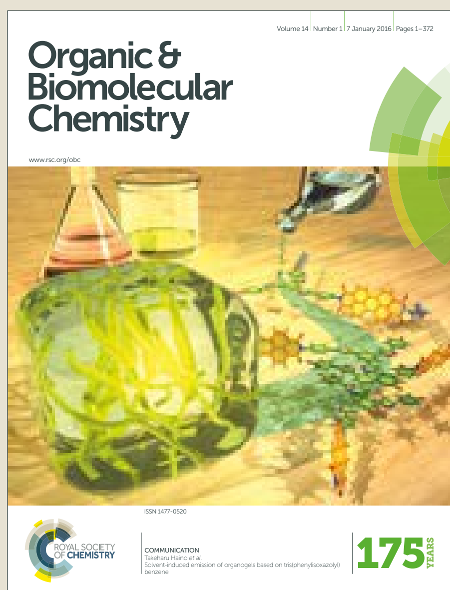
Check for updates

View Article Online
View Journal

Organic & Biomolecular Chemistry

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: M. M. Pichon, F. Stauffert, A. Bodlenner and P. Compain, *Org. Biomol. Chem.*, 2019, DOI: 10.1039/C9OB00826H.



This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the author guidelines.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the ethical guidelines, outlined in our author and reviewer resource centre, still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/obc

Organic & Biomolecular Chemistry

ROYAL SOCIETY OF CHEMISTRY
View Article Online
DOI: 10.1039/C9OB00826H

ARTICLE

Tight-binding Inhibition of Jack bean α-Mannosidase by Glycoimidazole Clusters

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Maëva M. Pichon, Fabien Stauffert, Anne Bodlenner, and Philippe Compain*

The best multivalent effects observed in glycosidase inhibition have been achieved so far with Jack bean α-mannosidase (JBα-man) using iminosugar clusters based on weakly binding mismatching active-site-directed inhibiting epitopes (inhitopes) in the D-*gluco* series. Here, we synthesize and evaluate as JBα-man inhibitors a series of mono- to 14-valent glycoimidazoles with inhitopes displaying inhibitions values up to the hundreds of nM range to study the impact of inhitope affinity on the multivalent effect. The most potent inhibitor of the series, a 14-valent mannoimidazole derivative, inhibits JBα-man with a nanomolar K_i value (2 ± 0.5 nM) and binding enhancements observed are, at best, relatively small (up to 25-fold on a valency-corrected basis). Results of this study support the fact that JBα-man-inhitope affinity and the strength of the inhibitory multivalent effect evolve in opposite direction. The major impact of the glycoimidazole-based inhitope is found on the binding scenario; most of the synthesized mannoimidazole clusters as well as a 14-valent glucoimidazole derivative prove to be tight binding inhibitors of JBα-man.

Introduction

Research in the field of multivalent glycosidase inhibitors has experienced an impressive growth in the last decade.[1] Yet multivalency has long been neglected as a solid option to achieve potent and selective glycosidase inhibition. It was the discovery of the first quantifiable[2] and large[3] binding enhancements achieved with multimeric mannosidase inhibitors that ended a long lag period. The stakes involved are high considering the therapeutic potential of glycosidase inhibitors, exemplified by the antiviral Tamiflu™ or the antidiabetic Glyset™.[4] Glycosidase-catalyzed carbohydrate hydrolysis is indeed a biologically widespread reaction involved in a diversity of key processes including cell wall degradation and the turnover of signalling molecules.[5] Attempts to assess the structural basis of the inhibitory multivalent effect were performed using physical methods. Most works focused on Jack bean α-mannosidase (JBα-man), a dimeric high-molecular-weight glycosidase which is by far the most sensitive to multivalent binding known to date.[1] Very recently, an important step has been taken with the disclosing of the first high-resolution crystal structure[6] of JBα-man in complex with **1**, the multimeric inhibitor displaying the largest binding enhancements reported so far (Fig. 1).[7] The exceptionally large affinity enhancements observed were explained by the formation of a sandwich-type complex in which the multivalent inhibitor simultaneously bridges the four catalytic sites of two dimeric JBα-man molecules, resulting in a strong chelate effect. Other arrangements may be obtained depending on the structure of the multimeric inhibitors. For example, the formation of discrete "S"-shaped arrangement resulting from the interaction of two molecules of the 9-valent inhibitor **2** with three molecules of JBα-man has been recently proposed based on TEM study.[8] In addition to the use of physical methods, structure-activity relationship investigations have been performed to get further insights into multimeric binding modes.[1] Such studies were motivated also by the objective of pushing the limits of the inhibitory multivalent effect and to assess its potential as a tool for modulating glycosidase activity in cells[9] and in vivo.[10] Over the last ten years, chemists have thus synthesized numerous multimeric systems from low-valency clusters to nanoparticle,[11] polymeric[12] and supramolecular[10] assemblies.[1] Structure-activity relationship studies focused on the impact of key structural features on the multivalent effect. The list includes the number, the nature and the spatial orientation of the active-site-directed inhibiting epitopes (i. e. inhitope,[13,14] by analogy with epitope-paratope interaction). Relative inhibition potency (rp) is estimated by a direct comparison of the inhibition constant values between the n-valent inhibitors and the corresponding monovalent control.

[a] Laboratoire d'Innovation Moléculaire et Applications (LIMA)
Univ. de Strasbourg | Univ. de Haute-Alsace | CNRS (UMR 7042)
Equipe de Synthèse Organique et Molécules Bioactives (SYBIO), ECPM
25 Rue Becquerel, 67000 Strasbourg, France
E-mail:philippe.compain@unistra.fr

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

Published on 21 May 2019. Downloaded by Syddansk Universitetsbibliotek on 5/22/2019 8:58:13 AM.

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C9OB00826H

Organic & Biomolecular Chemistry

ARTICLE

Published on 21 May 2019. Downloaded by Syddansk Universitetsbibliotek on 5/22/2019 8:58:13 AM.

Organic & Biomolecular Chemistry Accepted Manuscript

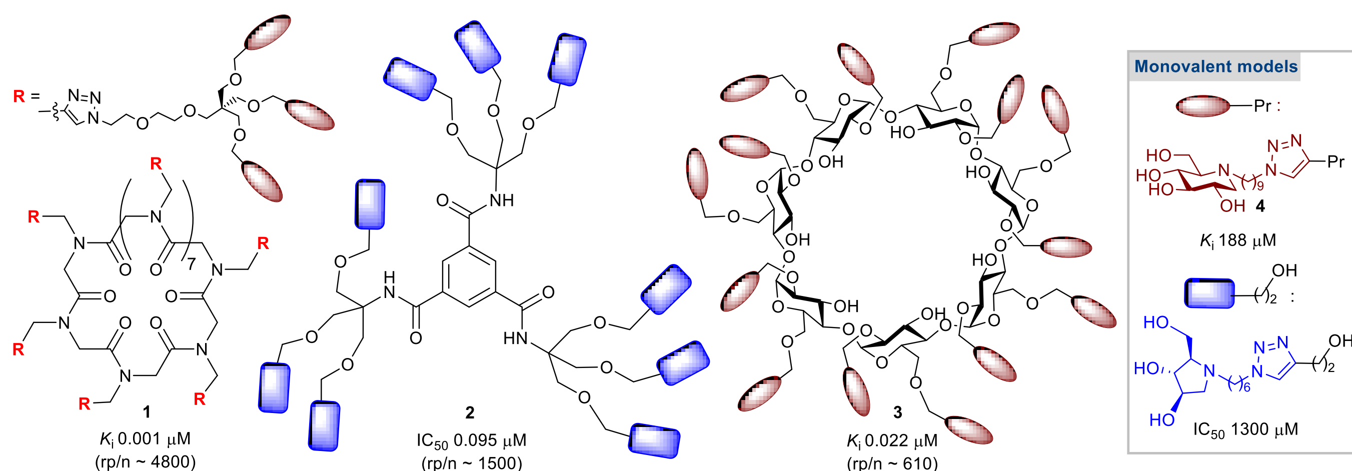


Fig. 1 Potent multivalent inhibitors of JBα-man and their corresponding monovalent controls.

A qualitative evaluation of the multivalent effect is thus provided by calculation of rp/n values. To date, the best multivalent effects were described with inhitopes displaying modest affinity for the targeted glycosidases.[1,7,8] For example, with regards to JBα-man inhibition, the largest rp/n values have been obtained with clusters based on weakly binding mismatching inhitopes in the *pseudo* D-*gluco* series (Fig. 1).[7,8,15] Multivalent inhibitors based on 1-deoxynojirimycin (DNJ) have been mainly used.[1] For such systems, the corresponding monovalent controls typically display inhibition in the hundreds of μM range. Even if clusters based on inhitopes in the matching D-*manno* series have already been evaluated,[13,22] the impact of inhitopes in the nM range has never been studied. Despite the impressive number of multivalent glycosidase inhibitors synthesized to date,[1] a puzzling question remains unanswered: what would be the impact of high affinity targeting inhitopes on the multivalent effect? Would this lead to changes in the binding modes or to improved relative inhibition potencies? To answer these questions, we have now designed neoglycoclusters based on glycoimidazole inhitopes. Here, we describe the full details of our study from the stereodivergent synthesis of two glycoimidazole-based inhitopes to the inhibition results obtained with the model glycosidase JBα-man.

Results and discussion

Neoglycocluster design. The choice of the inhitope structure was guided by different criteria. In addition to being a potent nanomolar inhibitor of JBα-man, the selected glycocluster head groups have to be accessible by a straightforward synthesis. The attachment of a linker to the inhitope in order to construct the multivalent systems by Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) must also be performed without negatively affecting binding to JBα-man active site. After an extensive review of the literature, mannoimidazole derivatives of type I were selected (Fig. 2). Related 2-alkyl substituted imidazoles are indeed potent inhibitors of JBα-man with inhibition values in the nanomolar range.[16,17]

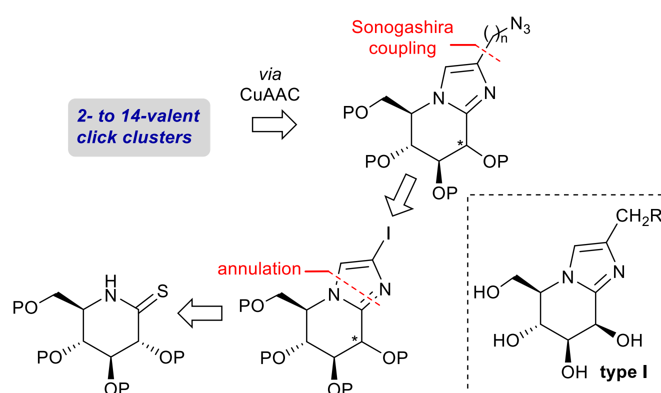


Fig. 2 Devised strategy towards click glycoimidazole clusters.

Our synthetic approach was partly based on the optimization of a strategy developed by Vasella *et al.* from readily prepared D-glucono-δ-thiolactam derivatives (Fig. 2).[18] In this stereodivergent synthesis, both D-*gluco*- and D-*manno*-

Please do not adjust margins

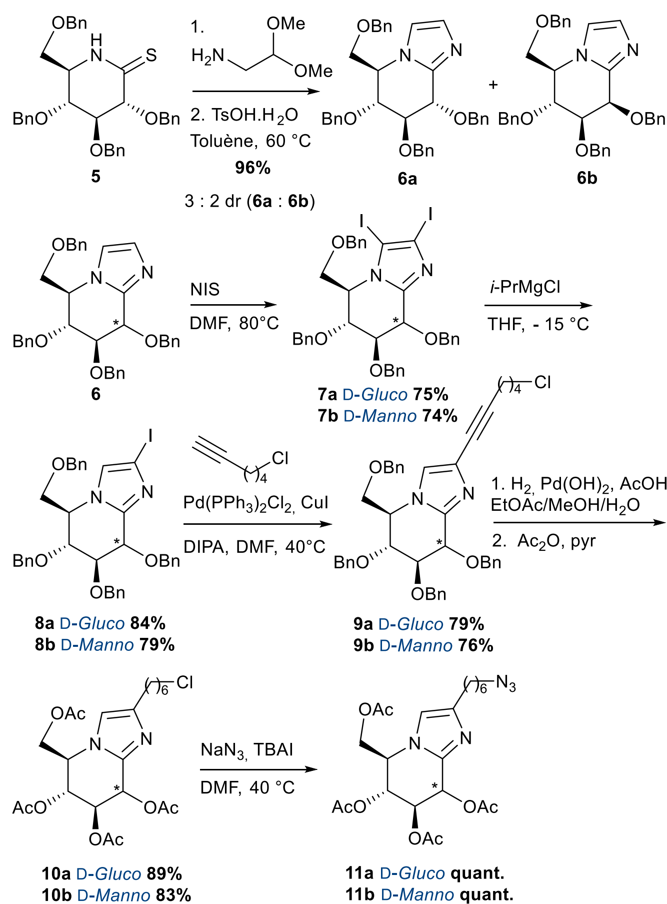
View Article Online
DOI: 10.1039/C9OB00836D

Published on 21 May 2019. Downloaded by Syddansk Universitetsbibliotek on 5/22/2019 8:58:13 AM.

Organic & Biomolecular Chemistry Accepted Manuscript

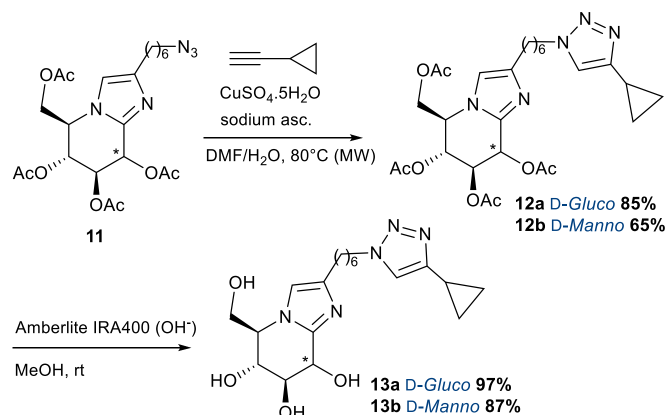
configurated glycoimidazole intermediates could be obtained. This offers the opportunity to access multivalent JBα-man inhibitors with inhitopes displaying inhibitions from the low µM range to the nM range.[16,17] Concerning the choice of the scaffold, β-cyclodextrin (β-CD) holds many advantages to build rapidly multivalent constructs that may display large multivalent effects with rp/n up to 620-fold (Fig. 1)[15] and will allow for direct comparison with previously synthesized clusters with different inhitopes.[15,22] Pentaerythritol was selected as well in the present study as a core to access di- to tetravalent systems. The objective was to evaluate if high affinity inhitopes could compensate for a reduced valency.

Neoglycocluster synthesis. The first key step of the synthesis is the annulation of an imidazole ring to D-gluconothiolactam **5** obtained by treatment with Lawesson's reagent of the corresponding D-glucono-δ-lactam derivative (Scheme 1).[18] The former compound was obtained in three steps on gram scale from commercially available tetra-*O*-benzyl-D-glucopyranose following a robust synthetic route developed recently in our group.[19] The key tetrahydroimidazopyridine skeleton was generated by a two-step process: nucleophilic addition of aminoacetaldehyde dimethyl acetal to the thiocarbonyl group of **5** followed by acid-mediated cyclization.



Scheme 1 Access to azide-armed glycoimidazole **11**.

The initial conditions described by Vasella *et al.*[18] which led in our hands to a 2:1 mixture of the *gluco/manno*-configurated imidazoles **6** in 77% yield were modified to increase the reaction yield as well as the amount of the *manno*-imidazole product **6b**. After a systematic study into the effects of reaction conditions, it was found that the best yields (up to 96%) were obtained using dry toluene and higher concentrations for the annulation step. Under these conditions, the amount of *manno*-imidazole product **6b** was slightly increased (**6a**/**6b** *ratio* 3:2). After separation by flash chromatography of diastereoisomers **6**, the synthesis was continued on both epimers separately. Diiodo derivatives **7** were obtained by treatment of **6** with an excess of *N*-iodo succinimide in DMF at 80°C.[17,20] In our hands, the mono-deiodination reaction using Vasella's protocol[17] (EtMgBr at room temperature) afforded the expected iodo derivative **8** in modest yield (57%) along with the di-deiodination product **6** in 32% yield. Increasing the reaction time or the amount of EtMgBr did not improve the product yield with possible decomposition of the Grignard reagent. After various attempts, the best results were obtained with 1.1 equivalent of *i*-PrMgCl at -15°C. Under these conditions, the desired mono-iodo derivatives **8** could be obtained in yields up to 84% on a gram scale. The precursor of the "clickable" linker was then introduced by way of a Sonogashira coupling.[17,20,21] Optimal reaction conditions using $Pd(PPh_3)_2Cl_2$ (5 mol%) in DMF in the presence of diisopropylamine (DIPA, 5 equivalents) and CuI (0.1 equivalent) afforded the 2-alkyl substituted imidazoles **9** in good yields. One-pot alkyne reduction/*O*-debenzylation followed by peracetylation of the corresponding crude tetrols provided **10** in high yields for the two steps. The use of acetate protecting groups is critical for the final challenging deprotection step in the synthesis of the targeted neoglycoclusters. The displacement of the chloride with sodium azide in the presence of TBAI was achieved in quantitative yields in DMF to afford the desired protected clickable glycoimidazole derivatives **11**. The protected monovalent controls **13** were then synthesized by treatment of glycoimidazoles **11** with ethynylcyclopropane in the presence of $CuSO_4.5H_2O$ and sodium ascorbate under microwave conditions (Scheme 2).



Scheme 2 Synthesis of monovalent models **13**.

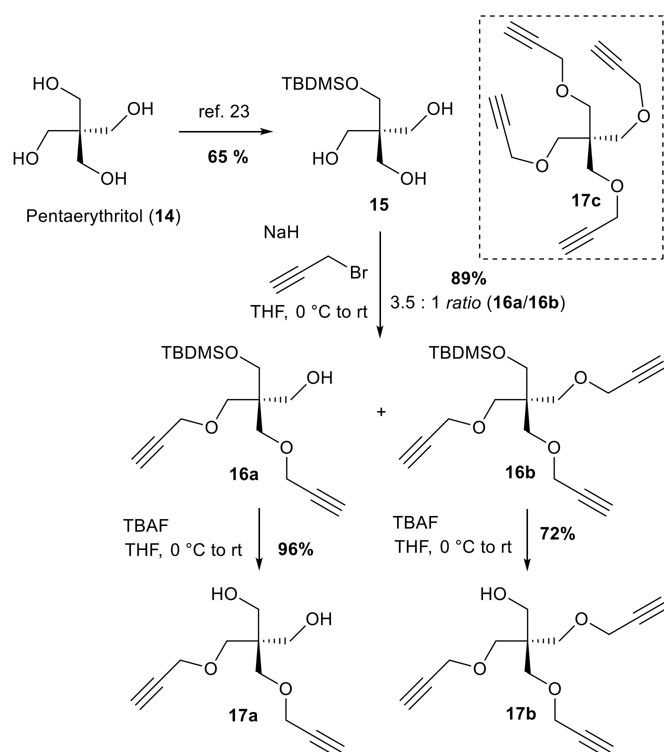
Please do not adjust margins

View Article Online
DOI: 10.1039/C9OB00826H

Published on 21 May 2019. Downloaded by Syddansk Universitetsbibliotek on 5/22/2019 8:58:13 AM.

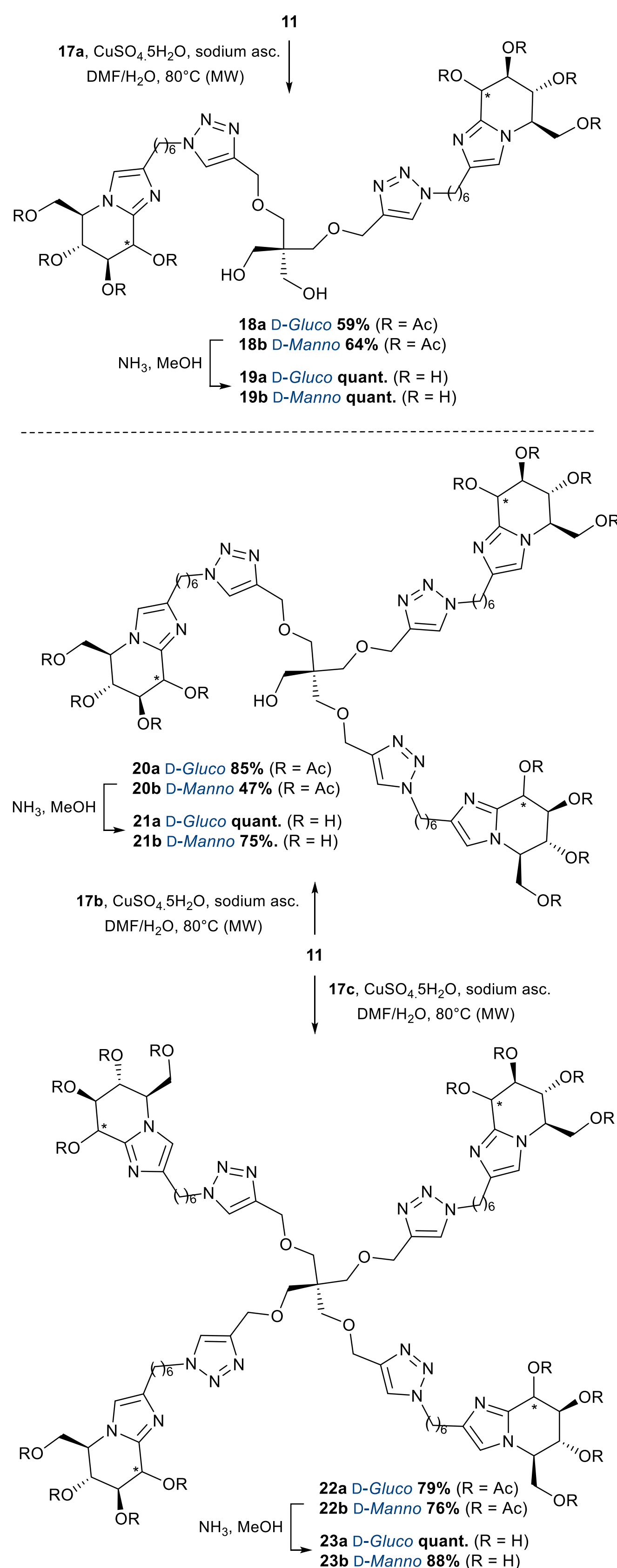
Organic & Biomolecular Chemistry Accepted Manuscript

The use of cyclopropylated alkyne in the CuAAC reaction avoided the formation of side-products arising from prior aerobic copper-catalyzed oxygenation at the propargylic methylene group in 1-pentyne.[14,22] The reaction proceeded in high yields for the conversion of glucoimidazole **11a**. However, in contrast to the previous steps of the synthesis, the CuAAC reaction showed a marked difference of reactivity between the D-*gluco* and the D-*manno* series, mannoimidazole **12b** being obtained in only 65% yield. The monovalent models **13** were then obtained by saponification of peracetates **12** according to a convenient method based on the use of anion exchange Amberlite IRA-400 (OH^-) resin.[15] Having in hands the clickable glycoimidazole derivatives **11**, we then focused our attention on the final steps of the neoglycoclusters synthesis. We first prepared the pentaerythritol-based scaffolds for the synthesis of di-, tri- and tetravalent glycoimidazole derivatives (Scheme 3).



Scheme 3 Synthesis of *O*-propargyl scaffolds **17**.

The first step in the synthesis of alkynes **17a,b** involved the monosylation of **14** to give **15.**[23] Complete propargylation of triol **15** proved difficult, dipropargyl ether **16a** being obtained as the predominant product with its tripropargyl ether analogue **16b**. Taking advantage of this incomplete reaction, the two desired pentaerythritol-based scaffolds **17a,b** could be readily synthesized by a divergent approach. After separation on silica gel, TBAF treatment of silyl ethers **16** provided the desired alcohols **17** in good to excellent yields. The tetrapropargyl ether **17c** was then prepared in one step from pentaerythritol (**14**) following Haag's protocol.[24] Microwave-assisted click conjugation of **11** with di-, tri- and tetrapropargyl ethers **17** provided the corresponding protected glycoimidazole clusters **18, 20** and **22** in 47 to 85% yields (Scheme 4).



Scheme 4 Synthesis of glycoimidazole clusters **19, 21** and **23**.

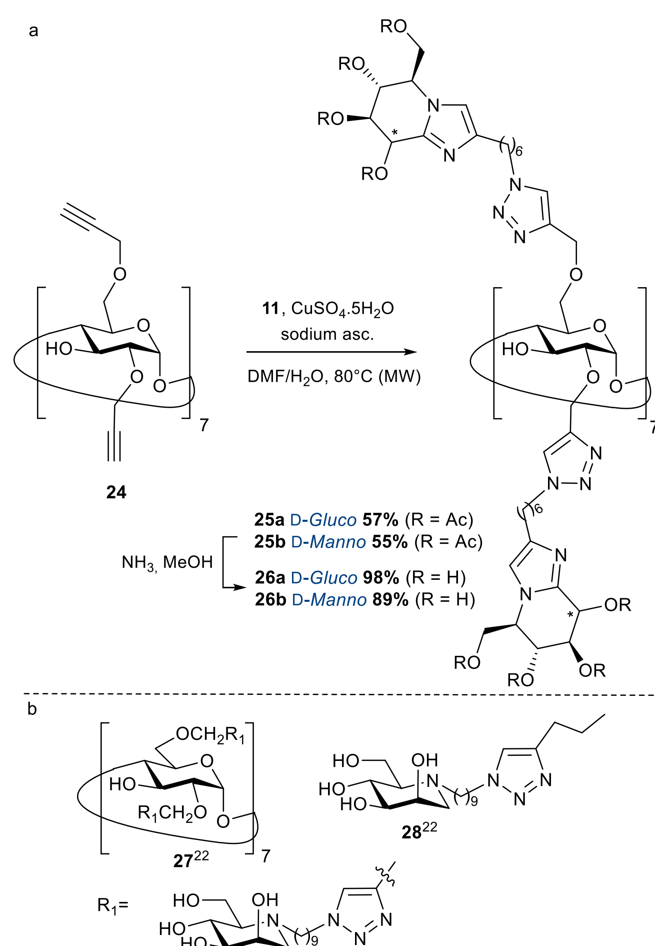
This journal is © The Royal Society of Chemistry 20xx

Please do not adjust margins

View Article Online
DOI: 10.1039/C9OB00826H

Published on 21 May 2019. Downloaded by Syddansk Universitetsbibliotek on 5/22/2019 8:58:13 AM.

Except in one case (clusters **20**), no marked difference of reactivity was observed between the azide-armed tetrahydroimidazopyridines in the D-*gluco*- and the D-*manno* series. Quite surprisingly, first attempts to perform *O*-deacetylation of glycoimidazole clusters **18, 20** and **22** using anion exchange Amberlite IRA-400 (OH^-) led to low yields of the desired deprotected clusters and to unidentified side-products. Alternatively, deacetylation of pentaerythritol-based clusters **18, 20** and **22** could be performed in good to high yields by treatment with ammonia in methanol at room temperature to provide the corresponding free-OH products **19, 21** and **23** (Scheme 4).[25] CuAAC was also performed with heptakis(2,6-di-*O*-propargyl) β-cyclodextrin (**24**),[26] leading to the desired tetradecavalent glycoimidazoles **26** after ammonia-mediated deprotection of the acetate groups in **25** (Scheme 5a).



Scheme 5 Synthesis of β-CD-based glycoimidazoles **26** and structure of the corresponding DMJ analogue **27** and its monovalent counterpart **28**.

Biological results. As indicated in the introduction, JBα-man has been widely used as a model probe to study multivalent glycosidase-inhibitor interactions.[1] The largest multivalent effects in glycosidase inhibition have been observed with this enzyme.[7] We first evaluated the inhibition potency of monovalent models **13** (Table 1). As expected, D-*manno*-configurated glycoimidazole **13b** has an inhibition constant (K_i) value in the hundreds of nM range. This is a gain of three orders of magnitude compared to the monovalent model **4** based on the prevalent DNJ inhitope. We were pleased to see that the D-*gluco*-configurated monovalent model **13a** displayed a K_i value in the low μM range, almost halfway between DNJ derivative **4** and mannoimidazole **13b**. Evaluation of di- to tetravalent tetrahydroimidazopyridines indicated clearly that the use of inhitopes displaying higher affinity than DNJ motifs did not lead to significant multivalent effects (Table 1). Moreover, the best rp/n values ranging from 2 to 3 were obtained for the clusters displaying the D-*gluco*-configurated inhitope. The use of the mannoimidazole inhitope had either no significant impact or even negative impact on the valency-corrected inhibition enhancements. Double-reciprocal Lineweaver-Burk plots revealed a competitive inhibition mode for the whole cluster series with the exception of tetravalent glucoimidazole **23a** which displayed a mixed-type inhibition mode of JBα-man. Interestingly, clusters **21b** and **23b** displaying three to four copies of the mannoimidazole-based inhitope were found to be competitive tight binding inhibitors of JBα-man. For those compounds, a similar trend was observed: Dixon curves showed a strong non linearity, and double-reciprocal Lineweaver-Burk plots were suggesting a pure non-competitive behaviour and the respective secondary curves (slope as a function of inhibitor concentration) were non-linear (see figures S92, S93, S97 and S98). Such a behaviour can be misleading and often hints a tight binding inhibition pattern.[27] In such cases, the classical steady-state kinetic model does not apply anymore. Enzyme (E) and inhibitor (I) concentrations are in the same range; inhibitor depletion due to the E.I complex formation cannot be neglected anymore and has therefore to be taken into account. Proper experiment design and data treatment for determination of accurate values of inhibition constants in the case of fast tight binding inhibition has been thoroughly discussed in the literature.[28] Inhibitor concentration range was chosen in order to have relevant data[28g,29] and the generic Morrison equation[28b] adapted by Cha[28d] for data analysis (eq. 1) was fitted to mean values of triplicate experiments in order to get the apparent inhibition constant K_i^{app} and then the real inhibition constant K_i from equation 2 in accordance to a competitive inhibitor type:

$$v_i = \frac{v_0\left(E-I-K_i^{app}+\sqrt{\left(E-I-K_i^{app}\right)^2+4EK_i^{app}}\right)}{2E} \quad \text{(eq. 1)}$$

$$K_i^{app} = K_i\left(1+\frac{S}{K_m}\right) \quad \text{(eq. 2)}$$

where v_i is the initial rate in presence of inhibitor and v_0 in the absence of inhibitor, E represents the total concentration of active sites, I the total inhibitor concentration, S the substrate concentration, K_i^{app} the apparent inhibition constant, K_i the real inhibition constant and K_m the Mickaelis-Menten constant determined independently. The initial fit using the Mickaelis Menten constant evaluated independently for each experiment and the calculated enzyme concentration as constants gave the K_i.

Organic & Biomolecular Chemistry Accepted Manuscript

Please do not adjust margins

Published on 21 May 2019. Downloaded by Syddansk Universitetsbibliotek on 5/22/2019 8:58:13 AM.

View Article Online
DOI: 10.1039/C9OB00896H

Table 1 JBα-man inhibitory activities (K_i, μM) for glycoimidazoles **13**, **19**, **21** and **23**.[a]

cluster	n	Inhitope	K_i	rp	rp/n	Inhibition mode
13a	1	*Gluco*-imidazole	2.23 ± 0.13	-	-	Competitive
19a	2	*Gluco*-imidazole	0.49 ± 0.64	4.5	2.3	Competitive
21a	3	*Gluco*-imidazole	0.25 ± 0.03	9	3	Competitive
23a	4	*Gluco*-imidazole	0.21 ± 0.05 1.00 ± 0.10	10.6	2.6	Mixed-type
13b	1	*Manno*-imidazole	0.11 ± 0.02	-	-	Competitive
19b	2	*Manno*-imidazole	0.037 ± 0.007	3	1.5	Competitive
21b	3	*Manno*-imidazole	0.037 ± 0.010	3	1	Competitive **Tight binding**
23b	4	*Manno*-imidazole	0.091 ± 0.017	1.2	0.3	Competitive **Tight binding**

[a] rp = K_i (monovalent reference)/K_i (glycocluster), n = number of inhitope units.

In our case, since K_i^{app} were higher than the enzyme concentration, the latter can not be evaluated from the first region of the curve but it has been demonstrated that errors on active enzyme concentration have no effect on the inhibition constant in such cases.[28g,f] We then turned our attention to glycoimidazole-cyclodextrin conjugates **26** (Table 2). The corresponding multivalent systems **3**[15] (Figure 1) and **27**[22] (scheme 5b) based on DNJ or 1-deoxymannojirimycin (DMJ) inhitopes have been included for comparative purposes. Both 14-valent glycoimidazole clusters **26a** and **26b** were found to be competitive, tight binding inhibitors of JBα-man with K_i values of 6 and 2 nM, respectively. The multivalent effects remained modest (rp/n of 26 and 4 respectively), although 14-valent glycoimidazole-based clusters **26** showed inhibition values similar to 36-valent iminosugar **1**[7] and a 120-valent cluster[30] which were both based on DNJ heads. Comparison of rp/n values of the 14-valent clusters **3**, **26** and **27** and the K_i values of the corresponding monovalent models revealed an interesting trend, showing that JBα-man-inhitope affinity and the strength of the inhibitory multivalent effect evolved in opposite direction. An increase of three orders of magnitude in the inhibition potency of the inhitopes led to a decrease of two orders of magnitude in the multivalent effect as judged by the rp/n values (Table 2, entries 1 and 4). A similar trend had been observed with sp^2-iminosugars 1-amino-5*N*,6*O*-oxomethylydenenojirimycin (1N-ONJ) and 1-amino-5*N*,6*O*-oxomethylydenemannojirimycin (1N-OMJ) in a different inhibitory scale. A shift in the monovalent inhibitory power from the hundreds of μM to the low μM led to a one-order magnitude reduction in rp/n values or to a total loss of the multivalent effect depending on the alkyl chain length.[13] In sharp contrast to our results, a study on the pentameric cholera toxin lectin showed that a strong multivalent effect (rp/n ~10000) was conserved by decreasing ligand IC_{50} values by 3 orders of magnitude.[32] An analysis of the inhibition modality in table 2 points out that tight binding inhibition is observed only for the glycoimidazole cluster series (entries 3 and 4). The development of tight binding inhibition appeared to be both dependent of JBα-man-inhitope affinity and cluster valency. With the exception of divalent derivative **19b**, tight binding inhibition is thus observed for all multimeric mannoimidazoles, i.e. clusters based on the inhitope displaying the highest affinity. When the inhibition potency of the inhitope is reduced by one order of magnitude as in the glucoimidazole cluster series, tight binding inhibition is observed only for the 14-valent cluster **26a**.

Conclusions

In conclusion, we have synthesized and evaluated as JBα-man inhibitors a series of multivalent glycoimidazole in the D-*gluco* and D-*manno* series. A special attention was paid to clusters based on strongly binding mannoimidazole inhitopes. Modest multivalent effects were observed for multimeric glycoimidazoles which were nevertheless found to be potent inhibitors of JBα-man with inhibition values up to 2 nM. A comparison study with iminosugar clusters based on DNJ or DMJ heads indicated that JBα-man-inhitope affinity and the strength of the inhibitory multivalent effect seemed to evolve in opposite direction. It seems from those results that the potential improvement of a strong inhibitor by multivalency is less important than for a loose inhibitor for JBα-man. Multivalency proved however to remain an interesting tool for modulation even in such cases. This trend still has to be confirmed on other systems. Interestingly, we found that mannoimidazole clusters (with the exception of **19b**) and 14-valent glucoimidazole cluster **26a** are tight binding inhibitors of JBα-man. To our knowledge, this is the first report of multimeric inhibitors that display tight binding inhibition of a carbohydrate-processing enzyme. These observations add new insights into the inhibitory multivalent effect. There is still space for improvements in finding appropriate arm length, geometry and rigidity while keeping those inhitopes with the help of molecular modelling thanks to the recently published JBα-man X-ray structure.[6]

Organic & Biomolecular Chemistry Accepted Manuscript

This journal is © The Royal Society of Chemistry 20xx

Please do not adjust margins

View Article Online
DOI: 10.1039/C9OB00826H

Organic & Biomolecular Chemistry

ARTICLE

Table 2. JBα-man inhibitory activities (Ki, µM) for 14-valent clusters **3**, **27** and **26** and their corresponding monovalent models[a]

	Monovalent derivatives				*14-valent derivatives*				
Entry	Monovalent models	Inhibition mode	Inhitope	K_i	rp/n	rp	K_i	Inhibition mode	14-valent clusters
1	**4**[15]	Competitive	DNJ	188[15]	610	8545	0.022[15]	Competitive	**3**[15]
2	**28**[22]	Competitive	DMJ	111[22]	115	1585	0.07[22]	Competitive	**27**[22]
3	**13a**	Competitive	Glucoimidazole	2.23	26	372	0.006 ± 0.001	Competitive Tight binding	**26a**
4	**13b**	Competitive	Mannoimidazole	0.11	4	55	0.0020 ± 0.0005	Competitive Tight binding	**26b**

[a] rp = K_i (monovalent reference)/K_i (glycocluster), n = number of inhitope units.

Published on 21 May 2019. Downloaded by Syddansk Universitetsbibliotek on 5/22/2019 8:58:13 AM.

Organic & Biomolecular Chemistry Accepted Manuscript

Experimental Section

Commercially available starting materials were purchased from commercial suppliers as Sigma-Aldrich Co., Merck Co., Alfa Aesar GmbH & Co., Acros Organics, Fluorochem, Carbosynth Limited or VWR and were used without further purification. When specified, anhydrous solvents were required. Tetrahydrofuran (THF) was distilled over sodium/benzophenone under argon. Dimethylformamide (DMF) was purchased anhydrous over molecular sieves. Toluene was distilled over CaH_2, pyridine and diisopropylamine were distilled over KOH and all of them were stored over KOH under argon. All reactions were performed in standard glassware or in vials adapted to a Biotage Initiator® microwave reactor. Reaction monitoring was achieved by Thin Layer Chromatography (TLC) on aluminum sheets coated with silica gel 60 F254 purchased from Merck KGaA. Crude mixtures were purified by flash column chromatography on silica gel 60 (230-400 mesh, 0.040-0.063 mm) purchased from Merck KGaA. Proton (^{1}H) and carbon (^{13}C) nuclear Magnetic Resonance (NMR) spectra were recorded at 298K on either Bruker Avance 300 MHz, Bruker Avance III HD 400 MHz with BBFO probe or Bruker 500 MHz Avance III HD with Prodigy BBO probe spectrometers. The chemical shifts are reported as δ values in parts per million (ppm) relative to residual solvent signals used as internal reference. Data are presented as followed: chemical shift, multiplicity (s = singlet, br s = broad singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublets of doublets, dt = doublet of triplets, t = triplet, td = triplet of doublets, q = quadruplet, qnt = quintuplet, m = multiplet), coupling constants (*J*) are expressed in Hz, integration value and assignment. The subscript Ar means aromatic. The superscript III means tertiary carbon and the superscript IV means quaternary carbon. Carbon multiplicities were assigned by Distortionless Enhancement by Polarization Transfer (DEPT) experiments. ^{1}H and ^{13}C signals were assigned by correlation spectroscopy (COSY), Heteronuclear Single Quantum Correlation (HSQC), Heteronuclear Multiple-Bond Correlation spectroscopy (HMBC) and Nuclear Overhauser Effect Spectroscopy (NOESY) when required. Optical rotations were measured at 589 nm (sodium lamp) and 20 °C on Anton Paar MCP 200 polarimeter with a path length of 1 dm. The concentration (c) is indicated in gram per deciliter. Infrared (IR) spectra were recorded neat or in solution on a Perkin-Elmer Spectrum Two FT-IR spectrometer. ESI-TOF high resolution mass spectra (HRMS) were carried out on a Bruker MicroTOF® mass spectrometer.

2,3,4,6-Tetra-*O*-benzyl-D-glucono-δ-thiolactam (5)

Lawesson's reagent (2.02 eq., 0.789 g, 1.89 mmol) was added to a mixture containing 2,3,4,6-Tetra-*O*-benzyl-D-glucono-δ-lactam (1 eq., 0.500 g, 0.93 mmol), pyridine (0.038 mL), freshly activated 4 Å molecular sieves and dry toluene (38.0 mL). The reaction mixture was stirred for 20 h. The mixture was stirred with MeOH (5 ml) for 2 h, filtered and the solvent were removed under reduced pressure. The residue was subjected to flash chromatography (1:6 EtOAc/petroleum ether) to afford product **5** (0.408 g, 0.737 mmol, 99%) as a pale orange solid. Analytical data were in accordance with those reported in literature.[31]

^{1}H NMR (400 MHz, $CDCl_3$) *δ* (ppm) 3.37 (dd, *J* = 9.8, 7.6 Hz, 1H), 3.56 (dd, *J* = 9.4, 4.6 Hz, 1H), 3.64 (dd, *J* = 9.8, 3.3 Hz, 1H), 3.85-3.93 (m, 2H), 4.35 (d, *J* = 11.5 Hz, 1H), 4.43-4.49 (m, 4H), 4.59 (d, *J*

Please do not adjust margins

= 11.5 Hz, 1H), 4.67 (d, J = 11.5 Hz, 1H), 4.74 (d, J = 11.6 Hz, 1H), 5.01 (d, J = 11.5 z, 1H), 7.11-7.44 (m, 20H), 8.05 (s, 1H).

Gluco- and manno-imidazole (6a and 6b)

Thiolactam **5** (1 eq., 517 mg, 0.935 mmol) was dissolved in $NH_2CH_2CH(OMe)_2$ (15 eq., 1.52 mL, 14 mmol) and stirred under argon atmosphere for 20h. At the beginning, the reaction was green-yellowish and turned progressively to orange and light brown solution. The reaction was diluted with Et_2O and water. The organic and aqueous phases were separated. Aqueous phase was extracted three times with Et_2O. Combined organic layers were washed with water and then with brine. The organic fraction was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude was used for the next step without purification.

TsOH. H_2O (1.87 eq., 333 mg, 0.312 mL, 1.75 mmol) was added to a solution of crude amidine (1 eq., 586 mg, 0.939 mmol) in dry toluene (13.4 mL). The solution was stirred at 60 °C for 63h under air. The cooler system was equipped with a drierite drying tube to avoid water in the medium. The solution was diluted with DCM and sat. $NaHCO_3$. Aqueous and organic phase were separated. Aqueous phase was extracted with DCM. Organic fractions were gathered and washed with water, brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (4:6 EtOAc/petroleum ether). Glucoimidazole **6a** (205 mg, 0.367 mmol, 39%) and mannoimidazole **6b** (291 mg, 0.52 mmol, 55%) were obtained as oils. Analytical data were in accordance with those reported in the literature.[18]

6a: $\boldsymbol{R_f}$ 0.35 (petroleum ether / EtOAc 1:1), **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm) 3.75 (dd, J = 10.4, 5.3 Hz, 1H, H6^a), 3.83-3.90 (m, 2H, H6^b, H3), 4.10 (dd, J = 7.6, 5.7 Hz, 1H, H4), 4.19 (ddd, J = 7.9, 5.7, 3.0 Hz, 1H, H5), 4.46 (d, J = 2.3 Hz, 2H, O$\underline{CH_2}$Ph), 4.51 (d, J = 11.2 Hz, 1H, O$\underline{CH_2}$Ph), 4.70 (d, J = 11.2 Hz, 1H, O$\underline{CH_2}$Ph), 4.75 (d, J = 5.7 Hz, 1H, H2), 4.83 (d, J = 10.4 Hz, 1H, O$\underline{CH_2}$Ph), 4.85 (d, J = 11.2 Hz, 1H, O$\underline{CH_2}$Ph), 4.88 (d, J = 11.7 Hz, 1H, O$\underline{CH_2}$Ph), 5.18(d, J = 11.7 Hz, 1H, O$\underline{CH_2}$Ph), 7.05 (d, J = 1.1 Hz, 1H, H7), 7.12 (d, J = 1.1 Hz, 1H, H8), 7.17-7.46 (m, 20H, H_{Ar}), **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm) 58.2, 68.5, 72.9, 73.4, 74.2, 74.4, 76.2, 82.3, 117.4, 127.7 (C7), 128.0, 128.02, 128.09, 128.1, 128.2, 128.3, 128.5, 128.51, 128.57, 128.60, 128.7 (C_{Ar}), 129.5 (C8), 137.4, 137.7, 138.0, 138.8 ($C^{IV}{}_{Ar}$), 144.1 (C1).

6b: $\boldsymbol{R_f}$ 0.21 (petroleum ether / EtOAc 1:1), **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm) 3.63 (dd, J = 9.9, 7.1 Hz, 1H, H6^a), 3.77 (dd, J = 10.1, 3.1 Hz, 1H, H6^b), 3.87 (dd, J = 9.4, 3.2 Hz, 1H, H3), 4.14 (td, J = 7.0, 2.9 Hz,1H, H5), 4.29 (dd, J = 9.6, 7.3 Hz, 1H, H4), 4.41-4.52 (m, 2H, O$\underline{CH_2}$Ph), 4.58 (d, J = 12.0 Hz, 1H, O$\underline{CH_2}$Ph), 4.63 (d, J = 11.2 Hz, 1H, O$\underline{CH_2}$Ph), 4.67 (d, J = 12.8 Hz, 2H, O$\underline{CH_2}$Ph), 4.74 (d, J = 12.2 Hz, 1H, O$\underline{CH_2}$Ph), 4.82 (d, J = 2.9 Hz, 1H, H2), 5.01 (d, J = 11.1 Hz, 1H, O$\underline{CH_2}$Ph), 7.07 (s ,1H, H7), 7.17 (s, 1H, H8), 7.23-7.43 (m, 20H, H^{Ar}), **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm) 56.0 (C5), 68.3 (C2), 70.6 (O$\underline{CH_2}$Ph), 71.2 (C6), 71.8, 73.3 (O$\underline{CH_2}$Ph), 74.3 (C4), 75.0 (O$\underline{CH_2}$Ph), 80.3 (C3), 119.5 (C7), 127.6, 127.8, 127.9, 127.9, 128.0, 128.3, 128.34, 128.4, 128.53, 128.54, 128.56, 128.6 ($C^{III}{}_{Ar}$), 129.3 (C8), 137.7, 138.0, 138.2, 138.3 ($C^{IV}{}_{Ar}$), 143.0 (C1).

View Article Online
DOI: 10.1039/C9OB00826H

Compound 7a

A solution of **6a** (1 eq., 0.48 g, 0.855 mmol) in DMF (9.3 mL) was treated with NIS (10 eq., 2.02 g, 8.55 mmol) and stirred under argon atmosphere at 80°C for 14 h. The brown mixture was cooled, diluted with Et_2O, and washed with a 10% $Na_2S_2O_3$ solution (120mL). The combined H_2O layers were extracted with Et_2O. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered and concentrated. Flash chromatography on silica gel (petroleum ether/EtOAc 7:1) gave product **7a** (515 mg, 0.634 mmol, 75%) as an oil. Analytical data were in accordance with those reported in literature.[20]

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 3.64 (dd, J = 9.5, 4.4 Hz, 1H, H6^a), 3.72 (t, J = 9.2 Hz, 1H, H6^b), 4.08 (t, J = 4.0 Hz, 1H, H3), 4.34 (dd, J = 4.3, 2.1 Hz, 1H, H4), 4.42 (s, 2H, O$\underline{CH_2}$Ph), 4.46 (m, 1H, H5), 4.48 (d, J = 11.7 Hz, 1H, O$\underline{CH_2}$Ph), 4.57 (dd, J = 11.9, 4.8 Hz, 2H, O$\underline{CH_2}$Ph), 4.65-4.71 (m, 2H, O$\underline{CH_2}$Ph and H2), 4.82 (d, J = 11.9 Hz, 1H, O$\underline{CH_2}$Ph), 5.1 (d, J = 12.0 Hz, 1H, O$\underline{CH_2}$Ph), 7.16-7.45 (m, 20H, H_{Ar}).

Compound 7b

A solution of **6b** (1 eq., 1.33 g, 2.37 mmol) in DMF (20 mL) was treated with NIS (10 eq., 5.62 g, 23.7 mmol) and stirred under argon atmosphere at 80°C for 20h. The brown mixture was cooled, diluted with Et_2O, and washed with sat. $Na_2S_2O_3$ (x3). The combined H_2O layers were extracted with Et_2O (x3). The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash column chromatography on silica gel (7:1 petroleum ether/EtOAc) and product **7b** (1.43 g, 1.76 mmol, 74%) was obtained as an orange oil. Analytical data were in accordance with those reported in literature.[17]

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 3.61 (dd, J = 9.4, 4.9 Hz, 1H), 3.71 (dd, J = 9.3, 8.7 Hz, 1H), 3.74 (dd, J = 7.6, 3.1 Hz, 1H), 3.76 (m, 2H), 4.35 (ddd, J = 8.7, 4.8, 2.4 Hz, 1H), 4.43 (d, J = 7.6 Hz, 1H), 4.45 (dd, J = 7.7, 2.4 Hz, 1H), 4.47-4.52 (m, 2H), 4.54 (d, J = 11.6 Hz, 1H), 4.57 (d, J = 11.6 Hz, 1H), 4.60 (d, J = 11.2 Hz, 1H), 4.73-4.78 (m, 3H), 7.20-7.39 (m, 20H).

Compound 8a

Isopropyl magnesium chloride (1.11 eq., 1.99 M, 0.82 mL, 1.63 mmol) in solution in THF was added dropwise for 15 min to a stirred solution of **7a** in freshly distilled THF (14 mL) at -15°C. Reaction was stopped 2h after the beginning of the addition. The mixture was treated with sat. NH_4Cl and diluted with Et_2O. The layers were separated, and the organic layer was washed with sat. NH_4Cl twice. The combined H_2O layers were extracted with Et_2O (x3). The combined organic layers were washed with H_2O twice and brine, dried over Na_2SO_4, filtered, and evaporated. The crude was purified by flash column chromatography on silica gel (Pentane/ EtOAc 9:1 to 8:2). Product **8a** (843 mg, 1.23 mmol, 84%) was isolated as an oil. Analytical data were in accordance with those reported in literature.[20]

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 3.69 (dd, J = 10.5, 5.6 Hz, 1H, H6a), 3.78 (dd, J = 10.3, 3.0 Hz, 1H, H6b), 3.80 (t, J = 7.5 Hz, 1H, H4),

Organic & Biomolecular Chemistry Accepted Manuscript

Published on 21 May 2019. Downloaded by Syddansk Universitetsbibliotek on 5/22/2019 8:58:13 AM.

This journal is © The Royal Society of Chemistry 20xx

View Article Online
DOI: 10.1039/C9OB00826H

4.07 (dd, J = 7.1, 5.1 Hz, 1H, H3), 4.17 (ddd, J = 8.1, 5.1, 2.9 Hz, 1H, H5), 4.40-4.51 (m, 3H, O<u>CH$_2$</u>Ph), 4.63 (d, J = 11.4 Hz, O<u>CH$_2$</u>Ph), 4.70 (d, J = 5.1 Hz, 1H, H2), 4.74-4.82 (m, 3H, O<u>CH$_2$</u>Ph), 5.11 (d, J = 11.6 Hz, O<u>CH$_2$</u>Ph), 7.09 (s, 1H, H7), 7.13-7.43 (m, 20 H, H_{Ar}).

Compound 8b

Isopropyl magnesium chloride (1.11 eq., 1.99 M, 0.065 mL, 0.123 mmol) in solution in THF was added dropwise for 15 min to a stirred solution of **7b** in freshly distilled THF (0.9 mL) at -15°C. Reaction was stopped 30 min after the beginning of the addition. The mixture was treated with sat. NH_4Cl and diluted with Et_2O. The layers were separated, and the organic layer was washed with sat. NH_4Cl twice. The combined H_2O layers were extracted with Et_2O (x3). The combined organic layers were washed with H_2O twice and brine, dried over Na_2SO_4, filtered, and evaporated. The crude was purified by flash column chromatography on silica gel (Pentane/ EtOAc 9:1). Product **8b** (61.2 mg, 0.089 mmol, 80%) was obtained as an oil. Analytical data were in accordance with those reported in literature.[17]

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 3.57 (dd, J = 10.0, 7.2 Hz, 1H, H6a), 3.71 (dd, J = 10.1, 2.7 Hz, 1H, H6b), 3.84 (dd, J = 9.3, 2.8 Hz, 1H, H3), 4.11 (td, J = 7.1, 2.9 Hz, 1H, H5), 4.26 (dd, J = 8.9, 7.4 Hz, 1H, H4), 4.46 (s, 2H, O<u>CH$_2$</u>Ph), 4.57-4.70 (m, 4H, O<u>CH$_2$</u>Ph), 4.76 (d, J = 13.2 Hz, 1H, O<u>CH$_2$</u>Ph), 4.78 (d, J = 2.8 Hz, 1H, H2), 4.99 (d, J = 11.2 Hz, 1H, O<u>CH$_2$</u>Ph), 7.21-7.45 (m, 20H, H_{Ar}).

Compound 9a

A degassed suspension of CuI (0.1 eq., 23.3 mg, 0.122 mmol) in DMF (20.4 mL) was added to **8a** (1 eq., 839 mg, 1.22 mmol) under argon. 6-chloro-1-hexyne (4 eq., 0.61 mL, 4.93 mmol) and $Pd(PPh_3)_2Cl_2$ (5.02%, 43.5 mg, 0.0614 mmol) were then added under argon. DIPA (5 eq., 0.87 mL, 6.16 mmol) was finally added and the reaction mixture was stirred at 40°C for 17 h. The solution was cooled to room temperature, diluted with Et_2O and washed with NH_4Cl (x3). The combined organic layers were extracted with Et_2O (x3). The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered and evaporated. The crude was purified by flash column chromatography on silica gel (15:85 EtOAc/pentane to 2:8 EtOAc/pentane) affording **9a** (0.65 g, 0.97 mmol, 79%) as an oil.

R$_f$ 0.2 (EtOAc/petroleum ether 2:8), $[\alpha]_D^{20}$= + 29 (c 1.0, $CHCl_3$), **IR** (neat) 3031, 2923, 2867, 1497, 1454, 1362, 1338, 1095, 1067, 1028, 737, 698 cm^{-1}, **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm) 1.76 (qnt, J = 7.4 Hz, 2H, H12), 1.97 (qnt, J = 7.1 Hz, H13), 2.47 (t, J = 6,9 Hz, 2H, H11), 3.60 (t, J = 6,5 Hz, 2H, H14), 3.71 (dd, J = 10.3, 5.6 Hz, 1H, H6^a), 3.79-3.84 (m, 2H, H4 and H6^b), 4.08 (dd, J = 7.3, 5.3 Hz, 1H, H3), 4.17 (m, 1H, H5), 4.42-4.51 (m, 3H, O<u>CH$_2$</u>Ph), 4.64 (d, J = 11.3 Hz, 1H, O<u>CH$_2$</u>Ph), 4.69 (d, J = 5.2 Hz, 1H, H2), 4.75-4.84 (m, 3H, O<u>CH$_2$</u>Ph), 5.15 (d, J = 11.7 Hz, 1H, O<u>CH$_2$</u>Ph), 7.15 (s, 1H, H7), 7.23-7.44 (m, 20H, H_{Ar}), **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm) 19.0 (C11), 25.9 (C12), 31.8 (C13), 44.8 (C14), 58.3 (C5), 68.5 (C6), 72.8, 73.4 (2x O<u>CH$_2$</u>Ph), 73.9 (C2), 74.0, 74.2 (O<u>CH$_2$</u>Ph), 76.1 (C4), 81.8 (C3), 121.0 (C10), 127.7 (C9), 128.0, 128.08, 128.09, 128.2, 128.3, 128.4, 128.57, 128.59 (C^{III}_{Ar}), 137.4, 137.6, 137.9, 138.3 (4xC^{IV}_{Ar}), 143.8 (C1), **HRMS** (ESI) m/z 675.2971 ($[M + H]^+$ calcd. For $C_{42}H_{44}ClN_2O_4$: 678.2984).

Compound 9b

A degassed suspension of DMF (4.3 mL) and CuI (0.1 eq., 4.8 mg, 0.0252 mmol) was added to **8b** (1 eq., 175 mg, 0.255 mmol) under argon. Then 6-chloro-1-hexyne (4.12 eq., 0.13 mL, 1.05 mmol) and $Pd(PPh_3)_2Cl_2$ (4.98%, 9 mg, 0.0127 mmol) were added under argon. DIPA (5 eq., 0.18 mL, 1.27 mmol) was finally added and the reaction mixture was stirred at 40°C for 16 h. The solution was cooled to room temperature, diluted with Et_2O and washed with NH_4Cl (x3). The combined organic layers were extracted with Et_2O (x3). The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered and evaporated. The crude was purified by flash column chromatography on silica gel (2:8 EtOAc/pentane). Product **9b** (131 mg, 0.194 mmol, 76%) was obtained as an oil.

R$_f$ 0.3 (EtOAc/petroleum ether 2:8), $[\alpha]_D^{20}$= -31 (c 1,0, $CHCl_3$), **IR** (neat) 3063, 3030, 2925, 2865, 1497, 1454, 1365, 1028, 1114, 1027, 913, 737, 698 cm^{-1}, **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm) 1.74 (qnt, J = 7.4 Hz, 2H, H12), 1.95 (qnt, J = 7.2 Hz, 2H, H13), 2.45 (t, J = 7.0 Hz, 2H, H11), 3.54-3.61 (m, 3H, H14 and H6a), 3.71 (dd, J = 10.0, 2.9 Hz, 1H, H6b), 3.83 (dd, J = 9.3, 3.0 Hz, 1H, H3), 4.08 (td, J = 7.1, 2.7 Hz, 1H, H5), 4.25 (dd, J = 9.3, 7.4 Hz, 1H, H4), 4.44 (s, 2H, O<u>CH$_2$</u>Ph), 4.54-4.68 (m, 4H, O<u>CH$_2$</u>Ph), 4.71-4.76 (m, 2H, O<u>CH$_2$</u>Ph and H2), 4.98 (d, J = 11.2 Hz, 1H, O<u>CH$_2$</u>Ph), 7.19-7.43 (m, 20H, H_{Ar}), **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm) 18.8 (C11), 25.8 (C12), 31.6 (C13), 44.7 (C14), 60.1 (C5), 68.4 (C2), 70.7 (O<u>CH$_2$</u>Ph), 70.9 (C6), 71.8, 73.2 (2 x O<u>CH$_2$</u>Ph), 73.9 (C4), 74.6 (C9), 75.0 (O<u>CH$_2$</u>Ph), 80.0 (C3), 89.4 (C10), 122.6 (C8), 127.6-128.5 (C^{III}_{Ar}), 137.4, 137.8, 138.0, 138.1, (4 x C^{IV}_{Ar}), 142.8 (C1), **HRMS** (ESI) m/z 675.2988 ($[M + H]^+$ calcd. For $C_{42}H_{44}ClN_2O_4$: 675.2984).

Compound 10a

$Pd(OH)_2/C$ (4,55 eq., 620 mg, 4.42 mmol) was placed in a round bottom flask. Three cycles vacuum/argon were done, followed by a vacuum/H_2 cycle. A solution of **9a** (1 eq., 653 mg, 0.968 mmol) in a 3:1:1 EtOAc/MeOH/H_2O mixture (29 mL) and AcOH (17.6 mL) were added on the activated catalyst, then degassed with H_2 and the reaction mixture was stirred under H_2 for 3 days. The solution was filtered over a pad of Silica with 85:15 DCM/MeOH (300 mL). The crude of was used without further purification for the next step.

Ac_2O (12.8 mL) and dry pyridine (12.5 mL) were added to the crude and the solution was stirred for 20 h under argon atmosphere. Water was slowly added at 0 °C. The aqueous phase was extracted with CH_2Cl_2 (x3). The combined organic layers were then washed with an aqueous solution of 2M HCl (x3), sat. $NaHCO_3$ solution, water (x3) and dried over Na_2SO_4, filtered and concentrated. Crude residue was purified by flash column chromatography on silica gel (6:4 EtOAc/pentane). Product **10a** (419 mg, 0.862 mmol, 89%) was obtained as an oil.

R$_f$ 0.4 (EtOAc/petroleum ether 6:4), $[\alpha]_D^{20}$= +25 (c 1,1, $CHCl_3$), **IR** (neat) 2934, 2858, 1749, 1455, 1370, 1219, 1035, 644, 602 cm^{-1}, **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm) 1.35-1.42 (m, 2H, H11), 1.42-1.51

Published on 21 May 2019. Downloaded by Syddansk Universitetsbibliotek on 5/22/2019 8:58:13 AM.

Organic & Biomolecular Chemistry Accepted Manuscript

Please do not adjust margins

Please do not adjust margins

(m, 2H, H12), 1.64 (m, 2H, H10), 1.77 (qnt, *J* = 7.0 Hz, 2H, H13), 2.08 (s, 3H, $C(O)CH_3$), 2.09 (s, 3H, $C(O)CH_3$), 2.10 (s, 3H, $C(O)CH_3$), 2.11 (s, 3H, $C(O)CH_3$), 2.57 (t, *J* = 7.8 Hz, 2H, H9), 3.52 (t, *J* = 6.7 Hz, 2H, H14), 4.30-4.46 (m, 3H, H6, H5), 5.39 (dd, *J* = 7.1, 5.3 Hz, 1H, H4), 5.50 (dd, *J* = 7.2, 5.3 Hz, 1H, H3), 6.0 (d, *J* = 5.2 Hz, 1H, H2), 6.72 (s, 1H, H7), **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm) 20.7, 20.8, 20.8, 21.1 (4 C(O)<u>CH$_3$</u>), 26.8 (C12), 28.5 (C9), 28.7 (C11), 29.2 (C10), 32.6 (C13), 45.2 (C14), 56.3 (C5), 62.2 (C6), 66.3 (C2), 66.5 (C4), 70.3 (C3), 113.5 (C7), 139.2 (C1), 144.9 (C8), 169.2, 169.5, 169.8, 170.4 (4 <u>C</u>(O)CH$_3$), **HRMS** (ESI) *m/z* 487.1838 ($[M + H]^+$ calcd. For $C_{22}H_{32}ClN_2O_8$: 487.1842).

Compound 10b

$Pd(OH)_2/C$ (454%, 667 mg, 4.75 mmol) was placed in a round bottom flask. Three cycles vacuum/argon were done, followed by a vacuum/H_2 cycle. A solution of **9b** (1 eq., 706 mg, 1.05 mmol) in a 3:1:1 EtOAc/MeOH/H_2O mixture (31.3 mL) and AcOH (19 mL) were added on the activated catalyst, then degassed with H_2 and the reaction mixture was stirred under H_2 for 3 days. The reaction was filtered over a pad of silica gel with 84:16 DCM/MeOH (250mL). The filtrate was evaporated to dryness and the crude was used for the next step without purification.

Ac_2O (13.8 mL) and dry pyridine (13.5 mL) were added to the crude. And the solution was stirred for 22 h under argon atmosphere. Water was slowly added at 0 °C. The aqueous phase was extracted with DCM (x3). The combined organic layers were then washed with 2M HCl (2 x 4 mL), sat. $NaHCO_3$ solution, water and dried over Na_2SO_4, filtered and concentrated. Crude residue was purified by flash column chromatography on silica gel (6:4 EtOAc/pentane). Product **10b** (421 mg, 0.865 mmol, 83%) was obtained as an oil.

R_f 0.3 (EtOAc/petroleum ether 6:4), $[\alpha]_D^{20}$= -39 (*c* 0.7, $CHCl_3$), **IR** (neat) 2932, 2857, 1749, 1455, 1370, 1221, 1054, 950 cm^{-1}, **^{1}H NMR** (500 MHz, $CDCl_3$) δ (ppm) 1.36 (qnt, *J* = 7.4 Hz, 2H, H11), 1.45 (qnt, *J* = 7.4 Hz, 2H, H12), 1.59-1.66 (m, 2H, H10), 1.76 (qnt, *J* = 7.1 Hz, 2H, H13), 2.03 (s, 3H, C(O)<u>CH$_3$</u>), 2.10 (s, 3H, C(O)<u>CH$_3$</u>), 2.11 (s, 6H, C(O)<u>CH$_3$</u>), 2.55 (td, *J* = 7.8, 2.4 Hz, 2H, H9), 3.51 (t, *J* = 6.7 Hz, 2H, H14), 4.24-4.33 (m, 2H, H5 and H6a), 4.53 (dd, *J* = 11.7, 4.0 Hz, 1H, H6b), 5.41 (dd, *J* = 8.9, 3.8 Hz, 1H, H3), 5.63 (dd, *J* = 8.9, 6.1 Hz, 1H, H4), 6.33 (d, *J* = 3.8 Hz, 1H, H2), 6.76 (s, 1H, H7), **^{13}C NMR** (125 MHz, $CDCl_3$) δ (ppm) 20.7, 20.78, 20.81, 20.9 (4 x C(O)<u>CH$_3$</u>), 26.7 (C12), 28.4 (C9), 28.7 (C11), 29.2 (C10), 32.6 (C13), 45.2 (C14), 57.3 (C5), 63.4 (C6), 63.6 (C2), 65.8 (C4), 68.9 (C3), 114.4 (C7), 139.1 (C1), 144.9 (C8), 169.6, 169.66, 169.67, 170.3 (4 x <u>C</u>(O)CH$_3$), **HRMS** (ESI) *m/z* 487.1845 ($[M + H]^+$ calcd. For $C_{22}H_{32}ClN_2O_8$: 487.1842).

Compound 11a

Substrate **10a** (1 eq., 276 mg, 0.567 mmol) was dissolved in DMF (3.2 mL). TBAI (0.201 eq., 42.1 mg, 0.114 mmol) and NaN_3 (6.08 eq., 224 mg, 3.45 mmol) were added. Reaction mixture was heated at 60°C for 20 h. The mixture was diluted with EtOAc and water. The aqueous phase was extracted with EtOAc (x3). Gathered organic phases were washed with water (x5) and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Compound **11a** (283 mg, 0.575 mmol, 100%) was obtained as a pure product without purification.

View Article Online
DOI: 10.1039/C9OB00826H

$[\alpha]_D^{20}$= +23 (*c* 1,1, $CHCl_3$), **IR** (neat) 2934, 2858, 1749, 1455, 1370, 1219, 1035, 644, 602 cm^{-1}, **^{1}H NMR** (500 MHz, $CDCl_3$) δ (ppm) 1.34-1.41 (m, 4H, H11 and H12), 1.54-1.65 (m, 4H, H10 and H13), 2.06 (s, 3H, C(O)<u>CH$_3$</u>), 2.07 (s, 3H, C(O)<u>CH$_3$</u>), 2.08 (s, 3H, C(O)<u>CH$_3$</u>), 2.09 (s, 3H, C(O)<u>CH$_3$</u>), 2.55 (t, *J* = 7.8 Hz, 2H, H9), 3.23 (t, *J* = 6.9 Hz, 2H, H14), 4.31 (dd, *J* = 10.9, 4.3 Hz, 1H, H6[a]), 4.35-4.44 (m, 2H, H5 and H6[b]), 5.38 (dd, *J* = 7.2, 5.4 Hz, 1H, H4), 5.48 (dd, *J* = 7.2, 5.4 Hz, 1H, H3), 5.98 (d, *J* = 5.2 Hz, 1H, H2), 6.71 (s, 1H, H7), **^{13}C NMR** (125 MHz, $CDCl_3$) δ (ppm) 20.70, 20.75, 20.8, 21.0 (4x C(O)<u>CH$_3$</u>), 26.6 (C12), 28.5 (C9), 28.8 (C13), 29.0 (C10), 29.2 (C11), 51.5 (C14), 56.3 (C5), 62.2 (C6), 66.2 (C2), 66.4 (C4), 70.3 (C3), 113.7 (C7), 139.2 (C1), 144.9 (C8), 169.2, 169.4, 169.8, 170.4 (4x <u>C</u>(O)CH$_3$), **HRMS** (ESI) *m/z* 494.2242 ($[M + H]^+$ calcd. For $C_{22}H_{32}N_5O_8$: 494.2245).

Compound 11b

Substrate **10b** (1 eq., 421 mg, 0.865 mmol) was dissolved in DMF (5 mL), TBAI (0.198 eq., 63.4 mg, 0.172 mmol) and NaN_3 (5.88 eq., 334 mg, 5.09 mmol) were added. Reaction mixture was heated at 60°C for 18h. Reaction was diluted with EtOAc and water. The aqueous phase was extracted with EtOAc (x3). The gathered organic phase was washed with water (x5) and then brine. Organic phase was then dried over Na_2SO_4, filtered and evaporated under reduced pressure. Compound **11b** (427 mg, 0.865 mmol, 100%) was obtained as a pure product without purification.

$[\alpha]_D^{20}$= -41 (*c* 1.1, $CHCl_3$), **IR** (neat) 2935, 2859, 2097, 1749, 1456, 1370, 1221, 1054, 950 cm^{-1}, **^{1}H NMR** (500 MHz, $CDCl_3$) δ (ppm) 1.33-1.43 (m, 4H, H11 and H12), 1.56-1.67 (m, 4H, H10 and H13), 2.04 (s, 3H, C(O)<u>CH$_3$</u>), 2.107 (s, 3H, C(O)<u>CH$_3$</u>), 2.114 (s, 3H, C(O)<u>CH$_3$</u>), 2.116 (s, 3H, C(O)<u>CH$_3$</u>), 2.56 (td, *J* = 7.8, 2.6 Hz, 2H, H9), 3.25 (t, *J* = 6.9 Hz, 2H, H14), 4.25-4.34 (m, 2H, H5 and H6[a]), 4.54 (dd, *J* = 11.8, 4.1 Hz, 1H, H6[b]), 5.42 (dd, *J* = 8.9, 3.7 Hz, 1H, H3), 5.64 (dd, *J* = 8.9, 6.1 Hz, 1H, H4), 6.34 (d, *J* = 3.7 Hz, 1H, H2), 6.76 (s, 1H, H7), **^{13}C NMR** (125 MHz, $CDCl_3$) δ (ppm) 20.7, 20.81, 20.85, 21.0 (4x C(O)<u>CH$_3$</u>), 26.6 (C12), 28.5 (C9), 28.8 (C13), 29.0 (C10), 29.2 (C11), 51.5 (C14), 57.3 (C5), 63.4 (C6), 63.6 (C2), 65.8 (C4), 69.0 (C3), 114.4 (C7), 139.2 (C1), 145.0 (C8), 169.6, 169.7, 170.3 (4x <u>C</u>(O)CH$_3$), **HRMS** (ESI) *m/z* 494.2250 ($[M + H]^+$ calcd. For $C_{22}H_{32}N_5O_8$: 494.2245).

Peracetylated monovalent 12a

To a solution of substrate **11a** (1 eq., 91.4 mg, 0.185 mmol) and cyclopropylacetylene (5 eq., 0.081 mL, 0.926 mmol) in DMF (4.92 mL) was added a solution of $CuSO_4.5H_2O$ (6.6 mg, 0.0264 mmol) and sodium ascorbate (10.4 mg, 0.0525 mmol) in H_2O (1.3 mL). The resulting solution was heated under microwave irradiation conditions at 80 °C for 30 min. Water was added, and the aqueous phase was extracted with EtOAc (3x). The organic phases were combined, washed with water (3x) and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Traces of copper salts were removed by filtration through a short pad of silica gel eluting with $CH_3CN/H_2O/NH_4OH$ (10:1:1). Crude residue was purified by flash column chromatography on silica gel (100 EtOAc). Product **12a** (88.4 mg, 0.158 mmol, 85%) was obtained as an oil.

Published on 21 May 2019. Downloaded by Syddansk Universitetsbibliotek on 5/22/2019 8:58:13 AM.

Organic & Biomolecular Chemistry Accepted Manuscript

 This journal is © The Royal Society of Chemistry 20xx

Please do not adjust margins

View Article Online
DOI: 10.1039/C9OB00826H

Published on 21 May 2019. Downloaded by Syddansk Universitetsbibliotek on 5/22/2019 8:58:13 AM.

RR_f 0.3 (DCM/MeOH 97:3), $[\alpha]_D^{20}$= +19 (*c* 0.9, $CHCl_3$), **IR** (neat) 2931, 2857, 1749, 1370, 1219, 1040 cm^{-1}, **^{1}H NMR** (500 MHz, $CDCl_3$) δ (ppm) 0.86 (dd, *J* =11.5, 4.5 Hz, 2H, H18), 0.95-1.00 (m, 2H, H18), 1.35-1.47 (m, 4H, H11 and H12), 1.61-1.69 (m, 2H, H10), 1.91 (qnt, *J* = 7.3 Hz, 2H, H13), 1.98 (m, 1H, H17), 2.12 (s, 3H, C(O)$\underline{CH_3}$), 2.131 (s, 3H, C(O)$\underline{CH_3}$), 2.135 (s, 3H, C(O)$\underline{CH_3}$), 2.15 (s, 3H, C(O)$\underline{CH_3}$), 2.59 (t, *J* = 7.9 Hz, 2H, H9), 4.31 (t, *J* = 7.2 Hz, 2H, H14), 4.38 (dd, *J* = 11.3, 4.3 Hz, 1H H6[a]), 4.43-4.51 (m, 2H, H5 and H6[b]), 5.44 (dd, *J* = 7.2, 5.4 Hz, 1H, H4), 5.54 (dd, *J* = 7.3, 5.3 Hz, 1H, H3), 6.04 (d, *J* = 5.2 Hz, 1H, H2), 6.78 (s, 1H, H7), 7.34 (s, 1H, H15), **^{13}C NMR** (125 MHz, $CDCl_3$) δ (ppm) 6.7 (C17), 7.7 (C18 and C18'), 20.6, 20.67, 20.73, 21.0 (4x C(O)$\underline{CH_3}$), 26.3 (C12), 28.3 (C9), 28.7 (C11), 29.0 (C10), 30.2 (C13), 50.1 (C14), 56.3 (C5), 62.1 (C6), 66.1 (C2), 66.3 (C4), 70.2 (C3), 113.8 (C7), 119.5 (C15), 139.1(C1), 144.5 (C8), 150.2 (C16), 169.1, 169.3, 169.7, 170.3 (4x $\underline{C}$(O)CH_3), **HRMS** (ESI) *m/z* 560.2720 ([M + H]$^+$ calcd. For $C_{27}H_{38}N_5O_8$: 560.2715).

Peracetylated monovalent 12b

To a solution of substrate **11b** (1 eq., 40.7 mg, 0.0825 mmol) and cyclopropylacetylene (5 eq., 0.036 mL, 0.412 mmol) in DMF (2.2 mL) was added a solution of $CuSO_4.5H_2O$ (4 mg, 0.016 mmol) and sodium ascorbate (6.5 mg, 0.0328 mmol) in H_2O (0.6 mL). The resulting solution was heated under microwave irradiation conditions at 80 °C for 30 min. Water was added, and the aqueous phase was extracted with EtOAc (3x). The organic phases were combined, washed with water (3x) and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Traces of copper salts were removed by filtration through a short pad of silica gel eluting with $CH_3CN/H_2O/NH_4OH$ (10:1:1). Crude residue was purified by flash column chromatography on silica gel (97:3 DCM/MeOH to 9:1). Product **12b** (30 mg, 0.054 mmol, 65%) was obtained as a beige oil.

R_f 0.3 (DCM/MeOH 97:3), $[\alpha]_D^{20}$= -34 (*c* 0.7, $CHCl_3$), **IR** (neat) 2934, 2857, 1749, 1460, 1433, 1370, 1221, 1052, 949 cm^{-1}, **^{1}H NMR** (500 MHz, $CDCl_3$) δ (ppm) 0.78-0.83 (m, 2H, H18), 0.89-0.95 (m, 2H, H18), 1.29-1.41 (m, 4H, H11 and H12), 1.6 (qnt, *J* = 7.2 Hz, 2H, H10), 1.86 (qnt, *J* = 7.1 Hz, 2H, H13), 1.90-1.95 (m, 1H, H17), 2.03 (s, 3H, C(O)$\underline{CH_3}$), 2.10 (s, 3H, C(O)$\underline{CH_3}$), 2.11 (s, 6H, C(O)$\underline{CH_3}$), 2.53 (td, *J* = 7.7, 3.4 Hz, 2H, H9), 4.22-4.33 (m, 4H, H14, H5, H6[a]), 4.54 (dd, *J* = 11.4, 3.6 Hz, 1H, H6[b]), 5.41 (dd, *J* = 9.0, 3.6 Hz, 1H, H3), 5.63 (dd, *J* = 9.0, 6.0 Hz, 1H, H4), 6.33 (d, *J* = 3.7 Hz, 1H, H2), 6.75 (s, 1H, H7), 7.19 (s, 1H, H15), **^{13}C NMR** (125 MHz, $CDCl_3$) δ (ppm) 6.8 (C17), 7.8 (C18, C18'), 20.7, 20.81, 20.82, 21.0 (4x C(O)$\underline{CH_3}$), 26.4 (C11 ou C12), 28.4 (C9), 28.8 (C11 ou C12), 29.1 (C10), 30.3 (C13), 50.2 (C14), 57.3 (C5), 63.4 (C6), 63.6 (C2), 65.8 (C4), 69.0 (C3), 114.4 (C7), 119.6 (C15), 139.2 (C1), 144.8 (C8), 150.3 (C16), 169.6, 169.7, 170.3 (4x $\underline{C}$(O)CH_3), **HRMS** (ESI) *m/z* 280.6399 ([M + 2H]$^+$ calcd. For $C_{27}H_{39}N_5O_8$: 280.6394).

Monovalent 13a

Substrate **12a** (1 eq., 23 mg, 0.0411 mmol) was dissolved in MeOH (4.6 mL) and freshly prepared Amberlite IRA-400 (OH^-) (1000 mg) was added. The suspension was smoothly stirred for 14 h. The resin was filtered and rinsed with MeOH and H_2O. The filtrate was concentrated under reduced pressure. Product **13a** (15.6 mg, 0.040 mmol, 97%) was obtained pure without purification.

$[\alpha]_D^{20}$= -13 (*c* 0.4, MeOH), **IR** (neat) 3338, 3142, 3096, 3004, 2928, 2857, 1563, 1459, 1106, 1023, 666 cm^{-1}, **^{1}H NMR** (400 MHz, CD_3OD) δ (ppm) 0.72-0.79 (m, 2H, H18), 0.92-0.98 (m, 2H, H18), 1.26-1.43 (m, 4H, H11 and H12), 1.61 (qnt, *J* = 7.4 Hz, 2H, H10), 1.87 (qnt, *J* = 7.3 Hz, 2H, H13), 1.92-1.99 (m, 1H, H17), 2.50 (t, *J* = 7.5 Hz, 2H, H9), 3.67 (t, *J* = 8.3 Hz, 1H, H3), 3.80 (dd, *J* = 17.1, 8.3 Hz, 1H, H4), 3.84 (dd, *J* = 8.4, 2.9 Hz, 1H, H5), 3.93 (dd, *J* = 11.9, 3.9 Hz, 1H, H6[a]), 4.16 (dd, *J* = 12.0, 2.2 Hz, 1H, H6[b]), 4.31 (t, *J* = 7.1 Hz, 2H, H14), 4.46 (d, *J* = 7.9 Hz, 1H, H2), 6.98 (s, 1H, H7), 7.66 (s, 1H, H15), **^{13}C NMR** (100 MHz, CD_3OD) δ (ppm) 7.3 (C17), 8.2 (C18 and C18'), 27.2 (C12), 28.9 (C9), 29.6 (C11), 30.2 (C10), 31.1 (C13), 51.2 (C14), 61.5 (C6), 62.6 (C5), 69.3 (C4), 69.8 (C2), 76.7 (C3), 114.5 (C7), 121.8 (C15), 143.5 (C16), 146.9 (C1), 151.4 (C8), **HRMS** (ESI) *m/z* 392.2290 ([M + H]$^+$ calcd. For $C_{19}H_{30}N_5O_4$: 392.2292).

Monovalent 13b

Substrate **12b** (1 eq., 29.5 mg, 0.0527 mmol) was dissolved in MeOH (5.9 mL) and freshly prepared Amberlite IRA-400 (OH^-) (1 g) was added. The suspension was smoothly stirred for 18 h. The resin was filtered and rinsed with MeOH and H_2O. The filtrate was concentrated under reduced pressure. Product **13b** (17.9 mg, 0.046 mmol, 87%) was obtained pure without purification as a solid.

$[\alpha]_D^{20}$= -18 (*c* 0.3, MeOH), **m.p.** 93 °C **IR** (neat) 3335, 3142, 3090, 2926, 2855, 1685, 1563, 1465, 1097, 901, 825 cm^{-1}, **^{1}H NMR** (400 MHz, CD_3OD) δ (ppm) 0.72-0.79 (m, 2H, H18), 0.92-0.98 (m, 2H, H18), 1.26-1.44 (m, 4H, H11, H12), 1.61 (qnt, *J* = 7.3 Hz, 2H, H10), 1.87 (qnt, *J* = 7,3 Hz, 2H, H13), 1.95 (m, 1H, H17), 2.5 (t, *J* = 7.6 Hz, 2H, H9), 3.76 (dd, *J* = 9.4, 3.7 Hz, 1H, H3), 3.80 (ddd, *J* = 7.9, 5.4, 2.7 Hz, 1H, H5), 3.89 (dd, *J* = 11.9, 5.5 Hz, 1H, H6[a]), 4.09 (dd, *J* = 9.4, 7.8 Hz, 1H, H4), 4.16 (dd, *J* = 11.7, 2.8 Hz, 1H, H6[b]), 4.31 (t, *J* = 7.1 Hz, 2H, H14), 4.78 (d, *J* = 3.7 Hz, 1H, H2), 7.04 (s, 1H, H7), 7.66 (s, 1H, H15), **^{13}C NMR** (100 MHz, CD_3OD) δ (ppm) 7.3 (C17), 8.2 (C18, C18'), 27.2 (C12), 28.8 (C9), 29.6 (C11), 30.3 (C10), 31.1 (C13), 51.2 (C14), 63.0 (C6), 63.5 (C5), 65.7 (C2), 67.1 (C4), 73.2 (C3), 115.5 (C7), 121.8 (C15), 143.5 (C8), 145.9 (C1), 151.4 (C16), **HRMS** (ESI) *m/z* 392.2291 ([M + H]$^+$ calcd. For $C_{19}H_{30}N_5O_4$: 392.2292).

Compounds 16a and 16b

A solution of **15** (1 eq., 3.2 g, 12.8 mmol) in dry THF was cooled to 0 °C under argon atmosphere. NaH (4.0 eq., 2.05 g, 51.3 mmol) was added portion-wise and after stirring for 30 min, propargyl bromide (6.04 eq., 8.6 mL, 77.2 mmol) was added dropwise over 45 min. The reaction was allowed to warm to room temperature and was stirred for 16 h. After quenching the excess of NaH with NH_4Cl, the solvent was evaporated under reduced pressure. The residue was diluted with Et_2O and washed with water. The aqueous phase was extracted with Et_2O (2x). The combined organic layers were washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (100:0 to 8:2 petroleum ether/EtOAc) to give **16a** (2,92 g, 8.94 mmol, 70%) as a yellow oil and **16b** (0.9 g,

Organic & Biomolecular Chemistry Accepted Manuscript

Please do not adjust margins

2.47 mmol, 19%) as a yellow oil. Analytical data of **16b** were in accordance with those reported in the literature.[26]

16a: ***R**$_f$* 0.35 (DCM/MeOH 9:1), **IR** (neat) 3372 cm^{-1}, **^{1}H NMR** (400 MHz, $CDCl_3$) *δ* (ppm) 0.05 (s, 6H, CH_3), 0.89 (s, 9H, *t*-Bu), 2.41 (t, *J* = 2.4 Hz, 2H, C-H), 2.66 (t, *J* =5.9 Hz, 1H, OH), 3.54 (m, 4H, CH_2-O), 3.64 (s, 2H, CH_2-OSi), 3.70 (d, *J* =5.9 Hz, 2H, $\underline{CH_2}$-OH), 4.12 (d, *J* = 2.4 Hz, 4H, CH_2), **^{13}C NMR** (100 MHz, $CDCl_3$) *δ* (ppm) -5.6, 18.3, 25.9, 45.2, 58.8, 63.5, 65.5, 70.1, 74.5, 79.8, **HRMS** (ESI) *m/z* 349.180 ([M + Na]$^+$ calcd. For $C_{17}H_{30}O_4SiNa$: 349.181).

16b: ^{1}H NMR (400 MHz, $CDCl_3$) *δ* (ppm) 0.03 (s, 6H, CH_3), 0.88 (s, 9H, *t*-Bu), 2.38 (t, *J* = 2.4 Hz, 3H, C-H), 3.49 (s, 6H, CH_2), 3.56 (s, 2H, CH_2-OSi), 4.11 (d, *J* = 2.3 Hz, 6H, CH_2).

Compound 17a[33]

To a solution of **16a** (1 eq., 197 mg, 0.603 mmol) in dry THF (10 mL) at 0 °C was added dropwise a 1 M solution of TBAF (5 eq., 3.02 mL, 3.02 mmol) in THF over a period of 20 min. The reaction mixture was allowed to warm to room temperature and stirred for 5 h under argon atmosphere. The solvent was evaporated under reduced pressure. The residue was diluted with EtOAc and washed with water (2x) and brine. The organic layers were dried over Na_2SO_4, and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (55:45 EtOAc/pentane) to afford **17a** (122 mg, 0.579 mmol, 96%) as a colorless oil.

IR (neat) 3373 cm^{-1}, **^{1}H NMR** (400 MHz, $CDCl_3$) *δ* (ppm) 2.34 (br s, 2H, OH), 2.45 (t, *J* = 2.4 Hz, 2H, H5), 3.60 (s, 4H, H2’), 3.68 (s, 4H, H2), 4.15 (d, *J* = 2.4 Hz, 4H, H3). **^{13}C-NMR** (75.5 MHz, $CDCl_3$) δ (ppm) 45.0, 58.9, 64.3, 71.0, 74.9, 79.6, **HRMS** (ESI) *m/z* 235.0969 ([M + Na]$^+$ calcd. For $C_{11}H_{16}O_4Na$: 235.0941).

Compound 17b

To a solution of **16b** (1 eq., 54.3 mg, 0.149 mmol) in dry THF (2.9 mL) at 0 °C was added dropwise a solution of TBAF (5 eq., 1 M, 0.745 mL, 0.745 mmol) in THF over a period of 30 min. Solution turned orange directly after TBAF addition. The reaction mixture was allowed to warm to room temperature and was stirred for 5 h under argon atmosphere. The solvent was evaporated under reduced pressure. The residue was diluted with EtOAc and washed with water (2x) and brine. The organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (8:2 to 7:3 petroleum ether/EtOAc) to afford **17b** (27 mg, 0.11 mmol, 72%) as a colorless oil. Analytical data were in accordance with those reported in literature.[23]

^{1}H NMR (400 MHz, $CDCl_3$) *δ* (ppm) 2.42 (t, *J* = 2.4 Hz, 3H), 3.56 (s, 6H), 3.69 (s, 2H), 4.13 (d, *J* = 2.4 Hz, 6H).

General procedure A

Azide substrate **11** (1.1 eq by alkyne) and platform **17** or **24** (1 eq.) were dissolved in DMF (20 mL/mmol of alkyne) in a microwave vial. Solution was stirred till the solution was homogeneous. A solution of sodium ascorbate (0.2 eq. by alkyne) and $CuSO_4.5H_2O$ (0.1 eq. by alkyne) in H_2O (0.25 mL per mL of DMF) was added. The mixture was stirred and heated under microwave irradiation at 80°C for 30-40 min. Water was added, and the aqueous phase was extracted with EtOAc (3x). The organic phases were combined, washed with water (3x) and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Traces of copper salts were removed by filtration through a short pad of silica gel eluting with a 10:1:1 $CH_3CN/H_2O/NH_4OH$ solution. The crude residue was purified by flash column chromatography on silica gel (97:3 to 9:1 DCM/MeOH).

Peracetylated divalent cluster 18a

Product **18a** (67.8 mg, 0.0565 mmol, 59%) was obtained following general procedure A starting from **11a** (111 mg, 0.225 mmol) and **17a** (20.5 mg, 0.0966 mmol).

***R**$_f$* 0.25 (DCM/MeOH 95:5), $[\alpha]_D^{20}$= +19 (*c* 0.8, $CHCl_3$), **m.p.** 67-70 °C **IR** (neat) 2932, 2860, 1750, 1462, 1371, 1222, 1048 cm^{-1}, **^{1}H NMR** (500 MHz, $CDCl_3$) *δ* (ppm) 1.26-1.42 (m, 8H, H11 and H12), 1.54-1.64 (m, 4H, H10), 1.85-1.94 (m, 4H, H13), 2.02-2.14 (m, 24H, C(O)$\underline{CH_3}$), 2.51 (t, *J* = 7.6 Hz, 4H, H9), 3.54 (s, 4H, H18), 3.62 (s, 4H, H18’), 4.28-4.35 (m, 6H, H14 and H6[a]), 4.60 (s, 4H, H17), 5.35-5.40 (m, 2H, H4), 5.44-5.50 (m, 2H, H3), 5.98 (d, *J* = 5.0 Hz, 2H, H2), 6.71 (s, 2H, H7), 7.52 (s, 2H, H15), **^{13}C NMR** (125 MHz, $CDCl_3$) *δ* (ppm) 20.7, 20.73, 20.8, 21.0 (8x C(O)$\underline{CH_3}$), 26.2 (C12), 28.3 (C9), 28.7 (C11), 29.0 (C10), 30.2 (C13), 45.2 (C19), 50.3 (C14), 56.3 (C5), 62.1 (C6), 64.4 (C18’), 65.0 (C17), 66.1 (C2), 66.4 (C4), 70.3 (C3), 71.0 (C18), 113.8 (C7), 122.3 (C15), 139.2 (C1), 144.6 (C8), 145.1 (C16), 169.2, 169.4, 169.8, 170.4 (8x $\underline{C}$(O)CH_3), **HRMS** (ESI) *m/z* 1199.5470 ([M + H]$^+$ calcd. For $C_{55}H_{79}N_{10}O_{20}$: 1199.5467).

Peracetylated divalent cluster 18b

Product **18b** (57.1 mg, 0.0476 mmol, 64%) was obtained following general procedure A starting from **11b** (82.2 mg, 0.167 mmol) and **17a** (15.9 mg, 0.0749 mmol).

***R**$_f$* 0.2 (DCM/MeOH 95:5), $[\alpha]_D^{20}$= -35 (*c* 0.8, $CHCl_3$), **m.p.** 60 °C **IR** (neat) 2928, 2860, 1749, 1461, 1370, 1222, 1052, 950 cm^{-1}, **^{1}H NMR** (400 MHz, $CDCl_3$) *δ* (ppm) 1.25-1.40 (m, 8H, H11 and H12), 1.60 (qnt, *J* = 7.3 Hz, 4H, H10), 1.90 (qnt, *J* = 7.2 Hz, 4H, H13), 2.01 (s, 6H, C(O)$\underline{CH_3}$), 2.08 (s, 6H, C(O)$\underline{CH_3}$), 2.088 (s, 6H, C(O)$\underline{CH_3}$), 2.09 (s, 6H, C(O)$\underline{CH_3}$), 2.51 (td, *J* = 7.6, 2.6 Hz, 4H, H9), 3.53 (s, 4H, H18), 3.61 (s, 4H, H18’), 4.25-4.35 (m, 8H, H14, H5 and H6[a]), 4.50-4.57 (m, 2H, H6[b]), 4.59 (s, 4H, H17), 5.41 (dd, *J* = 9.0, 3.7 Hz, 2H, H3), 5.64 (dd, *J* = 9.0, 6.1 Hz, 2H, H4), 6.33 (d, *J* = 3.7 Hz, 2H, H2), 6.75 (s, 2H, H7), 7.52 (s, 2H, H15), **^{13}C NMR** (100 MHz, $CDCl_3$) *δ* (ppm) 20.6, 20.7, 20.8, 20.9 (8x C(O)$\underline{CH_3}$), 26.2 (C12), 28.2 (C9), 28.6 (C11), 29.0 (C10), 30.1 (C13), 45.2 (C19), 50.3 (C14), 57.2 (C5), 63.2 (C6), 63.5 (C2), 64.2 (C18’), 64.9 (C17), 65.7 (C4), 68.9 (C3), 70.9 (C18), 114.4 (C7), 122.2 (C15), 139.1 (C1), 144.6 (C8), 145.0 (C16), 169.5, 169.62, 169.65, 170.3 (8x $\underline{C}$(O)CH_3), **HRMS** (ESI) *m/z* 1221.5290 ([M + Na]$^+$ calcd. For $C_{55}H_{78}N_{10}NaO_{20}$: 1221.5286).

Peracetylated trivalent cluster 20a

Product **20a** (71.4 mg, 0.0413 mmol, 85%) was obtained following general procedure A starting from **11a** (81 mg, 0.164 mmol) and **17b** (12.1 mg, 0.0483 mmol).

This journal is © The Royal Society of Chemistry 20xx

Please do not adjust margins

View Article Online
DOI: 10.1039/C9OB00736J

Published on 21 May 2019. Downloaded by Syddansk Universitetsbibliotek on 5/22/2019 8:58:13 AM.

Organic & Biomolecular Chemistry Accepted Manuscript

***R**_f_* 0.45 (DCM/MeOH 94:6), $[\alpha]_D^{20}$= +21 (*c* 0.5, $CHCl_3$), **m.p.** 65-67 °C **IR** (neat) 2930, 2859, 1749, 1461, 1370, 1220, 1049, 756 cm^{-1}, **^{1}H NMR** (500 MHz, $CDCl_3$) δ (ppm) 1.29-1.43 (m, 12H, H11 and H12), 1.56-1.65 (m, 6H, H10), 1.89 (qnt, *J* = 7.3 Hz, 6H, H13), 2.06 (s, 9H, $C(O)\underline{CH_3}$), 2.075 (s, 9H, $C(O)\underline{CH_3}$), 2.077 (s, 9H, $C(O)\underline{CH_3}$), 2.10 (s, 9H, $C(O)\underline{CH_3}$), 2.53 (t, *J* = 7.7 Hz, 6H, H9), 3.50 (s, 6H, H18), 3.61 (s, 2H, H18'), 4.29-4.35 (m, 9H, H14 and H6^a), 4.37-4.45 (m, 6H, H5 and H6^b), 4.56 (s, 6H, H17), 5.38 (dd, *J* = 7.2, 5.6 Hz, 3H, H4), 5.48 (dd, *J* = 7.3, 5.3 Hz, 3H, H3), 5.99 (d, *J* = 5.3 Hz, 3H, H2), 6.77 (s, 3H, H7), 7.53 (s, 3H, H15), **^{13}C NMR** (125 MHz, $CDCl_3$) δ (ppm) 20.7, 20.75, 20.8, 21.1 (12x $C(O)\underline{CH_3}$), 26.3 (C12), 28.4 (C9), 28.8 (C11), 29.1 (C10), 30.3 (C13), 45.3 (C19), 50.4 (C14), 56.3 (C5), 62.2 (C6), 64.5 (C18'), 65.1 (C17), 66.2 (C2), 66.4 (C4), 70.3 (C3), 70.4 (C18), 113.8 (C7), 122.4 (C15), 139.2 (C1), 144.6 (C8), 145.2 (C16), 169.2, 169.4, 169.8, 170.4 (12x $\underline{C}(O)CH_3$), **HRMS** (ESI) *m/z* 865.8943 ($[M + 2H]^{2+}$ calcd. For $C_{80}H_{113}N_{15}O_{28}$: 865.8934).

Peracetylated trivalent cluster 20b

Product **20b** (38.0 mg, 0.0219 mmol, 47%) was obtained as a white solid following general procedure A starting from **11b** (79.9 mg, 0.157 mmol) and **17b** (11.8 mg, 0.0471 mmol).

***R**_f_* 0.4 (DCM/MeOH 94:6), $[\alpha]_D^{20}$= -39 (*c* 1.8, $CHCl_3$), **m.p.** 83-85 °C **IR** (neat) 2934, 2860, 1747, 1461, 1370, 1220, 1051, 950, 753 cm^{-1}, **^{1}H NMR** (500 MHz, $CDCl_3$) δ (ppm) 1.29-1.41 (m, 12H, H11 and H12), 1.60 (qnt, *J* = 7.2 Hz, 6H, H10), 1.89 (qnt, *J* = 7.2 Hz, 6H, H13), 2.02 (s, 9H, $C(O)\underline{CH_3}$), 2.09 (s, 9H, $C(O)\underline{CH_3}$), 2.098 (s, 9H, $C(O)\underline{CH_3}$), 2.10 (s, 9H, $C(O)\underline{CH_3}$), 2.51 (td, *J* = 7.6, 3.1 Hz, 6H, H9), 3.50 (s, 6H, H18), 3.61 (s, 2H, H18'), 4.25-4.34 (m, 12H, H5, H14 and H6^a), 4.51-4.58 (m, 9H, H17 and H6^b), 5.40 (dd, *J* = 9.1, 3.7 Hz, 3H, H3), 5.64 (dd, *J* = 9.1, 6.3 Hz, 3H, H4), 6.32 (d, *J* = 3.7 Hz, 3H, H2), 6.75 (s, 3H, H7), 7.53 (s, 3H, H15), **^{13}C NMR** (125 MHz, $CDCl_3$) δ (ppm) 20.7, 20.8, 21.0 (12x $C(O)\underline{CH_3}$), 26.3 (C12), 28.4 (C9), 28.8 (C11), 29.1 (C10), 30.3 (C13), 45.2 (C19), 50.3 (C14), 57.2 (C5), 63.3 (C6), 63.6 (C2), 64.5 (C18'), 65.1 (C17), 65.7 (C4), 69.1 (C3), 70.4 (C18), 144.4 (C7), 122.4 (C15), 139.2 (C1), 144.8 (C8), 145.2 (C16), 169.6, 169.7, 170.3 (12x $\underline{C}(O)CH_3$), **HRMS** (ESI) *m/z* 865.8928 ($[M + 2H]^{2+}$ calcd. For $C_{80}H_{113}N_{15}O_{28}$: 865.8934).

Peracetylated tetravalent cluster 22a

Product **22a** (77.2 mg, 0.0341 mmol, 79%) was obtained following general procedure A starting from **11a** (97.2 mg, 0.191 mmol) and **17c** (12.4 mg, 0.043 mmol).

***R**_f_* 0.35 (DCM/MeOH 94:6), $[\alpha]_D^{20}$= +20 (*c* 1.0, $CHCl_3$), **m.p.** 82-85 °C **IR** (neat) 2933, 2859, 1747, 1461, 1370, 1217, 1048, 750 cm^{-1}, **^{1}H NMR** (500 MHz, $CDCl_3$) δ (ppm) 1.30-1.41 (m, 16H, H11 and H12), 1.54-1.63 (m, 8H, H10), 1.88 (qnt, *J* = 7.2 Hz, H13), 2.05 (s, 12H, $C(O)\underline{CH_3}$), 2.06 (s, 24H, $C(O)\underline{CH_3}$), 2.08 (s, 12H, $C(O)\underline{CH_3}$), 2.52 (t, *J* = 7.8 Hz, 8H, H9), 3.44 (s, 8H, H18), 4.27-4.34 (m, 12H, H14 and H6^a), 4.36-4.44 (m, 8H, H5 and H6^b), 4.52 (s, 8H, H17), 5.37 (dd, *J* = 7.2, 5.6 Hz, 4H, H4), 5.47 (dd, *J* = 7.3, 5.3 Hz, 4H, H3), 5.98 (d, *J* = 5.3Hz, 4H, H2), 6.72 (s, 4H, H7), 7.54 (s, 4H, H15), **^{13}C NMR** (125 MHz, $CDCl_3$) δ (ppm) 20.67, 20.71, 20.8, 21.0 (16x $C(O)\underline{CH_3}$), 26.4 (C12), 28.4 (C9), 28.8 (C11), 29.1 (C10), 30.3 (C13), 45.3 (C19), 50.3 (C14), 56.3 (C5), 62.1 (C6), 65.1 (C17), 66.1 (C2), 66.4 (C4), 69.2 (C18), 70.3 (C3), 113.8 (C7), 122.5 (C15), 139.1 (C1), 144.6 (C8), 145.3 (C16), 169.1, 169.4, 169.7, 170.3 (16x $\underline{C}(O)CH_3$), **HRMS** (ESI) *m/z* 754.6768 ($[M + 3H]^{3+}$ calcd. For $C_{105}H_{147}N_{20}O_{36}$: 754.6757).

Peracetylated tetravalent cluster 22b

Product **22b** (70.0 mg, 0.0309 mmol, 76%) was obtained as a solid following general procedure A starting from **11b** (91.1 mg, 0.180 mmol) and **17c** (11.8 mg, 0.0409 mmol).

***R**_f_* 0.3 (DCM/MeOH 94:6), $[\alpha]_D^{20}$= -31 (*c* 0.8, $CHCl_3$), **m.p.** 75 °C **IR** (neat) 2931, 2859, 1748, 1460, 1370, 1221, 1051, 950, 755 cm^{-1}, **^{1}H NMR** (500 MHz, $CDCl_3$) δ (ppm) 1.28-1.42 (m, 16H, H11 and H12), 1.60 (qnt, *J* = 7.2 Hz, 8H, H10), 1.89 (qnt, *J* = 6.9 Hz, 8H, H13), 2.02 (s, 12H, $C(O)\underline{CH_3}$), 2.09 (s, 12H, $C(O)\underline{CH_3}$), 2.096 (s, 12H, $C(O)\underline{CH_3}$), 2.10 (s, 12H, $C(O)\underline{CH_3}$), 2.51 (td, *J* = 7.6, 3.2 Hz, 8H, H9), 3.44 (s, 8H, H18), 4.25-4.34 (m, 16H, H5, H14 and H6^a), 4.51-4.57 (m, 12H, H17 and H6^b), 5.39 (dd, *J* = 9.2, 3.7 Hz, 4H, H3), 5.63 (dd, *J* = 9.1, 6.2 Hz, 4H, H4), 6.32 (s, *J* = 3.7 Hz, 4H, H2), 6.76 (s, 4H, H7), 7.54 (s, 4H, H15), **^{13}C NMR** (125 MHz, $CDCl_3$) δ (ppm) 20.7, 20.8, 20.9 (16x $C(O)\underline{CH_3}$), 26.4 (C12), 28.4 (C9), 28.8 (C11), 29.2 (C10), 30.4 (C13), 45.3 (C19), 50.3 (C14), 57.2 (C5), 63.3 (C6), 63.6 (C2), 65.2 (C17), 65.7 (C4), 69.0 (C3), 69.2 (C18), 144.4 (C7), 122.5 (C15), 139.1 (C1), 144.8 (C8), 145.4 (C16), 169.6, 169.7, 170.3 (16x $\underline{C}(O)CH_3$), **HRMS** (ESI) *m/z* 754.6767 ($[M + 3H]^{3+}$ calcd. For $C_{105}H_{147}N_{20}O_{36}$: 754.6757).

Peracetylated tetradecavalent cluster 25a

Product **25a** (22.9 mg, 0.0027 mmol, 57%) was obtained as an oil following general procedure A starting from **11a** (36.8 mg, 0.074 mmol) and **24** (7.8 mg, 0.0047 mmol).

***R**_f_* 0.65 (DCM/MeOH 9:1), $[\alpha]_D^{20}$= +27 (*c* 1.15, MeOH), **IR** (neat) 3452, 3139, 2929, 2857, 1750, 1462, 1440, 1371, 1223, 1047, 604 cm^{-1}, **^{1}H NMR** (500 MHz, $CDCl_3$) δ (ppm) 1.28-1.44 (m, 56H, H11 and H12), 1.53-1.66 (m, 28H, H10), 1.82-1.95 (m, 28H, H13), 2.03-2.12 (m, 168H, $\underline{CH_3}(O)C$), 2.52 (t, *J* = 7.0 Hz, 28H, H9), 3.34-3.44 (m, 7H, H23), 3.44-3.50 (m, 7H, H21), 3.58-3.75 (m, 21H, H19 and H18), 3.92 (t, *J* = 8.9 Hz, 7H, H22), 4.26-4.47 (m, 70H, H5, H6 and H14), 4.53 (m, 14H, H17), 4.76 (br s, 7H, H20), 4.86-5.03 (m, 14 H, H17'), 5.36-5.42 (m, 14H, H4), 5.45-5.51 (m, 14H, H3), 5.99 (d, *J* = 5.4 Hz, 14H, H2), 6.75 (br s, 14H, H7), 7.66 (s, 7H, H15'), 7.74 (s, 7H, H15), **^{13}C NMR** (125 MHz, $CDCl_3$) δ (ppm) 20.7, 20.78, 20.83, 21.1 ($\underline{CH_3}(O)C$), 26.4, 26.5 (C12 and C12'), 28.6 (C9), 28.9, 29.0 (C11 and C11'), 29.2 (C10), 30.5 (C13), 50.3, 50.4 (C14 and C14'), 56.3 (C5), 62.1 (C6), 64.9 (C17), 65.2 (C17'), 66.3 (C2), 66.4 (C4), 68.8 (C18), 70.4 (C3), 70.6 (C19), 73.4 (C22), 79.1 (C21), 83.1 (C23), 101.7 (C20), 113.8 (C7), 123.0 (C15'), 123.7 (C15), 139.2 (C1), 144.1 (C16), 144.7 (C8), 169.2, 169.4, 169.8, 170.4 ($CH_3(O)\underline{C}$), **HRMS** (ESI) *m/z* 1072.4607 ($[M + 8H]^{8+}$ calcd. For $C_{392}H_{540}N_{70}O_{147}$: 1072.4611).

Peracetylated tetradecavalent cluster 25b

Product **25b** (29.0 mg, 0.0034 mmol, 55%) was obtained as an oil following general procedure A starting from **11b** (49.2 mg, 0.0987 mmol) and **24** (10.3 mg, 0.0062 mmol).

***R**_f_* 0.5 (DCM/MeOH 9:1), $[\alpha]_D^{20}$= -169 (*c* 0.5, $CHCl_3$), **IR** (neat) 3417, 2930, 2860, 1749, 1457, 1370, 1222, 1051, 949, 755, 600 cm^{-1}, **^{1}H NMR** (500 MHz, $CDCl_3$) δ (ppm) 1.27-1.43 (m, 56 H, H11 and H12),

View Article Online DOI: 10.1039/C9OB00626H

Published on 21 May 2019. Downloaded by Syddansk Universitetsbibliotek on 5/22/2019 8:58:13 AM.

Organic & Biomolecular Chemistry Accepted Manuscript

Please do not adjust margins

View Article Online
DOI: 10.1039/C9OB00835B

1.53-1.66 (m, 28H, H10), 1.80-1.95 (m, 28H, H13), 2.00-2.13 (m 168H, $\underline{CH_3}$(O)C), 2.45-2.55 (m, 28H, H9), 3.33-3.43 (m, 7H, H21), 3.43-3.57 (m, 14H, H19 and H23), 3.60-3.74 (m, 14H, H18), 3.86-3.96 (m, 7H, H22), 4.23-4.40 (m, 56H, H14, H5 and H6a), 4.46-4.60 (m, 21H, H6b and H17), 4.76 (s, 7H, H20), 4.83-5.05 (m, 14H, H17'), 5.35-5.45 (m, 14H, H3), 5.59-5.68 (m, 14H,H4), 6.32 (s, 14H, H2), 6.80 (s, 14H, H7), 7.67 (s, 7H, H15'), 7.75 (s, 7H, H15), **^{13}C NMR** (125 MHz, $CDCl_3$) δ (ppm) 20.7, 20.8, 20.9 ($\underline{CH_3}$(O)C), 26.4 (C12), 28.3 (C9), 28.9 (C11), 29.2 (C10), 30.4 (C13), 50.3, 50.4 (C14 and C14'), 57.3 (C5), 63.2 (C6), 63.5 (C2), 64.9, 65.2 (C17 and C17'), 65.7 (C4), 68.9 (C3), 70.4, 70.6 (C18 and C18'), 73.0 (C22), 75.5 (C19), 79.1 (C21), 83.0 (C23), 101.7 (C20), 114.6 (C7), 123.1 (C15'), 123.8 (C15), 139.1 (C1), 144.0 (C8), 144.6 (C16), 169.6, 169.7, 170.3 (CH_3(O)$\underline{C}$), **HRMS** (ESI) *m/z* 1225.5263 ($[M + 7H]^{7+}$ calcd. For $C_{392}H_{539}N_{70}O_{147}$: 1225.5259).

General procedure B

To the peracetylated multivalent cluster (1 eq.) dissolved in MeOH (55.5 mL/mmol), a 2M ammonia solution in MeOH (110 eq.) was added. The reaction was stirred for 16-20h under argon atmosphere and then concentrated. Pure deprotected cluster was obtained after acetamide removal by co-evaporation with toluene and then MeOH.

Divalent cluster 19a

Product **19a** (11.6 mg, 0.0135 mmol, 100%) was obtained as an oil following general procedure B, starting from **18a** (16.2 mg, 0.0135 mmol).

$[\alpha]_D^{20}$= -14 (*c* 0.6, MeOH), **IR** (neat) 3326, 3142, 2926, 2857, 1664, 1461, 1094, 1056, 1031, 778, 640 cm^{-1}, **^{1}H NMR** (500 MHz, CD_3OD) δ (ppm) 1.28-1.42 (m, 8H, H11 and H12), 1.61 (qnt, *J* = 7.4 Hz, 4H, H10), 1.90 (qnt, *J* = 7.3 Hz, 4H, H13), 2.50 (t, *J* = 7.5 Hz, 4H, H9), 3.45 (s, 4H, H18), 3.54 (s, 4H, H18'), 3.67 (t, *J* = 8.5 Hz, 2H, H3), 3.79 (t, *J* = 8.8 Hz, 2H, H4), 3.84 (dt, *J* = 11.0, 3.0 Hz, 2H, H5), 3.93 (dd, *J* = 12.1, 4.0 Hz, 2H, H6^a), 4.15 (dd, *J* = 12.0, 2.3 Hz, 2H, H6^b), 4.38 (t, *J* = 7.1 Hz, 4H, H14), 4.46 (d, *J* = 7.9 Hz, 2H, H2), 4.55 (s, 4H, H17), 6.97 (s, 2H, H7), 7.93 (s, 2H, H15), **^{13}C NMR** (125 MHz, CD_3OD) δ (ppm) 27.2 (C12), 28.8 (C14), 29.6 (C11), 30.2 (C10), 31.1 (C13), 46.9 (C19), 51.3 (C14), 61.4 (C6), 62.6 (C18'), 62.7 (C5), 65.3 (C17), 69.3 (C4), 69.8 (C2), 70.4 (C18), 76.7 (C3), 114.6 (C7), 124.8 (C15), 143.4 (C8), 146.1 (C16), 146.9 (C1), **HRMS** (ESI) *m/z* 863.4621 ($[M + H]^+$ calcd. For $C_{39}H_{63}N_{10}O_{12}$: 863.4621).

Divalent cluster 19b

Product **19b** (30.1 mg, 0.035 mmol, 100%) was obtained as a beige solid, following general procedure B, starting from **18b** (41.8 mg, 0.035 mmol).

$[\alpha]_D^{20}$= -16 (*c* 1.6, MeOH), **m.p.** 63-65 °C, **IR** (neat) 3308, 3142, 2926, 2858, 1658, 1462, 1090, 1059, 901, 773 cm^{-1}, **^{1}H NMR** (500 MHz, CD_3OD) δ (ppm) 1.27-1.43 (m, 8H, H11 and H12), 1.61 (qnt, *J* = 7.6 Hz, 4H, H10), 1.90 (qnt, *J* = 7.2 Hz, 4H, H13), 2.50 (t, *J* = 7.6 Hz, 4H, H9), 3.45 (s, 4H, H18), 3.54 (s, 4H, H18'), 3.77 (dd, *J* = 9.3, 3.7 Hz, 2H, H3), 3.81 (m, 2H, H5), 3.89 (dd, *J* = 11.8, 5.4 Hz, 2H, H6^a), 4.09 (dd, *J* = 9.2, 7.8 Hz, 2H, H4), 4.16 (dd, *J* = 11.9, 2.8 Hz, 2H, H6^b), 4.38 (t, *J* = 7.0 Hz, 4H, H14), 4.55 (s, 4H, H17), 4.76 (d, *J* = 3.7 Hz, 2H, H2), 7.04 (s, 2H, H7), 7.93 (s, 2H, H15), **^{13}C NMR** (125 MHz, CD_3OD) δ (ppm) 27.2 (C12), 28.7 (C9), 29.6 (C11), 30.2 (C10), 31.1 (C13), 46.9 (C19), 51.3 (C14), 62.6 (C18'), 62.9 (C6), 63.5 (C5), 65.3 (C17), 65.7 (C2), 67.1 (C4), 70.4 (C18), 73.2 (C3), 115.6 (C7), 124.8 (C15), 143.3 (C8), 145.9 (C1), 146.1 (C16), **HRMS** (ESI) *m/z* 863.4633 ($[M + H]^+$ calcd. For $C_{39}H_{63}N_{10}O_{12}$: 863.4621).

Trivalent cluster 21a

Product **21a** (13.8 mg, 0.0113 mmol, 100%) was obtained as a white solid following general procedure B, starting from **20a** (19.5 mg, 0.0113 mmol).

$[\alpha]_D^{20}$= -24 (*c* 1.0, MeOH), **m.p.** 125 °C, **IR** (neat) 3325, 3142, 2926, 2858, 1567, 1461, 1092, 1059, 1024, 778, 649 cm^{-1}, **^{1}H NMR** (500 MHz, CD_3OD) δ (ppm) 1.27-1.41 (m, 12H, H11 and H12), 1.60 (qnt, *J* = 7.5 Hz, 6H, H10), 1.89 (qnt, *J* = 7.2 Hz, 6H, H13), 2.49 (t, *J* = 7.7 Hz, 6H, H9), 3.43 (s, 6H, H18), 3.52 (s, 2H, H18'), 3.67 (dd, *J* = 8.7, 8.2 Hz, 3H, H3), 3.76-3.85 (m, 6H, H4 and H5), 3.92 (dd, *J* = 12.0, 3.9Hz, 3H, H6^a), 4.15 (dd, *J* = 12.1, 2.5 Hz, 3H, H6^b), 4.37 (t, *J* = 7.0 Hz, 6H, H14), 4.46 (d, *J* = 7.9Hz, 3H, H2), 6.97 (s, 3H, H7), 7.92 (s, 3H, H15), **^{13}C NMR** (125 MHz, CD_3OD) δ (ppm) 27.2 (C12), 28.8 (C9), 29.6 (C11), 30.2 (C10), 31.2 (C13), 46.2 (C19), 51.3 (C14), 61.5 (C6), 62.3 (C18'), 62.6 (C5), 65.3 (C17), 69.3 (C4), 69.8 (C2), 70.1 (C18), 76.7 (C3), 114.5 (C7), 124.9 (C15), 143.5 (C8), 146.1 (C16), 146.9 (C1), **HRMS** (ESI) *m/z* 409.5577 ($[M + 3H]^{3+}$ calcd. For $C_{56}H_{90}N_{15}O_{16}$: 409.5558).

Trivalent cluster 21b

Product **21b** (19.3 mg, 0.0157 mmol, 75%) was obtained as a white solid following general procedure B, starting from **20b** (36.5 mg, 0.0211 mmol).

$[\alpha]_D^{20}$= -18 (*c* 1.3 MeOH), **m.p.** 82-85 °C, **IR** (neat) 3325, 3142, 2926, 2857, 1659, 1462, 1088, 1060, 900 cm^{-1}, **^{1}H NMR** (500 MHz, CD_3OD) δ (ppm) 1.26-1.43 (m, 12H, H11 and H12), 1.60 (qnt, *J* = 7.4 Hz, 6H, H10), 1.89 (qnt, *J* = 7.3 Hz, 6H, H13), 2.49 (t, *J* = 7.5 Hz, 6H, H9), 3.43 (s, 6H, H18), 3.52 (s, 2H, H18'), 3.76 (dd, *J* = 9.4, 3.7 Hz, 3H, H3), 3.79 (ddd, *J* = 8.0, 5.1, 2.6 Hz, 3H, H5), 3.89 (dd, *J* = 11.8, 5.4 Hz, 3H, H6^a), 4.09 (dd, *J* = 9.4, 8.0 Hz, 3H, H4), 4.16 (dd, *J* = 11.8, 2.8 Hz, 3H, H6^b), 4.37 (t, *J* = 7.1 Hz, 6H, H14), 4.51 (s, 6H, H17), 4.78 (d, *J* = 3.8 Hz, 3H, H2), 7.03 (s, 3H, H7), 7.92 (s, 3H, H15), **^{13}C NMR** (125 MHz, CD_3OD) δ (ppm) 27.2 (C12), 28.8 (C9), 29.6 (C11), 30.3 (C10), 31.2 (C13), 46.7 (C19), 51.3 (C14), 62.3 (C18'), 62.9 (C6), 63.5 (C5), 65.4 (C17), 65.7 (C2), 67.1 (C4), 70.2 (C18), 73.2 (C3), 115.5 (C7), 124.9 (C15), 143.5 (C8), 145.9 (C1), 146.1 (C16), **HRMS** (ESI) *m/z* 409.5547 ($[M + 3H]^{3+}$ calcd. For $C_{56}H_{90}N_{15}O_{16}$: 409.5558).

Tetravalent cluster 23a

Product **23a** (42 mg, 0.0264 mmol, 100%) was obtained as a white solid following general procedure B, starting from **22a** (58.4 mg, 0.0258 mmol).

$[\alpha]_D^{20}$= -32 (*c* 0.4, MeOH/H_2O 2:1), **m.p.** 122-125 °C, **IR** (neat) 3307, 2925, 2857, 1565, 1455, 1083, 1021, 775, 638 cm^{-1}, **^{1}H NMR** (500 MHz, CD_3OD) δ (ppm) 1.27-1.41 (m, 16H, H11 and H12), 1.59 (qnt, *J* = 7.6 Hz, 8H, H10), 1.88 (qnt, *J* = 7.3 Hz, 8H, H13), 2.48 (t, *J* = 7.6 Hz,

Published on 21 May 2019. Downloaded by Syddansk Universitetsbibliotek on 5/22/2019 8:58:13 AM.

Organic & Biomolecular Chemistry Accepted Manuscript

This journal is © The Royal Society of Chemistry 20xx

Please do not adjust margins

8H, H9), 3.42 (s, 8H, H18), 3.67 (dd, J = 8.7, 7.9 Hz, 4H, H3), 3.78 (dd, J = 8.8, 8.5 Hz, 4H, H4), 3.82 (m, 4H, H5), 3.92 (dd, J = 12.1, 3.9 Hz, 4H, H6[a]), 4.15 (dd, J = 12.0, 2.3 Hz, 4H, H6[b]), 4.36 (t, J = 7.1 Hz, 8H, H14), 4.46 (d, J = 7.9 Hz, 4H, H2), 4.48 (s, 8H, H17), 6.97 (s, 4H, H7), 7.90 (s,4H, H15), **^{13}C NMR** (125 MHz, CD_3OD) δ (ppm) 27.2 (C12), 28.9 (C9), 29.6 (C11), 30.2 (C10), 31.2 (C13), 46.0 (C19), 51.3 (C14), 61.5 (C6), 62.6 (C5), 65.4 (C17), 69.3 (C4), 69.8 (C2), 69.9 (C18), 76.7 (C3), 144.5 (C7), 124.9 (C15), 145.5 (C8), 146.1 (C16), 146.9 (C1), **HRMS** (ESI) m/z 530.6219 ($[M + 3H]^{3+}$ calcd. For $C_{73}H_{115}N_{20}O_{20}$: 530.6193).

Tetravalent cluster 23b

Product **23b** (15.4 mg, 0.0097 mmol, 88%) was obtained as a beige solid following general procedure B, starting from **22b** (24.9 mg, 0.011 mmol).

$[\alpha]_D^{20}$= -19 (c 0.7, MeOH), **m.p.** 128 °C (decomp) **IR** (neat) 3338, 3142, 2926, 2858, 1462, 1087 cm^{-1}, **^{1}H NMR** (500 MHz, CD_3OD) δ (ppm) 1.26-1.41 (m, 16H, H12 and H11), 1.59 (qnt, J = 7.2 Hz, 8H, H10), 1.88 (qnt, J = 7.2 Hz, 8H, H13), 2.49 (t, J = 7.5 HZ, 8H, H9), 3.42 (s, 8H, H18), 3.77 (dd, J = 9.3, 3.7 Hz, 4H, H3), 3.80 (ddd, J = 7.8, 5.5, 2.8 Hz, 4H, H5), 3.89 (dd, J = 11.8, 5.5 Hz, 4H, H6[a]), 4.09 (dd, J = 9.2, 7.8 Hz, 4H, H4), 4.15 (dd, J = 11.8, 2.8 Hz, 4H, H6[b]), 4.36 (t, J = 7.0 Hz, 8H, H14), 4.45 (s, 8H, H17), 4.79 (d, J = 3.7 Hz, 4H, H2), 7.04 (s, 4H, H7), 7.90 (s, 4H, H15), **^{13}C NMR** (125 MHz, CD_3OD) δ (ppm) 27.2 (C12), 28.8 (C9), 29.6 (C11), 30.2 (C10), 31.2 (C13), 46.4 (C19), 51.3 (C14), 63.0 (C6), 63.5 (C5), 65.4 (C17), 65.7 (C2), 67.2 (C4), 69.9 (C18), 73.1 (C3), 115.6 (C7), 124.9 (C15), 143.3 (C8), 145.9 (C1), 146.1 (C16), **HRMS** (ESI) m/z 530.6181 ($[M + 3H]^{3+}$ calcd. For $C_{73}H_{115}N_{20}O_{20}$: 530.6193).

Tetradecavalent cluster 26a

Product **26a** (5 mg, 0.0008 mmol, 98%) was obtained as a white solid following general procedure B, starting from **25a** (7 mg, 0.00082 mmol).

$[\alpha]_D^{20}$= +7 (c 0.07, DMSO), **m.p.** 205 °C (decomp), **IR** (neat) 3343, 2925, 2860, 1733, 1456, 1369, 1227, 1084, 1044, 731 cm^{-1}, **^{1}H NMR** (500 MHz, DMSO-d_6) δ (ppm) 1.13-1.30 (m, 56H, H11 and H12), 1.41-1.51 (m, 28H, H10), 1.67-1.80 (m, 28H, H13), 2.29-2.39 (m, 28H, H9), 3.45-3.52 (m, 14H, H3), 3.52-3.59 (m, 14H, H4), 3.62-3.73 (m, 28H, H5 and H6a), 3.95-4.03 (m, 14H, H6b), 4.18-4.33 (m, 42H, H14 and H2), 4.33-4.44 (m, 7H, H19), 4.68-4.79 (m, 14H, H23 and H18a), 4.80-4.92 (m, 14H, H22 and H18b), 5.00-5.10 (m, 7H, H21), 5.33-5.45 (m, 28H, H17 and H17'), 5.76-5.88 (m, 7H, H20), 6.91 (s, 14H, H7), 8.02 (s, 7H, H15'), 8.10 (s, 7H, H15), **^{13}C NMR** (125 MHz, DMSO-d_6) δ (ppm) 25.7 (C2), 27.9 (C9), 28.2 (C11), 28.8 (C10), 29.7 (C13), 49.3 (C14), 60.0 (C6), 60.9 (C5), 63.6 (C17), 64,8 (C17'), 67.6 (C4), 68.2 (C2), 69.9 (C22), 73.0 (C19), 75.1 (C3), 79.2 (C21), 82.3 (C23), 100.6 (C20),112.6 (C7), 123.6 (C15'), 124.1 (C15), 141.3 (C8), 143.4 (C16'), 143.8 (C16), 145.8 (C1) *carbon C18 is not visible*, **HRMS** (ESI) m/z 1037.5147 ($[M + 3H]^{6+}$ calcd. For $C_{280}H_{426}N_{70}O_{91}$: 1037.5138).

Tetradecavalent cluster 26b

Product **26b** (11.4 mg, 0.0018 mmol, 89%) was obtained as an orange solid following general procedure B, starting from **25b** (17.7 mg, 0.0021 mmol).

m.p. 170 °C (decomp), **IR** (neat) 3342, 2927, 2857, 1735, 1460, 1370, 1225, 1085, 1044, 732 cm^{-1}, **^{1}H NMR** (500 MHz, DMSO-d_6) δ (ppm) 1.15-1.31 (m, 56H, H11 and H12), 1.43-1.52 (m, 28H, H10), 1.69-1.80 (m, 28H, H13), 2.31-2.41 (m, 28H, H9), 3.26-3.52 (m, 63H, H21, H23, H17, H17',H19 and H18), 3.62-3.71 (m, 42H, H3, H5 and H6a), 3.90-4.00 (m, 28H, H4 and H6b), 4.19-4.26 (m, 14H, H14), 4.27-4.34 (m, 14H, H14'), 4.36-4.43 (m, 7H, H22), 4.63 (s, 14H, H2), 4.83-4.92 (m, 7H, H20), 6.98 (s, 14H H7), 8.02 (s, 7H, H15'), 8.10 (s, 7H, H15), **^{13}C NMR** (125 MHz, DMSO-d_6) δ (ppm) 25.7, 25.8 (C12 and C12'), 27.4, 27.5 (C9 and C9'), 28.1, 28.3 (C11 and C11'), 28.6, 28.7 (C10 and C10'), 29.7 (C13), 49.2, 49.3 (C14 and C14'), 61.6 (C6), 62.3 (C5), 63.6 (C22), 64.0 (C2), 65.7 (C4), 69.8 (C18), 71.3 (C3), 72.3, 72.4 (C17 and C17'), 74.6 (C19), 79.1 (C21), 82.4 (C23), 100.2 (C20), 113.9 (C7), 123.6, 124.0 (C15 and C15'), 140.3 (C8), 143.3, 143.8 (C16 and C16'), 144.7 (C1), **HRMS** (ESI) m/z 622.9111 ($[M + 10H]^{10+}$ calcd. For $C_{280}H_{430}N_{70}O_{91}$: 622.9112).

Inhibition assay on α-mannosidase of Jack-bean

p-nitrophenyl-α-D-mannopyranoside and α-mannosidase (EC 3.2.1.24, from Jack bean) were purchased from Sigma Aldrich. Inhibition constants were determined by spectrophotometrically measuring the residual hydrolytic activities of the mannosidase against p-nitrophenyl-α-D-mannopyranoside in the presence and absence of inhibitor. Each well was filled with a total volume of 100 µL containing 0.2 M acetate buffer pH 5, inhibitor, substrate and enzyme. All kinetics are performed between 25 and 27 °C and started by enzyme addition. After 30-40 min incubation, the reaction was quenched by addition of 100 µL of 1M Na_2CO_3. The absorbance of the resulting solution was determined at 405 nm. K_i values were determined in duplicate or triplicate, using the Dixon and Lineweaver Burk graphical method with Microsoft Excel, or using non-linear regression with GraphPad Prism Software. When inhibitors were only partially soluble in water, stock solutions in 1:1 DMSO/buffer were prepared. The final DMSO content was under 2.5 %. The stability of the enzyme in presence of the same concentrations of DMSO was controlled and the enzyme activity was unaffected.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors are grateful to financial supports from the CNRS (UMR 7042), the University of Strasbourg, and the International Centre for Frontier Research in Chemistry (icFRC). M.M.P thank the French Department of Research for a doctoral fellowship. The authors express their gratitude to Robin Henches and Alexis Taponard for assistance with synthetic work and to Dr Camille Decroocq for the preparation of compound **24**.

Published on 21 May 2019. Downloaded by Syddansk Universitetsbibliotek on 5/22/2019 8:58:13 AM.

View Article Online DOI: 10.1039/C9OB00825H

Organic & Biomolecular Chemistry Accepted Manuscript

Please do not adjust margins

View Article Online
DOI: 10.1039/C9OB00826H
Published on 21 May 2019. Downloaded by Syddansk Universitetsbibliotek on 5/22/2019 8:58:13 AM.
Organic & Biomolecular Chemistry Accepted Manuscript

Notes and references

1 For reviews see: (a) P. Compain and A. Bodlenner, *ChemBioChem* **2014**, *15,* 1239-1251; (b) S. Gouin, *Chem. Eur. J.,* 2014, **20**, 11616-11628; (c) R. Zelli, J.-F. Longevial, P. Dumy and A. Marra, *New. J. Chem.,* 2015, **39**, 5050-5074; (d) N. Kanfar, E. Bartolami, R. Zelli, A. Marra, J.-Y. Winum and P. Dumy, *Org. Biomol. Chem.*, 2015, **13**, 9894-9906; (e) A. Matassini, C. Parmeggiani, F. Cardona and A. Goti, *Tetrahedron Lett.,* 2016, **57** 5407-5415; (f) J. M. Garcia Fernández, J.-F. Nierengarten and C. Ortiz Mellet, *J. Mater. Chem. B,* 2017, **5**, 6428-6436; (g) P. Compain, *Chem. Rec.,* 2019, *in press.*

2 J. Diot, M. I. Garcia-Moreno, S. G. Gouin, C. Ortiz Mellet, K. Haupt and J. Kovensky, *Org. Biomol. Chem.,* 2009, **7***,* 357-363.

3 P. Compain, C. Decroocq, J. Iehl, M. Holler, D. Hazelard, T. Mena Barragán, C. Ortiz Mellet and J.-F. Nierengarten, *Angew. Chem. Int. Ed.,* 2010, **49**, 5753-5756.

4 For recent reviews see: (a) T. M. Gloster and G. J. Davies, *Org. Biomol. Chem.,* 2010, **8**, 305-320; (b) A. E. Stütz and T. M. Wrodnigg, *Adv. Carbohydr. Biochem.,* 2011, **66**, 187-298; (c) U. Ghani, *Eur. J. Med. Chem.,* 2015, **103**, 133-162; (d) A. Singha, N. Mhlongoa and M. E. S. Soliman, *Anti-Cancer Agents Med. Chem.,* 2015, **15**, 933-946.

5 (a) G. J. Davies, T. M. Gloster and B. Henrissat, *Curr. Opin. Struct. Biol.,* 2005, **15**, 637–645; (b) G. J. Davies and B. Henrissat, *Structure,* 1995, **3**, 853-859.

6 E. Howard, A. Cousido-Siah, M. L. Lepage, J. P. Schneider, A. Bodlenner, A. Mitschler, A. Meli, I. Izzo, A. Alvarez, A. Podjarny and P. Compain, *Angew. Chem. Int. Ed.,* 2018*,* **57***,* 8002-8006.

7 M. L. Lepage, J. P. Schneider, A. Bodlenner, A. Meli, F. De Riccardis, M. Schmitt, C. Tarnus, N.-T. Nguyen-Huynh, Y.-N. Francois, E. Leize-Wagner, C. Birck, A. Cousido-Siah, A. Podjarny, I.Izzo and P. Compain, *Chem. Eur. J.,* 2016, **22**, 5151-5155.

8 S. Mirabella, G. D'Adamio, C. Matassini, A. Goti, S. Delgado, A. Gimeno, I. Robina, A. J. Moreno-Vargas, S. Šesták, J. Jimenez-Barbero and F. Cardona, *Chem. Eur. J.,* 2017, **23***,* 14585-14596.

9 (a) C. Decroocq, D. Rodríguez-Lucena, K. Ikeda, N. Asano and P. Compain, *ChemBioChem,* 2012, **13**, 661-664; (b) P. Compain, C. Decroocq, A. Joosten, J. de Sousa, D. Rodriguez-Lucena, T. D. Butters, J. Bertrand, R. Clément, C. Boinot, F. Becq and C. Norez, *ChemBioChem,* 2013, **14**, 2050-2058; (c) A. Joosten, C. Decroocq, J. de Sousa, J. Schneider, E. Etamé, A. Bodlenner, T. D. Butters and P. Compain*, ChemBioChem,* 2014, **15**, 309-319; (d) E. Laigre, D. Hazelard, J. Casas, J. Serra-Vinardell, H. Michelakakis, I. Mavridou, J. M. F. G. Aerts, A. Delgado and P. Compain, *Carbohydr. Res.,* 2016, **429**, 98–104.

10 (a) M. LI, K.-R. Wang, J.-X. Yang, Y.-T. Peng, Y.-X. Liu, H.-X. Zhang and X.-L. Li, *J. Mater. Chem. B,* 2019, **7**, 1379-1383; (b) J.-J. LI, K.-R. Wang, R.-F. Li, J.-X. Yang, M. LI, H.-X. Zhang, Z.-R. Cao and X.-L. Li, *J. Mater. Chem. B,* 2019, **7**, 1270-1275.

11 (a) C. Bonduelle, J. Huang, T. Mena-Barragán, C. Ortiz Mellet, C. Decroocq, E. Etamé, A. Heise, P. Compain and S. Lecommandoux, *Chem. Commun.,* 2014, **50***,* 3350-3352 ; (b) C. Matassini, M. Marradi, F. Cardona, C. Parmeggiani, I. Robina, A. J. Moreno-Vargas, S. Penadés and A. Goti, *RSC Adv.*, 2015, 5, 95817-95822; (c) D. Alvarez-Dorta, Y. Brissonnet, A. Saumonneau, D. Deniaud, J. Bernard, X. Yan, C. Tellier, F. Daligault and S. G. Gouin, *Chem. Select*, 2017, **2**, 9552-9556; (d) C. Matassini, C. Vanni, A. Goti, A. Morrone, M. Marradi and F. Cardona, *Org. Biomol. Chem.*, 2018, **16**, 8604-8612.

12 (a) Y. Brissonnet, S. Ladevèze, D. Tezé, E. Fabre, D. Deniaud, F. Daligault, C. Tellier, S. Šesták, M. Remaud-Simeon, G. Potocki-Veronese and S. G. Gouin, *Bioconjugate Chem.,* 2015, **26**, 766-772; (b) E. T. Sletten, R. S. Loka, F. Yu and H. M. Nguyen, *Biomacromol.,* 2017, **18**, 3387-3399.

13 R. Rísquez-Cuadro, J. M. García Fernández, J.-F. Nierengarten and C. Ortiz Mellet, *Chem Eur. J.,* 2013, **19***,* 16791-16803.

14 F. Stauffert, A. Bodlenner, T. M. N. Trinh, M. I. García-Moreno, C. Ortiz Mellet, J.-F. Nierengarten and P. Compain*, New J. Chem.,* 2016, **40**, 7421-7430.

15 C. Decroocq, D. Rodríguez-Lucena, V. Russo, T. Mena Barragán, C. Ortiz Mellet and P. Compain, *Chem. Eur. J.,* 2011, **17**, 13825-13831.

16 N. Panday and A. Vasella, *Synthesis,* 1999, 1459–1468.

17 M. Terinek and A. Vasella, *Helvetica Chimica Acta,* 2003, **86**, 3482–3509.

18 T. Granier, N. Panday and A. Vasella, *Helvetica Chimica Acta*, 1997, **80**, 979–987.

19 F. Stauffert, M. L. Lepage, M. M. Pichon, D. Hazelard, A. Bodlenner and P. Compain, *Synthesis* 2016, **48**, 1177–1180.

20 N. Panday and Y. Canac, A. Vasella, *Helvetica Chimica Acta,* 2000, **83**, 58–79.

21 T. Li, L. Guo, Y. Zhang, J. Wang, Z. Zhang, J. Li, W. Zhang, J. Lin, W. Zhao and P. G. Wang, *Bioorganic & Medicinal Chemistry* 2011, **19**, 2136–2144.

22 C. Decroocq, A. Joosten, R. Sergent, T. Mena Barragán, C. Ortiz Mellet and P. Compain, *ChemBioChem,* 2013, **14**, 2038-2049.

23 A. Mollard and I. Zharov, *Inorganic Chemistry,* 2006, ***45***, 10172–10179.

24 I. Papp, J. Dernedde, S. Enders and R. Haag, *Chem. Commun.,* 2008, 5851-5853.

25 A. Marra, R. Zelli, G. D'Orazio, B. La Ferla and A. Dondoni, *Tetrahedron*, 2014, **70**, 9387–9393.

26 S. F. Ortega-Caballero, J. J. Giménez-Martínez and A. Vargas-Berenguel, *Org. Lett.,* 2003, **5**, 2389-2392.

27 (a) J. M. Fominaya, J. M. García-Segura, M. Ferreras and J. G. Gavilanes, *Biochem. J.*, 1988, **253**, 517–522; (b) R. A. Copeland, *Enzymes: A Practical Introduction to Structure, Mechanism, and Data Analysis.*, Wiley-VCH, Inc., New York, 2000, p 305-317.

28 (a) W. W. Ackermann and V. R. Potter, *Proc. Soc. Exp. Biol. Med.*, 1949, **72**, 1–9; (b) J. F. Morrison, *Biochim. Biophys. Acta*, 1969, **185**, 269–86; (c) P. J. F. Henderson, *Biochem. J.*, 1972, **127**, 321–333; (d) S. Cha, *Biochem. Pharmacol.*, 1975, **24**, 2177–2185; (e) W. R. Greco and M. T. Hakala, *J. Biol. Chem.*, 1979, **254**, 12104–12109; (f) P. Kuzmič, K. C. Elrod, L. M. Cregar, S. Sideris, R. Rai and J. W. Janc, *Anal. Biochem.*, 2000, **286**, 45–50; (g) D. J. Murphy, *Anal. Biochem.*, 2004, **327**, 61–67; (h) P. Kuzmič (2015) History, variants and usage of the "Morrison equation" in *enzyme inhibition kinetics, BioKin Technical Note TN-2015-01*, BioKin Ltd., Watertown MA, www.biokin.com/TN/2015/01.

29 R. A. Copeland, *evaluation of enzyme inhibitors in drug discovery. A guide for Medicinal Chemists and Pharmacologists*, Wiley, John Wiley & Sons, Inc., 2013, p 253-282.

30 J. F. Nierengarten, J. P. Schneider, T. M. Nguyet Trinh, A. Joosten, M. Holler, M. L. Lepage, A. Bodlenner, M. I. García-Moreno, C. Ortiz Mellet and P. Compain, *Chem. Eur. J.*, 2018, **24**, 2483–2492.

31 R. Hoos, A. B. Naughton, W. Thiel, A. Vasella, W. Weber, K. Rupitz and S. G. Withers, *Helv. Chim. Acta*, 1993, **76**, 2666–2686.

32 H. Zuilhof, *Acc. Chem. Res.*, 2016, **49**, 274–285. and references cited there.

This journal is © The Royal Society of Chemistry 20xx

33 M. Ortega-Muñoz, F. Perez-Balderas, J. Morales-Sanfrutos, F. Hernandez-Mateo, J. Isac-García and F. Santoyo-Gonzalez, *Eur. J. Org. Chem.*, 2009, 2454–2473.

View Article Online
DOI: 10.1039/C9OB00826H

Published on 21 May 2019. Downloaded by Syddansk Universitetsbibliotek on 5/22/2019 8:58:13 AM.

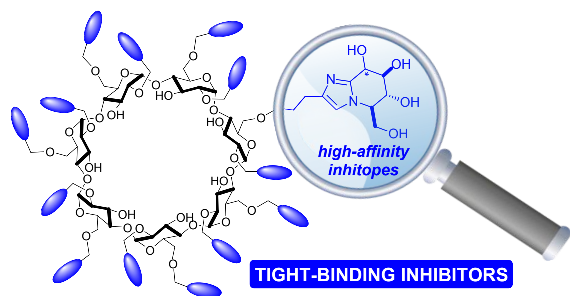
Organic & Biomolecular Chemistry Accepted Manuscript

Please do not adjust margins

View Article Online
DOI: 10.1039/C9OB00826H

Published on 21 May 2019. Downloaded by Syddansk Universitetsbibliotek on 5/22/2019 8:58:13 AM.

Organic & Biomolecular Chemistry Accepted Manuscript



Examples of multimeric inhibitors displaying tight binding inhibition of a carbohydrate-processing enzyme are presented.