Tri- and hexavalent mannoside clusters as potential inhibitors of type 1 fimbriated bacteria using pentaerythritol and triazole linkages

Mohamed Touaibia, Tze Chieh Shiao, Alex Papadopoulos, Jonathan Vaucher, Qingan Wang, Karima Benhamioud and René Roy*

Received (in Cambridge, UK) 30th August 2006, Accepted 31st October 2006
First published as an Advance Article on the web 23rd November 2006
DOI: 10.1039/b612471b

Several oligomannoside clusters having a hundred-fold increase in affinities toward *E. coli* were synthesized by Cu(I)-catalyzed [1,3]-dipolar cycloadditions using pentaerythritol scaffolds bearing either alkyne or azide functionalities.

In many cases, adhesions of pathogens to host tissues constitute the prerequisite for infections.[1] The initial contacts are mediated by generally weak carbohydrate–protein interactions that are amplified by multiple copies of both the ligands and the receptors.[2] One such important family of bacterial receptors is part of the so-called type 1 fimbriae (FimH) at the surface of *Escherichia coli* that utilize carbohydrates for specific adhesion of the bacteria to the host cells glycocalyx. X-Ray crystal-structure studies have recently revealed that the lectin domain of *E. coli* FimH possesses a carbohydrate recognition domain (CRD) at its tip, which can accommodate one α-D-mannopyranoside residue.[3,4]

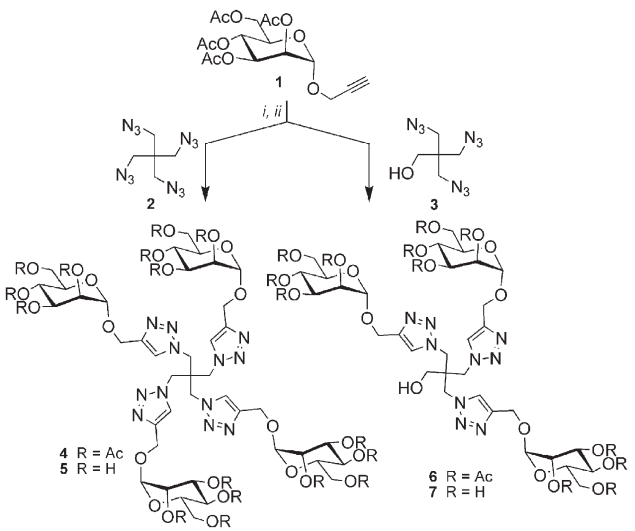
Toward our ongoing research program aimed at the synthesis and biological evaluation of multivalent glycomimetic inhibitors against bacterial adhesion,[5] we choose pentaerythritol and dipentaerythritol-based mannopyranosides. Pentaerythritol is an interesting molecule that allows the attachment of four identical or different groups, two pairs of which being tilted at 90°. Hence, it serves as a versatile scaffold for the construction of highly branched structures.[6–8] For instance, galabiosides built on pentaerythritol clusters proved to be excellent *in vitro* inhibitors against the hemagglutination of the Gram-positive bacterium *Streptococcus suis* at low nanomolar concentrations.[9] Inhibition of bacterial adhesion of fimbriated *E. coli.* to α-D-mannopyranoside residues has also been often addressed, albeit without systematic structure–activity relationships.[10–12]

Additionally, viral infections by Ebola and HIV-1 can also be impeded by hyperbranched mannoside dendrimers.[13,14] Properly designed inhibitors of these interactions may thus potentially represent new antiviral therapies. Consequently, we describe herein the efficient and systematic synthesis of a family of mannoside clusters built on pentaerythritol scaffolds using click chemistry. We decided to explore the recently described Cu(I)-catalysed azide–alkyne [1,3]-dipolar cycloaddition,[15] as it proceeds in high yields and with complete regioselectivity with multivalent systems.[16]

A retrosynthetic analysis reveals two possibilities for the synthesis of mannoside clusters bearing 1,2,3-triazole linkages: the azide or the alkyne functions can be located on either the pentaerythritol core or on the mannoside moiety. Thus, treatment of azides **2**[17] and **3**[18] with prop-2-ynyl α-D-mannopyranoside **1**[19] using Cu(I) catalysed click reactions, provided tetramer **5** and trimer **7** in good yields after deacetylation (NaOMe, MeOH) (Scheme 1). Generally, the condition in which the Cu(I) catalyst was generated *in situ* provided slightly better yields than that using the Cu(I) species (CuI) directly.

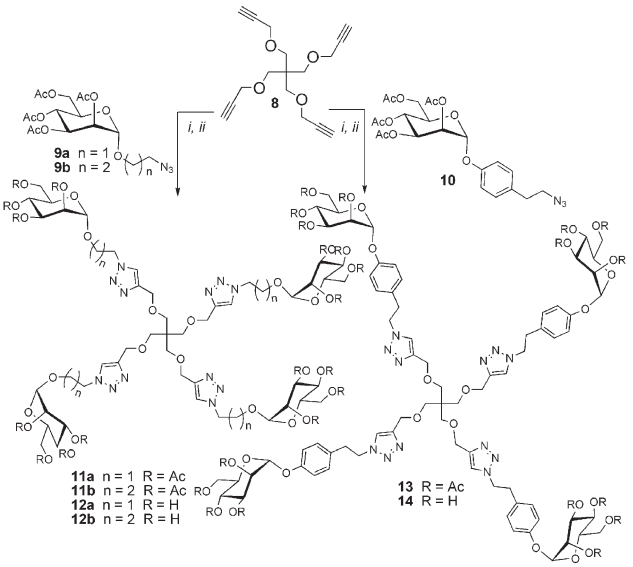
As shown in Scheme 2, treatment of the known tetrakis(2-propynyloxymethyl)methane **8**,[20] prepared by our modified nucleophilic substitution of the tetratosylates by a propargylate, with 2-azidoethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside **9a**[21] or 3-azidopropyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside **9b**[22] under the same conditions described above, provided excellent yields of the extended cluster analogues **12a** and **12b** after deacetylation (NaOMe, MeOH, 90%, 93%). Tetramannoside **14**, bearing a more rigid aromatic spacer, was similarly obtained from **8** and **10**[23] (Scheme 2).

To provide higher flexibility and FimH accessibilities, while keeping an hydrophobic residue in the mannoside aglycon (triazole) into the CRD's active site near tyrosine-48 and -137,[4] the four arms of the cluster **5** were equipped with a tri(ethylene glycol) spacer. To this end, the synthesis of **18** was initiated. As outlined in Scheme 3, nucleophilic substitution between pentaerythritol and toluene 4-sulfonic acid 2-[2-(2-azidoethoxy)ethoxy] ethyl ester (**15**),[24] furnished tetraazide **16** (68%).



Scheme 1 *Reagents and conditions*: (i) CuI, DIEPA, THF, rt, 12 h; **4** (80%), **6** (82%); or $CuSO_4$, Na ascorbate, THF–H_2O, rt, 12 h; **4** (92%), **6** (90%); (ii) MeONa, MeOH, rt, 4 h; **5** (90%), **7** (93%).

Department of Chemistry, Université du Québec à Montréal, P.O. Box 8888, Succ. Centre-Ville Montreal, Québec, Canada H3C 3P8. E-mail: roy.rene@uqam.ca; Fax: +1 514 987 4054; Tel: +514 987 3000x2546

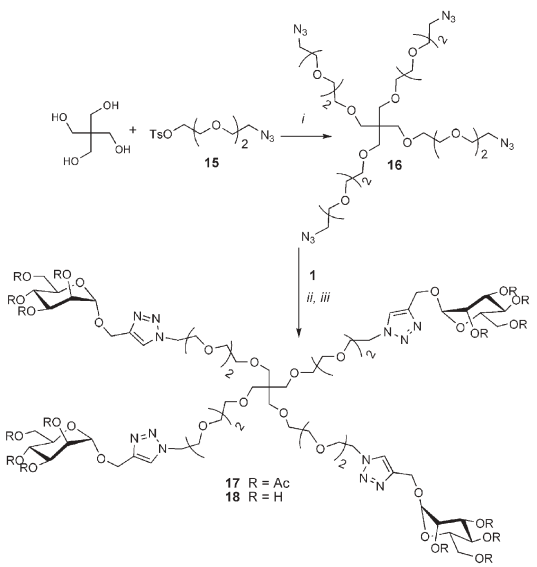


Scheme 2 *Reagents and conditions*: (i) $CuSO_4$, Na ascorbate, THF–H_2O, rt, 12 h; **11a** (89%), **11b** (77%), **13** (89%); (ii) MeONa, MeOH, rt, 4 h; **12a** (92%), **12b** (92%), **14** (98%).

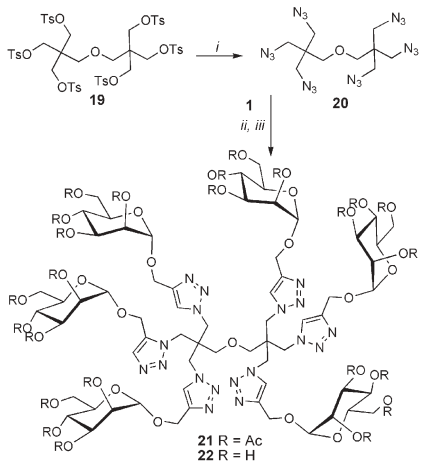
Compound **16** was then transformed into the triazole with propargyl α-D-mannopyranoside **1**, using the same standard conditions previously described, which after deacetylation under Zemplén conditions (NaOMe, MeOH) gave tetravalent cluster **18** in 92% yield.

Further dendritic growth offers promising new multiarmed clusters having more flexibility and various geometries. As shown in Scheme 4, hexatosylated dipentaerythritol **19**[25] was converted in to the novel hexaazide **20** (NaN_3, DMF, 70%). The cycloaddition between **20** and **1** afforded **21**, which gave the hexacluster **22** after deacetylation (Scheme 4).

Furthermore, treatment of triazide **3** and ditosylates **23a** or **23b** under basic conditions (KOH, DMSO) provided new hexaazido



Scheme 3 *Reagents and conditions*: (i) KOH, DMSO, 40 °C, 12 h, 68%; (ii) $CuSO_4$, Na ascorbate, THF–H_2O, rt, 12 h, 90%; (iii) MeONa, MeOH, rt, 4 h, 92%.

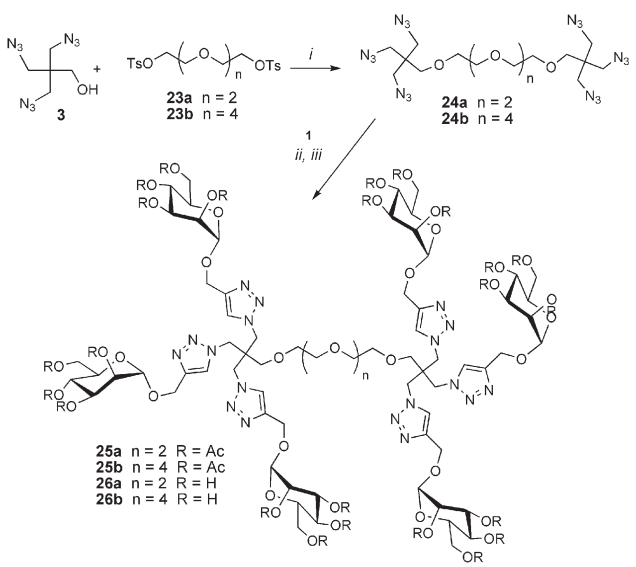


Scheme 4 *Reagents and conditions*: (i) NaN_3, DMF, 80 °C, 12 h, 70%; (ii) $CuSO_4$, Na ascorbate, THF–H_2O, rt, 12 h, 72%; (iii) MeONa, MeOH, rt, 4 h, 85%.

pentaerythritol scaffolds **24a** and **24b** in good yields (Scheme 5). Their cycloaddition with propargyl α-D-mannopyranoside **1** followed by deacetylation as above furnished hexavalent clusters **26a** (95%) and **26b** (92%) showing a distance of 11 and 18 Å between each the tripodal mannoside moieties, respectively.

In all cases, analysis of the ^1H NMR spectra of the mannoside clusters revealed calculated integrations for the triazole protons respective to the anomeric protons, complete disappearance of the acetylenic signals, thus confirming, together with MS and IR data, completion of the multivalent Cu(I)-catalysed azide–alkyne cycloadditions.

Interestingly, preliminary data from this family of clusters indicated that they were approximately a hundred times more efficient in the inhibition of agglutination[26] of *E. coli* x7122[27] by Baker's yeast than the monomer D-mannose. Indeed, inhibition



Scheme 5 *Reagents and conditions*: (i) KOH, DMSO, 40 °C, 12 h, 70%; (ii) $CuSO_4$, Na ascorbate, THF–H_2O, rt, 12 h; **25a** (72%), **25b** (75%); (iii) MeONa, MeOH, rt, 4 h; **26a** (95%), **26b** (92%).

titers of 370 and 3.96 mM were obtained for D-mannose and compound **5**, respectively.[28]

In summary, we have presented an efficient and systematic route toward the syntheses of a series of tri- to hexa-mannopyranoside clusters, built on the pentaerythritol scaffold and bearing aliphatic and aromatic spacers, by using triazole chemistry.

We acknowledge financial support from CORPAQ (Québec) and the Natural Sciences and Engineering Research Council of Canada (NSERC).

Notes and references

1 N. Sharon, *Biochim. Biophys. Acta*, 2006, **1760**, 527.
2 R. Roy, *Curr. Opin. Struct. Biol.*, 1996, **6**, 692; L. L. Kiessling and N. L. Pohl, *Chem. Biol.*, 1996, **3**, 71; R. Roy, *Top. Curr. Chem.*, 1997, **187**, 241; M. Mammen, S. K. Choi and G. M. Whitesides, *Angew. Chem., Int. Ed.*, 1998, **37**, 2754.
3 C.-S. Hung, J. Bouckaert, D. Hung, J. Pinkner, C. Widberg, A. DeFusco, C. G. Auguste, R. Strouse, S. Langerman, G. Waksman and S. J. Hultgren, *Mol. Microbiol.*, 2002, **44**, 903.
4 J. Bouckaert, J. Berglund, M. Schembri, E. De Genst, L. Cools, M. Wuhrer, C. S. Hung, J. Pinkner, R. Slattegard, A. Zavialov, D. Choudhary, S. Langerman, S. J. Hultgren, L. Wyns, P. Klemm, S. Oscarson, S. D. Knight and H. De Greve, *Mol. Microbiol.*, 2005, **55**, 441.
5 N. Nagahori, R. T. Lee, S. I. Nishimura, D. Pagé, R. Roy and Y. C. Lee, *ChemBioChem*, 2002, **3**, 836; R. Roy, *Trends Glycosci. Glycotechnol.*, 2003, **15**, 291.
6 J. Katajisto, P. Heinonen and H. Loennberg, *Curr. Org. Chem.*, 2004, **8**, 977.
7 G. R. Newkome, J. K. Young, G. R. Baker, R. L. Potter, L. Audoly, D. Cooper and C. D. Weis, *Macromolecules*, 1993, **26**, 2394.
8 S. Hanessian, D. Qiu, H. Prabhanjan, G. V. Reddy and B. Lou, *Can. J. Chem.*, 1996, **74**, 173.
9 H. C. Hansen, S. Haatja, J. Finne and G. Magnusson, *J. Am. Chem. Soc.*, 1997, **119**, 6974.
10 T. K. Lindhorst, M. Dubber, U. Krallmann-Wenzel and U. S. Ehlers, *Eur. J. Org. Chem.*, 2000, 2027.
11 P. Langer, S. J. Ince and S. L. Ley, *J. Chem. Soc., Perkin Trans. 1*, 1998, 3913.
12 H. Al-Mughaid and T. B. Grindley, *J. Org. Chem.*, 2006, **71**, 1390.
13 G. Tabarani, J. J. Reina, C. Ebel, C. Vivès, H. Lortat-Jacob, J. Rojo and F. Fieschi, *FEBS Lett.*, 2006, **580**, 2402.
14 B. Dey, D. L. Lerner, P. Lusso, M. R. Boyd, J. H. Elder and E. A. Berger, *J. Virol.*, 2000, **74**, 4562.
15 H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004.
16 F. G. Calvo-Flores, J. Isac-García, F. Hernández-Mateo, F. Pérez-Balderas, J. A. Calvo-Asín, E. Sanchéz-Vaquero and F. Santoyo-González, *Org. Lett.*, 2000, **2**, 2499; F. Pérez-Balderas, M. Ortega-Muñoz, J. Morales-Sanfrutos, F. Hernández-Mateo, F. G. Calvo-Flores, A. Calvo-Asín Jose, J. Isac-García and F. Santoyo-González, *Org. Lett.*, 2003, **5**, 1951; F. Pérez-Balderas, F. Hernández-Mateo and F. Santoyo-Gonzaález, *Tetrahedron*, 2005, **61**, 9338; S. Chittaboina, F. Xie and Q. Wang, *Tetrahedron Lett.*, 2005, **46**, 2331; E. Fernandez-Megia, J. Correa, I. Rodriguez-Meizoso and R. Riguera, *Macromolecules*, 2006, **39**, 2113; P. Wu, M. Malkoch, J. N. Hunt, R. Vestberg, E. Kaltgrad, M. G. Finn, V. V. Fokin, K. B. Sharpless and C. J. Hawker, *Chem. Commun.*, 2005, 5775.
17 W. Hayes, H. M. I. Osborn, S. D. Osborne, R. A. Rastall and B. Romagnoli, *Tetrahedron*, 2003, **59**, 7983.
18 E. R. Wilson and M. B. Frankel, *J. Org. Chem.*, 1985, **50**, 3211.
19 S. K. Das, M. C. Trono and R. Roy, *Methods Enzymol.*, 2003, **362**, 3.
20 S. E. Korostova, A. I. Mikhaleva, S. G. Shevchenko, L. N. Sobenina, V. D. Fel'dman and N. I. Shishov, *Zh. Prikl. Khim.*, 1990, **63**, 234.
21 A. Y. Chernyak, G. V. Sharma, L. O. Kononov, P. R. Krishna, A. B. Levinsky, N. K. Kochetkov and A. V. R. Rao, *Carbohydr. Res.*, 1992, **223**, 303; E. Arce, P. M. Nieto, V. Díaz, R. García Castro, A. Bernad and J. Rojo, *Bioconjugate Chem.*, 2003, **14**, 817.
22 C. C. M. Appeldoorn, J. A. F. Joosten, F. A. el Maate, U. Dobrindt, J. Hacker, R. M. J. Liskamp, A. S. Khan and R. J. Pieters, *Tetrahedron: Asymmetry*, 2005, **16**, 361.
23 Compound **10** was obtained by $BF_3{\cdot}Et_2O$ catalyzed glycosidation of penta-*O*-acetyl-α,β-D-mannopyranose with *p*-$HOC_6H_4CH_2CH_2N_3$ itself obtained from thyramine prepared by the diazo transfer reaction. See: J. T. Lundquist, IV and J. C. Pelletier, *Org. Lett.*, 2001, **3**, 781.
24 W. K. C. Park, S. J. Meunier, D. Zanini and R. Roy, *Carbohydr. Lett.*, 1995, **1**, 179; I. Lundt, A. J. Steiner, A. E. Stuetz, C. A. Tarling, S. Ully, S. G. Withers and T. M. Wrodnigg, *Bioorg. Med. Chem.*, 2006, **14**, 1737; T. Nabeshima, T. Saiki, J. Iwabuchi and S. Akine, *J. Am. Chem. Soc.*, 2005, **127**, 5507.
25 A. A. Shukla, S. S. Bae, J. A. Moore, K. A. Barnthouse and S. M. Cramer, *Ind. Eng. Chem. Res.*, 1998, **37**, 4090; D. Laliberté, T. Maris, A. Sirois and J. D. Wuest, *Org. Lett.*, 2003, **5**, 478.
26 Inhibition of agglutination of Baker's yeast was made according to: E. A. Kabat and M. M. Mayer, in *Experimental Immunochemistry*, C. C. Thomas, Springfield, IL, USA, 1971.
27 A. Olsen, A. Jonsson and S. Normark, *Nature (London)*, 1989, **338**, 652.
28 Using surface plasmon resonance (SPR) for the bioassay against *E. coli* K-12 FimH (see ref. 4), compounds **5**, **7**, and **12a** had K_d of 14 nM, 25 nM and 53 nM, respectively (compared to D-mannose, K_d = 2.3 μM).

