DOI: 10.1002/chem.201102052

The Inhibition of Liposaccharide Heptosyltransferase WaaC with Multivalent Glycosylated Fullerenes: A New Mode of Glycosyltransferase Inhibition

Maxime Durka,[a] Kevin Buffet,[a] Julien Iehl,[b] Michel Holler,[b] Jean-François Nierengarten,*[b] and Stéphane P. Vincent*[a]

Abstract: L,D-Heptosides (L-glycero-D-manno-heptopyranoses) are found in important bacterial glycolipids such as lipopolysaccharide (LPS), the biosynthesis of which is targeted for the development of novel antibacterial agents. This work describes the synthesis of a series of fullerene hexa-adducts bearing 12 copies of peripheral sugars displaying the mannopyranose core structure of bacterial L,D-heptoside. The multimers were assembled through an efficient copper-catalyzed alkyne–azide cycloaddition reaction as the final step. The final fullerene sugar balls were assayed as inhibitors of heptosyltransferase WaaC, the glycosyltransferase catalyzing the incorporation of the first L-heptose into LPS. Interestingly, the inhibition of the final molecules was found in the low micromolar range (IC_{50} = 7–45 μM), whereas the corresponding monomeric glycosides displayed high micromolar to low millimolar inhibition levels (IC_{50} always above 400 μM). When evaluated on a "per-sugar" basis, these inhibition data showed that, in each case, the average affinity of a single glycoside of the fullerenes towards WaaC was significantly enhanced when displayed as a multimer, thus demonstrating an unexpected multivalent effect. To date, such a multivalent mode of inhibition had never been evidenced with glycosyltransferases.

Keywords: cell wall · fullerenes · heptose · inhibitors · multivalence · virulence

Introduction

Fullerene derivatives substituted with sugar residues, that is, glycofullerenes, exhibit a combination of interesting properties related to water solubility and biological relevance.[1–2] Most of the glycofullerenes reported to date[3–4] are however amphiphilic molecules, with a hydrophobic fullerene subunit occupying a part of the outer architecture of the molecule. As a result, low solubility in aqueous media and/or aggregation phenomena remain major limitations for some biological applications with such compounds. Recently, we have shown that the amphiphilic character of glycofullerenes can be avoided[5] by taking advantage of the unique globular structure of fullerene hexakis-adducts with a T_h-symmetrical octahedral addition pattern.[6] These glycoclusters display twelve sugar residues on their periphery in a globular topology.[5] Importantly, the high local concentration of carbohydrates around the C_{60} core in such derivatives has been found perfectly suited to the binding of lectins[7–8] and dramatic multivalent effects have been observed in some cases.[8] On the other hand, surprising effects have been evidenced for the inhibition of carbohydrate-processing enzymes with a fullerene hexakis-adduct decorated with 12 iminosugar residues.[9] Specifically, multivalent effects have been observed with isomaltase of baker's yeast and α-mannosidase of Jack bean with K_i values improved by two and three orders of magnitude, respectively, over the corresponding monomeric iminosugar. This first evidence of dramatic multivalent effects in glycosidase inhibition not only opens a new field of research, but also reveals that the multivalent approach may be an appealing tool for innovative drug discoveries. As part of this research, we now report the synthesis of dodecaglycosylated fullerenes and their evaluation as a new class of multivalent inhibitors of a biologically relevant bacterial glycosyltransferase (GT). Indeed, the biosynthesis of complex glycans involves the participation of a huge number of GTs that catalyzes the regio- and stereoselective transfer of a saccharide from a donor (usually a nucleotide sugar) to an acceptor (mainly a "growing" oligosaccharide, a lipid or a protein; Figure 1).

Many of these enzymes are involved in key biological processes such as cell adhesion and recognition, signaling, and some of them represent attractive targets for the development new treatments of various pathologies such as cancer, inflammation and infectious diseases.[10–12] Given their biological significance, the discovery of GT inhibitors[13] as well

[a] Dr. M. Durka, K. Buffet, Prof. S. P. Vincent
Chemistry Department, University of Namur (FUNDP)
rue de Bruxelles 61, 5000 Namur (Belgium)
Fax: (+32) 81-72-45-17
E-mail: stephane.vincent@fundp.ac.be

[b] Dr. J. Iehl, Dr. M. Holler, Dr. J.-F. Nierengarten
Laboratoire de Chimie des Matériaux Moléculaires
Université de Strasbourg et CNRS (UMR 7509)
Ecole Européenne de Chimie, Polymères et Matériaux (ECPM)
25 rue Becquerel, 67087 Strasbourg Cedex 2 (France)
Fax: (+33) 368-85-27-74
E-mail: nierengarten@unistra.fr

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201102052.

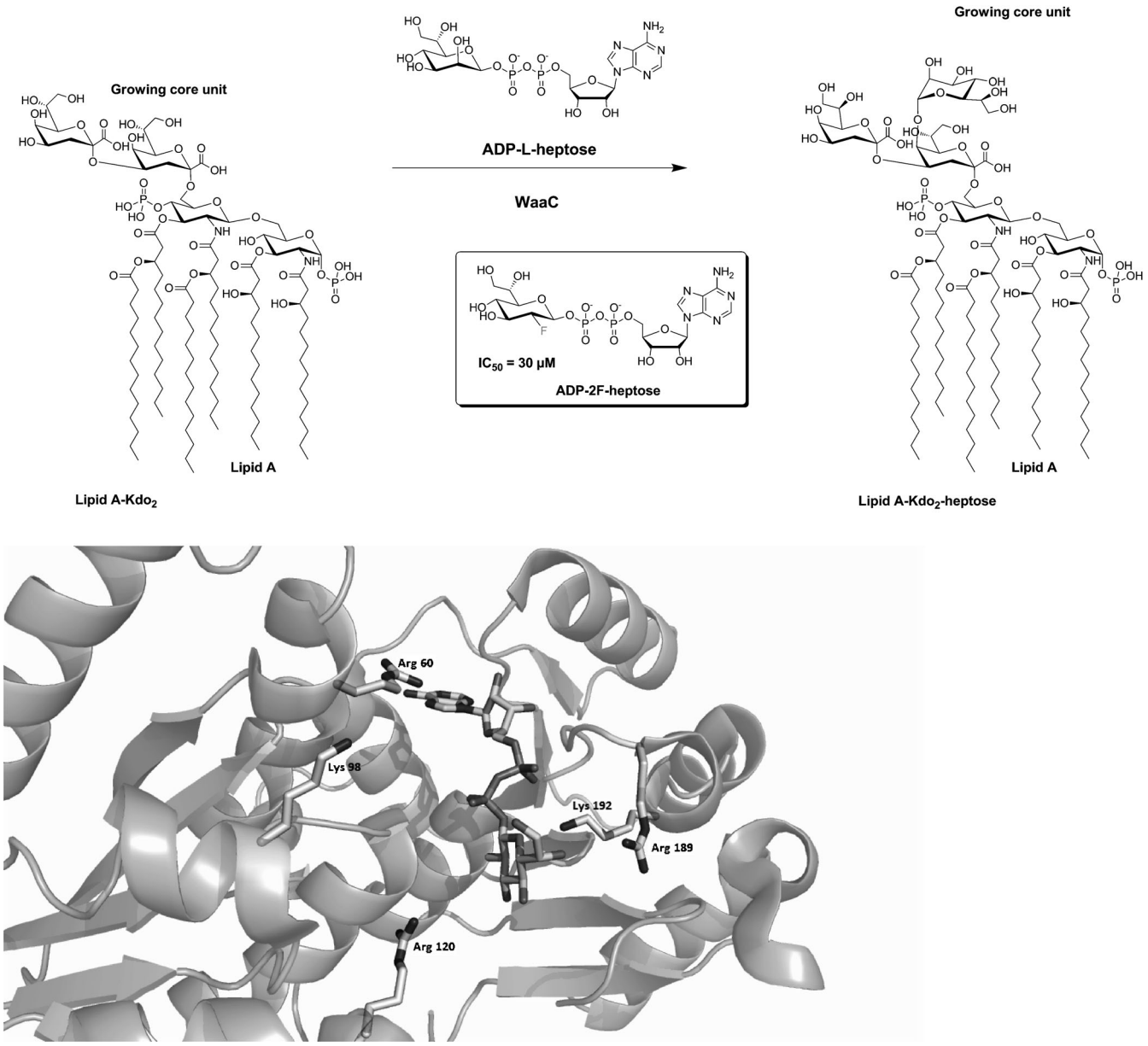


Figure 1. Top: Heptosylation of Lipid A-Kdo_2 catalyzed by heptosyltransferase WaaC. Bottom: Partial view of the crystallographic structure of heptosyltransferase WaaC complexed with ADP-2F-heptose (pdb 2H1H) highlighting the proximity of the heptose unit of the substrate with the amine of lysine residues (Lys 192) and the presence of cationic residues in the binding pocket (Lys98, Arg120, Arg 60, Arg189).

as the understanding of their intimate mechanism[14] are of major importance but still represent challenging tasks.[15] Indeed, very potent inhibitors have been only obtained in a few cases which is in deep contrast with other glycosyl-processing enzymes or lectins for which many low nanomolar or even picomolar inhibitors have been developed.[16]

Taking into account the medicinal relevance of GTs, novel strategies to discover and develop potent inhibitors are clearly needed.

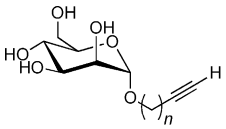
Results and Discussion

The targeted glycosyltransferase and design of the multivalent inhibitors: Lipopolysaccharide (LPS) is a key component of the outer membrane of Gram-negative bacteria.[17] From a pharmaceutical viewpoint, its biosynthesis has become a very important research field if one considers that the mortality of many infectious diseases is closely related to the amount of circulating LPS found in patient sera.[18] LPS is an amphipathic molecule that can be decomposed into three main substructures: lipid A, the oligosaccharide core, and the O-antigen. The oligosaccharide core unit can itself be divided into two parts: the inner core, which is

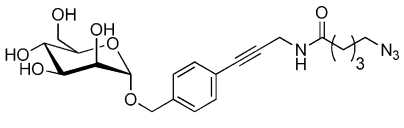
formed at least by one molecule of 3-deoxy-α-D-manno-oct-2-ulopyranosonic acid (Kdo) and two molecules of L-glycero-α-D-manno-heptopyranose (heptose), and the outer core which is composed of hexoses. Lipid A and one Kdo is the minimal structure for maintaining cell viability. Gram-negative bacteria lacking the heptose units display the deep-rough phenotype[19] and show a reduction in outer membrane protein content, an increased sensitivity towards detergents or hydrophobic antibiotics and are much more susceptible to phagocytosis by macrophages.[20]

Therefore, the heptosyltransferases implied in the LPS biosynthesis represent attractive targets, the inhibition of which could attenuate the virulence of diverse bacterial strains. As depicted in Figure 1, heptosyltransferase WaaC catalyzes the incorporation of the first heptosyl-subunit onto the growing core moiety of LPS.[21]

To provide a proof-of-principle that fullerene sugar balls would be inhibitors of WaaC, we have decided to decorate the fullerene core with L-heptose moieties (Figure 2, molecules $\mathbf{C_{60}(A)_{12}}$ and $\mathbf{C_{60}(B)_{12}}$) sharing the D-mannopyranose substructure found in ADP-heptose, the donor substrate of WaaC or in the product LipidA-Kdo_2-heptose. As outlined in Figure 1, WaaC is an inverting glycosyltransferase: the donor substrate being β-configured, thus the product is an α-heptoside. Having in hand the three α-mannosides **1**–**3** and their corresponding fullerenes,[7] we decided to synthesize α-configured heptosides and octosides to compare a homogeneous series and evaluate whether WaaC could be inhibited in a multivalent fashion.



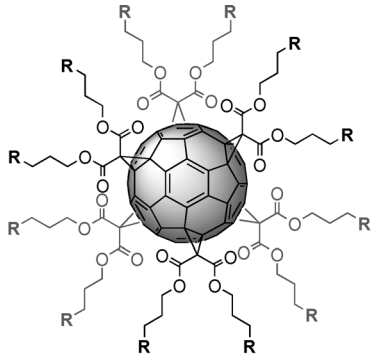
1 $n = 1$
2 $n = 4$



3

Based on the knowledge of the contacts between the heptose residue and the enzyme deduced from the crystallographic structure of heptosyltransferase WaaC in a complex with ADP-2F-heptose (Figure 1),[22] we have also decided to explore the binding properties of an octose bearing an additional ionic functionality (Figure 2, molecule $\mathbf{C_{60}(C)_{12}}$). Indeed, the amine of a lysine residue is located at the vicinity of the 6-position of the heptose (Figure 1)[23] and a properly positioned carboxylate group in the inhibitor structure might provide additional strong coulombic interactions.

To complete the study, we also assayed compounds $\mathbf{C_{60}(D)_{12}}$, $\mathbf{C_{60}(E)_{12}}$, and $\mathbf{C_{60}(F)_{12}}$ bearing peripheral mannosyl subunits. Finally, to define whether these multimers display a multivalent effect or not, the corresponding monomeric structures **A** and **C**–**F** were also investigated. Furthermore, fullerene derivative $\mathbf{C_{60}(Ph)_{12}}$ lacking the carbohydrate residues was used as a negative control compound for the biological assays.



Glycofullerene derivatives

Control Monomers

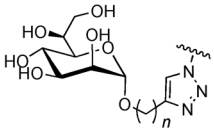
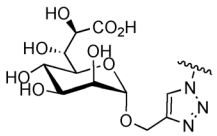
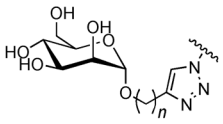
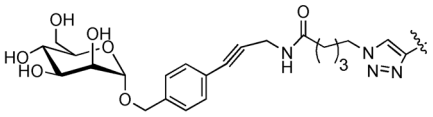
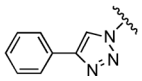
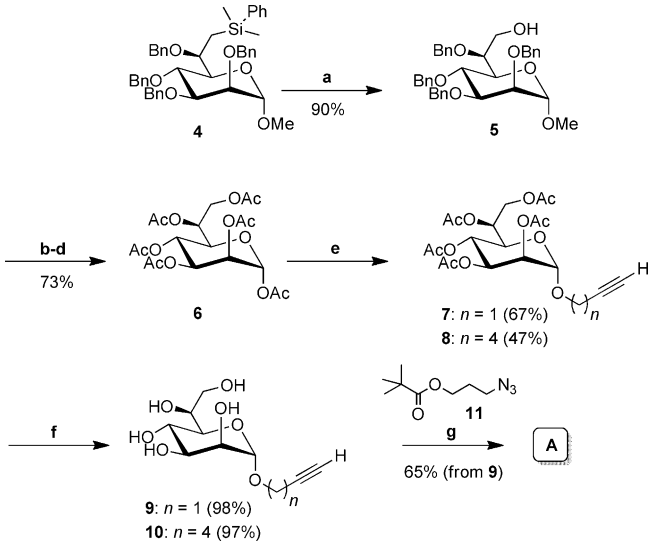
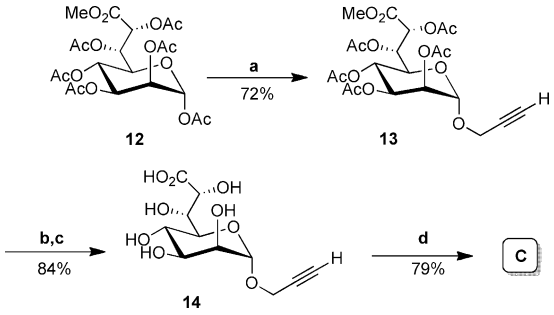
R		Fullerene	Monomer
	n=1	$\mathbf{C_{60}(A)_{12}}$	**A**
	n=4	$\mathbf{C_{60}(B)_{12}}$	–
		$\mathbf{C_{60}(C)_{12}}$	**C**
	n=1	$\mathbf{C_{60}(D)_{12}}$	**D**
	n=4	$\mathbf{C_{60}(E)_{12}}$	**E**
		$\mathbf{C_{60}(F)_{12}}$	**F**
		$\mathbf{C_{60}(Ph)_{12}}$	–

Figure 2. Targeted multimeric glycofullerenes and their corresponding control monomers.

Synthesis: The synthesis of glycofullerenes $\mathbf{C_{60}(A–F)_{12}}$ relies on the direct grafting of unprotected sugar derivatives onto preconstructed fullerene hexa-adduct derivatives under the copper-catalyzed azide–alkyne cycloaddition (CuAAC) conditions.[5] The mannoside precursors bearing the appropriate alkyne or azide function allowed the preparation of $\mathbf{C_{60}(D–F)_{12}}$ (compounds **1–3**). The preparation of the alkyne building blocks requested to prepare $\mathbf{C_{60}(A–C)_{12}}$ is shown in Scheme 1 and Scheme 2. Among the various synthetic procedures that have been developed for the construction of L-heptoses,[24] the most appealing strategy for us was the sequence developed by Van Boom and collaborators.[25] Compound **4** (Scheme 1) was obtained by the addition of a silylated Grignard reagent to the corresponding aldehyde precursor. In the original procedure of Van Boom, compound **5** was prepared from **4** under Fleming–Tamao oxidation condi-



Scheme 1. Synthesis of L,D-heptoside building blocks **9** and **10**, and preparation of model compound **A**. Reagents and conditions: a) AcOK, $Hg(TFA)_2$, AcOOH, AcOH, 10°C to RT; b) Ac_2O, Py, DMAP, RT; c) Ac_2O, AcOH, H_2SO_4, 0°C; d) Pd/C 10%, H_2, MeOH/H_2O 5:1, RT then Ac_2O, Py, DMAP, RT; e) Propargyl alcohol ($n=1$) or hexynyl alcohol ($n=4$), $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0°C to RT; f) MeONa, MeOH, RT; g) CuI, DIEA, DMF, microwave irradiation 80°C.

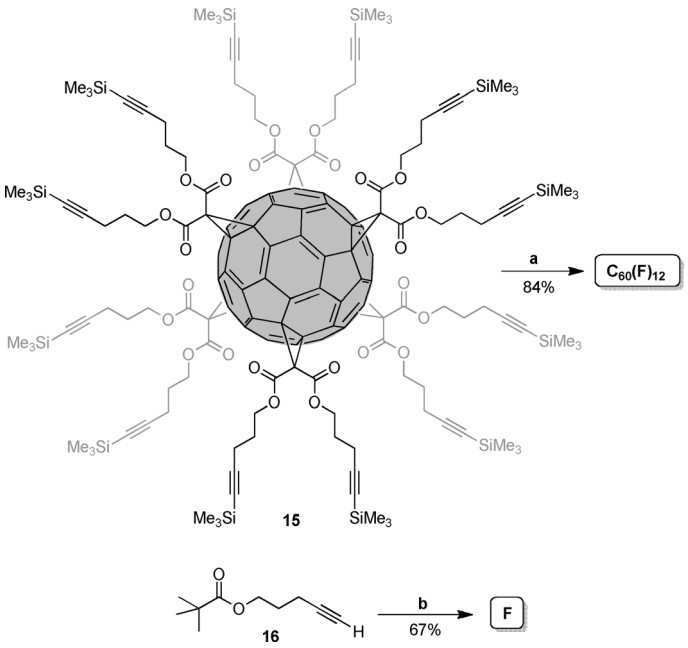


Scheme 2. Synthesis of propargyl octoside **14** and preparation of model compound **C**. Reagents and conditions: a) Propargyl alcohol, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0°C to RT; b) MeONa, MeOH, RT; c) LiOH 1M, RT; d) **11**, CuI, DIEA, DMF, microwave irradiation.

tions. We found that the scaling-up of the latter step was highly problematic. Actually, we have recently reported a modification of the Fleming–Tamao procedure allowing the synthesis of L,D-heptoside **5** on a multigram scale,[26] this compound was thus selected as the starting material for the preparation of alkynylated heptoses **9** and **10** (Scheme 1).

Precursor **5** was engaged in an acetylation of its primary alcohol function followed by acetolysis, hydrogenolysis, and peracetylation to afford **6**. Subsequent glycosylations with propargyl alcohol and 5-hexynyl alcohol gave alkynes **7** and **8**, respectively. Subsequent deacetylation yielded the two unprotected heptosides **9** and **10**, ready for ligation to the fullerene core. The copper-catalyzed cycloaddition of azide **11** with alkyne **9** gave model compound **A** in 65% yield. The synthesis of the targeted octoside building block **14** was performed from the known molecule **12**[26] (Scheme 2). Peracetate **12** was coupled to propargyl alcohol under standard conditions to give intermediate **13** in 72% yield. Deacetylation under Zemplén conditions followed by saponification of the methyl ester group afforded fully unprotected octoside **14** that was further coupled to azide **11** under CuAAC conditions to yield model compound **C**.

The preparation of fullerene sugar balls $\mathbf{C_{60}(A–F)_{12}}$ is depicted in Scheme 3 and Scheme 4. The hexa-substituted fullerene building blocks **15**[27] and **17**[28] were prepared according to reported procedures. The grafting of azide **3** onto the hexa-substituted fullerene core was achieved under the CuAAC conditions we have optimized for the preparation of fullerene sugar balls.[5] Compound **15** was treated with a slight excess of azide **3** (13 equiv) in the presence of tetrabutylammonium fluoride (TBAF), $CuSO_4{\cdot}5H_2O$ and sodium ascorbate (AscNa) in DMSO. At the end of the reaction, the product was precipitated by addition of MeOH, filtered and extensively washed with MeOH and CH_2Cl_2. To remove eventual copper-based impurities remaining from the CuAAC reaction, glycoconjugate $\mathbf{C_{60}(F)_{12}}$ was further purified by filtration on a Sephadex™ column (H_2O), precipitated with MeOH and dried under high vacuum. Compound $\mathbf{C_{60}(F)_{12}}$ was thus isolated in 84% yield. Finally, the corresponding model compound (**F**) was prepared in 67% yield from **16** and **3**.



Scheme 3. Preparation of glycocluster $\mathbf{C_{60}(F)_{12}}$ and of model compound **F**. Reagents and conditions: a) **3**, $CuSO_4{\cdot}5H_2O$, AscNa, DMSO, RT; b) **3**, CuI, DIEA, DMF, microwave irradiation 80°C.

The chemical structure of compound $\mathbf{C_{60}(F)_{12}}$ was confirmed by ^1H and ^{13}C NMR analyses. As shown in Figure 2, the ^{13}C NMR spectrum of fullerene hexakis-adduct $\mathbf{C_{60}(F)_{12}}$ is in full agreement with its *T*-symmetrical structure and shows all the expected signals for the six equivalent malo-

nate addends (Figure 3). Importantly, the signals corresponding to the sp C atoms detected at $\delta = 85.6$ and 105.0 ppm for precursor **15** are not present anymore for

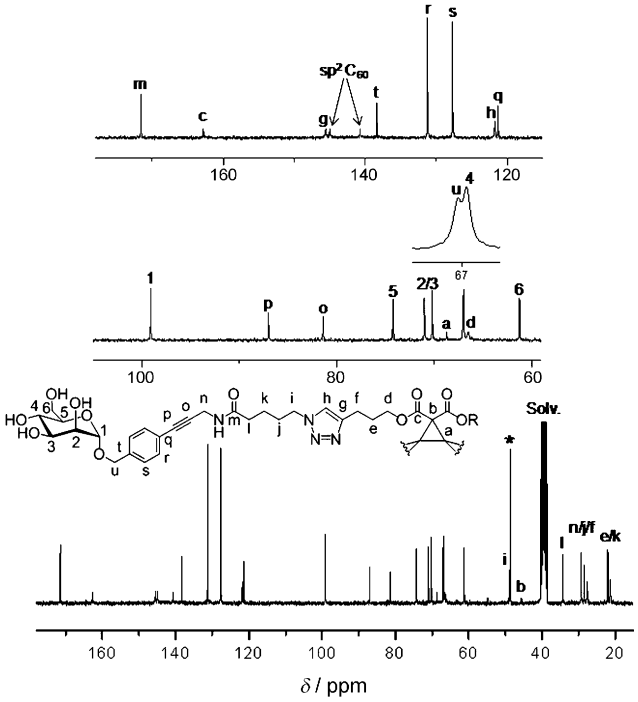
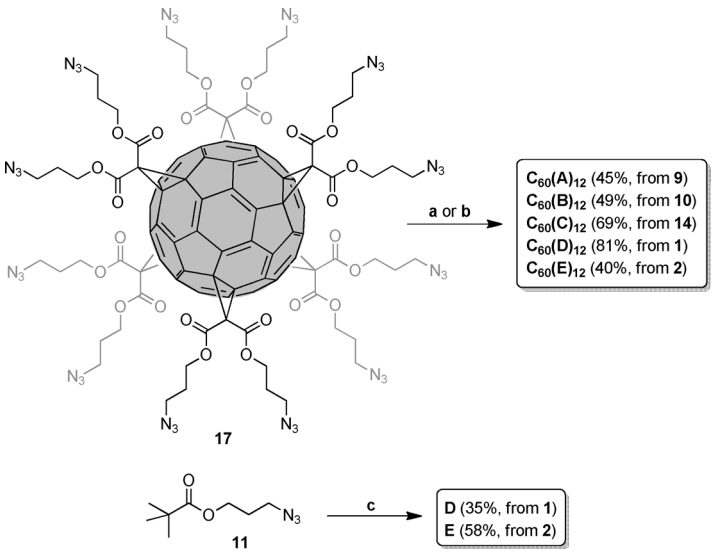


Figure 3. ^{13}C NMR spectrum of $\mathbf{C_{60}(F)_{12}}$ recorded in [D_6]DMSO (100 MHz, 25 °C); unambiguous assignment was achieved with the help of the corresponding DEPT spectrum (★ = CH_2Cl_2).

$\mathbf{C_{60}(F)_{12}}$, whereas two additional resonances are detected at $\delta = 121.7$ and 145.6 ppm. The latter signals are attributed to the sp^2 C atoms of the 12 equivalent 1,2,3-triazole subunits. Finally, only three signals out of the five expected ones are observed for the fullerene C atoms ($\delta = 68.8$ for the sp^3 C atom; $\delta = 140.7$ and 145.0 ppm for the sp^2 C atoms). Indeed, these three signals are reminiscent of those of the three non-equivalent fullerene C atoms of the hexakis-adduct carrying achiral addends (overall T_h symmetry). No influence of the overall symmetry of $\mathbf{C_{60}(F)_{12}}$ (T symmetry) could be deduced and the two pairs of diastereotopic sp^2 C atoms are pseudo-equivalent. Similar observations have been already reported for related C_{60} derivatives.[5]

The preparation of compounds $\mathbf{C_{60}(A–E)_{12}}$ required the use of polyazide **17**[28] (Scheme 4). As stated in our previous reports,[8b,28,29] compound **17** must be handled with special care owing to its high number of azide residues. Upon evaporation, compound **17** has never been extensively dried under high vacuum and the use of metallic spatula avoided. Furthermore, this compound has been always prepared on a small scale (less than 500 mg). It can also be noted that compound **17** cannot be stored in the solid state as slow decomposition into insoluble polymers occurs. The latter observation is most probably associated to the slow thermolysis or



Scheme 4. Preparation of glycoclusters $\mathbf{C_{60}(A–C)_{12}}$ and of model compounds **D** and **E**. Reagents and conditions: a) $CuSO_4 \cdot 5H_2O$, AscNa, DMSO/CH_2Cl_2/H_2O (1:1:1), RT $\mathbf{C_{60}(D–E)_{12}}$; b) $CuSO_4 \cdot 5H_2O$, AscNa, DMSO/CH_2Cl_2/H_2O (1:1:1), microwave irradiation 100 °C, $\mathbf{C_{60}(A–C)_{12}}$; c) CuI, DIEA, DMF, microwave irradiation 80 °C.

photolysis of azide groups and the nitrene residues thus generated are then responsible of the intermolecular reactions leading to polymers. Cycloaddition of the azide group onto the hexasubstituted fullerene core can also be at the origin of this slow polymerization process. Upon purification, the best is to use polyazide **17** for the CuAAC reactions within the next 24 h to obtain good yields. Reaction of freshly prepared **17** with alkynes **1**, **2**, **9**, **10**, and **14** was first attempted under the classical CuAAC conditions previously optimized for the preparation of fullerene sugar balls ($CuSO_4 \cdot 5H_2O$/AscNa in a CH_2Cl_2/H_2O/DMSO (1:1:1) mixture at room temperature).[5] These conditions were efficient for the preparation of $\mathbf{C_{60}(D)_{12}}$ and $\mathbf{C_{60}(E)_{12}}$ from alkynylated mannosides **1** and **2**, respectively. In contrast, compounds $\mathbf{C_{60}(A–C)_{12}}$ thus prepared were always polluted with defected by-products even after one week of reaction. Indeed, their IR spectra revealed minor traces of azide groups (typical signal observed at ca. 2092 cm^{-1}). Furthermore, as in the case of building block **17**, these compounds became completely insoluble after a few days of storage. In other words, the polymerization reactions occurred due to the presence of unreacted azide subunits.

This prompted us to attempt the CuAAC reactions of **17** with **9**, **10**, and **14** under microwave irradiation. Interestingly, complete functionalization was thus achieved in 2 h. The IR spectrum of $\mathbf{C_{60}(A–C)_{12}}$ prepared under microwave irradiation confirmed that no azide residues remain in the final products (Figure 4). This was further confirmed by the stability of the prepared compounds. Finally, it is also worth noting that glycoconjugates $\mathbf{C_{60}(A–E)_{12}}$ were all purified by precipitation followed by filtration on a Sephadex™ column to remove residual copper-based impurities from the CuAAC reactions. Actually, in most of the cases, two well

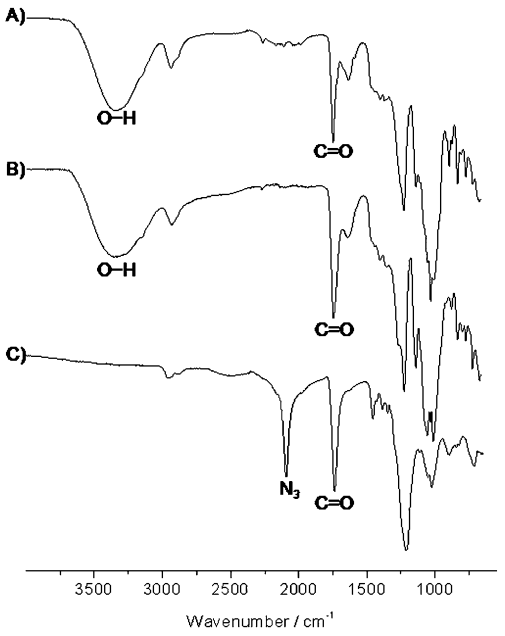


Figure 4. IR spectra of compounds $\mathbf{C_{60}(A)_{12}}$ (A), $\mathbf{C_{60}(C)_{12}}$ (B) and **17** (C).

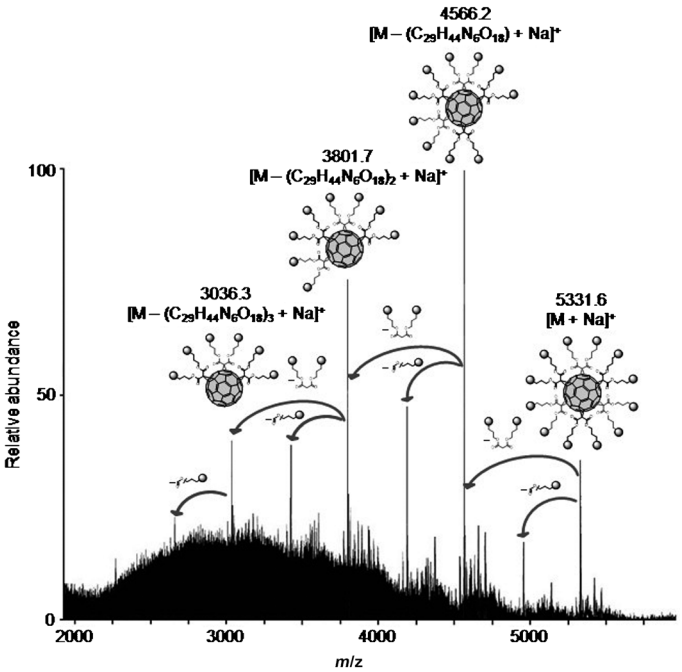


Figure 5. MALDI TOF mass spectrum of compound $\mathbf{C_{60}(A)_{12}}$. A series of typical fragments resulting from successive retro-Bingel reactions are observed. Further fragments resulting from the cleavage of a malonic ester unit followed by a decarboxylation are also observed.

separated fractions were collected upon Sephadex™ filtration. The high molecular weight one was orange and corresponded to the glycofullerene whereas the low molecular weight one was pale blue and contained the residual copper salts.

Additionally, model compounds **D** and **E** were prepared from **11** and the corresponding alkylynated mannose derivatives **1** and **2**.

The structures of the final compounds $\mathbf{C_{60}(A–E)_{12}}$ were confirmed by their ^{1}H and ^{13}C NMR spectra. Inspection of the ^{1}H NMR spectra shows the typical signal of the triazole unit at $\delta = 7.81$–8.06 ppm. As discussed for $\mathbf{C_{60}(F)_{12}}$, the ^{13}C NMR spectra of fullerene hexakis-adducts $\mathbf{C_{60}(A\text{-}E)_{12}}$ were particularly helpful to evidence their *T*-symmetrical structure. In all the cases, the expected signals for the hexasubstituted fullerene core and the six equivalent malonate addends were clearly observed.

The structure of glycoclusters $\mathbf{C_{60}(A–E)_{12}}$ was also confirmed by their MALDI-TOF mass spectra. The analysis of glycoclusters of high molecular weight such as $\mathbf{C_{60}(A–E)_{12}}$ is generally rather difficult due to high degree of fragmentation and/or to the formation of matrix adducts.[30] Despite these problems, the expected molecular ion peak could be evidenced in the case of compounds $\mathbf{C_{60}(A–E)_{12}}$. As a typical example, the MALDI-TOF mass spectrum of compound $\mathbf{C_{60}(A)_{12}}$ is shown in Figure 5. In addition to the expected molecular ion peak at $m/z = 5331.6$ $[M+\mathrm{Na}]^+$, a series of typical fragments resulting from successive retro-Bingel reactions[31] are observed ($[M+\mathrm{Na}-(C_{29}H_{44}N_6O_4)_n]^+$, with $n =$ 1 to 3). This fragmentation pathway is indeed classical for hexasubstituted fullerene derivatives.[27–29] It is also worth noting that an additional peak is always associated with the ones corresponding to $[M+\mathrm{Na}-(C_{29}H_{44}N_6O_4)_n]^+$. The latter corresponds to the hydrolysis of a malonic ester unit followed by a decarboxylation, leading to a loss of $C_{14}H_{22}N_3O_9$ with respect to the parent peak.[32] Despite the high level of fragmentation observed in the MALDI-TOF spectrum of $\mathbf{C_{60}(C)_{12}}$, no significant signals corresponding to defected products could be observed thus providing definitive conclusion about the monodispersity of the isolated product as already suggested from its IR spectrum in which no azide residues could be detected.

Inhibition of WaaC: The inhibition of WaaC by the glycofullerenes and their control monomers was measured by the assay we previously developed and applied to measure the IC_{50} of ADP-2F-heptose (Figure 1).[22,23] In brief, the specific activity of WaaC was followed by a coupled enzymatic assay involving pyruvate kinase to transform the ADP reaction product into ATP. The concentration of the latter was then monitored either by luminescence using luciferase or by fluorescence using lactate dehydrogenase. The results are summarized in Table 1. Interestingly, the glycosylated fullerenes $\mathbf{C_{60}(A–F)_{12}}$ displayed all low micromolar inhibition levels with IC_{50}'s ranging from 7 to 47 μM (Table 1, entries 1, 2, 5, 8, 10, and 12). By itself, this result is remarkable when analyzed in the global context of GTs inhibition. Effectively, the best GT inhibitors bind generally these enzymes with low μM K_i's, typically in the range of the K_m of the donor substrate.[13]

In the particular case of heptosyltransferase WaaC, the IC_{50} of the donor analogue ADP-2F-heptose is 30 μM

Table 1. Inhibition data.

Entry		IC_{50} [μM]	Inhibition enhancement	Enhancement per sugar[d]
1	$\mathbf{C_{60}(A)_{12}}$	11 ± 0.3	> 45	> 3.7
2	$\mathbf{C_{60}(B)_{12}}$	47 ± 6.6	> 11	> 0.9
3	**A**	19 %[a]		
4	**10**	0 %[b]		
5	$\mathbf{C_{60}(C)_{12}}$	6.7 ± 2.0	61	5.1
6	**14**	0 %[b]		
7	**C**	409 ± 28		
8	$\mathbf{C_{60}(D)_{12}}$	18 ± 1.3	> 28	> 2.3
9	**D**	19 %[a]		
10	$\mathbf{C_{60}(E)_{12}}$	36 ± 2.1	> 14	> 2.2
11	**E**	18 %[a]		
12	$\mathbf{C_{60}(F)_{12}}$	9.5 ± 0.3	> 53	> 4.4
13	**F**	8 %[a]		
14	$\mathbf{C_{60}(Ph)_{12}}$	0 %[c]	–	–

[a] Inhibition percentage at 500 μM. [b] No inhibition at 500 μM. [c] No inhibition at 250 μM. For solubility reasons, this fullerene could not be assayed at 500 μM. [d] Calculated on a per-sugar basis = IC_{50}monomer/(IC_{50}fullerene/12) with IC_{50}monomer = 500 when this value could not be measured above 500 μM.

(Figure 1). Moreover, the most potent GT inhibitors are always charged species that provide strong coulombic interactions with the protein. In our case, the grafting of neutral monosaccharides (except for fullerene $\mathbf{C_{60}(C)_{12}}$) is sufficient to allow a tight binding with the transferase. As a negative control, we measured the inhibition of WaaC by fullerene $\mathbf{C_{60}(Ph)_{12}}$ bearing phenyl rings in place of carbohydrates (Table 1, entry 14). No inhibition could be observed within the solubility limits of this molecule, thus showing that the origin of the inhibition of WaaC by the glycofullerenes does not originate from the fullerene core structure.

The peripheral residues of glycofullerenes $\mathbf{C_{60}(A)_{12}}$, $\mathbf{C_{60}(C)_{12}}$, and $\mathbf{C_{60}(D)_{12}}$ are all mannopyranosides attached to the central core through the same spacer. The only structural variation between these molecules is the nature of the substituent at the 5-position: CH_2OH ($\mathbf{C_{60}(D)_{12}}$), CH(OH)-CH_2OH ($\mathbf{C_{60}(A)_{12}}$) and $CH(OH)CH(OH)CO_2H$ ($\mathbf{C_{60}(C)_{12}}$). The observed decrease in IC_{50} along the series when going from the hexose derivative to the octose one thus demonstrates the beneficial effect of additional interactions of the substituent at the 5-position of the carbohydrate unit on WaaC inhibition. Octoside $\mathbf{C_{60}(C)_{12}}$ displayed the best inhibition profile of the whole series (IC_{50} = 6.7 μM, Table 1, entry 5). This result was anticipated based on the X-ray crystal structure of the enzyme. As illustrated in Figure 1, several cationic residues (lysines 98 and 192 and arginines 60, 120, and 189) are located at the vicinity of the binding pocket thus allowing positive electrostatic interactions with the carboxylate groups of $\mathbf{C_{60}(C)_{12}}$. Overall, if one considers that these molecules are α-configured, the observed inhibition might be related to product inhibition (see Figure 1).

A comparison of the inhibition properties of the two multivalent heptosides $\mathbf{C_{60}(A)_{12}}$ and $\mathbf{C_{60}(B)_{12}}$ bearing the same peripheral heptose units (Table 1, entries 1 and 2) indicates that the fullerene with the shortest spacer is a better inhibitor. A similar trend is observed by comparing the IC_{50} values obtained with the two dodecamannosides $\mathbf{C_{60}(D)_{12}}$ and $\mathbf{C_{60}(E)_{12}}$ with different spacers (Table 1 entries 8 and 10). Moreover, $\mathbf{C_{60}(F)_{12}}$ is the most potent mannosylated inhibitor (Table 1, entry 12). Thus, it appears that the incorporation of an aromatic moiety in the spacer improves the binding properties.

To assess whether some multivalent effect operates for the inhibition of heptosyltransferase WaaC with glycofullerenes $\mathbf{C_{60}(A–F)_{12}}$, the binding properties of the corresponding monomeric triazoles **A** to **F** (Figure 2) were evaluated. For comparison purposes, heptoside **10** and octoside **14** bearing an alkyne functionality were also assessed. The model compounds **A**–**F** bear an additional arm functionalized with a pivaloate ester to mimic as closely as possible the attachment to the fullerene core. The monomeric structures **A–F** only weakly inhibited WaaC. Interestingly, the two alkynylated derivatives **10** and **14** did not display any inhibition properties at concentrations up to 500 μM (Table 1, entries 4 and 6). The latter observation suggests an active role of the aromatic triazole subunit in the recognition process, this is also in line with the fact that $\mathbf{C_{60}(F)_{12}}$ possessing an aromatic subunit in the linker is the most potent mannosylated inhibitor (see below). The IC_{50} of the best monomeric inhibitor, octoside **C**, is 409 μM, a value 61-fold higher than for the corresponding multivalent fullerene derivative $\mathbf{C_{60}(C)_{12}}$, which corresponds to a 5.1 enhancement factor of inhibition per octose residue. For the other monomeric structures, the IC_{50} values were too high to be accurately measured under our experimental conditions. Therefore, we only report here the inhibition percentages measured at an inhibitor concentration of 500 μM and only a minimal value of the enhancement factors for the multimers on a "per-sugar" basis could be estimated. Globally, a clear multivalent effect is observed for all the fullerene derivatives with binding affinities enhanced by at least one order of magnitude when compared with the corresponding monomeric derivatives. Such an enhancement resulting from the multivalent presentation of carbohydrates around a central core structure had never been observed or even evidenced for glycosyltransferase inhibition.

In contrast to lectins, the inhibition of enzymes by multivalent inhibitors has been poorly explored to date. The only reported examples are multimeric iminosugars that have been assayed against glycosidases. In the two first pioneering studies, a nojirimycine[33] and a azafagomine tetramer[34] were found to be micromolar glycosidase inhibitors but a multivalent inhibition effect could not be evidenced. A similar result was later obtained on the enzymes involved in the glucosylceramide biosynthesis with a dimeric nojirimycine,[35a] and against E. coli β-galactosidase by multimeric thiogalactosides.[35b] Importantly, Gouin and Kovensky described the synthesis of a trimeric iminosugar that displayed a low micromolar inhibition against Jack bean α-mannosidase and, for the first time, an inhibition enhancement factor of 2.5 per iminosugar unit.[36] More recently, Compain and Nierengarten described a fullerene iminosugar ball that was assayed against a range of glycosidases and displayed a relative inhibition potency of 179 compared with the monomeric

structure, also against Jack bean α-mannosidase.[9] These literature data clearly indicate that all enzymes are not prone to multivalent inhibition and that the nature of both the central core structure and the spacer unit tethering the inhibitory monomers play critical roles in the inhibition process. The inhibition data presented in this study demonstrate that heptosyltransferase WaaC is the second enzyme ever reported for which significant levels of multivalent inhibition have been measured. In contrast to many lectins that are themselves multimeric and for which multimeric inhibitors were found extremely potent thanks to a "cluster effect", multivalent enzyme inhibition is much more difficult to rationalize.[16h,37] WaaC is a monomeric enzyme that has the specific property to have a glycolipid (Lipid A-Kdo_2) as the acceptor substrate (Figure 1). Due to the lipidic nature of the substrate, the reaction takes place at a surface: either of a vesicle for in vitro experiments or at the cytoplasmic inner membrane (in vivo). Therefore, clustering or local concentration effects might explain why multimeric structures such as glycofullerenes do inhibit WaaC in a multivalent manner. At the molecular level, inspection of the structure of WaaC in complex with an inhibitor shows that the binding pockets (donor and acceptor) are buried in a large open cleft that could allow quick rebinding processes with heptosides presented in the multimeric fashion. However, given the complexity of both the glycosyltransferase and the fullerenes, it is not reasonable, at this stage, to speculate on a specific binding mode. A novel series of multimeric glycosides, especially β-glycosides, will certainly be helpful to delineate the binding strength and selectivity of the sugar balls with WaaC.

Conclusion

Although the mechanism by which WaaC is inhibited by these multimeric structures remains obscure, we are convinced that these results are significant because glycosyltransferases, in general, constitute a fantastic class of targets for drug development, and, in particular, because the glycosyltransferases of the LPS biosynthesis have been overlooked for the discovery of novel antibacterial agents. This work paves the way to novel generations of glycosyltransferase inhibitors for systematic studies on the role of the central core and on the peripheral ligands. Work in this direction is under progress in our laboratories.

Experimental Section

General techniques: Reagents and solvents were purchased as reagent grade and used without further purification. All reactions were performed using purified and dried solvents: tetrahydrofuran (THF) was refluxed over sodium-benzophenone, dichloromethane (CH_2Cl_2), triethylamine (NEt_3), and pyridine were refluxed over calcium hydride (CaH_2). All reactions were monitored by thin-layer chromatography (TLC) carried out on Merck aluminum roll silica gel 60-F254 using UV light and a molybdate-sufuric acid solution as revelator. Detailed description of the spectroscopic apparatus used for the characterization of the compounds is given in the Supporting Information.

Synthesis of compounds: Compounds $C_{60}(Ph)_{12}$,[28] **1**,[38] **2**,[39] **3**,[7] **4**,[26] **5**,[26] **11**,[7] **12**,[26] and **15**[27] were prepared according to previously reported procedures, whereas the preparation of compounds **6**, **7**, **8**, **9**, **10**, **13**, and **14** is given in the Supporting Information.

*Compound **A***: A mixture of **9** (15 mg, 60.7 μmol), **11** (13 mg, 72.8 μmol), diisopropyl ethylamine (DIEA, 16 mg, 124 μmol) and CuI (5 mg, 0.26 μmol) in dry DMF (120 μL) was heated for one hour under microwave irradiation (80 °C). The solvent was evaporated under reduced pressure. Column chromatography (SiO_2, EtOAc to EtOAc/MeOH 9:1) gave **A** (17 mg, 65 %) as a yellow oil. $[\alpha]_D^{20} = +25.9$ (MeOH, $c = 0.5$). ^{1}H NMR (400 MHz, D_2O): $\delta = 7.94$ (s, 1H), 4.83 (s, 1H), 4.65 (AB, $J = 12$ Hz, 1H), 4.53 (AB, $J = 12$ Hz, 1H), 4.43 (t, $J = 6$ Hz, 2H), 3.98 (t, $J = 6$ Hz, 2H), 3.90 (app t, $J = 6.5$ Hz, 1H), 3.77 (app brs, 1H), 3.73 (app t, $J = 10$ Hz ,1H), 3.64 (dd, $J = 10$ and 3 Hz, 1H), 3.55 (dd, $J = 11$ and 8 Hz, 1H), 3.49 (m, 2H), 2.18 (quint., $J = 6$ Hz, 2H), 0.98 ppm (s, 9H). ^{13}C NMR (100 MHz, D_2O): $\delta = 181.8$, 143.6, 125.3, 99.6, 71.4, 70.7, 70.0, 68.7, 66.0, 62.8, 62.7, 59.8, 48.2, 38.5, 28.3, 26.2 ppm. MS (ESI): *m/z*: 456.2 $[M+Na]^+$; HRMS (ESI): calcd for $C_{18}H_{31}N_3O_9Na$ $[M+Na]^+$: 456.1953; found: 456.1970.

*Compound **C***: As described for **A**, with **11** (12 mg, 0.07 mmol) and **14** (15 mg, 0.06 mmol). Column chromatography (SiO_2, EtOAc to EtOAc/EtOH 7:3, 0.05 % NEt_3) afforded the triethylammonium salt of **C** (19 mg, 79 %) as a yellow oil. $[\alpha]_D^{20} = +9.3$ (MeOH, $c = 0.8$); ^{1}H NMR (400 MHz, D_2O): $\delta = 7.98$ (s, 1H), 4.83 (brs, 1H), 4.74–4.53 (m, 2H), 4.43 (t, $J =$ 6 Hz, 2H), 3.97 (t, $J = 5.5$ Hz, 2H), 3.77–3.66 (m, 4H), 3.05 (q, $J = 7$ Hz, 2H), 2.18 (tt, $J = 5.5$ and 6 Hz, 2H), 1.13 (t, $J = 7$ Hz, 3H), 0.95 ppm (s, 9H); ^{13}C NMR (101 MHz, D_2O): $\delta = 181.8$, 99.0, 71.6, 70.5, 69.7, 69.1, 62.7, 59.6, 58.8, 48.18, 46.7, 38.5, 28.2, 26.2, 8.24 ppm; MS (ESI+): *m/z* (%): 478.2 $[M+H]^+$ (100), 500.2 $[M+Na]^+$ (95), HRMS (ESI+): calcd for $C_{19}H_{32}O_{11}N_3$ $[M+H]^+$: 478.2031; found: 478.2050.

*Compound **D***: As described for **A**, with **1** (22 mg, 0.1 mmol, 1 equiv) and **11** (18 mg, 0.12 mmol, 1.2 equiv). Column chromatography (SiO_2, EtOAc to EtOAc/EtOH 8:2) gave **D** (14 mg, 35 %) as a yellow oil. $[\alpha]_D^{20}$ (MeOH, $c = 0.5$) $= +26.2$. ^{1}H NMR (400 MHz, D_2O): $\delta = 7.95$ (s,1H), 4.82 (d, $J =$ 1.5 Hz, 1H), 4.62 (AB, $J = 12$ Hz, 2H), 4.43 (t, $J = 6$ Hz, 2H), 3.97 (t, $J =$ 5.5 Hz, 2H), 3.78 (dd, $J = 1.5$ and 3 Hz, 1H), 3.71 (m, 1H), 3.64–3.58 (m, 1H), 3.62 (m, 1H), 3.52 (t, $J = 10$ Hz, 1H), 3.54–3.48 (m, 1H), 2.17 (quint., $J = 6$ Hz, 2H), 0.95 ppm (s, 9H); ^{13}C NMR (100 MHz, D_2O): $\delta =$ 181.8, 125.4, 99.5, 72.9, 70.5, 69.9, 66.7, 62.6, 60.8, 59.7, 48.1, 38.5, 28.2, 26.18 ppm. MS (TOF-MS-ES+): *m/z* (%) 242.1 $[M-C_6H_{11}O_6+NH_4]^+$ (100), 404.2 $[M+H]^+$ (88). HRMS: calcd for $C_{17}H_{30}N_3O_8$ $[M+H]^+$: 404.2027, found: 404.2041.

*Compound **E***: As described for **A**, with **2** (15 mg, 0.058 mmol, 1 equiv) and **11** (12 mg, 0.069 mol, 1.2 equiv). Column chromatography (SiO_2, EtOAc to EtOAc/EtOH 9:1) gave **E** (15 mg, 58 %) as a yellow oil. $[\alpha]_D^{20}$ (MeOH, $c = 0.2$) $= +4.7$. ^{1}H NMR (400 MHz, D_2O): $\delta = 7.73$ (s,1H), 4.74 (s, 1H), 4.29 (t, $J = 6$ Hz, 2H), 3.99 (t, $J = 5.5$ Hz, 2H), 3.82 (m, 1H), 3.75 (d, $J = 12$ Hz, 1H), 3.69–3.62 (m, 3H), 3.57–3.43 (m, 3H), 2.65 (t, $J =$ 7 Hz, 2H), 2.20 (m, 2H), 1.67–1.51 (m, 4H), 1.00 ppm (s, 9H). ^{13}C NMR (100 MHz, D_2O): $\delta = 181.7$, 99.7, 72.7, 70.6, 70.1, 67.4, 66.7, 62.6, 60.9, 48.0, 38.5, 28.2, 27.9, 26.2, 25.2, 24.3 ppm. MS (TOF-MS-ES+): *m/z* (%): 468.23 $[M+Na]^+$ (100), 913.47 $[2M+Na]^+$ (21); HRMS: calcd for $C_{20}H_{35}N_3O_8Na$): 468.2316 $[M+Na]^+$; found: 468.2292.

*Compound **F***: As described for **A**, with **3** (67 mg, 0.15 mmol, 1 equiv) and **16** (63 mg, 0.37 mmol). Column chromatography (SiO_2, EtOAc to EtOAc/EtOH 85/15) gave **F** (41 mg, 67 %) as a yellow oil. $[\alpha]_D^{20}$ (MeOH, $c = 1.2$) $= +20.2$. ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.74$ (s, 1H), 7.36 (d, $J = 8.5$ Hz, 2H), 7.31 (d, $J = 8.5$ Hz, 2H), 4.81 (d, $J = 1.5$ Hz, 1H), 4.61 (AB, $J = 12$ Hz, 2H), 4.37 (t, $J = 7$ Hz, 2H), 4.15 (s, 2H), 4.07 (t, $J = 6$ Hz, 2H), 3.82 (m, 1H), 3.81 (dd, $J = 2$ and 3 Hz, 1H), 3.70 (dd, $J = 4$ and 9 Hz, 1H), 3.67 (m, 1H), 3.60 (brt, $J = 10$ Hz, 1H), 3.54 (ddd, $J = 2$, 6 and 10 Hz, 1H), 2.74 (t, $J = 8$ Hz, 2H), 2.26 (t, $J = 7$ Hz, 2H), 2.00–1.88 (m, 4H), 1.61 ppm (quint., $J = 7$ Hz, 2H), 1.17 (s, 9H). ^{13}C NMR (100 MHz, CD_3OD): $\delta = 178.7$, 173.8, 138.2, 131.3, 127.7, 122.2, 99.5, 84.9, 81.8, 73.7, 71.3, 70.8, 68.0, 67.3, 63.4, 61.6, 49.6, 34.6, 29.4, 28.9, 28.2, 26.2, 22.3, 21.6 ppm. MS (TOF-MS-ES+): *m/z* (%): 617.32 $[M+H]^+$ (100), 639.30

$[M+Na]^+$ (100); HRMS: calcd for $C_{31}H_{45}N_4O_9$ $[M+H]^+$: 617.3181, found: 617.3189.

Compound $\mathbf{C_{60}(A)_{12}}$: A mixture of **17** (166 mg, 0.071 mmol), **9** (230 mg, 0.926 mmol), $CuSO_4{\cdot}5H_2O$ (1 mg, 0.007 mmol) and sodium ascorbate (4 mg, 0.021 mmol) in CH_2Cl_2/H_2O/DMSO (1:1:1, 3 mL) was heated 2 h under microwave irradiation (100 °C). The product was precipitated by addition of acetone (20 mL). The precipitate was washed with MeOH, then CH_2Cl_2 and dried under reduced pressure. Compound $\mathbf{C_{60}(A)_{12}}$ was further purified by filtration on a Sephadex™ column (H_2O), precipitated with MeOH and dried under high vacuum in order to totally remove eventual traces of residual copper-based impurities from the CuAAC reactions. Compound $\mathbf{C_{60}(A)_{12}}$ (169 mg, 45 %) was thus obtained as a red solid. ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 8.02$ (s, 12H), 4.61 (m, 24H), 4.49 (24H), 4.07 (m, 12H), 4.61 (m, 48H), 4.49 (m, 48H), 4.07 (m, 12H), 3.35 (m, 36H), 3.13 (m, 24H), 2.04 ppm (m, 24H). ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 162.7$, 145.2, 143.6, 140.6, 124.2, 98.9, 71.2, 70.1, 68.6, 65.9, 64.3, 62.5, 58.8, 46.1, 28.6 ppm. IR (neat): $\tilde{\nu} = 3340$ (O–H), 1739 cm^{-1} (C=O). UV/Vis (H_2O): 232 (66000), 326 (sh, 24000), 379 nm (sh, 9500 mol^{-1} dm^3 cm^{-1}). MALDI-TOF MS: 5331.6 $[M+Na]^+$, 4956.3 $[M-C_{14}H_{22}N_3O_9+Na]^+$, 4566.2 $[M-C_{29}H_{44}N_6O_{18}+Na]^+$, 4190.9 ($[M-C_{43}H_{66}N_9O_{24}+Na]^+$), 3801.7 $[M-C_{58}H_{88}N_{12}O_{36}+Na]^+$, 3426.5 $[M-C_{72}H_{110}N_{15}O_{45}+Na]^+$, 3036.3 $[M-C_{87}H_{132}N_{18}O_{54}+Na]^+$, 2662.1 $[M-C_{101}H_{154}N_{21}O_{63}+Na]^+$.

Compound $\mathbf{C_{60}(B)_{12}}$: As described for $\mathbf{C_{60}(A)_{12}}$, with **17** (100 mg, 0.043 mmol) and **10** (162 mg, 0.56 mmol). Compound $\mathbf{C_{60}(B)_{12}}$ (122 mg, 49 %) was thus obtained as a red–orange solid. ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 7.81$ (s, 12H), 4.61 (m, 12H), 4.30 (m, 72H), 3.76 (m, 24H), 3.62 (m, 48H), 3.35 (m, 96H), 2.18 (m, 24H), 1.55 ppm (m, 48H). ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 162.7$, 146.9, 145.0, 140.6, 121.7, 99.7, 71.3, 71.2, 70.7, 70.4, 68.7, 65.9, 65.8, 64.3, 62.6, 48.5, 46.4, 45.9, 28.5, 25.7, 24.8 ppm. IR (neat): $\tilde{\nu} = 3362$ (O-H), 1740 cm^{-1} (C=O). UV/Vis (H_2O): 232 (64000), 326 (sh, 22000), 379 nm (sh, 8500 mol^{-1} dm^3 cm^{-1}).MALDI-TOF MS: 5851.5 $[M+K]^+$, 5434.0 $[M-C_{17}H_{28}N_3O_9+K]^+$, 5419.9 $[M-C_{17}H_{28}N_3O_9+Na]^+$, 5002.7 $[M-C_{35}H_{56}N_6O_{18}+K]^+$, 4987.8 $[M-C_{35}H_{56}N_6O_{18}+Na]^+$, 4154.1 $[M-C_{70}H_{112}N_{12}O_{36}+K]^+$, 3736.9 $[M-C_{87}H_{139}N_{15}O_{45}+K]^+$, 3305.6 $[M-C_{105}H_{168}N_{18}O_{54}+K]^+$.

Compound $\mathbf{C_{60}(C)_{12}}$: As described for $\mathbf{C_{60}(A)_{12}}$, with **17** (90 mg, 0.038 mmol) and **14** (147 mg, 0.502 mmol). Compound $\mathbf{C_{60}(C)_{12}}$ (156 mg, 69 %) was thus obtained as a dark red solid. ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 8.01$ (s, 12H), 4.66 (m, 12H), 4.45 (m, 12H), 4.37 (m, 36H), 4.29 (m, 12H), 3.95 (m, 12H), 3.80 (m, 72H, partially masked by the H_2O signal), 2.22 ppm (m, 24H). ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 175.1$, 163.2, 145.6, 144.8, 141.1, 124.7, 99.5, 75.7, 72.2, 71.5, 71.0, 70.4, 69.9, 64.4, 59.4, 46.6, 45.7, 29.2 ppm. IR (neat): $\tilde{\nu} = 3355$ (O–H), 1736 cm^{-1} (C=O). UV/Vis (H_2O): 232 (65000), 326 (sh, 24000), 379 nm (sh, 8900 mol^{-1} dm^3 cm^{-1}). MALDI-TOF MS: 5860.0 [M+Na]$^+$, 5439.8 $[M-C_{15}H_{22}N_3O_{11}+Na]^+$, 5007.6 $[M-C_{31}H_{44}N_6O_{22}+Na]^+$, 4586.7 $[M-C_{46}H_{66}N_9O_{33}+Na]^+$, 4154.5 $[M-C_{62}H_{88}N_{12}O_{44}+Na]^+$, 3734.4 $[M-C_{77}H_{110}N_{15}O_{55}+Na]^+$.

Compound $\mathbf{C_{60}(D)_{12}}$: A mixture of **17** (176 mg, 0.072 mmol), **1** (203 mg, 0.93 mmol), $CuSO_4{\cdot}5H_2O$ (1 mg, 0.007 mmol) and sodium ascorbate (4 mg, 0.021 mmol) in CH_2Cl_2/H_2O/DMSO (1:1:1, 3 mL) was stirred at RT for 96 h. Methanol (15 mL) was added to the mixture and the resulting orange precipitate filtered, extensively washed with methanol then CH_2Cl_2 and dried under high vacuum. Compound $\mathbf{C_{60}(D)_{12}}$ was further purified by filtration on a Sephadex™ column (H_2O), precipitated with MeOH and dried under high vacuum to totally remove eventual traces of residual copper-based impurities from the CuAAC reactions. Compound $\mathbf{C_{60}(D)_{12}}$ (261 mg, 73 %) was thus obtained as a red–orange powder. ^{1}H NMR ([D$_6$]DMSO, 300 MHz): $\delta = 8.06$ (s, 12H), 4.81 (m, 12H), 4.71 (m, 12H), 4.16 (m, 24H), 4.38 (m, 48H), 3.20 (m, 48H partially masked by the H_2O signal), 2.07 ppm (m, 24H). ^{13}C NMR ([D$_6$]DMSO, 75 MHz): $\delta = 163.0$, 145.3, 143.9, 140.8, 124.4, 99.3, 74.1, 71.0, 70.3, 68.9, 67.1, 64.6, 61.4, 59.3, 46.5, 46.0, 28.8 ppm. IR (neat): $\tilde{\nu} = 3332$ (O–H), 1740 cm^{-1} (C=O). UV/Vis (H_2O): 245 (77700), 268 (62200), 282 (57500), 321 (sh, 35800), 341 nm (sh, 26400 mol^{-1} dm^3 cm^{-1}). MALDI-TOF MS: 4971.4 $[M+Na]^+$, 4625.9 $[M-C_{13}H_{20}N_3O_8+Na]^+$, 4266.5 $[M-C_{27}H_{40}N_6O_{16}+Na]^+$, 3921.3 $[M-C_{39}H_{60}N_9O_{24}+Na]^+$, 3562.0 $[M-C_{54}H_{80}N_{12}O_{32}+Na]^+$, 3215.9 $[M-C_{67}H_{100}N_{15}O_{40}+Na]^+$, 2856.7 $[M-C_{81}H_{120}N_{18}O_{48}+Na]^+$.

Compound $\mathbf{C_{60}(E)_{12}}$: As described for $\mathbf{C_{60}(D)_{12}}$, with **17** (295 mg, 0.127 mmol) and **2** (360 mg, 1.65 mmol). Compound $\mathbf{C_{60}(E)_{12}}$ (509 mg, 81 %) was thus obtained as a red–orange powder. ^{1}H NMR ([D$_6$]DMSO, 300 MHz):$\delta = 7.81$ (s, 12H), 4.70 (m, 24H), 4.55 (m, 12H), 4.43 (m, 12H), 4.33 (m, 60H), 3.62 (m, 24H), 3.40 (m, 24H partially masked by the H_2O signal), 2.59 (m, 24H), 2.19 (m, 24H), 1.55 ppm (m, 48H). ^{13}C NMR ([D$_6$]DMSO, 75 MHz): $\delta = 162.6$, 146.9, 145.0, 140.6, 121.7, 99.7, 73.8, 71.0, 70.3, 68.6, 67.0, 65.9, 64.3, 61.2, 48.5, 45.9, 45.2, 28.5, 25.7, 24.7 ppm. IR (neat): $\tilde{\nu} = 3332$ (O–H), 1740 cm^{-1} (C=O). UV/Vis (H_2O): 247 (79700), 269 (64300), 283 (58700), 320 (sh, 37200), 337 nm (sh, 29200 mol^{-1} dm^3 cm^{-1}). MALDI-TOF MS: 5476.8 $[M+Na]^+$, 5088.0 $[M-C_{16}H_{26}N_3O_8+Na]^+$, 4688.3 $[M-C_{33}H_{52}N_6O_{16}+Na]^+$, 4299.9 $[M-C_{49}H_{78}N_9O_{24}+Na]^+$, 3898.5 $[M-C_{66}H_{104}N_{12}O_{32}+Na]^+$, 3511.2 $[M-C_{82}H_{130}N_{15}O_{40}+Na]^+$), 3110.0 $[M-C_{99}H_{156}N_{18}O_{48}+Na]^+$, 3721.8 $[M-C_{115}H_{182}N_{21}O_{56}+Na]^+$.

Compound $\mathbf{C_{60}(F)_{12}}$: A 1 M solution of TBAF in THF (0.37 mL, 0.37 mmol) was added to a mixture of **15** (80 mg, 0.027 mmol), **3** (156 mg, 0.35 mmol), $CuSO_4{\cdot}5H_2O$ (0.4 mg, 0.003 mmol) and sodium ascorbate (1.6 mg, 0.008 mmol) in CH_2Cl_2/MeOH/DMSO (1:1:1, 3 mL). The resulting mixture was stirred at room temperature. After 24 h, methanol (10 mL) was added to the mixture and the resulting orange precipitate filtered, extensively washed with methanol then CH_2Cl_2 and dried under high vacuum. Compound $\mathbf{C_{60}(F)_{12}}$ was further purified by filtration on a Sephadex™ column (H_2O), precipitated with MeOH and dried under high vacuum to totally remove eventual traces of residual copper-based impurities from the CuAAC reactions. Compound $\mathbf{C_{60}(F)_{12}}$ (168 mg, 84 %) was thus obtained as a red solid. ^{1}H NMR ([D$_6$]DMSO, 300 MHz): $\delta = 8.33$ (br s, 12H), 7.82 (br s, 12H), 7.26–7.46 (m, 48H), 4.40–4.80 (m, 84H), 4.20–4.38 (m, 36H), 4.04–4.16 (m, 24H), 3.60–3.75 (m, 24H), 3.47–3.57 (m, 24H), 3.33–3.45 (m, 36H), 2.55–2.75 (m, 24H), 2.10–2.20 (m, 24H), 1.88–2.00 (m, 24H), 1.70–1.85 (m, 24H), 1.40–1.55 ppm (m, 24H). ^{13}C NMR ([D$_6$]DMSO, 100 MHz): $\delta = 171.5$, 162.7, 145.6, 145.0, 140.7, 138.3, 131.2, 127.7, 121.7, 121.3, 99.1, 87.0, 81.4, 74.2, 71.0, 70.2, 68.8, 67.1, 67.0, 66.5, 61.2, 48.9, 45.6, 40.3, 34.3, 29.2, 28.5, 27.6, 22.0, 21.3 ppm. IR (neat): $\tilde{\nu} = 3385$ (O–H), 2129 (C≡C), 1740 (C=O), 1648 (C=O) cm^{-1}.

Enzymatic assays: For determination of specific activity, WaaC activity was monitored by a coupled assay involving pyruvate kinase (PK) and luciferase as coupling enzymes.[22,23] Basically, the reaction product ADP was converted by PK to ATP, which was detected by luciferase activity. The reaction took place in a white 96-well plate (Costar) in a final volume of 60 µL. The assay buffer contained Hepes (50 mM, pH 7.5), $MgCl_2$ (10 mM), KCl (50 mM), dithiothreitol (1 mM), myelin basic protein (0.1 µM; Sigma) and Triton X-100 (0.1 % (v/v)), which generated supporting micelles for Re-LPS. The pre-incubation mixture contained 3 µL of inhibitors (at eight different concentrations) in DMSO and of WaaC (27 µL; 0.1 nM final concentration). After 30 min of preincubation at room temperature, ADP-heptose (1 µM) and Re-LPS (1 µM; Sigma, purified from Salmonella minnesota) were added and the resulting reaction mixture (60µL) was incubated at room temperature until a 30 % conversion rate was reached. The following readout mixture (100 µL) was finally added in each well: pyruvate kinase (5 units mL^{-1}, Sigma), phosphoenolpyruvate (50 µM; Sigma), luciferase (Sigma), 20 µM luciferin (10000 units mL^{-1}, Sigma), *N*-acetylcysteamine (0.1 mM, Aldrich). The luminescence intensity was read on a Luminoskan (Thermo). IC_{50} curves were fit to a classical Langmuir equilibrium model using XLFIT (IDBS).

Acknowledgements

The authors thank the EU ("Marie Curie Training Network" grant, "Dynamic Interactive Chemical Biology & Biomedicine", MRTN-CT-2005–019561), the FNRS (FRFC grant 2.4.625.08.F and "Crédit au Chercheur " 1.5.167.10), the CNRS (UMR 7509) and the University of Strasbourg for financial support. We also thank V. Oerthel for the initial preparation of

compound $C_{60}(D)_{12}$. We further thank M. Schmitt for NMR measurements and Julien Dewinter (UMH) for having recorded the mass spectra.

[1] Y. M. Chabre, R. Roy, *Adv. Carbohydrate Chem. and Biochem.* **2010**, *63*, 165.
[2] For reviews on biological applications of fullerenes, see: a) T. Da Ros, M. Prato, *Chem. Commun.* **1999**, 663; b) E. Nakamura, H. Isobe, *Acc. Chem. Res.* **2003**, *36*, 807; c) S. Bosi, T. Da Ros, G. Spalluto, M. Prato, *Eur. J. Med. Chem.* **2003**, *38*, 913; d) A. Bianco, T. Da Ros in *Fullerenes: Principles and Applications* (Eds.: F. Langa, J.-F. Nierengarten), RSC Nanoscience and Nanotechnology Series, Cambridge, **2007**, pp. 301–328.
[3] a) A. Vasella, P. Uhlmann, C. A. A. Waldraff, F. Diederich, C. Thielgen, *Angew. Chem.* **1992**, *104*, 1383; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1388; b) Y. Wang, J. Cao, D. I. Schuster, S. R. Wilson, *Tetrahedron Lett.* **1995**, *36*, 6843; c) M. R. Banks, J. I. G. Cadogan, I. Gosney, P. K. G. Hodgson, P. R. R. Langridge-Smith, J. R. A. Millar, A. T. Taylor, *J. Chem. Soc. Chem. Commun.* **1995**, 885; d) A. Yashiro, Y. Nishida, M. Ohno, S. Eguchi, K. Kobayashi, *Tetrahedron Lett.* **1998**, *39*, 9031; e) F. Cardullo, F. Diederich, L. Echegoyen, T. Habicher, N. Jayaraman, R. M. Leblanc, J. F. Stoddart, S. Wang, *Langmuir* **1998**, *14*, 1955; f) H. Kato, A. Yashiro, A. Mizuno, Y. Nishida, K. Kobayashi, H. Shinohara, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2935; g) A. Dondoni, A. Marra, *Tetrahedron Lett.* **2002**, *43*, 1649; h) Y. Mikata, S. Takagi, M. Tanahashi, S. Ishii, M. Obata, Y. Miyamoto, K. Wakita, T. Nishisaka, T. Hirano, T. Ito, M. Hoshino, C. Ohtsuki, M. Tanihara, S. Yano, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3289; i) H. Ito, T. Tada, M. Sudo, Y. Ishida, T. Hino, K. Saigo, *Org. Lett.* **2003**, *5*, 2643; j) H. Isobe, H. Mashima, H. Yorimitsu, E. Nakamura, *Org. Lett.* **2003**, *5*, 4461; k) Y. Nishida, A. Mizuno, H. Kato, A. Yashiro, T. Ohtake, K. Kobayashi, *Chem. Biodiversity* **2004**, *1*, 1452; l) H. Kato, N. Kaneta, S. Nii, K. Kobayashi, N. Fukui, H. Shinohara, Y. Nishida, *Chem. Biodiversity* **2005**, *2*, 1232; m) H. Kato, C. Böttcher, A. Hirsch, *Eur. J. Org. Chem.* **2007**, 2659; n) S. Tanimoto, S. Sakai, S. Matsumura, D. Takahashi, K. Toshima, *Chem. Commun.* **2008**, 5767.
[4] For glycofullerenes prepared under CuAAC conditions, see: a) H. Isobe, K. Cho, N. Solin, D. B. Werz, P. H. Seeberger, E. Nakamura, *Org. Lett.* **2007**, *9*, 4611; b) G. Rocha Pereira, L. J. Santos, I. Luduvico, R. Brondi Alves, R. Pereira de Freitas, *Tetrahedron Lett.* **2010**, *51*, 1022.
[5] J.-F. Nierengarten, J. Iehl, V. Oerthel, M. Holler, B. M. Illescas, A. Munoz, N. Martin, J. Rojo, M. Sanchez-Navarro, S. Cecioni, S. Vidal, K. Buffet, M. Durka, S. P. Vincent, *Chem. Commun.* **2010**, *46*, 3860.
[6] For a review on fullerene hexa-adducts, see: A. Hirsch, O. Vostrowsky, *Eur. J. Org. Chem.* **2001**, 829.
[7] M. Durka, K. Buffet, J. Iehl, M. Holler, J.-F. Nierengarten, J. Taganna, J. Bouckaert, S. P. Vincent, *Chem. Commun.* **2011**, *47*, 1321.
[8] a) M. Sánchez-Navarro, A. Muñoz, B. M. Illescas, J. Rojo, N. Martín, *Chem. Eur. J.* **2011**, *17*, 766; b) S. Cecioni, V. Oerthel, J. Iehl, M. Holler, D. Goyard, J.-P. Praly, A. Imberty, J.-F. Nierengarten, S. Vidal, *Chem. Eur. J.* **2011**, *17*, 3252.
[9] P. Compain, C. Decroocq, J. Iehl, M. Holler, D. Hazelard, T. Mena Barragán, C. Ortiz Mellet, J.-F. Nierengarten, *Angew. Chem.* **2010**, *122*, 5889; *Angew. Chem. Int. Ed.* **2010**, *49*, 5753.
[10] D. H. Dube, C. R. Bertozzi, *Nat. Rev. Drug Discov.* **2005**, *4*, 477.
[11] E. E. Simanek, G. J. McGarvey, J. A. Jablonowski, C. H. Wong, *Chem. Rev.* **1998**, *98*, 833.
[12] S. Berg, D. Kaur, M. Jackson, P. J. Brennan, *Glycobiology* **2007**, *17*, 35r.
[13] a) P. Compain, O. R. Martin, *Bioorg. Med. Chem.* **2001**, *9*, 3077; b) M. L. Mitchell, F. Tian, L. V. Lee, C. H. Wong, *Angew. Chem.* **2002**, *114*, 3167; *Angew. Chem. Int. Ed.* **2002**, *41*, 3041; c) S. H. Khan, S. C. Crawley, O. Kanie, O. Hindsgaul, *J. Biol. Chem.* **1993**, *268*, 2468; d) B. Müller, C. Schaub, R. R. Schmidt, *Angew. Chem.* **1998**, *110*, 3021; *Angew. Chem. Int. Ed.* **1998**, *37*, 2893; e) M. M. Palcic, L. D. Heerze, O. P. Srivastava, O. Hindsgaul, *J. Biol. Chem.* **1989**, *264*, 17174; f) T. Kajimoto, M. Node, *Synthesis* **2009**, 3179; g) M. D. Burkart, S. P. Vincent, C.-H. Wong, *Chem. Commun.* **1999**, 1525; h) H. Y. Sun, S. W. Lin, T. P. Ko, J. F. Pan, C. L. Liu, C. N. Lin, A. H. J. Wang, C. H. Lin, *J. Biol. Chem.* **2007**, *282*, 9973; i) K. Takaya, N. Nagahori, M. Kurogochi, T. Furuike, N. Miura, K. Monde, Y. C. Lee, S. I. Nishimura, *J. Med. Chem.* **2005**, *48*, 6054; j) L. V. Lee, M. L. Mitchell, S. J. Huang, V. V. Fokin, K. B. Sharpless, C. H. Wong, *J. Am. Chem. Soc.* **2003**, *125*, 9588; k) K. Hosoguchi, T. Maeda, J. Furukawa, Y. Shinohara, H. Hinou, M. Sekiguchi, H. Togame, H. Takemoto, H. Kondo, S. I. Nishimura, *J. Med. Chem.* **2010**, *53*, 5607; l) T. Pesnot, R. Jorgensen, M. M. Palcic, G. K. Wagner, *Nat. Chem. Biol.* **2010**, *6*, 321; m) N. L. Rose, G. C. Completo, S.-H. Lin, M. McNeil, M. Palcic, T. L. Lowary, *J. Am. Chem. Soc.* **2006**, *128*, 6721; n) M. B. Lazarus, Y. Nam, J. Jiang, P. Sliz, S. Walker, *Nature* **2011**, *469*, 564.
[14] a) J. C. Errey, S. S. Lee, R. P. Gibson, C. M. Fleites, C. S. Barry, P. M. J. Jung, A. C. O'Sullivan, B. G. Davis, G. J. Davies, *Angew. Chem.* **2010**, *122*, 1256; *Angew. Chem. Int. Ed.* **2010**, *49*, 1234; b) J. F. May, R. A. Splain, C. Brotschi, L. L. Kiessling, *Proc. Nat. Acad. Sci. USA* **2009**, *106*, 11851; c) L. L. Lairson, B. Henrissat, G. J. Davies, S. G. Withers, *Annu. Rev. Biochem.* **2008**, *77*, 521; d) C. Breton, L. Snajdrova, C. Jeanneau, J. Koca, A. Imberty, Glycobiology **2006**, *16*, 29R; e) T.-S. A. Wang, T. J. Lupoli, Y. Sumida, H. Tsukamoto, Y. Wu, Y. Rebets, D. E. Kahne, S. Walker, *J. Am. Chem. Soc.* **2011**, *133*, 8528.
[15] a) S. G. Withers, *Carbohydr. Polym.* **2001**, *21*, 325; b) L. L. Lairson, C. P. C. Chiu, H. D. Ly, S. He, W. W. Wakarchuk, N. C. J. Strynadka, S. G. Withers, *J. Biol. Chem.* **2004**, *279*, 28339; c) M. D. Burkart, S. P. Vincent, A. Düffels, B. W. Murray, S. V. Ley, C.-H. Wong, *Bioorg. Med. Chem.* **2000**, *8*, 1937; d) P. A. Frantom, J. K. Coward, J. S. Blanchard, *J. Am. Chem. Soc.* **2010**, *132*, 6626.
[16] a) M. Lahmann, *Top Curr. Chem.* **2009**, *288*, 17; b) J. J. Lundquist, E. J. Toone, *Chem. Rev.* **2002**, *102*, 555; c) R. J. Pieters, *Org. Biomol. Chem.* **2009**, *7*, 2013; d) M. Touaibia, R. Roy in *Application of Multivalent Mannosylated Dendrimers in Glycobiology, Vol. 3* (Ed.: J. P. Kamerling), Elsevier, Oxford, **2007**, pp. 781–829; e) V. H. Lillelund, H. H. Jensen, J. Liang, M. Bols, *Chem. Rev.* **2002**, *102*, 515; f) S. Cecioni, O. A. Argintaru, T. Docsa, P. Gergely, J. P. Praly, S. Vidal, *New J. Chem.* **2009**, *33*, 148; g) A. Imberty, Y. M. Chabre, R. Roy, *Chem. Eur. J.* **2008**, *14*, 7490; h) L. L. Kiessling, J. E. Gestwicki, L. E. Strong, *Angew. Chem.* **2006**, *118*, 2408; *Angew. Chem. Int. Ed.* **2006**, *45*, 2348.
[17] a) S. Gronow, H. Brade, *J. Endotoxin Res.* **2001**, *7*, 3; b) A. Silipo, A. Molinaro, T. Ierano, A. De Soyza, L. Sturiale, D. Garozzo, C. Aldridge, P. A. Corris, C. M. A. Khan, R. Lanzetta, M. Parrilli, *Chem. Eur. J.* **2007**, *13*, 3501.
[18] C. M. Kahler, A. Datta, Y.-L. Tzeng, R. W. Carlson, D. S. Stephens, *Glycobiology* **2005**, *15*, 409.
[19] E. T. Rietschel, T. Kirikae, F. U. Schade, U. Mamat, G. Schmidt, H. Loppnow, A. J. Ulmer, U. Zähringer, U. Seydel, F. Di Padova, M. Schreier, H. Brade, *FASEB J.* **1994**, *8*, 217.
[20] a) W. G. Coleman, Jr., L. Leive, *J. Bacteriol.* **1979**, *139*, 899; b) S. Escaich, *Curr. Opin. Chem. Biol.* **2008**, *12*, 400; c) F. Moreau, N. Desroy, J. M. Genevard, V. Vongsouthi, V. Gerusz, G. Le Fralliec, C. Oliveira, S. Floquet, A. Denis, S. Escaich, K. Wolf, M. Busemann, A. Aschenbrenner, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4022.
[21] A. Zamyatina, S. Gronow, C. Oertelt, M. Puchberger, H. Brade, P. Kosma, *Angew. Chem.* **2000**, *112*, 4322; *Angew. Chem. Int. Ed.* **2000**, *39*, 4150.
[22] H. Dohi, R. Périon, M. Durka, M. Bosco, Y. Roué, F. Moreau, S. Grizot, A. Ducruix, S. Escaich, S. P. Vincent, *Chem. Eur. J.* **2008**, *14*, 9530.
[23] S. Grizot, M. Salem, V. Vongsouthi, L. Durand, F. Moreau, H. Dohi, S. Vincent, S. Escaich, A. Ducruix, *J. Mol. Biol.* **2006**, *363*, 383.
[24] a) S. Oscarson in *Glycoscience Synthesis of Oligosaccharides and Glycoconjugates, Vol. 186* (Eds.: H. Driguez, J. Thiem), Springer, Berlin, **1997**, p. 171; b) S. Oscarson, *Carbohydr. Polym.* **2001**, *44*, 305; c) T. Ohara, A. Adibekian, D. Esposito, P. Stallforth, P. H. See-

berger, *Chem. Commun.* **2010**, 4106; d) D. Crich, A. Banerjee, *J. Am. Chem. Soc.* **2006**, *128*, 8078.

[25] a) G. J. P. H. Boons, M. Overhand, G. A. van der Marel, J. H. van Boom, *Carbohydr. Res.* **1989**, *192*, c1; b) G. J. P. H. Boons, M. Overhand, G. A. van der Marel, J. H. van Boom, *Angew. Chem.* **1989**, *101*, 1538; *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 1504; c) G. J. P. H. Boons, G. A. van der Marel, J. H. van Boom, J. T. Poolman, *Recl. Trav. Chim. Pays-Bas* **1989**, *108*, 339.

[26] M. Durka, A. Tikad, R. Périon, M. Bosco, M. Andaloussi, S. Floquet, E. Malacain, F. Moreau, M. Oxoby, V. Gerusz, S. P. Vincent, *Chem. Eur. J.* **2011**, *17*, 11305.

[27] J. Iehl, J. F. Nierengarten, *Chem. Eur. J.* **2009**, *15*, 7306.

[28] J. Iehl, R. Pereira de Freitas, B. Delavaux-Nicot, J.-F. Nierengarten, *Chem. Commun.* **2008**, 2450.

[29] a) J. Iehl, J.-F. Nierengarten, *Chem. Commun.* **2010**, *46*, 4160; b) J. Iehl, M. Holler, J.-F. Nierengarten, K. Yoosaf, J. M. Malicka, N. Armaroli, J.-M. Strub, A. Van Dorsselaer, B. Delavaux-Nicot, *Aust. J. Chem.* **2011**, *64*, 153; c) P. Fortgang, E. Maisonhaute, C. Amatore, B. Delavaux-Nicot, J. Iehl, J.-F. Nierengarten, *Angew. Chem.* **2011**, 123, 2412; *Angew. Chem. Int. Ed.* **2011**, *50*, 2364.

[30] D. J. Harvey, *Mass Spectrom. Rev.* **1999**, *18*, 349.

[31] a) R. Kessinger, J. Crassous, A. Herrmann, M. Rüttiman, L. Echegoyen, F. Diederich, *Angew. Chem.* **1998**, *110*, 2022; *Angew. Chem. Int. Ed.* **1998**, *37*, 1919; b) N. N. P. Moonen, C. Thilgen, L. Echegoyen, F. Diederich, *Chem. Commun.* **2000**, 335.

[32] a) D. Felder, H. Nierengarten, J.-P. Gisselbrecht, C. Boudon, E. Leize, J.-F. Nicoud, M. Gross, A. Van Dorsselaer, J.-F. Nierengarten, *New J. Chem.* **2000**, *24*, 687; b) Y. Rio, G. Enderlin, C. Bourgogne, J.-F. Nierengarten, J.-P. Gisselbrecht, M. Gross, G. Accorsi, N. Armaroli, *Inorg. Chem.* **2003**, *42*, 8783; c) M. Gutierrez-Nava, G. Accorsi, P. Masson, N. Armaroli, J.-F. Nierengarten, *Chem. Eur. J.* **2004**, *10*, 5076; d) H. Herschbach, K. Hosomizu, U. Hahn, E. Leize, A. Van Dorsselaer, H. Imahori, J.-F. Nierengarten, *Anal. Bioanal. Chem.* **2006**, *386*, 46; e) J. Iehl, M. Vartanian, M. Holler, J.-F. Nierengarten, B. Delavaux-Nicot, J.-M. Strub, A. Van Dorsselaer, Y. Wu, J. Mohanraj, K. Yoosaf, N. Armaroli, *J. Mater. Chem.* **2011**, *21*, 1562.

[33] B. A. Johns, C. R. Johnson, *Tetrahedron Lett.* **1998**, *39*, 749.

[34] A. Lohse, K. B. Jensen, K. Lundgren, M. Bols, *Bioorg. Med. Chem.* **1999**, *7*, 1965.

[35] a) T. Wennekes, R. J. B. H. N. van den Berg, K. M. Bonger, W. E. Donker-Koopman, A. Ghisaidoobe, G. A. van der Marel, A. Strijland, J. M. F. G. Aerts, H. S. Overkleeft, *Tetrahedron: Asymmetry* **2009**, *20*, 836; b) A. J. Cagnoni, O. Varela, S. G. Gouin, J. Kovensky, M. L. Uhrig, *J. Org. Chem.* **2011**, *76*, 3064.

[36] J. Diot, M. I. Garcia-Moreno, S. G. Gouin, C. Ortiz Mellet, K. Haupt, J. Kovensky, *Org. Biomol. Chem.* **2009**, *7*, 357.

[37] J. E. Gestwicki, C. W. Cairo, L. E. Strong, K. A. Oetjen, L. L. Kiessling, *J. Am. Chem. Soc.* **2002**, *124*, 14922;

[38] C. Ferrandiz-Huertas, J. Isac-García, F. Pérez-Balderas, F. Santoyo-González, *Synthesis* **2005**, *6*, 939.

[39] Y. Yamada, K. Matsuura, K. Kobayashi, *Bioorg. Med. Chem.* **2005**, *13*, 1913.

Received: July 4, 2011
Published online: December 6, 2011

