DOI: 10.1002/cmdc.201000282

Dansyl *C*-Glucoside as a Novel Agent Against Endotoxic Shock

Barbara La Ferla,[a] Valerio Spinosa,[a] Giuseppe D'Orazio,[a] Marco Palazzo,[b] Andrea Balsari,[c] Anastasia A. Foppoli,[d] Cristiano Rumio,[b] and Francesco Nicotra*[a]

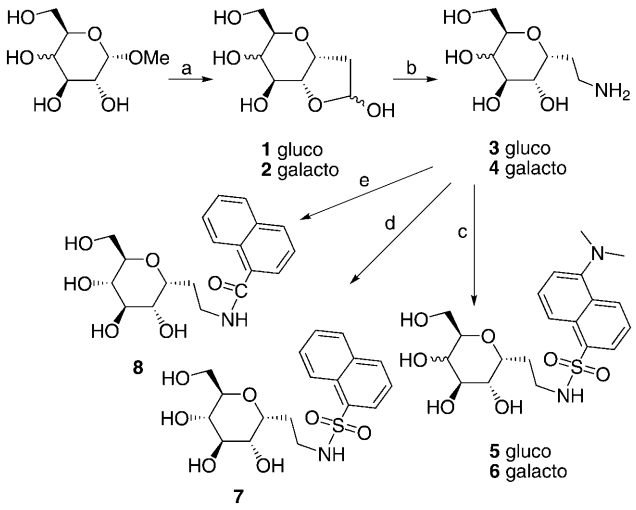
Dedicated to Prof. Saverio Florio on the occasion of his 70th birthday.

The biological role of carbohydrates in a variety of recognition processes of pharmacological relevance has stimulated interest in glycomimetics as potential drugs.[1] Relenza, Tamiflu, and Glyset are some successful examples of approved drugs belonging to this class of compounds. In the field of *C*-glycosides, which are glycomimetics where the anomeric oxygen is replaced with a carbon atom to give improved chemical and metabolic stability at the anomeric centre, no relevant bioactivity has been reported until now. We have found that the dansyl *C*-glucoside **5** is a novel and potent agent against endotoxic shock, fully protecting mice from sepsis.

Some of us found that, in a murine model of endotoxic shock induced by LPS, oral administration of glucose (2.5 g kg^{-1}) protects all mice tested from sepsis.[2] This protection is mediated by the activation of the sodium-dependent glucose transporter-1 (SGLT-1). A major drawback of this treatment is that a high concentration of glucose, which impacts metabolism, must be used. Therefore, our goal was to identify glucomimetics that, at pharmacological concentrations, were able to achieve protection against LPS-induced damage while avoiding the side effects associated with the administration of a high concentration of glucose.

Very little is known about the structure of SGLT-1 and its mechanism of action,[3–6] although some aryl β-glucosides have been patented as SGLT-1 inhibitors. Therefore, the only information available for the rational design of SGLT-1 ligands is based on the structure of D-glucose (or D-galactose, which is also transported) and an aromatic aglycon. In order to generate compounds that would mimic glucose but could not be metabolised, a *C*-glucoside is the key intermediate of choice. We also looked for a procedure that allows the stereoselective generation of this *C*-glucosidic key intermediate, and then its derivatisation to generate a library, avoiding protection and deprotection steps. A strong limitation in the development of glyco-drugs arises from the need for protection/deprotection steps and the control of stereochemistry. An orthogonal functional group suitable for chemoselective derivatisation of a polyhydroxylated compound like a sugar is the primary amino group.

We identified 2-(α-D-glucopyranosyl)ethanamine **3** (Scheme 1) as a key *C*-glycosyl intermediate, and generated this compound by stereoselective C-allylation[7] of methyl α-D-glucopyranoside, followed by ozonolysis and reductive amination of the obtained aldehyde (present as hemiacetal **1**).[8]



Scheme 1. *Reagents and conditions*: a) BSTFA, CH_3CN, 80 °C, 1 h; then, TMSOTf, Me_3Si(allyl), RT, 12 h; then O_3, MeOH/H_2O (1:1), −78 °C, 75 min; then Me_2S, 5 min;[8] b) $AcONH_4$, $NaCNBH_3$, MeOH, RT, 1 h, (**3**) 55%, (**4**) 57% (two steps); c) MeOH, THF, K_2CO_3, dansyl-Cl, RT, 2 h, (**5**) 50%, (**6**) 58%; d) MeOH, THF, K_2CO_3, naphthylsulphonyl-Cl, RT, 2 h, 52%; e) MeOH, THF, K_2CO_3, naphthylcarboxy-Cl, RT, 2 h, 46%.

The amino group of compound **3** was chemoselectively functionalised in different ways in the presence of the free sugar hydroxy groups. The choice of pharmacophores to be linked to the amine in **3** was essentially based on the limited data reported in the literature on molecules able to interact with SGLT-1, such as phloridzin, a natural β-glucoside with an aromatic aglycon.[9] We started to derivatise compound **3** by reaction with fluorescent dansyl chloride, and luckily dansyl *C*-glucoside **5** displayed a very interesting anti-inflammatory activity in vitro (see below, Figures 1 and 2).

Encouraged by this result, and to identify some structural requirements for biological activity, we made a number of significant changes in the structure of compound **5**. We generated naphthyl sulphonamide **7**, which lacks a dimethylamino group,

[a] *Dr. B. La Ferla, V. Spinosa, G. D'Orazio, Prof. F. Nicotra*
Department of Biotechnology and Bioscience & CINMPIS, Università degli studi di Milano Bicocca, Piazza della Scienza 2, 20126 Milano (Italy)
Fax: (+39) 026-448-3565
E-mail: francesco.nicotra@unimib.it

[b] *Dr. M. Palazzo, Prof. C. Rumio*
Department of Human Morphology, Università degli studi di Milano, Via Mangiagalli 31, 20133 Milano (Italy)

[c] *Prof. A. Balsari*
Institute of Pathology, Università degli studi di Milano, Via Mangiagalli 31, 20133 Milano (Italy)

[d] *Dr. A. A. Foppoli*
Department of Pharmaceutical Sciences "P. Pratesi", Università degli studi di Milano, Via Mangiagalli 25, 20133 Milano (Italy)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.201000282.

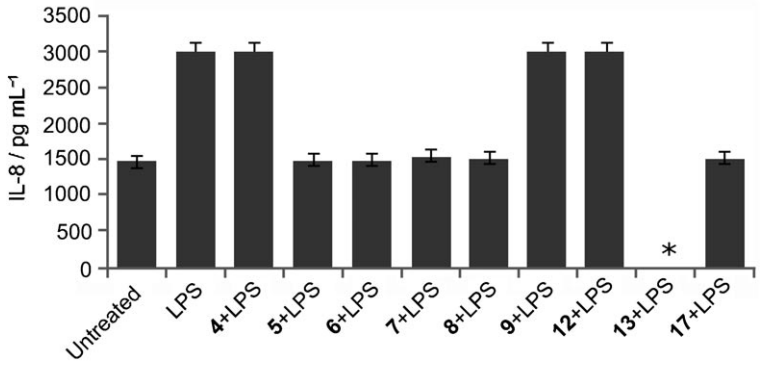


Figure 1. IL-8 production in response to LPS (1 μg mL^{-1}) and compounds **4**, **5**, **6**, **7**, **8**, **9**, **12**, **17** (50 mg L^{-1}). * **13**+LPS was below the level of detection.

and naphthyl amide **8**, which has an amide group in place of the sulphonamide group (Scheme 1).

Taking into account that galactose is recognised and transported by SGLT-1,[10] we synthesised a galacto-derivative **6**, exploiting the same procedure described for compound **5.** We also elongated the spacer between the dansyl moiety and the sugar in compound **17** (Scheme 3), and changed the position of the dansyl group in compound **13**. The synthesis of compound **13** (Scheme 2) was performed, once more avoiding the protection/deprotection steps. Methyl α-D-glucopyranoside was reduced with triethylsilane, affording 1-deoxy-D-glucose **9**. Then selective tosylation of the primary hydroxy group, treatment of the tosylate **10** with NaN_3, reduction of the obtained azide **11** with $H_2/Pd(OH)_2$, and finally treatment of the amine **12** with dansyl chloride, afforded compound **13**.

The synthesis of compound **17** required the insertion of a longer spacer between the sugar and the dansyl moiety. We started from α-allyl *C*-glucoside **14**,[7] and exploited the cross-metathesis (CM) reaction for elongation. To perform the CM on our unprotected substrate, we exploited a recent protocol that describes the use of conventional hydrophobic ruthenium catalysts in homogeneous aqueous solvent mixtures.[11,12] CM between α-allyl *C*-glucoside **14** and *cis*-2-butene-1,4-diphthalimide,[13] using commercial Hoyveda–Grubbs 2nd generation catalyst (MeOH/$CHCl_3$ (1:1) at 40 °C for 36 h), afforded a reasonable quantity of compound **15** (57%, 80% conversion, 8:2 *E*/*Z*). Catalytic hydrogenation followed by hydrazinolysis generated amine **16**, which was converted into the dansyl derivative **17**.

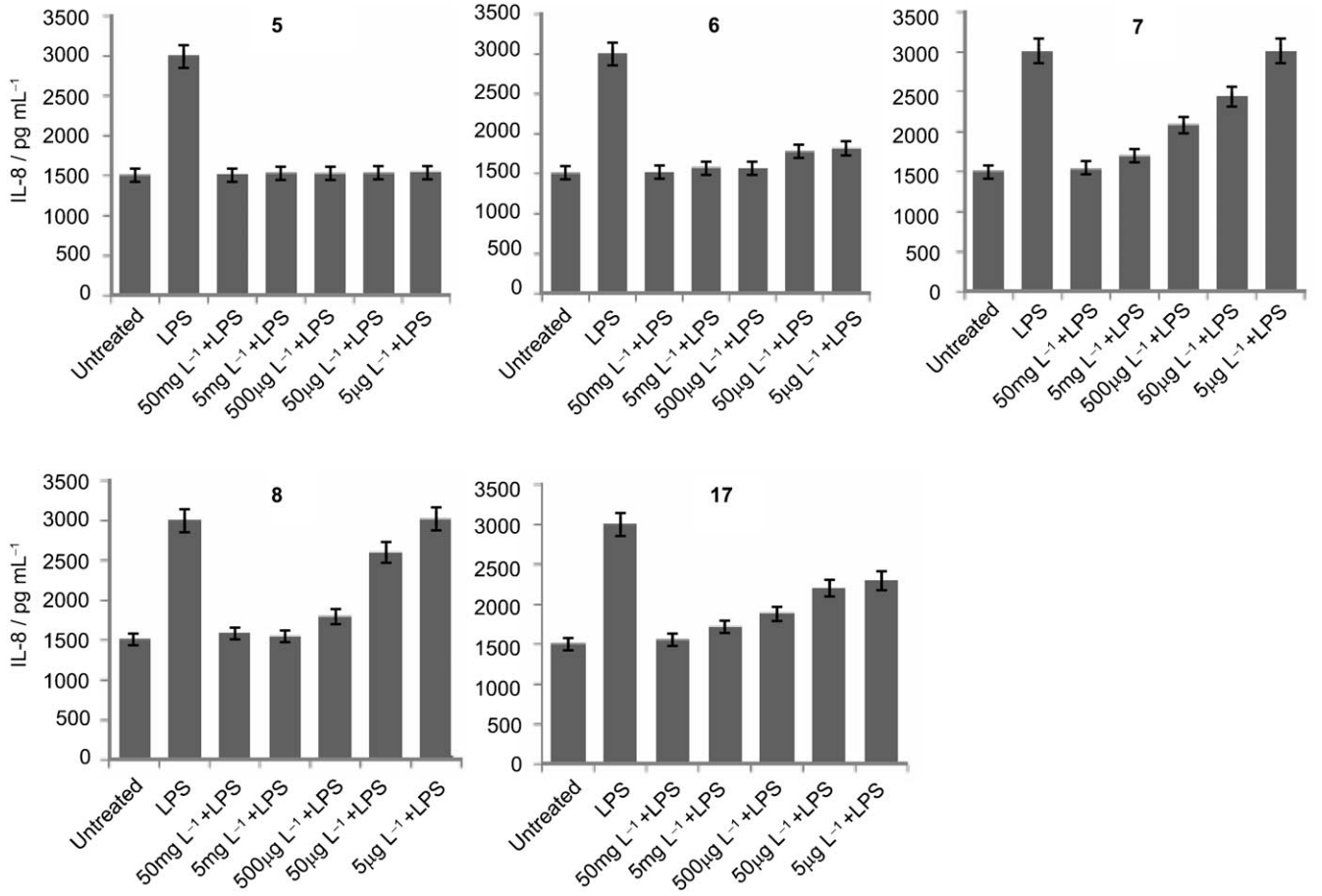
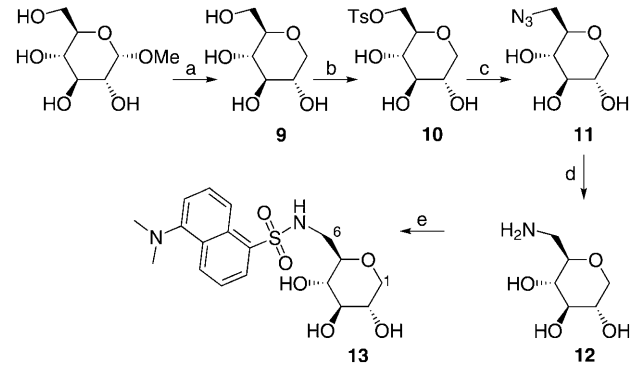
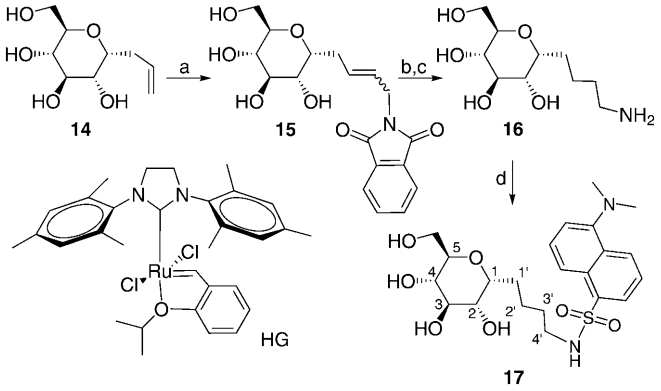


Figure 2. IL-8 production in response to LPS (1 μg mL^{-1}) and compounds **5**, **6**, **7**, **8**, **17** (50 mg L^{-1} to 5 μg L^{-1}).



Scheme 2. *Reagents and conditions*: a) BSTFA, CH_3CN, 80 °C, 1 h; then, TMSOTf, Et_3SiH, RT, 12 h;[7] b) TsCl, Py, 0 °C→RT, 5 h, 80% (two steps); c) NaN_3, TBAI, DMF, 80 °C, 24 h, 90%; d) H_2, $Pd(OH)_2$, MeOH, RT, 12 h, quant.; e) Dansyl-Cl, K_2CO_3, MeOH, THF, RT, 2 h, 42%.



Scheme 3. *Reagents and conditions*: a) HG, *cis*-2-butene-1,4-diphthalimide, $CHCl_3$/MeOH (1:1), 40 °C, 36 h, 55%; b) H_2, $Pd(OH)_2$, MeOH, RT, 3 h; c) N_2H_4, MeOH, 50 °C, 4 h, 78% (two steps); d) Dansyl-Cl, K_2CO_3, MeOH, THF, RT, 2 h, 77%.

IL-8 production by the human cell line, HT29, was used as a functional, preliminary screen of the anti-inflammatory activity of members of the library. The HT29 cells were incubated for 18 h in complete culture media alone or in media containing 50 mg L^{-1} of our compounds. Cells were then stimulated for 6 h with 1 μg mL^{-1} of LPS from *Salmonella enterica*, *serovar abortus equi*. Variations in IL-8 concentration in the culture media were evaluated by ELISA (Figure 1).

From this preliminary evaluation, it was clear that compounds **4**, **9**, **12**, simple nonmetabolisable glucose/galactose derivatives lacking the naphthyl moiety, did not affect LPS-induced IL-8 production, while for compounds **5**, **6**, **7**, **8**, **17**, each with a naphthyl group linked to the *C*-glycosidic appendage, a significant reduction in IL-8 was observed. Compound **13** was actually toxic to the HT29 cells. The dose-dependent activity of compounds **5**, **6**, **7**, **8** and **17** was also investigated, decreasing the concentration from 50 mg L^{-1} down to 5 μg L^{-1}. Compound **5** was able to maintain IL-8 suppression at all concentrations tested, even at 5 μg L^{-1}. The activity of compounds **6**, **7**, **8** and **17** decreased in a dose-dependent manner, but at the lowest concentration tested (5 μg L^{-1}), compounds **6** and **17** maintained a low efficacy, while **7** and **8** completely lost their suppressive activity (Figure 2).

From the ELISA data, it can be argued that the presence of the dimethylamino substituent on the naphthyl group is crucial for anti-inflammatory activity. Notably, the length of the spacer between the sugar residue and the naphthyl pharmacophore is also relevant.

To determine the effective involvement of the glucose transporter-1 (SGLT-1) in the observed reduction of HT29-derived IL-8, we repeated the experiments with the most active compound **5**, using an SGLT-1 knockdown cell line. Using small interfering RNA (siRNA), SGLT-1 expression was silenced in the HT29 cell line. These cells were then treated with LPS, and the level of IL-8 was measured by ELISA. As shown in Figure 3a

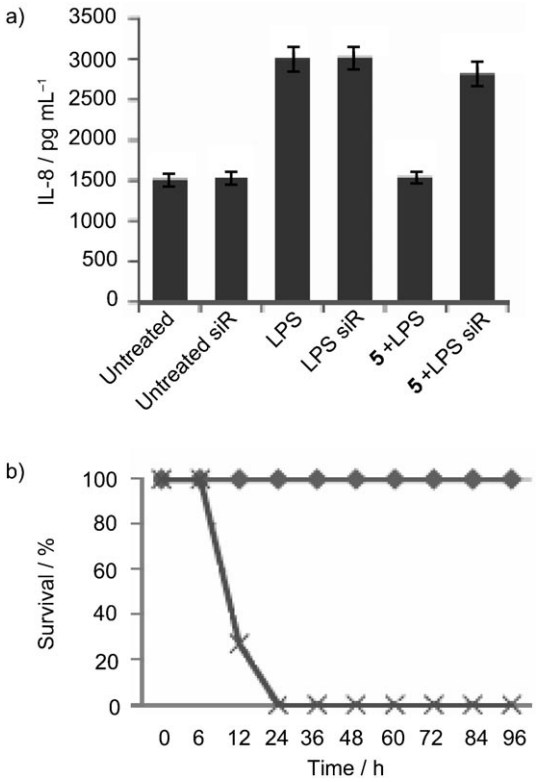


Figure 3. a) IL-8 production in response to LPS (1 μg mL^{-1}) and compound **5**, 50 mg L^{-1}, with or without siRNA (siR). b) Percent survival of mice treated with LPS/$GalNH_2$ and with (—◆—), or without (—x—) compound **5**. The animals were monitored for four days after treatment. Experimental protocols were approved by the Ethics Committee for Animal Experimentation of the Instituto Nazionale Tumori (Milan, Italy) and carried out according to guidelines of the United Kingdom Co-ordinating Committee on Cancer Research for animal welfare in experimental neoplasia (1998).

(last column), the activity of compound **5** is lost when SGLT-1 expression is knocked down, proving that the biological activity of compound **5** is mediated by its interaction with SGLT-1.

Finally, we investigated the biological activity of compound **5** in vivo. These studies were carried out with a mouse model of lethal septic shock induced by intraperitoneal (i.p.) administration of LPS and galactosamine ($GalNH_2$).[14] One group of mice was treated only with LPS (250 μg kg^{-1}) and $GalNH_2$

(1 g kg^{-1}); another group was injected with LPS (250 μg kg^{-1}), $GalNH_2$ (1 g kg^{-1}) and compound **5** (25 μg kg^{-1}), administrated by oral gavage; a third group of mice was given only sterile water; a final group was treated only with compound **5** (25 μg kg^{-1}). For each experimental group, 10 mice were used. As expected, in the mice treated with only LPS/$GalNH_2$, 100% of the animals developed and died from septic shock within 24 h. In contrast, 100% of the mice survived in the group treated with LPS/$GalNH_2$ and compound **5** (Figure 3b), and in the two control groups (see Supporting Information). This finding confirms that compound **5** can effectively inhibit bacteria-induced inflammation and has the potential to be a life-saving treatment for septic shock.

In conclusion, a new class of *C*-glucosides displaying strong anti-inflammatory activity have been synthesised using straightforward chemoselective procedures that did not require protection, including the Sakurai reaction and cross-metathesis. These compounds can interact with SGLT-1, a newly identified component in the inflammatory cascade. In particular, compound **5** displays a very potent activity, and its administration, at a dose of 25 μg kg^{-1}, results in a 100% survival of mice that are treated with LPS/$GalNH_2$. The identification of highly active anti-inflammatory compounds that act as agonists for SGLT-1 is intriguing, as studies published examining this transporter have sought to block its activity to prevent glucose absorption.[15]

Acknowledgements

The CINMPIS Consortium supported this work through a fellowship awarded to V. Spinosa.

Keywords: *C*-glycosides · chemoselectivity · endotoxic shock · glycomimetics · sepsis

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Received: July 9, 2010
Published online on August 19, 2010

