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**COMMUNICATION**

# Potent divalent inhibitors with rigid glucose click spacers for *Pseudomonas aeruginosa* lectin LecA†

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**The synthesis of a new rigid spacer based on carbohydrate-triazole repeating units and their incorporation into divalent systems is described. Inhibition studies showed that a well-matched system with a rigid spacer with flexible ends leads to the most potent inhibition of *Pseudomonas aeruginosa* lectin LecA.**

Carbohydrate–protein interactions play a key role in many important biological processes and often involve multivalency to increase binding to biologically relevant levels.[1] Selective interfering with these interactions may enable the development of therapeutics for inflammation,[2] bacterial toxins,[3] bacterial infections,[4] cancer,[5] flu,[6] and AIDS.[7] A logical approach towards inhibitors of protein–carbohydrate interactions is to make them multivalent. This approach has seen numerous successful examples with multivalency effects of several orders of magnitude *e.g.* with bacterial toxins and multisite lectins.[8] For potent inhibition the length of the spacer connecting the ligands is a known factor of importance.[9] The calculation of the 'effective length' is a useful optimization guide for the commonly used flexible spacers that tend to fold.[8a] Typically flexible PEG-based spacers have to be made three times longer than the distance spanned by an extended conformation for an optimal match.[8a] Besides the length, spacer rigidity is another important factor, which has received far less attention in the design of multivalent ligands.[10] This is due to challenges in creating a spacer that is of optimal length, maintains its distance and is sufficiently soluble. We here explored these issues in the design and synthesis of rigid spacers that contain flexible ends to allow some adjustment of the attached ligands (Fig. 1). The spacers were outfitted with galactose units and their inhibition of the *Pseudomonas aeruginosa* adhesion lectin and virulence factor LecA was evaluated.[11]

In the design of the spacer, hydrophilic carbohydrates were incorporated to enhance the solubility. In order to prevent folded conformations, glucose moieties were connected by 1,4-triazole units using direct equatorial linkages at positions 1 and 4 of the sugar, as can be seen in structure **E** (Fig. 2).

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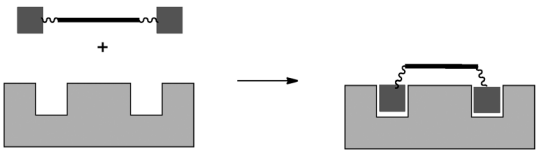


**Fig. 1** A divalent ligand with a rigid spacer containing flexible ends binding to a protein with two binding sites.

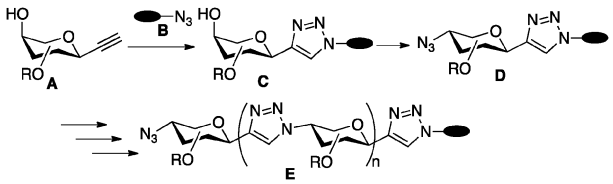
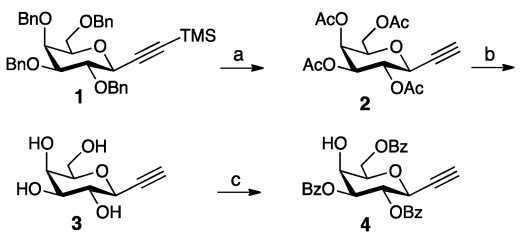



**Fig. 2** Synthesis and elongation strategy of the spacer.

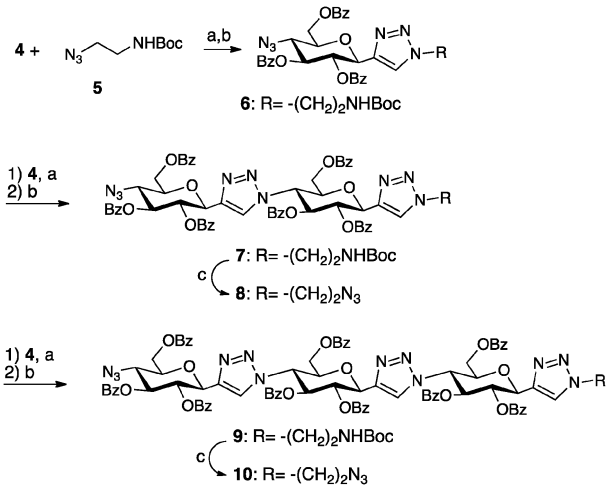
Rotation of bonds within the spacer is possible but the overall shape remains close to linear according to modelling.[12a] Its synthesis strategy involved a copper catalyzed azide–alkyne cycloaddition (CuAAC) between a suitable azide **B** and a building block **A**, followed by the conversion of the free axial hydroxyl group present in product **C** to an equatorial azide in **D**. Repeating this process allowed chain elongation.

The actual synthesis of building block **4** is shown in Scheme 1 and started with **1**.[13] Its benzyl and trimethylsilyl groups were removed using $BCl_3{\cdot}SMe_2$ which was followed by acetylation to give **2** in good yield. After removal of the acetyl groups, **3** was selectively benzoylated at low temperature to give **4** along with other isomers that were recycled.

In order to build the spacer, a starting point **B** (Fig. 2) was needed. Compound **5** (Scheme 2) was used for this purpose and it was linked to **4** to give the 'click' product in high yield.




**Scheme 1** Preparation of the building block **4**. *Reagent and conditions*: (a) (i) $BCl_3{\cdot}SMe_2$, $CH_2Cl_2$, microwave, 80 °C, 20 min; (ii) $Ac_2O$, Py, rt, 14 h, 70% over 2 steps; (b) MeONa, MeOH, quant.; (c) BzCl, Py, −40 °C, 67%.




**Scheme 2** Synthesis of the spacers. *Reagent and conditions*: (a) $CuSO_4{\cdot}5H_2O$, Na-ascorbate, DMF, 10% $H_2O$, microwave, 80 °C, 30 min, 85–91%; (b) (i) $Tf_2O$, Py, $CH_2Cl_2$, 0 °C, 1 h; (ii) $NaN_3$, DMF, rt, 4 h, 80–85% over 2 steps; (c) (i) TFA, $CH_2Cl_2$; (ii) imidazole-1-sulfonyl azide, $K_2CO_3$, $CuSO_4{\cdot}5H_2O$, MeOH, 82–85% over 2 steps.

Its axial 4-hydroxyl was converted, *via* the triflate, to an equatorial azido group, by sodium azide, thus yielding **6**. By reiteration of this procedure compounds **7** and **9** were synthesized with two and three carbohydrate units, respectively. Finally, the Boc-protected amino groups were deprotected and converted to azido groups to give **8** and **10**.[14]

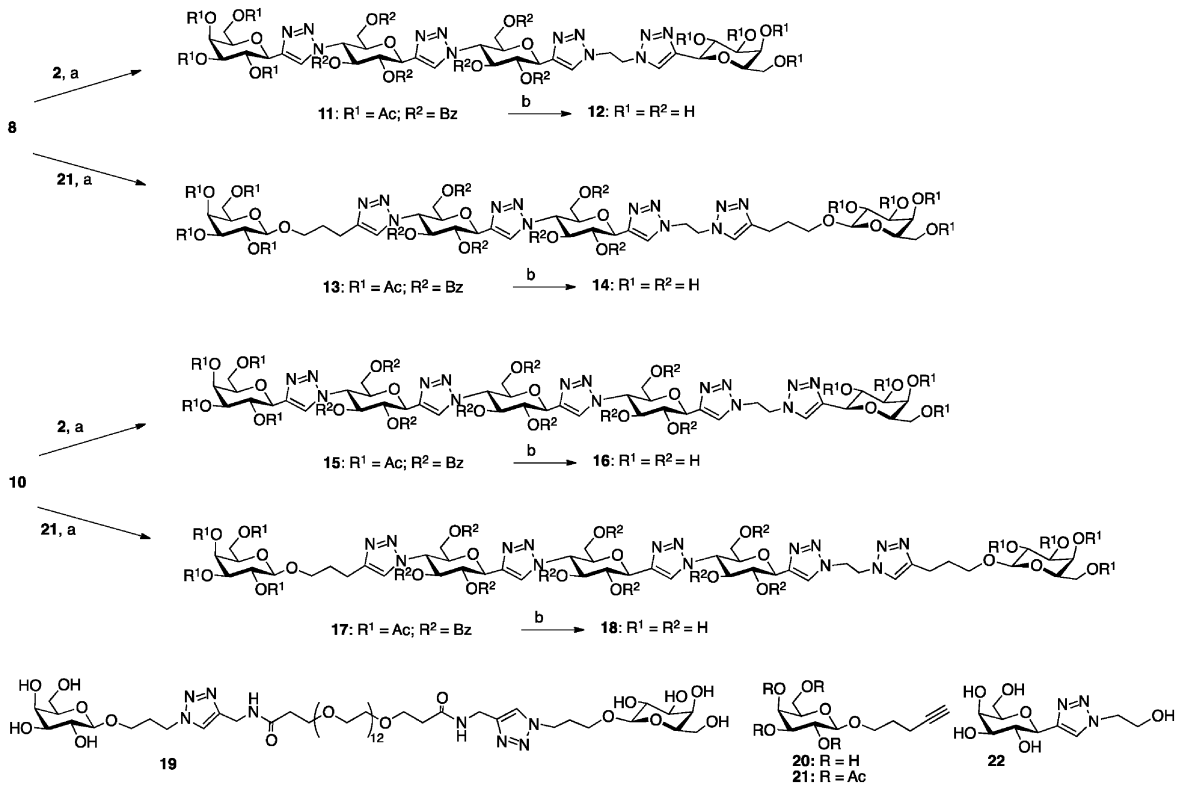
Both **8** and **10** contain two azido groups, allowing a double "click" reaction to introduce the carbohydrate ligands (Scheme 3). Since the target was LecA, a galactose binding lectin, the two galactosides **2** and **21**, differing in their aglycon, were chosen for attachment. In **21** the alkyne moiety is connected to the sugar *via* a flexible four atom chain, while in **2** the alkyne moiety is directly connected. Respective 'click' reactions yielded the divalent ligands **12**, **14**, **16** and **18** in good yield after purification by preparative HPLC.

**Table 1** Inhibitory potency of mono and divalent galactosides on LecA binding[a]

| Compound | Valency | $IC_{50}$/M | Relative potency (per sugar) |
|---|---|---|---|
| **20** | 1 | 12 (±6.0) × $10^{-5}$ | 1(1) |
| **14** | 2 | 22 (±2.3) × $10^{-8}$ | 545(272) |
| **18** | 2 | 38 (±6.1) × $10^{-8}$ | 315(158) |
| **19** | 2 | 20 (±5.9) × $10^{-7}$ | 60(30) |
| **22** | 1 | 92 (±37) × $10^{-6}$ | 1(1) |
| **12** | 2 | 31 (±6.2) × $10^{-5}$ | 0.30(0.15) |
| **16** | 2 | 17 (±3.4) × $10^{-7}$ | 54(27) |

[a] FITC-labeled LecA, 20 μg $mL^{-1}$ binding to a galactoside functionalized surface.[16]

These compounds were employed in LecA inhibition studies and their inhibitory potencies were compared to that of the monovalent reference compounds and the divalent **19**. The latter contains the long flexible PEG-based spacer, which is expected to span the 26 Å between neighbouring binding sites of LecA (see ESI†).[15] Inhibitory potencies were determined by observing the binding of fluorescently labelled LecA to a galactose-displaying chip surface[16] (Table 1).




**Scheme 3** *Reagent and conditions*: (a) $CuSO_4{\cdot}5H_2O$, Na-ascorbate, DMF, 10% $H_2O$, microwave, 80 °C, 30 min, 83–85%; (b) MeONa, MeOH, 70–87%.

In this assay two monovalent galactosides were used as reference compounds to account for the different aglycon part of the divalent ligands. Compound **20**, with a propylene aglycon, and **22**, with a triazole aglycon, showed $IC_{50}$'s of 120 and 93 μM, respectively. With the exception of compound **12**, all divalent ligands were more potent. Compound **14** was the most potent with an $IC_{50}$ of 220 nM, a 545-fold improvement over the monovalent ligand **20**. The related compound **18**, containing an extra spacer unit, was almost as potent with an $IC_{50}$ of 380 nM. The flexible divalent PEG-based ligand **19** showed an $IC_{50}$ of 2 μM which is 60 times better than the monovalent ligand **20**.

A dramatic decrease in potency was observed with **12**, a relative of **14** just lacking 2 propyloxy units, which showed an $IC_{50}$ of 310 μM, clearly not showing divalent binding. Compound **16**, a relative of **12** just containing an extra spacer unit, showed major improvement with an $IC_{50}$ of 1.7 μM, which is 182 times better than **12**.

To conclude, a series of divalent inhibitors, based on carbohydrate-triazole spacers, were synthesized in a multiple step synthesis, using azide–alkyne "click" chemistry in high yield. These compounds were evaluated as LecA inhibitors. Considering that a triazole-glucose unit contributes *ca.* 7 Å to the spacer length, the prepared compounds were expected to show potency variation. Multivalency effects were observed as almost all divalent compounds showed improved potency over the monovalent. It was shown that **14** and **18** both containing rigid moieties were considerably more potent than the PEG based **19**. Compounds **14** and **18** contained more flexibility in its spacer than **12** and **16**, which feature a direct attachment to the sugar. For **12** and **16** the potency is critically dependent on the spacer length, where one spacer unit too few, as in **12**, leads to the abolishment of divalent binding.[12b] For such rigid compounds a perfect design could lead to high potency and more importantly to very high specificity. Naturally we can only include whole building blocks and no partial ones. Therefore not all distances can be prepared with a rigid design, so flexible end groups are needed. In the compounds containing the most flexible spacer ends, *i.e.* **14** and **18**, more forgiving inhibition behaviour was observed with respect to design imperfections. Besides the enhanced potency of the compounds containing rigid spacer units, effective non-folding spacers can also be considerably shorter. There were 61 atoms present between the sugar anomeric carbons of **19**, and between 22 and 37 atoms for **12**, **14**, **16** and **18**. Additionally, the use of sugars in the spacer is likely to enhance the biocompatibility[17] and their rigid nature should enhance their selectivity. Overall the design strategy has led to some of the most potent LecA inhibitors that rival those of greater valency and size.[18]

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## Notes and references


1 (*a*) J. J. Lundquist and E. J. Toone, *Chem. Rev.*, 2002, **102**, 555–578; (*b*) R. J. Pieters, *Org. Biomol. Chem.*, 2009, **7**, 2013–2025; (*c*) R. T. Lee and Y. C. Lee, *Glycoconjugate J.*, 2000, **17**, 543–551; (*d*) N. Jayaraman, *Chem. Soc. Rev.*, 2009, **38**, 3463–3483; (*e*) T. K. Dam, T. A. Gerken and C. F. Brewer, *Biochemistry*, 2009, **48**, 3822–3827; (*f*) J. E. Gestwicki, C. W. Cairo, L. E. Strong, K. A. Oetjen and L. L. Kiessling, *J. Am. Chem. Soc.*, 2002, **124**, 14922–14933; (*g*) M. Cloninger, *Curr. Opin. Chem. Biol.*, 2002, **6**, 742–748; (*h*) F. Sansone, L. Baldini, A. Casnati and R. Ungaro, *New J. Chem.*, 2010, **34**, 2715–2728.

2 C. Kneuer, C. Ehrhardt, M. W. Radomski and U. Bakowsky, *Drug Discovery Today*, 2006, **11**, 1034–1040.

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 (*a*) A. Imberty and A. Varrot, *Curr. Opin. Struct. Biol.*, 2008, **18**, 567–576; (*b*) R. J. Pieters, *Med. Res. Rev.*, 2007, **27**, 796–816.

5 (*a*) G. Ragupathi, F. Koide, P. O. Livingston, Y. S. Cho, A. Endo, Q. Wan, M. K. Spassova, S. J. Keding, J. Allen, O. Ouerfelli, R. M. Wilson and S. J. Danishefsky, *J. Am. Chem. Soc.*, 2006, **128**, 2715–2725; (*b*) Y. Li and P. J. Cozzi, *Curr. Cancer Drug Targets*, 2007, **7**, 259–271.

6 (*a*) J. Stevens, O. Blixt, T. M. Tumpey, J. K. Taubenberger, J. C. Paulson and I. A. Wilson, *Science*, 2006, **312**, 404–410; (*b*) M. J. Kiefel and M. von Itzstein, *Chem. Rev.*, 2002, **102**, 471–490.

7 L. X. Wang, *Curr. Opin. Drug Discovery Dev.*, 2006, **9**, 194–206.

8 (*a*) E. Fan, Z. Zhang, W. E. Minke, Z. Hou, C. L. M. J. Verlinde and W. G. J. Hol, *J. Am. Chem. Soc.*, 2000, **122**, 2663–2664; (*b*) P. I. Kitov, J. M. Sadowska, G. Mulvey, G. D. Armstrong, H. Ling, N. S. Pannu, Read and D. R. Bundle, *Nature*, 2000, **403**, 669–672; (*c*) H. M. Branderhorst, R. M. J. Liskamp, G. M. Visser and R. J. Pieters, *Chem. Commun.*, 2007, 5043–5045; (*d*) D. Schwefel, C. Maierhofer, J. G. Beck, S. Seeberger, K. Diederichs, H. M. Möller, W. Welte and V. Wittmann, *J. Am. Chem. Soc.*, 2010, **132**, 8704–8719.

9 P. Braun, B. Nägele, V. Wittmann and M. Drescher, *Angew. Chem., Int. Ed.*, 2011, **50**, 8428–8431.

10 D. Deniaud, K. Julienne and S. G. Gouin, *Org. Biomol. Chem.*, 2011, **9**, 966–979.

11 (*a*) A. Imberty, M. Wimmerová, E. P. Mitchell and N. Gilboa-Garber, *Microbes Infect.*, 2004, **6**, 221–228; (*b*) G. Cioci, E. P. Mitchell, C. Gautier, M. Wimmerová, D. Sudakevitz, S. Pérez, N. Gilboa-Garber and A. Imberty, *FEBS Lett.*, 2003, **555**, 297–301.

12 (*a*) Both computer modeling and CPK model building indicate this. More detailed studies including conformational analysis are ongoing; (*b*) Modeling indicates that **12** is indeed too short for divalent binding (see ESI†).

13 (*a*) T. Lowary, M. Meldal, A. Helmboldt, A. Vasella and K. Bock, *J. Org. Chem.*, 1998, **63**, 9657–9668; (*b*) A. Ernst and A. Vasella, *Helv. Chim. Acta*, 1996, **79**, 1279–1294.

14 E. D. Goddard-Borger and R. V. Stick, *Org. Lett.*, 2007, **9**, 3797–3800.

15 Measured between the anomeric oxygens of bound galactosides. X-ray structure 1OKO, see ref. 11*b*.

16 As the galactose-displaying chip surface, our previously reported glycodendrimer surface was used as described in: (*a*) N. Parera-Pera, H. M. Branderhost, R. Kooij, C. Maierhofer, M. van der Kaanden, R. M. J. Liskamp, V. Wittman, R. Ruijtenbeek and R. J. Pieters, *ChemBioChem*, 2010, **11**, 1896; (*b*) H. M. Branderhorst, R. Ruijtenbeek, R. M. J. Liskamp and R. J. Pieters, *ChemBioChem*, 2008, **9**, 1836–1844.

17 The benefits of carbohydrate units in a multivalent scaffold were previously recognized, see M. Dubber and T. K. Lindhorst, *J. Org. Chem.*, 2000, **65**, 5275–5281.

18 (*a*) S. Cecioni, R. Lalor, B. Blanchard, J.-P. Praly, A. Imberty, S. E. Matthews and S. Vidal, *Chem.–Eur. J.*, 2009, **15**, 13232–13240; (*b*) R. U. Kadam, M. Bergmann, M. Hurley, D. Garg, M. Cacciarini, M. A. Swiderska, C. Nativi, M. Sattler, A. R. Smyth, P. Williams, M. Cámara, A. Stocker, T. Darbre and J.-L. Reymond, *Angew. Chem., Int. Ed.*, 2011, **50**, 10631–10635; (*c*) S. Cecioni, V. Oerthel, J. Iehl, M. Holler, D. Goyard, J.-P. Praly, A. Imberty, J.-F. Nierengarten and S. Vidal, *Chem.–Eur. J.*, 2011, **17**, 3252–3261; (*d*) Y. M. Chabre, D. Giguere, B. Blanchard, J. Rodrigue, S. Rocheleau, M. Neault, S. Rauthu, A. Papadopoulos, A. Arnold, A. Imberty and R. Roy, *Chem.–Eur. J.*, 2011, **17**, 6545–6562.


 