Synthesis of heteroglycoclusters by using orthogonal chemoselective ligations

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Letter

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Abstract

Synthetic heteroglycoclusters are being subjected to increasing interest due to their potential to serve as selective ligands for carbohydrate-binding proteins. In this paper, we describe an expedient strategy to prepare cyclopeptides displaying well-defined distributions and combinations of carbohydrates. By using both oxime ligation and copper(I)-catalyzed alkyne–azide cycloaddition, two series of compounds bearing binary combinations of αMan, αFuc or βLac in an overall tetravalent presentation, and either 2:2 or 3:1 relative proportions, have been prepared.

Introduction

Multivalent interactions between carbohydrates and proteins play key roles in diverse biological events, including fertilization, cell–cell communication, host–pathogen interactions, immune response and cancer metastasis [1]. Synthetic molecules displaying multiple copies of a sugar binding motif, called (homo)glycoclusters, represent attractive tools for studying these complex recognition processes as well as for developing biological applications, for example, the inhibition of infections by pathogens such as viruses or bacteria [2-5]. In a suitable density and spatial display, clusters of carbohydrate indeed allow multiple contacts with a target protein, thus increasing avidity by means of the "glycoside cluster effect" [6,7]. While the recent progress in glycomics has led to the design of glycoclusters active at nanomolar concentration [8-10], the achievement of selective binding remains challenging because of the close structural similarities of the binding sites of proteins specific for the same carbohydrate moiety.
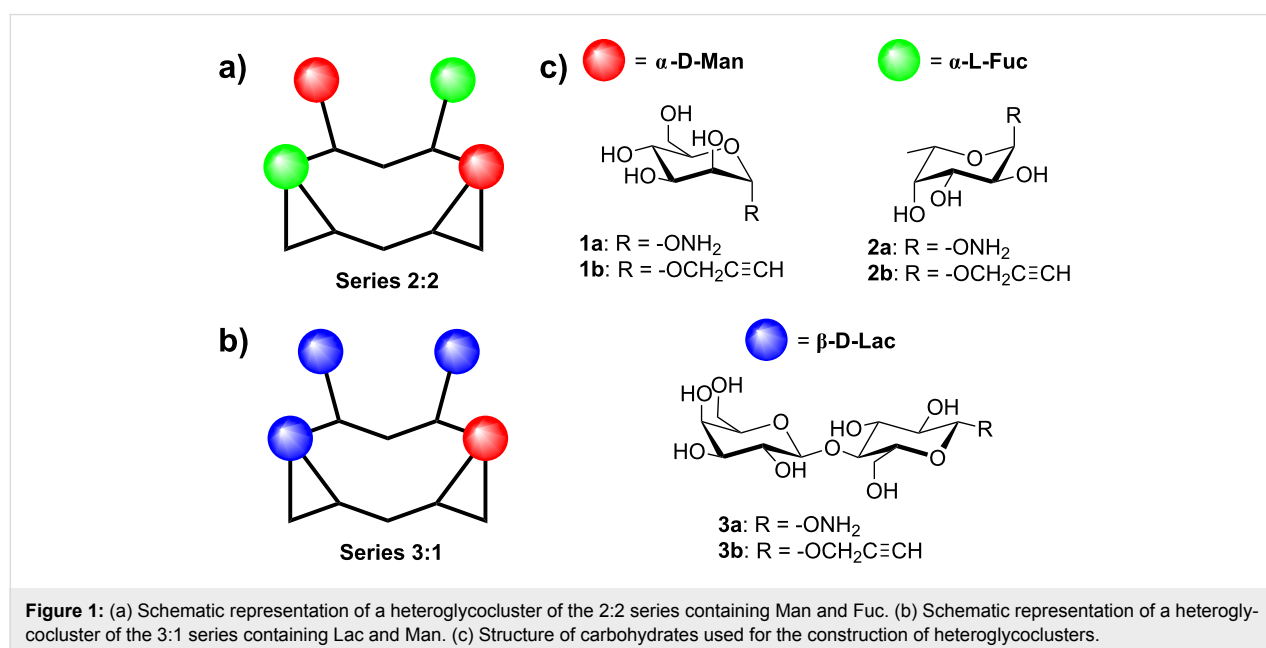
Interestingly, recent reports have highlighted that the association of different sugar units instead of a single motif, namely heteroglycocluster, reflects the presence of sugars found in native biological systems more closely than homoglyco-cluster does. Although recognition mechanisms are not fully understood, these studies suggest that heteroglycoclusters should interact with proteins through distinct binding sites, which may influence both affinity and selectivity [11-25]. In this context, we previously reported a combinatorial procedure to prepare libraries of heteroglycoclusters displaying sugars and/or amino acids at randomized positions on a topological cyclopeptide scaffold [26]. Deconvolution of the resulting libraries by affinity chromatography allowed the selection of heteroglycoclusters that were proved to be useful for exploring the surrounding regions of the binding pocket in a model lectin. Although it is easy to handle, this combinatorial procedure leads to the formation of inseparable mixtures of regioisomers, which precludes their utilization for further assays with relevant bio-logical targets. In order to circumvent this drawback, we herein report the synthesis of similar heteroglycoclusters by using a protocol based on orthogonal chemoselective ligations. Two series of compounds containing different combinations of two different sugars have been designed (Figure 1). In one series (heteroglycocluster 2:2), two αMan, αFuc and/or βLac are conjugated at alternate positions into the tetravalent cyclopep-tide sequence, whereas the second series (heteroglycocluster 3:1) contains one of a given sugar and three of another.
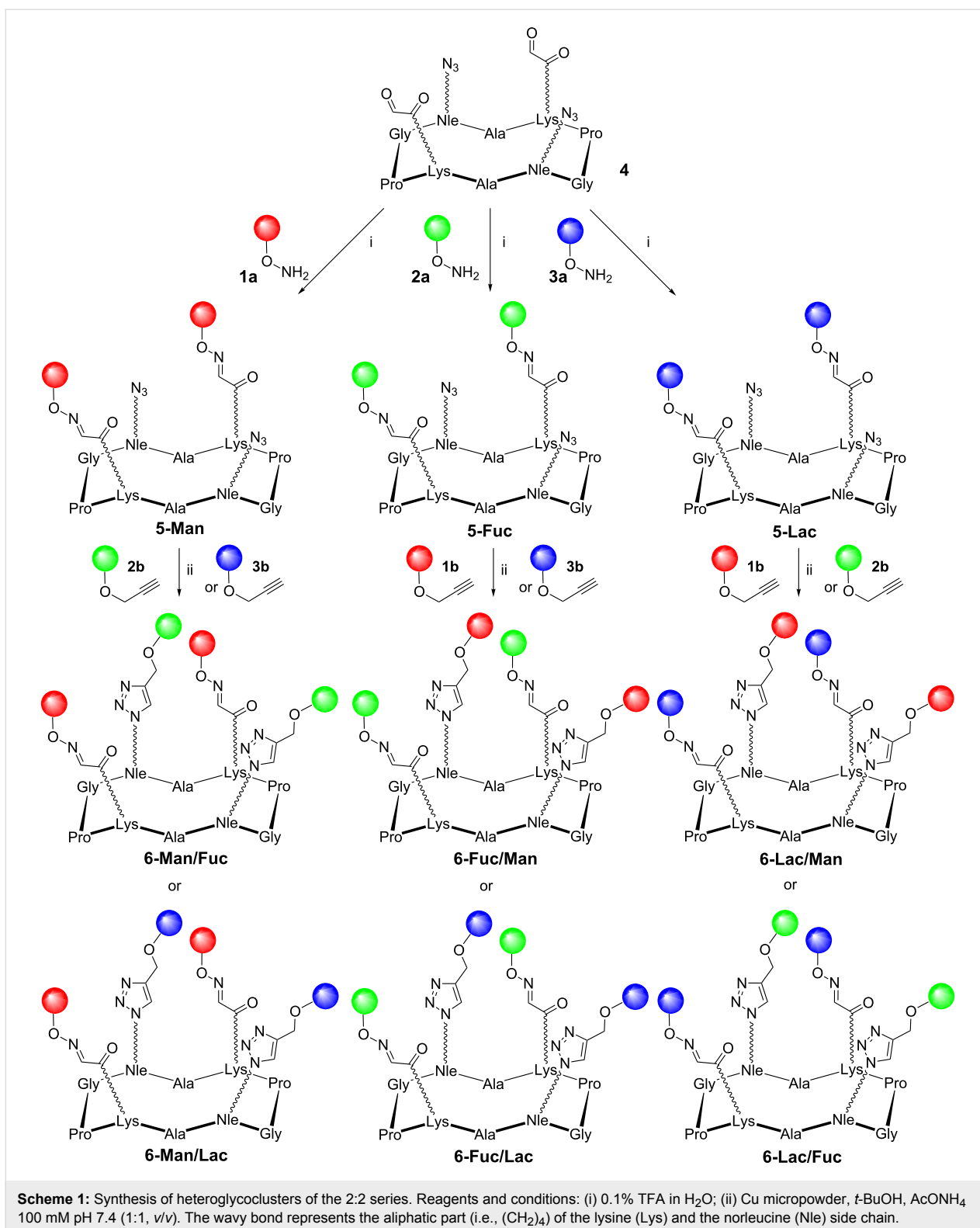
Results and Discussion

Glycoclusters are classically constructed from a molecular scaf-fold containing multiple anchoring sites that can be functional-ized with sugars by using a single coupling reaction. By contrast, the chemical access of heteroglycoclusters is not trivial because it requires the controlled conjugation of different sugars at a precise position into the scaffold to obtain a well-defined distribution. Taking advantage of our experience in bioconjuga-tion methods, we decided to explore two chemoselective strate-gies to achieve this purpose. We first selected the oxime liga-tion since we have previously used this approach successfully for the preparation of sophisticated molecular systems, such as synthetic vaccines [27,28], immunomodulators [29], lectin ligands [30] or vectors of hepatocytes [31]. As the second strategy, we have chosen the well-known copper(I)-catalyzed alkyne–azide cycloaddition (CuAAC) [32,33], which is inten-sively exploited for the conjugation of sugars to both molecular and biological systems [34,35]. Besides being fully compatible with carbohydrate and peptide chemistries, oximation and CuAAC reactions offer the advantage of being orthogonal [36,37], therefore allowing a controlled assembly process with a minimized risk of side reactions. The 2:2 series of heteroglyco-clusters was prepared from the aminooxy [38-40] and propargyl [41] glycosides **1-3** (Figure 1) and the cyclopeptide **4** (Scheme 1). This scaffold containing two lysines (Lys) func-tionalized with an aldehyde and two norleucines (Nle) bearing an azide group was prepared by using a strategy adapted from the procedure described previously [42].

In the first step, two copies of aminooxy αMan **1a**, αFuc **2a** and βLac **3a** were coupled to **4**, affording divalent oxime-linked compounds **5-Man**, **5-Fuc**, and **5-Lac**, respectively. The oxime ligation was performed at 37 °C in aqueous acidic buffer with 2 equiv of sugars per anchoring site. After 3 h, complete reac-

Figure 1: (a) Schematic representation of a heteroglycocluster of the 2:2 series containing Man and Fuc. (b) Schematic representation of a heterogly-cocluster of the 3:1 series containing Lac and Man. (c) Structure of carbohydrates used for the construction of heteroglycoclusters.

Scheme 1: Synthesis of heteroglycoclusters of the 2:2 series. Reagents and conditions: (i) 0.1% TFA in H$_2$O; (ii) Cu micropowder, *t*-BuOH, AcONH$_4$ 100 mM pH 7.4 (1:1, *v/v*). The wavy bond represents the aliphatic part (i.e., (CH$_2$)$_4$) of the lysine (Lys) and the norleucine (Nle) side chain.

tions were observed by analytical HPLC. The excess of sugar was then quenched by the addition of acetone, and the resulting crude mixtures were used for CuAAC without further purification. The efficiency of CuAAC clearly depends on the experimental conditions [34,35]. The choice of the solvent and of the copper(I) catalyst (delivered either using CuI, copper micropowder, or CuSO$_4$ and sodium ascorbate as reducing agent) and the utilization of microwave or ultrasonic irradiation are para-

meters that can influence the reaction kinetics, improve the yields and sometimes prevent side reactions. In a previous study, we observed that a tetravalent glycocluster can be obtained in good yields and as a unique 1,4-regioisomer by using a catalytic amount of copper micropowder in a mixture of isopropanol and ammonium acetate buffer [42]. Therefore we decided to follow similar conditions in this study with propargyl glycosides αMan **1b**, αFuc **2b** and βLac **3b**. Here again, each reaction occurred quantitatively, as shown in RP-HPLC profiles of the crude reaction mixtures (see Supporting Information File 1). After removal of solid copper by filtration and semi-preparative HPLC, six tetravalent heteroglycoclusters combining two sugars (e.g., **6-Man/Fuc**, **6-Man/Lac**, **6-Fuc/Man**, **6-Fuc/Lac**, **6-Lac/Man** and **6-Lac/Fuc**) were obtained in excellent conversion rate and purity and unambiguously characterized by mass spectrometry (Table 1).

To demonstrate the reliability of our protocol, a new series of 3:1 heteroglycoclusters was prepared from the same carbohydrate building blocks and the cyclopeptide **7**. Similar experimental conditions were followed, with the exception of the stoichiometry of reagents (see Experimental section). In this series, one oxime linkage was formed from **7** by using aminooxy αMan **1a**, αFuc **2a** and βLac **3a**, and three CuAAC reactions were subsequently performed with propargyl glycosides αMan **1b**, αFuc **2b** and βLac **3b** as described above (Scheme 2).

No difference in reactivity from the previous 2:2 series was observed, thereby confirming the efficiency of this strategy for the preparation of well-defined heteroglycoclusters.

Conclusion

In this paper we have described an expedient and controlled assembly protocol to prepare heteroglycoclusters similar to those obtained previously from randomized combinatorial libraries [26]. Following two orthogonal chemoselective reactions, cyclopeptides **4** and **7** were successively reacted with aminooxy glycosyls **1–3a** and propargyl glycosides **1–3b.** Twelve novel oxime and triazole linked-heteroglycoclusters displaying well-defined distributions and combinations of carbohydrates were thus obtained in excellent yields and purity.

Experimental
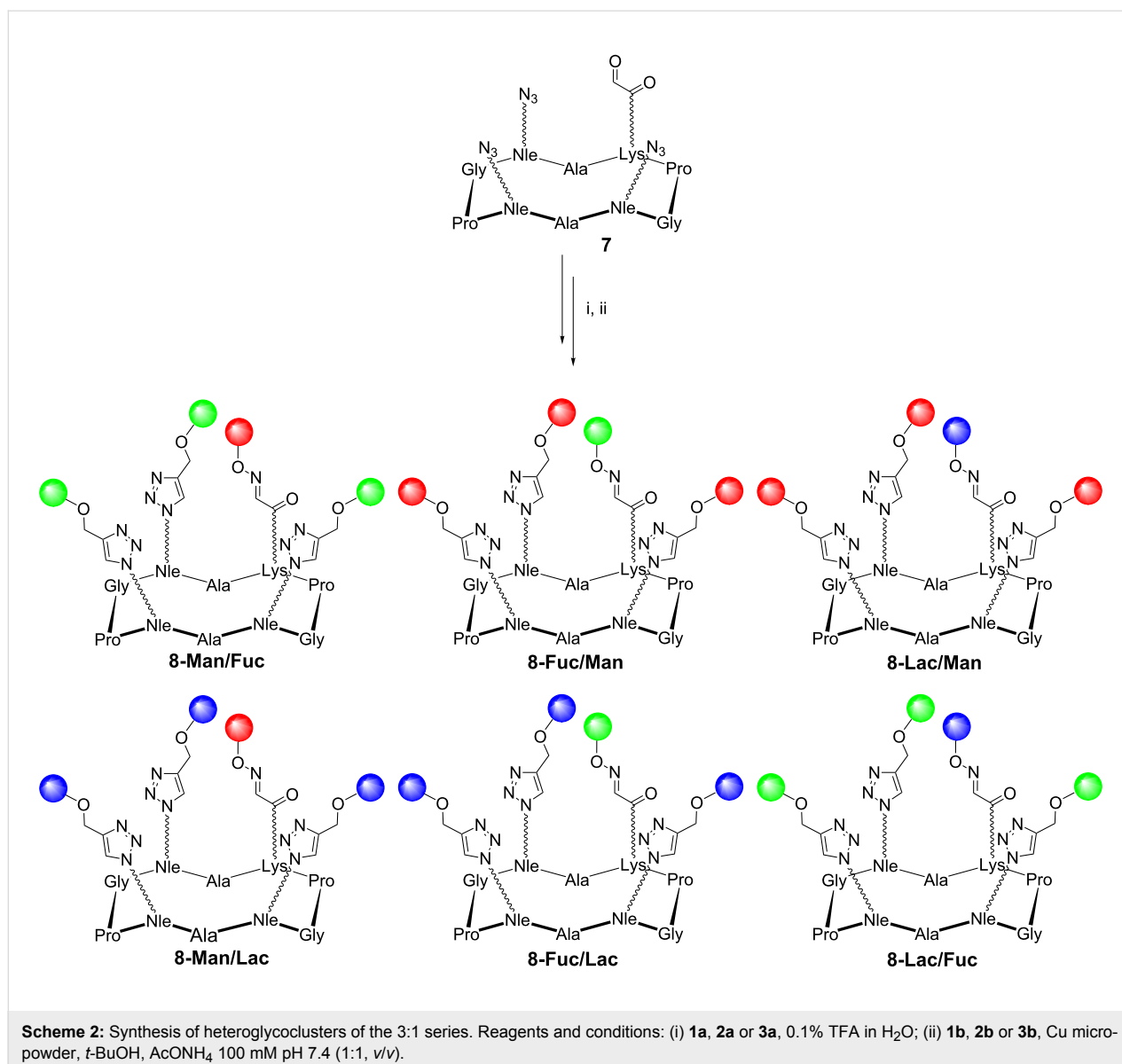### Standard procedures for the heteroglycocluster assembly
Series 2:2

A solution of **4** (4.0 mg, 3.4 μmol) and **2a** (2.6 mg, 14.2 μmol, 4 equiv) was stirred at 37 °C in 0.1% TFA in H_2O (400 μL). After 3 h, analytical HPLC revealed the total conversion of **4a** into **5-Fuc**. Analytical HPLC: t_R 9.34 min (gradient: 5 to 100% B in 20 min); ESI$^+$–MS (*m/z*): [M + Na]$^+$ calcd for $C_{60}H_{96}N_{20}O_{22}Na$, 1471.7; found, 1471.8. After the addition of acetone (100 μL) to the crude, the reaction mixture was lyophilized, then resuspended with *t*-BuOH/AcONH$_4$ 100 mM pH 7.4 (500 μL, 1:1 *v/v*). Compound **1b** (4.0 mg, 10.5 μmol, 3 equiv) and copper micropowder (455 μg, 7.0 μmol) were next added to the solution and the resulting mixture was left under stirring at room temperature. After 4 h, copper was removed by centrifugation and the supernatant purified by semipreparative HPLC. Compound **6-Fuc/Man** was obtained in 91% yield

Table 1: Outcome of the orthogonal ligation procedure.

compound	yield[a]	MS calcd[b]	MS found[c]	t_R (min)[d]
6-Man/Fuc	83%	1885.9 ($C_{78}H_{124}N_{20}O_{34}$)	1886.0	7.79
6-Man/Lac	99%	2242.9 ($C_{90}H_{145}N_{20}O_{46}$)	2242.3	7.43
6-Fuc/Man	98%	1885.9 ($C_{78}H_{124}N_{20}O_{34}$)	1886.0	7.73
6-Fuc/Lac	98%	2210.0 ($C_{90}H_{145}N_{20}O_{44}$)	2210.3	7.62
6-Lac/Man	94%	2242.9 ($C_{90}H_{145}N_{20}O_{46}$)	2242.2	7.31
6-Lac/Fuc	93%	2210.0 ($C_{90}H_{145}N_{20}O_{44}$)	2210.3	7.60
8-Man/Fuc	85%	1880.9 ($C_{79}H_{126}N_{21}O_{32}$)	1881.1	7.88
8-Man/Lac	87%	2415.0 ($C_{97}H_{156}N_{21}O_{50}$)	2415.4	7.30
8-Fuc/Man	91%	1912.9 ($C_{79}H_{126}N_{21}O_{34}$)	1913.2	7.66
8-Fuc/Lac	90%	2400.0 ($C_{97}H_{156}N_{21}O_{49}$)	2399.3	7.46
8-Lac/Man	89%	2092.0 ($C_{85}H_{136}N_{21}O_{40}$)	2091.2	7.46
8-Lac/Fuc	91%	2043.9 ($C_{85}H_{136}N_{21}O_{37}$)	2043.1	7.83

[a]Yields were calculated by integrating the peak corresponding to the expected compound in the crude HPLC profile. Isolated yields are given in the Experimental section. [b]Calculated mass for [M + H]$^+$. [c]MS analysis was performed by electrospray ionization method in positive mode. [d]RP-HPLC retention time using a linear gradient A/B, 95:5 to 0:100 in 20 min, flow: 1.0 mL/ min, λ = 214 nm and 250 nm (column: nucleosil 300-5 C$_{18}$; solvent A: 0.09% TFA in H_2O, solvent B: 0.09% TFA in 90% acetonitrile).

Scheme 2: Synthesis of heteroglycoclusters of the 3:1 series. Reagents and conditions: (i) **1a**, **2a** or **3a**, 0.1% TFA in H$_2$O; (ii) **1b**, **2b** or **3b**, Cu micropowder, *t*-BuOH, AcONH$_4$ 100 mM pH 7.4 (1:1, *v/v*).

(5.9 mg). Analytical RP-HPLC: t_R 7.73 min (gradient: 5 to 100% B in 20 min); ^{1}H NMR (400 MHz, D$_2$O) δ 8.07 (s, 1H, H$_{trz}$), 8.06 (s, 1H, H$_{trz}$), 7.81 (s, 1H, H$_{ox}$), 7.80 (s, 1H, H$_{ox}$), 5.62 (d, $J_{1,2}$ = 4.0 Hz, 1H, H-1$_{Fuc}$), 5.60 (d, $J_{1,2}$ = 4.0 Hz, 1H, H-1$_{Fuc}$), 4.99 (bs, 2H, 2H-1$_{Man}$), 4.86–4.68 (m, 6H, 2H$_{αLys/Nle}$, 2CH$_{2proparpyl}$), 4.47–4.35 (m, 10H, 2H$_{αLys/Nle}$, 2CH$_{2εNle}$, 2H$_{αAla}$, 2H$_{αPro}$), 4.16–3.63 (m, 28H, 2CH$_{2αGly}$, 2CH$_{2δPro}$, 2H-2$_{Fuc}$, 2H-3$_{Fuc}$, 2H-4$_{Fuc}$, 2H-5$_{Fuc}$, 2H-2$_{Man}$, 2H-3$_{Man}$, 2H-4$_{Man}$, 2H-5$_{Man}$, 2CH$_2$-6$_{Man}$), 3.36–3.20 (m, 4H, 4H$_{εLys}$), 2.39–2.29 (m, 2H, 2H$_{βPro}$), 2.16–1.27 (m, 36H, 4CH$_{2βLys/Nle}$, 4CH$_{2δLys/Nle}$, 4CH$_{2γLys/Nle}$, 2H$_{βPro}$, 2CH$_{2γPro}$, 2CH$_{3Ala}$), 1.22 (d, $J_{5,6}$ = 6.0 Hz, 6H, 2CH$_{3Fuc}$); ESI$^+$–MS (*m/z*): [M + H]$^+$ calcd for C$_{78}$H$_{124}$N$_{20}$O$_{34}$, 1885.9; found, 1886.0. Compounds **6-Man/Fuc**, **6-Man/Lac**, **6-Fuc/Lac**, **6-Lac/Man** and **6-Lac/Fuc** were prepared following the same experimental conditions.

Series 3:1

A solution of **7** (6.6 mg, 5.9 μmol) and **2a** (2.2 mg, 1.2 μmol, 2 equiv) was stirred at 37 °C in 0.1% TFA in H$_2$O (600 μL). After 3 h, analytical HPLC revealed the total conversion of **7** into the corresponding monovalent intermediate. Analytical HPLC t_R 10.57 min (gradient: 5 to 100% B in 20 min); ESI$^+$–MS (*m/z*): [M + H]$^+$ calcd for C$_{52}$H$_{84}$N$_{21}$O$_{17}$, 1274.6; found, 1274.8. After the addition of acetone (100 μL) to the crude, the reaction mixture was lyophilized, then resuspended with *t*-BuOH/AcNH$_4$ 100 mM pH 7.4 (600 μL, 1:1 *v/v*). Compound **1b** (10 mg, 27 μmol, 4.5 equiv) and copper micropowder (600 μg, 10 μmol) were next added to the solution and the resulting mixture was left under stirring at room temperature. After 4 h, copper was removed by centrifugation and the supernatant purified by semipreparative HPLC. Compound

8-Fuc/Man was obtained in 88% yield (10.0 mg). Analytical RP-HPLC: t_R 7.66 min (gradient: 5 to 100% B in 20 min); ^{1}H NMR (400 MHz, D_2O) δ 8.05 (s, 1H, H_{trz}), 8.02 (s, 2H, H_{trz}), 7.79 (s, 1H, H_{ox}), 5.61 (d, $J_{1,2}$ = 4.0 Hz, 1H, H-1$_{Fuc}$), 5.60 (d, $J_{1,2}$ = 4.0 Hz, 1H, H-1$_{Fuc}$), 4.99 (bs, 3H, 3H-1$_{Man}$), 4.85–4.66 (m, 8H, 2H$_{\alpha Lys/Nle}$, 3CH$_{2propargyl}$), 4.48–4.32 (m, 12H, 2H$_{\alpha Lys/Nle}$, 3CH$_{2\varepsilon Nle}$, 2H$_{\alpha Ala}$, 2H$_{\alpha Pro}$), 4.15–3.64 (m, 30H, 2CH$_{2\alpha Gly}$, 2CH$_{2\delta Pro}$, H-2$_{Fuc}$, H-3$_{Fuc}$, H-4$_{Fuc}$, H-5$_{Fuc}$, 3H-2$_{Man}$, 3H-3$_{Man}$, 3H-4$_{Man}$, 3H-5$_{Man}$, 3CH$_2$-6$_{Man}$), 3.29–3.19 (m, 2H, 2H$_{\varepsilon Lys}$), 2.40–2.30 (m, 2H, 2H$_{\beta Pro}$), 2.15–1.28 (m, 36H, 4CH$_{2\beta Lys/Nle}$, 4CH$_{2\delta Lys/Nle}$, 4CH$_{2\gamma Lys/Nle}$, 2H$_{\beta Pro}$, 2CH$_{2\gamma Pro}$, 2CH$_{3Ala}$), 1.22 (d, $J_{5,6}$ = 6.6 Hz, 3H, CH$_{3Fuc}$); ESI$^+$–MS (*m/z*): [M + H]$^+$ calcd for C$_{79}$H$_{126}$N$_{21}$O$_{34}$, 1912.9; found, 1913.2. Compounds **8-Man/Fuc**, **8-Man/Lac**, **8-Fuc/Lac**, **8-Lac/Man** and **8-Lac/Fuc** were prepared under the same experimental conditions.

Supporting Information

The Supporting Information file contains analytical details of all heteroglycoclusters of series 2:2 (**6-Man/Fuc**, **6-Man/Lac**, **6-Fuc/Man**, **6-Fuc/Lac**, **6-Lac/Man** and **6-Lac/Fuc**) and 3:1 (**8-Man/Fuc**, **8-Man/Lac**, **8-Fuc/Man**, **8-Fuc/Lac**, **8-Lac/Man** and **8-Lac/Fuc**) described in this article.

Supporting Information File 1
Crude RP-HPLC profiles and ESI-MS spectra for the heteroglycoclusters.
[http://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-8-47-S1.pdf]

Acknowledgements

References

1. Varki, A. *Glycobiology* **1993,** *3,* 97–130. doi:10.1093/glycob/3.2.97
2. Chabre, Y. M.; Roy, R. *Adv. Carbohydr. Chem. Biochem.* **2010,** *63,* 165–393. doi:10.1016/S0065-2318(10)63006-5
3. Imberty, A.; Chabre, Y. M.; Roy, R. *Chem.–Eur. J.* **2008,** *14,* 7490–7499. doi:10.1002/chem.200800700
4. Pieters, R. J. *Org. Biomol. Chem.* **2009,** *7,* 2013–2025. doi:10.1039/b901828j
5. Renaudet, O.; Spinelli, N., Eds. *Synthesis and biological applications of glycoconjugates;* Bentham Science Publishers Ltd.: U.A.E., 2011.
6. Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995,** *28,* 321–327. doi:10.1021/ar00056a001
7. Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002,** *102,* 555–578. doi:10.1021/cr000418f
8. Kitov, P. I.; Sadowska, J. M.; Mulvey, G.; Armstrong, G. D.; Ling, H.; Pannu, N. S.; Read, R. J.; Bundle, D. R. *Nature* **2000,** *403,* 669–672. doi:10.1038/35001095
9. Touaibia, M.; Wellens, A.; Shiao, T. C.; Wang, Q.; Sirois, S.; Bouckaert, J.; Roy, R. *ChemMedChem* **2007,** *2,* 1190–1201. doi:10.1002/cmdc.200700063
10. Joosten, J. A. F.; Loimaranta, V.; Appeldoorn, C. C. M.; Haataja, S.; El Maate, F. A.; Liskamp, R. M. J.; Finne, J.; Pieters, R. J. *J. Med. Chem.* **2004,** *47,* 6499–6508. doi:10.1021/jm049476+
11. Horan, N.; Yan, L.; Isobe, H.; Whitesides, G. M.; Kahne, D. *Proc. Natl. Acad. Sci. U. S. A.* **1999,** *96,* 11782–11786. doi:10.1073/pnas.96.21.11782
12. Gómez-García, M.; Benito, J. M.; Rodríguez-Lucena, D.; Yu, J.-X.; Chmurski, K.; Ortiz Mellet, C.; Gutiérrez Gallego, R.; Maestre, A.; Defaye, J.; Garcia Fernández, J. M. *J. Am. Chem. Soc.* **2005,** *127,* 7970–7971. doi:10.1021/ja050934t
13. Ramström, O.; Lehn, J.-M. *ChemBioChem* **2000,** *1,* 41–48. doi:10.1002/1439-7633(20000703)1:1<41::AID-CBIC41>3.0.CO;2-L
14. Ramström, O.; Lohmann, S.; Bunyapaiboonsri, T.; Lehn, J.-M. *Chem.–Eur. J.* **2004,** *10,* 1711–1715. doi:10.1002/chem.200305551
15. Gómez-García, M.; Benito, J. M.; Gutiérrez-Gallego, R.; Maestre, A.; Ortiz Mellet, C.; García Fernández, J. M.; Jiménez Blanco, J. L. *Org. Biomol. Chem.* **2010,** *8,* 1849–1860. doi:10.1039/b920048g
16. Ortega-Muñoz, M.; Perez-Balderas, F.; Morales-Sanfrutos, J.; Hernandez-Mateo, F.; Isac-García, J.; Santoyo-Gonzalez, F. *Eur. J. Org. Chem.* **2009,** 2454–2473. doi:10.1002/ejoc.200801169
17. Deguise, I.; Lagnoux, D.; Roy, R. *New J. Chem.* **2007,** *31,* 1321–1331.
18. Katajisto, J.; Karskela, T.; Heinonen, P.; Lönnberg, H. *J. Org. Chem.* **2002,** *67,* 7995–8001. doi:10.1021/jo026053b
19. Patel, A.; Lindhorst, T. K. *Eur. J. Org. Chem.* **2002,** 79–86. doi:10.1002/1099-0690(20021)2002:1<79::AID-EJOC79>3.0.CO;2-1
20. Geng, J.; Mantovani, G.; Tao, L.; Nicolas, J.; Chen, G.; Wallis, R.; Mitchell, D. A.; Johnson, B. R. G.; Evans, S. D.; Haddleton, D. M. *J. Am. Chem. Soc.* **2007,** *129,* 15156–15163. doi:10.1021/ja072999x
21. Ladmiral, V.; Mantovani, G.; Clarkson, G. J.; Cauet, S.; Irwin, J. L.; Haddleton, D. M. *J. Am. Chem. Soc.* **2006,** *128,* 4823–4830. doi:10.1021/ja058364k
22. Fiore, M.; Chambery, A.; Marra, A.; Dondoni, A. *Org. Biomol. Chem.* **2009,** *7,* 3910–3913. doi:10.1039/b912686d
23. Lindhorst, T. K.; Bruegge, K.; Fuchs, A.; Sperling, O. *Beilstein J. Org. Chem.* **2010,** *6,* 801–809. doi:10.3762/bjoc.6.90
24. Liang, C.-H.; Wang, S.-K.; Lin, C.-W.; Wang, C.-C.; Wong, C.-H.; Wu, C.-Y. *Angew. Chem., Int. Ed.* **2011,** *50,* 1608–1612. doi:10.1002/anie.201003482
25. Gómez-García, M.; Benito, J. M.; Butera, A. P.; Ortiz Mellet, C.; García Fernández, J. M.; Jiménez Blanco, J. L. *J. Org. Chem.* **2012,** *77,* 1273–1288. doi:10.1021/jo201797b
26. Duléry, V.; Renaudet, O.; Wilczewski, M.; Van der Heyden, A.; Labbé, P.; Dumy, P. *J. Comb. Chem.* **2008,** *10,* 368–371. doi:10.1021/cc800029v
27. Renaudet, O.; BenMohamed, L.; Dasgupta, G.; Bettahi, I.; Dumy, P. *ChemMedChem* **2008,** *3,* 737–741. doi:10.1002/cmdc.200700315

28. Renaudet, O.; Dasgupta, G.; Bettahi, I.; Shi, A.; Nesburn, A. B.;
Dumy, P.; BenMohamed, L. *PLoS One* **2010,** *5,* e11216.
doi:10.1371/journal.pone.0011216

29. Renaudet, O.; Křenek, K.; Bossu, I.; Dumy, P.; Kádek, A.; Adámek, D.;
Vaněk, O.; Kavan, D.; Gažák, R.; Šulc, M.; Bezouška, K.; Křen, V.
J. Am. Chem. Soc. **2010,** *132,* 6800–6808. doi:10.1021/ja101296t

30. Bossu, I.; Šulc, M.; Křenek, K.; Dufour, E.; Garcia, J.; Berthet, N.;
Dumy, P.; Křen, V.; Renaudet, O. *Org. Biomol. Chem.* **2011,** *9,*
1948–1959. doi:10.1039/c0ob00772b

31. Pujol, A. M.; Cuillel, M.; Renaudet, O.; Lebrun, C.; Charbonnier, P.;
Cassio, D.; Gateau, C.; Dumy, P.; Mintz, E.; Delangle, P.
J. Am. Chem. Soc. **2011,** *133,* 286–296. doi:10.1021/ja106206z

32. Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002,** *67,*
3057–3064. doi:10.1021/jo011148j

33. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B.
Angew. Chem., Int. Ed. **2002,** *41,* 2596–2599.
doi:10.1002/1521-3773(20020715)41:14<2596::AID-ANIE2596>3.0.CO
;2-4

34. Dondoni, A. *Chem.–Asian J.* **2007,** *2,* 700–708.
doi:10.1002/asia.200700015

35. Jewett, J. C.; Bertozzi, C. R. *Chem. Soc. Rev.* **2010,** *39,* 1272–1279.
doi:10.1039/b901970g

36. Galibert, M.; Renaudet, O.; Dumy, P.; Boturyn, D.
Angew. Chem., Int. Ed. **2011,** *50,* 1901–1904.
doi:10.1002/anie.201006867

37. Clavé, G.; Volland, H.; Flaender, M.; Gasparutto, D.; Romieu, A.;
Renard, P.-Y. *Org. Biomol. Chem.* **2010,** *8,* 4329–4345.
doi:10.1039/c0ob00133c

38. Cao, S.; Tropper, F. D.; Roy, R. *Tetrahedron* **1995,** *51,* 6679–6686.
doi:10.1016/0040-4020(95)00325-3

39. Renaudet, O.; Dumy, P. *Org. Biomol. Chem.* **2006,** *4,* 2628–2636.
doi:10.1039/b604391g

40. Duléry, V.; Renaudet, O.; Philouze, C.; Dumy, P. *Carbohydr. Res.*
2007, *342,* 894–900. doi:10.1016/j.carres.2007.02.003

41. Fernandez-Megia, E.; Correa, J.; Rodriguez-Meizoso, I.; Riguera, R.
Macromolecules **2006,** *39,* 2113–2120. doi:10.1021/ma052448w

42. Bossu, I.; Berthet, N.; Dumy, P.; Renaudet, O. *J. Carbohydr. Chem.*
2011, *30,* 458–468. doi:10.1080/07328303.2011.590260