemistry strategy was extended to the preparation of four heteroglycoclusters using a tris(hydroxymethyl)-aminomethane (TRIS) central scaffold. One unit of either the conventional 1-deoxy-D-nojirymicin iminosugar or its' C-5 epimeric L-gulo isomer, were incorporated along with three D-glucopyranose units linked through either C-1 or C-6.

Keywords: multivalency, glycoclusters, heteroglycoclusters, iminosugars, CuAAC click reaction.

1. Introduction

The multivalent nature of cell surface glycoconjugates underlies cellular interaction processes with the extracellular matrix, microorganisms and other cells.[1] Lectins, typically multivalent carbohydrate-binding proteins, are particularly important in these events because their sugar binding sites interact specifically with carbohydrate ligands. Therefore, multivalent carbohydrate clusters with different architectures have been synthesised to understand glycan clustering effects.[2-4] Regardless of specific application, glycocluster affinity and selectivity can be achieved by an appropriate balance between valency, topology and the nature of both the carbohydrate and the linker that connects the latter to a central scaffold.[5,6.]

In recent literature, several studies have reported the synthesis of multivalent iminosugars, for instance based on deoxynojirimycin (DNJ **1**), presented on various scaffolds, such as dendrimers,[7,8] cyclodextrins,[9,10] fullerenes,[11] tetravalent porphyrins,[12] linear and branched dextran,[13] cyclo-peptoid clusters,[14,15] calixarenes[16] and self-assembled scaffolds (biomimetic nanoparticles).[17] In order to enhance their relative potency (rp) against glycoside hydrolase enzymes, a trivalent DNJ conjugate **2** was constructed and showed a strong multivalent effect (MVE) on jack bean α-mannosidase (JBman) (Fig. 1).[7] In a similar sense, heteroglycoclusters bearing more than one type of carbohydrate epitope have demonstrated

altered interactions between concanavalin A and α-mannopyranoside clusters containing additional non-ligand sugars (β-D-glucopyranose or β-lactose) for this lectin.[18] On a broader front, aiming to hit multiple binding sites on lectins/glycosidases, a range of pseudodisaccharide iminosugar and oligosaccharide glycosidase inhibitors, such as MDL73945 **3**,[19] acarbose **4**[20,21] and triazole-iminosugar pseudodisaccharide **5**,[22] have been investigated.

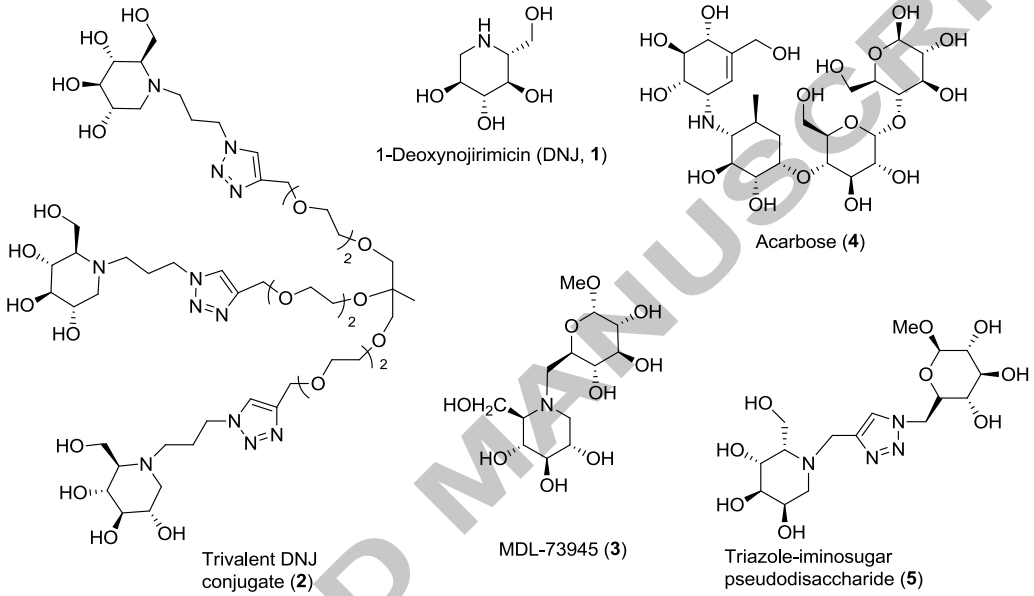


Figure 1. Chemical structures of glycosidase inhibitors including 1-deoxinojirimicin **1**, a trivalent DNJ-based glycocluster **2**, pseudodisaccharide MDL-73945 **3**, acarbose **4** and triazole-iminosugar pseudodisaccharide **5**.

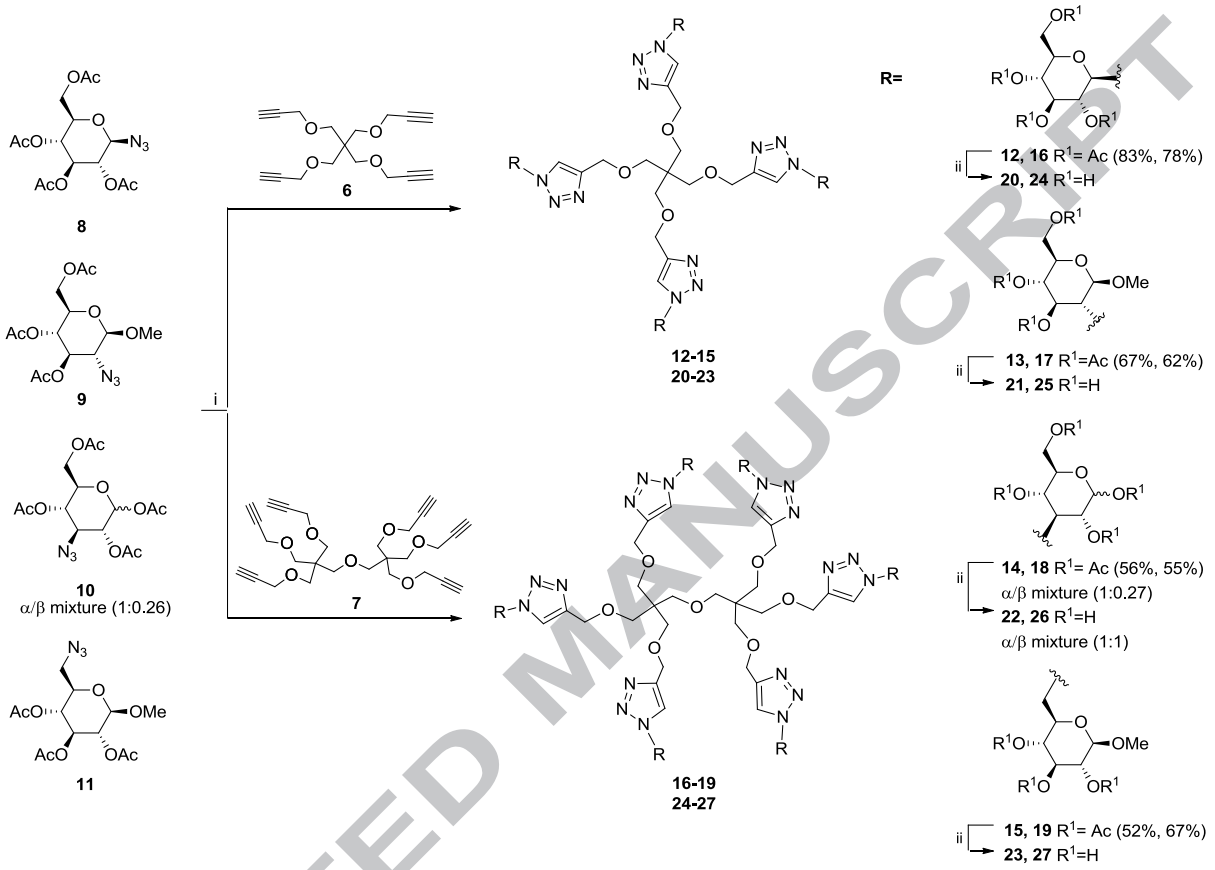
Building on our previous work on multivalent glycans[23-25] synthesised using CuAAC click chemistry,[26,27] we set out to explore new multivalent glycan assemblies based on pentaerythritol and dipentaerythritol as molecular scaffolds, in order to access tetra- and hexa-valent cluster glycosides. Furthermore, our intent was to explore systematic modification of D-glucopyranoside units, by coupling through the sugar C-1, C-2, C-3 or C-6 positions. Despite the plethora of complex multivalent lectin and glycosidase binding abilities described above, to the best of our knowledge, only one example of glycoclusters bearing six copies of glucose, trehalose, lactose or galactose presented on a dipentaerythritol scaffold has been reported.[28]

2. Results and Discussion

2.1 Synthesis of cluster glycosides

Initially, symmetric pentaerythritol **6** containing four equivalent propargyl functional groups was selected as the central scaffold for the synthesis of tetravalent cluster glycosides.

To this core, four Glc units were introduced in alternative orientations (coupled *via* the glucose C-1, C-2, C-3 or C-6 positions). A similar approach was applied to hexa-propargyl dipentaerythritol **7**, to produce hexavalent glycoclusters (Scheme 1).



Scheme 1. New 1,2,3-triazole-based glycoclusters obtained from 1,3-dipolar cycloaddition reactions. Reagents and conditions: i. Building blocks **6** or **7** (0.025 mmol), azidosugars **8**-**11** (1.1 eq. for each alkyne termination), $CuSO_4$ (0.05 eq.), Na ascorbate (0.1 eq.), DMF (0.1 mL), microwave heating at 80 °C, 15 min. (2x or 3x) ii. NaOMe (1 M in methanol), MeOH (1 mL). 1.5 h, rt, then DOWEX® 50WX4-50.

The attachment of four propargyl groups on the pentaerythritol scaffold was achieved with propargyl bromide in anhydrous DMF/KOH to afford the corresponding tetravalent core 6 in 38% yield (Scheme **1**).[29] Despite repeated attempts to prepare alkylated dipentaerythritol **7** using similar conditions, better results were obtained with the same alkylating reagent but using phase-transfer catalysis conditions, according to previous reports (31% yield).[30] The azido-functionalised D-glucopyranoses modified at C-1, C-2, C-3 or C-6 (**8**-**11**), required for subsequent CuAAC reactions, were prepared according to reported procedures, except for the commercially available compound **8**. Briefly, methyl 2-azido-2-deoxy-β-D-glucopyranoside **9** was obtained in three steps from D-glucosamine hydrochloride in 23% overall yield.[31,32] The synthesis of 3-azido-functionalised glucopyranose **10** started from commercially available 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose, which was initially converted into the corresponding azide *via* a triflation/azide displacement sequence. Subsequent treatment of the

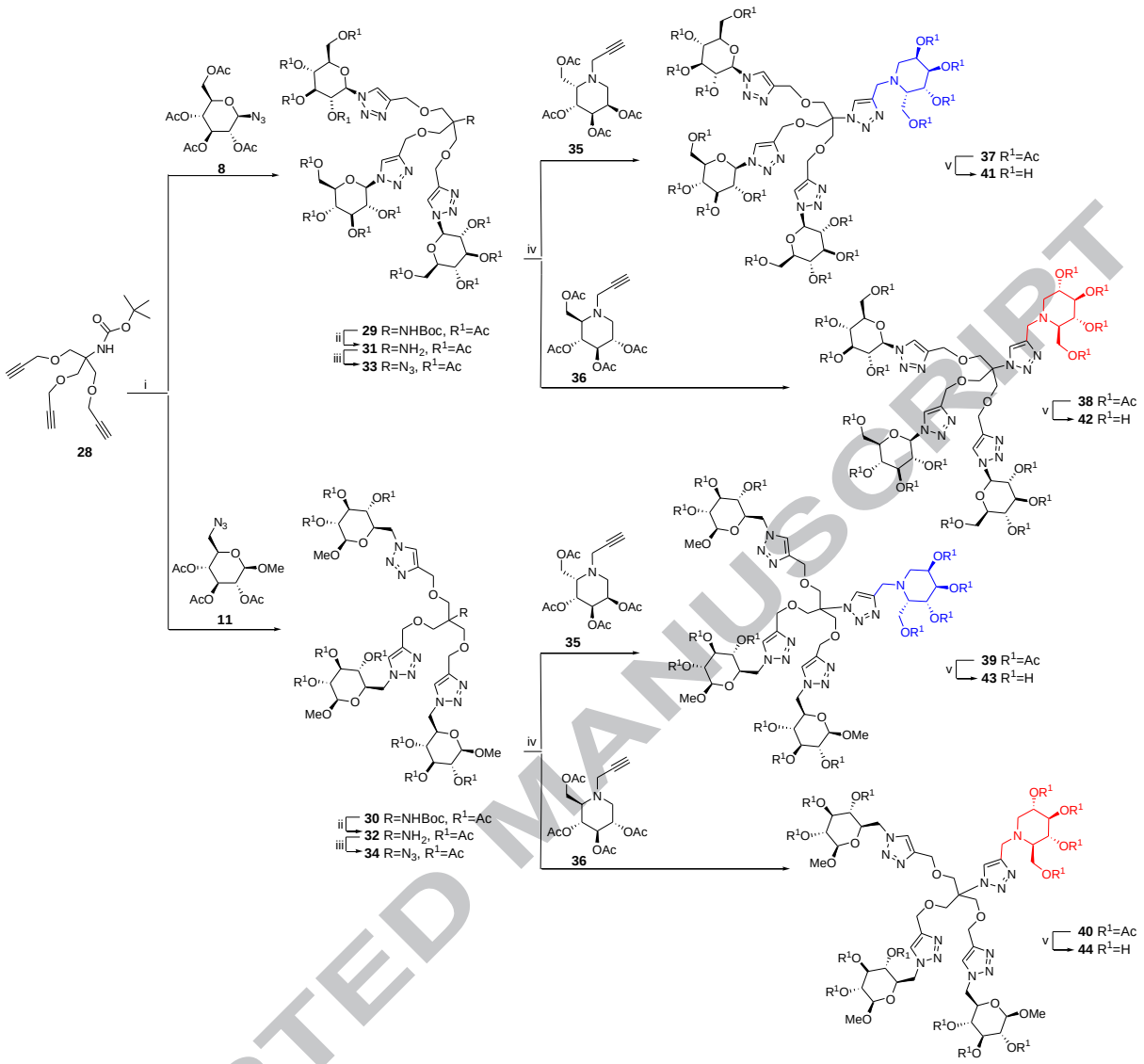
intermediate furanose diacetonide with aqueous trifluoroacetic acid followed by complete acetylation of the crude product by treatment with acetic anhydride and pyridine produced the pyranose acetate **10** as a mixture of anomers (α/β 4:1).[33,34] Finally the preparation of methyl 6-azido-6-deoxy-β-D-glucopyranoside 11 was carried out following the modified method described by Carvalho and co-workers,[35] with formation of the methyl 6-*O*-tosyl-β-D-glucopyranoside intermediate using a one-pot tosylation/acetylation reaction (43%). An improved yield was achieved by performing this reaction at low temperature and with dropwise addition of a solution of tosyl chloride in pyridine (68%). The synthesis was completed by displacement of the 6-*O*-tosylate group with NaN_3 in DMF, affording azidosugar (11) in 77% yield.[35,36]

Next, the propargylated scaffolds **6** and **7** were coupled with azidosugars **8-11**, *via* copper-catalysed azide and alkyne cycloaddition (CuAAC) to give a panel of eight tetra- and hexavalent protected glycoclusters (**12-19**) in good yields (52-83%) (Scheme 1). The reaction was conducted on a small scale (0.025 mM of alkyne) with a 10 mol% excess of the azidosugar to each alkyne and with copper sulfate/sodium ascorbate for *in situ* generation of the Cu(I) catalyst.[35] Typically, the coupling reactions were conducted in a sealed tube under microwave-assisted conditions, with heating at 80 °C in DMF (0.1 mL), with the reaction progress being monitored by TLC. After completion of the reaction, residual solvent was co-evaporated with toluene and the crude product was purified by flash chromatography. The structures of peracetylated 1,2,3-triazole-glycoclusters (**12-19**, Fig. 1) were confirmed by ^{1}H and ^{13}C NMR spectroscopy, which showed the presence of a single 1,4-disubstituted regioisomer and complete functionalization of the scaffold (four or six positions). Hydrogen chemical shifts of the triazole-linked glucose were observed downfield compared to the precursor azide **8-11** and the triazole hydrogen was observed in the range δ 7.6-8.1 ppm, with integration of 4 H (**12-15**) or 6 H (**16-19**), while the triazole carbon not bonded to hydrogen was detected at 144.9-146.0 ppm. Subsequently, *O*-deacetylation with NaOMe (1M in methanol) afforded the deprotected glycoclusters **20-27** in near quantitative yield. Their structures were confirmed by ^{1}H NMR spectroscopy which showed absence of singlets for the $COCH_3$ groups. HRESI-MS analysis showed the characteristic adducts of $[M+Na]^+$ or $[M+H]^+$ for compounds **20-27**, although the hexavalent glycoclusters (**24-27**) were more ms-sensitive and gave some in-source fragmentation during the analyses.

2.2 Synthesis of heteroglycoclusters

Using the same click chemistry approach described above, the synthesis of four tetravalent heteroglycoclusters was conducted on a bifunctional dendrimer scaffold to enable

the presentation of two non-identical ligands in a 3:1 ratio[37] (e.g. three glucopyranose units and one DNJ-derived moiety), as outlined in Scheme 2. The synthesis of known dendrimer scaffold **28**[38] was performed in two steps from commercially available tris(hydroxymethyl)aminomethane (TRIS). The preparation of trivalent clusters connected at glucopyranose C-1 (**29**) or C-6 (**30**) was carried out in a similar manner to glycoclusters **12-19**, using azidosugars **8** and **11**, allowing the formation of peracetylated products **29** and **30** in good (82%) and moderate yields (58%), respectively. Next, the introduction of azide functionality on the cluster cores of **29** and **30**, for further decoration with a second type of carbohydrate ligand, was performed in two steps by quantitative removal of the Boc protecting group with TFA to afford intermediates **31** and **32**, which undergo a diazo-transfer reaction with imidazole-1-sulfonyl azide[39] and $CuSO_4$ without further purification. The azide-functionalized dendrimers **33** (78%) and **34** (61%) were obtained in good yields over two steps. Their structures were confirmed by ^{1}H NMR and ^{13}C NMR spectroscopy, supported by the appearance of a 2100 cm^{-1} IR stretch consistent with the presence of azido groups, which was further substantiated by HRESI-MS analysis ($[M+H]^+$ 1381.4605 for compound **33** and $[M+H]^+$ 1297.4700 for **34**, respectively).



Scheme 2. Reagents and conditions: i. **28** (0.15 mmol), sugars **8** or **11** (1.1 eq. for each alkyne termination), $CuSO_4$ (0.03 eq.), Na ascorbate (0.1 eq.), DMF (0.1 mL), microwave heating at 80 °C, 15 min. (3x). ii. **29** or **30** (0.11 mmol), CH_2Cl_2/TFA 80% (2 mL, v/v), 2 h, rt. iii. $CuSO_4$ (0.023 mmol), imidazole-1-sulfonyl azide hydrosulfate (1.6 eq.), $NaHCO_3$ (0.23 mmol), MeOH (4 mL). iv. Azide-functionalized dendrimer **33** or **34** (0.05 mmol), iminosugars **35** or **36** (0.05 mmol), $CuSO_4$, (0.03 eq.), Na ascorbate (0.1 eq.), DMF (0.1 mL) microwave heating at 80 °C, 15 min. (3x). v. NaOMe (1 M in methanol), MeOH (1 mL). 1.5 h, rt, then DOWEX® 50WX4-50.

Finally, the preparation of heteroglycoclusters was achieved by the coupling of azide-functionalized dendrimers **33** and **34** with *N*-propargylated iminosugars **35**[22] or **36**[40,41] under the CuAAC conditions reported above. The four peracetylated glycoclusters 37-40 showed characteristic ^{1}H and ^{13}C NMR signals related to two distinct types of sugars in a 3:1 ratio. For instance, the ^{1}H NMR spectrum of compound **37** exhibited two characteristic signals of the triazole rings at δ 7.99 (3H, s) and δ 7.78 (1H, s) and a downfield shift was observed for the central CH_2 signal due to the influence of a new triazole ring, changing from δ 3.63 (compound **33**) to δ 3.98 in glycocluster **37**. The final deprotection step (NaOMe/MeOH) afforded compounds **41-44** in quantitative yields. HRESI-MS analysis showed the characteristic adducts of $[M+H]^+$ for all these compounds (protected and deprotected

derivatives). Preliminary assessment of yeast α-glucosidase and almond β-glucosidase inhibitory activities with heteroclusters **41**-**44** revealed that compounds **41** and **43** showed some inhibition toward yeast α-glucosidase (IC_{50} 225 and 210 μM) compared to DNJ as a reference compound (110 μM). On the other hand, non-iminosugar glycoclusters **20**-**27** bearing four or six copies of D-glucopyranose attached to pentaerythritol or dipentaerythritol scaffolds, including those linked by C-1 or C-6 sugar positions as observed in heteroclusters, proved to be inactive against both yeast α-glucosidase and almond β-glucosidase at 1000 μM concentration.

3. Conclusion

Using CuAAC click chemistry, four and six glucopyranose units were successfully coupled to per-*O*-propargylated penta- and dipenta-erythritol scaffolds *via* the glucose C-1, C-2, C-3 or C-6 positions, to produce multivalent glycoclusters presenting the carbohydrate in alternative arrangements. CuAAC coupling was achieved under microwave-assisted conditions, which allowed the ligation of sugar units to the central core through 1,2,3-triazole linkers in good yields. Thus, a single unit of glycopyranose can afford structurally diverse glycoclusters with defined spatial orientations for further use as tools to study essential carbohydrate-protein interactions. The usefulness of this approach also enabled the preparation of heteroclusters, bearing three glucopyranose units and one iminosugar attached to a bifunctional dendrimer scaffold. These adducts partially resemble pseudooligosaccharides and as such have potential as glycosidase inhibitors. The detailed biological evaluation of the glycoclusters will be reported in due course.

Acknowledgements

We acknowledge financial support from Brazilian agencies: FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo-Brazil 2013/27038-8) and CNPQ (Conselho Nacional de Desenvolvimento Científico e Tecnológico). Work in the UK was supported by the BBSRC Institute Strategic Program on Understanding and Exploiting Metabolism (MET) [BB/J004561/1] and the John Innes Foundation.

Supplementary data

The Supporting Information contains a description of the methods used and the structural characterization of compounds including ^{1}H and ^{13}C NMR and two-dimensional spectra,

along ESI-MS analyses of the new intermediates and all products. Supplementary data associated with this article can be found, in the online version, at:

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Cluster glycosides and heteroglycoclusters presented in alternative arrangements

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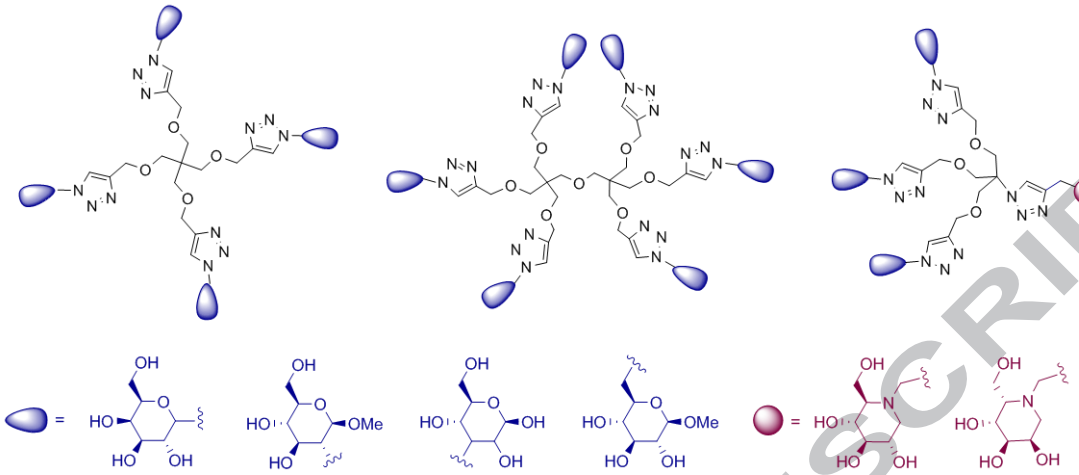

Highlights:

Tetra and hexavalent glycoclusters containing D-glucopyranose cores were synthesised

Sugars are linked in alternate arrangements through C-1, C-2, C-3 or C-6 positions

Heteroglycoclusters with non-identical ligands were also obtained

The iminosugar glycoclusters have trivalent D-glucose attached at C-1 or C-6 positions

These adducts have potential to resemble pseudooligosaccharides.