Strong inhibition of cholera toxin binding by galactose dendrimers†

Hilbert M. Branderhorst,[a] Rob M. J. Liskamp,[a] Gerben M. Visser[b] and Roland J. Pieters*[a]

Received (in Cambridge, UK) 19th July 2007, Accepted 7th September 2007
First published as an Advance Article on the web 24th September 2007
DOI: 10.1039/b711070g

Galactose-containing dendrimers with long spacer arms inhibit cholera toxin binding as strongly as the natural ganglioside GM1 oligosaccharide does.

Cholera toxin (CT) is the causative agent of cholera. This disease still causes major problems in the developing world, with over 100 000 reported cases per year.[1] The cholera toxin is a member of the AB_5 toxin family, and its B-subunits bind to the oligosaccharide portion of the GM1 ganglioside (GM1os) molecules present on the cell surfaces of the intestines. The binding process leads to toxin internalization[2] followed by disease initiation caused by the A-subunit.[3] Inhibitors of B-subunit binding have therapeutic potential and they may also be useful for toxin detection either in patient samples[4] or in materials suspected of having a terrorist origin.[5] Considering the pentameric architecture of the B-subunit (CTB_5), to which up to five GM1 oligosaccharide ligands can bind simultaneously, a multivalency approach is particularly attractive for inhibitor design. Several multivalent inhibitors have been designed for cholera toxin and related proteins of the AB_5 family.[6]

Recently we reported on a series of GM1os conjugated dendrimers that showed unprecedented affinity enhancements of up to 380 000-fold beyond GM1os derivatives.[7] The strong binding was attributed to the combined use of the strong GM1os ligand and its multivalent presentation on a dendrimer with long spacer arms.[6e] While these GM1os–dendrimer conjugates were very potent, the goal still remains to prepare inhibitors based on cheap bulk sugars like galactose with potencies equal to or better than the endogenous GM1os ligand. GM1os is very complex and difficult to prepare on a large scale. The design lessons learned in the GM1os study[7] were applied to the development of galactose-based multivalent CTB_5 inhibitors. We here report such inhibitors that exhibited similar CTB_5 inhibitory potencies to GM1os derivatives.

The CTB_5 ligand, galactose, was used since this is by far the most important residue for binding within GM1os.[8] Galactose was outfitted with a poly(ethylene glycol) unit, to crudely mimic the other sugar rings of the GM1os (Fig. 1). Furthermore, a lipophilic part was attached in order to keep this factor the same as in our GM1os-based system.[7]

Building block **5a** was prepared as shown in Scheme 1. The synthesis started with penta(ethylene glycol), which was mono tritylated to give **2**. The chain was elongated *via* an S_N2 reaction

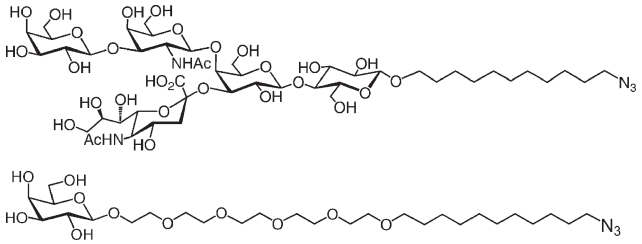
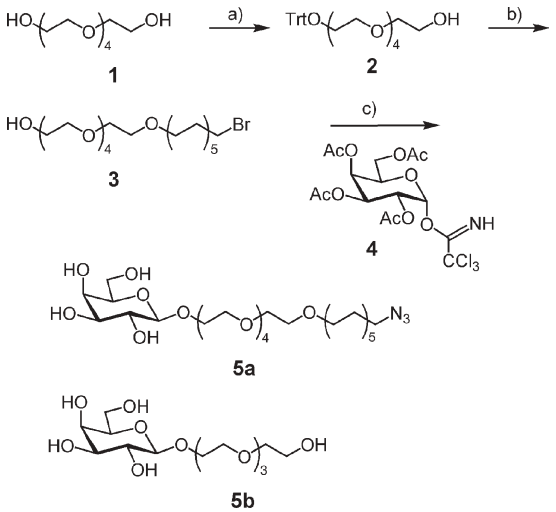


Fig. 1 Structure of the galactose building block used here (bottom) to mimic some of the features of the GM1os building block (top, ref. 7), such as the terminal galactose moiety, the spacer length and its hydro- and lipophilicity.

with 1,11-dibromo-undecane and the trityl group was cleaved by *p*-TsOH in MeOH to give **3**. Introduction of the galactose moiety was achieved with galactosyl donor **4** and $BF_3{\cdot}OEt_2$ as the promoter. An azide function for 'click' conjugation[9] was introduced by reaction of the coupling product with NaN_3 in DMF at elevated temperature and as the final step the acetyl protecting groups were removed by NaOMe in MeOH to afford the desired compound **5a**. The monovalent reference compound **5b** was prepared from per-acetylated galactose (see ESI†).

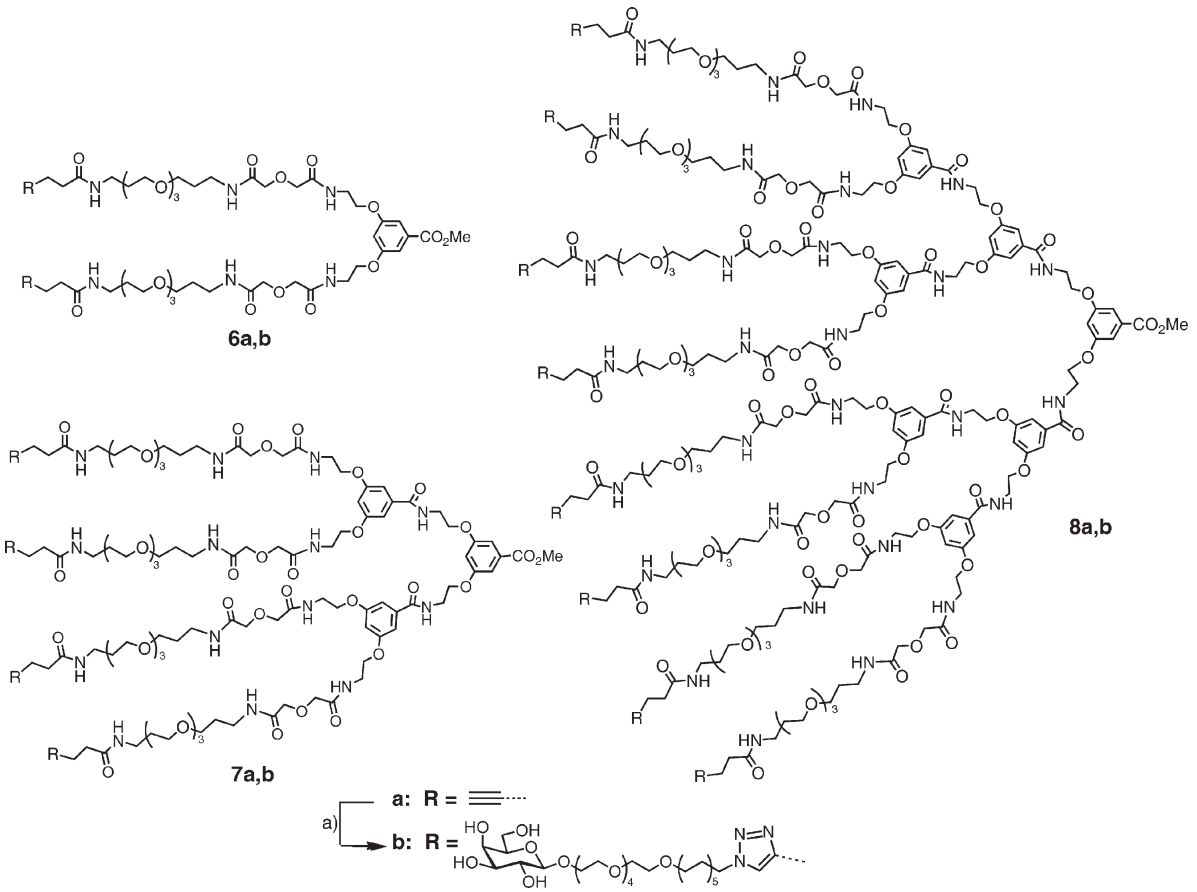
Ligation of **5a** to dendrimers **6a**, **7a** and **8a**[7] (Scheme 2) was performed using "click" chemistry.[9] Our recently developed protocol involving microwave heating at 80 °C was used[10] and the products **6b**, **7b** and **8b** were obtained after preparative HPLC purification in good yields and purity.



Scheme 1 Synthesis of galactose building block **5a** and the structure of **5b**; reagents and conditions: (a) trityl chloride, pyridine, 72%; (b) (i) NaH, $Br(CH_2)_{11}Br$, DMF, 66%; (ii) TsOH, MeOH, 95%; (c) (i) **4**, $BF_3{\cdot}Et_2O$, toluene, 44%; (ii) NaN_3, DMF, 84%; (ii) NaOMe, MeOH, 72%.

[a]*Department of Medicinal Chemistry and Chemical Biology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, PO Box 80082, 3508 TB Utrecht, The Netherlands. E-mail: R.J.Pieters@uu.nl; Fax: +31 30-2536655*
[b]*Laboratory of Organic Chemistry, Wageningen University, Dreijenplein 8, 6703HB Wageningen, The Netherlands*

† Electronic supplementary information (ESI) available: Synthesis and characterization details. See DOI: 10.1039/b711070g



Scheme 2 Synthetic galactose dendrimers; reagents and conditions: (a) **5a**, $CuSO_4$, sodium ascorbate, DMF–H_2O, 80 °C, 20 min, yields: 70% for **6b**, 78% for **7b**, 77% for **8b**.

The glycodendrimers were tested for cholera toxin inhibition in a well established ELISA-like assay.[7,11] A GM1 ganglioside coated 96 well plate was allowed to bind horseradish peroxidase labelled CTB_5 (CTB_5-HRP) and this binding was inhibited with a concentration range of dendrimeric inhibitors (Table 1). In this assay the monovalent galactose and its derivative **5b** exhibited the expected weak inhibitory power with IC_{50}s of 240 and 80 mM respectively. The divalent dendrimer **6b** showed a large increase in potency with an IC_{50} value of 130 μM. Tetravalent compound **7b** showed a further increase in potency (IC_{50} 25 μM) and the affinity of octavalent compound **8b** increased only by another factor of *ca.* 2 (IC_{50} 12 μM).

The potency increase of divalent **6b** is remarkable. However, the compound still binds more weakly than the monovalent GM1os derivative. The compounds of higher valency, **7b** and **8b**, showed IC_{50}s in the same range as the GM1os derivative. The multivalency effect, as expressed by the relative potency per sugar, still increased from di- to tetravalent (923 *vs.* 2400), while it remained basically the same at the octavalent stage (2500). Additional experiments, including the use of complementary experimental methods, will likely uncover the nature of the multivalency effects, *i.e.* chelation, aggregation or a combination thereof. In comparison to reported multivalent galactose-based CTB_5 inhibitors, the present system compares favourably. A reported pentavalent galactose-based system still fell short by a factor of *ca.* 80 relative to GM1.[6e] Another decavalent system seemed to come close, but no direct comparison with GM1os or its derivatives was made.[6c]

Table 1 Inhibitory potencies of the CTB_5 inhibitors

Compound	Valency	IC_{50}[a]/M	Relative potency (per sugar)
Galactose	1	$2.4 (\pm 0.5) \times 10^{-1}$	1 (1)
5b	1	$8.0 (\pm 0.2) \times 10^{-2}$	3 (3)
6b	2	$1.3 (\pm 0.2) \times 10^{-4}$	1846 (923)
7b	4	$2.5 (\pm 0.4) \times 10^{-5}$	9600 (2400)
8b	8	$1.2 (\pm 0.3) \times 10^{-5}$	20 000 (2500)
GM1os–$C_{11}H_{21}$ (ref. 7)	1	$1.9 (\pm 0.6) \times 10^{-5}$	12 632 (12 632)

[a] Determined in an ELISA experiment with 0.43 nM CTB_5-HRP and wells coated with 0.2 μg GM1.

To conclude, a galactose containing building block was synthesized in a straightforward synthesis from bulk galactose. This building block was efficiently coupled to di-, tetra- and octavalent dendrimers *via* 'click' chemistry. The potencies are very high, considering that **7b** and **8b** showed roughly equal IC_{50} values to the GM1os derivative in this assay. This result is an important step towards low cost potent ligands for cholera toxin with applications in therapy and detection.

The Utrecht Institute for Pharmaceutical Sciences (UIPS) is gratefully acknowledged for support. We thank the EU for fostering collaboration in this area: EU COST D34 working group 'Multivalent ligands for cholera toxin inhibition and sensing'.

Notes and references

1 World Health Organization (WHO), *Weekly Epidemiological Record*, 2006, **81**, 297–308.
2 M. L. Togersen, G. Skretting, B. van Deurs and K. Sandvig, *J. Cell Sci.*, 2001, **114**, 3737–3747.
3 E. Fan, E. A. Merritt, C. L. M. J. Verlinde and W. G. J. Hol, *Curr. Opin. Struct. Biol.*, 2000, **10**, 680–686.
4 S. Ahn-Yoon, T. R. DeCory, A. J. Baeumner and R. A. Durst, *Anal. Chem.*, 2003, **75**, 2256–2261.
5 (*a*) X. Song, J. Nolan and B. I. Swanson, *J. Am. Chem. Soc.*, 1998, **120**, 4873–4874; (*b*) A. K. Singh, S. H. Harrison and J. S. Schoeniger, *Anal. Chem.*, 2000, **72**, 6019–6024; (*c*) S. C. Clarke, *Br. J. Biomed. Sci.*, 2005, **62**, 40–46.
6 (*a*) J. P. Thompson and C.-L. Schengrund, *Glycoconjugate J.*, 1997, **14**, 837–845; (*b*) I. Vrasidas, N. J. de Mol, R. M. J. Liskamp and R. J. Pieters, *Eur. J. Org. Chem.*, 2001, 4685–4692; (*c*) Z. Zhang, E. A. Merritt, M. Ahn, C. Roach, Z. Hou, C. L. M. J. Verlinde, W. G. J. Hol and E. Fan, *J. Am. Chem. Soc.*, 2002, **124**, 12991–12998; (*d*) D. Arosio, I. Vrasidas, P. Valentini, R. M. J. Liskamp, R. J. Pieters and A. Bernardi, *Org. Biomol. Chem.*, 2004, **2**, 2113–2124; (*e*) Z. Zhang, J. C. Pickens, W. G. J. Hol and E. Fan, *Org. Lett.*, 2004, **6**, 1377–1380; (*f*) D. Arosio, M. Fontanello, L. Baldini, L. Mauri, A. Bernardi, A. Casnati, F. Sansone and R. Ungaro, *J. Am. Chem. Soc.*, 2005, **127**, 3660–3661; (*g*) P. I. Kitov, J. M. Sadowska, G. Mulvey, G. D. Arnstrong, H. Ling, N. S. Pannu, R. J. Read and D. R. Bundle, *Nature*, 2000, **403**, 669–672.
7 A. V. Pukin, H. M. Branderhorst, C. Sisu, C. A. G. M. Weijers, M. Gilbert, R. M. J. Liskamp, G. M. Visser, H. Zuilhof and R. J. Pieters, *ChemBioChem*, 2007, **8**, 1500–1503.
8 W. B. Turnbull, B. L. Precious and S. W. Homans, *J. Am. Chem. Soc.*, 2004, **126**, 1047–1054.
9 (*a*) V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596–2599; (*b*) C. W. Tornoe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057–3069.
10 (*a*) J. A. F. Joosten, N. T. H. Tholen, F. Ait El Maate, A. J. Brouwer, G. W. van Esse, D. T. S. Rijkers, R. M. J. Liskamp and R. J. Pieters, *Eur. J. Org. Chem.*, 2005, 3182–3185; (*b*) R. M. J. Liskamp, D. T. S. Rijkers, R. J. Pieters, A. J. Brouwer and J. A. F. Joosten, *Eur. Pat. Appl.* EP 1733742, 2006.
11 (*a*) A.-M. Svennerholm and J. Holmgren, *Curr. Microbiol.*, 1978, **1**, 19–23; (*b*) R. M. Dawson, *J. Appl. Toxicol.*, 2005, **25**, 30–38.