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Fullerenes

Glycosidase Inhibition with Fullerene Iminosugar Balls: A Dramatic Multivalent Effect**

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The electronic and structural properties of fullerene derivatives make them very attractive candidates for the construction of nanostructures that are potentially useful for applications in materials science and biological chemistry.[1] In particular, the C_{60} hexakis adducts with a T_h-symmetrical octahedral addition pattern initially developed by Hirsch and co-workers[2] are unique organic molecules with an appealing compact spherical scaffold for the construction of multifunctional nanomaterials.[3] However, the synthesis of functionalized fullerene hexakis adducts from malonates and C_{60} is difficult.[3,4] This major problem limits the applications of such systems and has been recently solved by the development of synthetic methodologies based on the postfunctionalization of easily accessible building blocks of fullerene hexakis adducts.[5,6] It has been shown that fullerene hexakis adducts that bear 12 peripheral carbohydrate moieties can be prepared in excellent yields by grafting unprotected sugar derivatives onto the fullerene core.[7] Although these fullerene sugar balls are obviously perfectly suited for applications in the field of carbohydrate–lectin interactions,[8] the evaluation of carbohydrate-processing enzyme inhibition with such multivalent derivatives is less obvious. Indeed, among the possible strategies to attain specific potent glycosidase inhibition, the concept of multivalent design has been clearly overlooked.[9] Most enzymes actually have a single, deep active site that is usually less accessible than the shallow binding pockets or grooves on the lectin surfaces.[10] Consequently, a limited number of binding mechanisms, including statistical rebinding, are possible, whereas multivalent ligands may interact with multiple receptors by additional mechanistic options (e.g., the chelate effect, receptor clustering).[9,11] It is likely that these factors may have hampered interest in projects directed towards the design of multivalent glycosidase inhibitors. In addition, the experimental results obtained to date were not particularly encouraging.[12] Di- to tetravalent analogues of 1-deoxynojirimycin, which is a well-known glycosidase inhibitor,[13] generally displayed comparable if not decreased inhibition compared with their monomeric counterparts. The best result reported to date was found for a trivalent iminosugar that showed a sixfold affinity enhancement towards Jack bean α-mannosidase.[14] Herein we report the synthesis of a fullerene hexakis adduct decorated with 12 iminosugar residues. The inhibition profile of this fullerene iminosugar ball has been systematically evaluated against various glycosidases,[15] and dramatic multivalent effects have been observed for the first time.

In order to explore the potential of multivalency on glycosidase inhibition with a globular polytopic ligand constructed around the fullerene scaffold, an N-alkyl analogue of 1-deoxynojirimycin was selected as the peripheral ligand. This class of compounds is indeed poorly selective and displays modest to good glycosidase inhibition.[13] It was thus anticipated that these compounds could be excellent models for the examination of the influence of multivalency on inhibition selectivity over a large range of glycosidases. In addition, the alkyl chain on the endocyclic nitrogen atom of the iminosugar is an ideal spacer that may allow for easy grafting onto the central C_{60} core by means of a cycloaddition reaction.[16] The synthesis of the azide building block is based on the optimization of a strategy reported independently by Overkleeft et al.[17] and Vasella and co-workers.[18] As shown in Scheme 1, the δ-hydroxy amide **2** was obtained directly from commercially available tetra-*O*-benzyl D-glucopyranose (**1**) in 78% yield by oxidative amidation with iodine in 30% aqueous ammonia (30%).[19] The main advantage of this one-pot process is that aldehyde oxidation and C–N bond formation are performed in a single synthetic step. Oxidation of the hydroxy group at C5 followed by intramolecular

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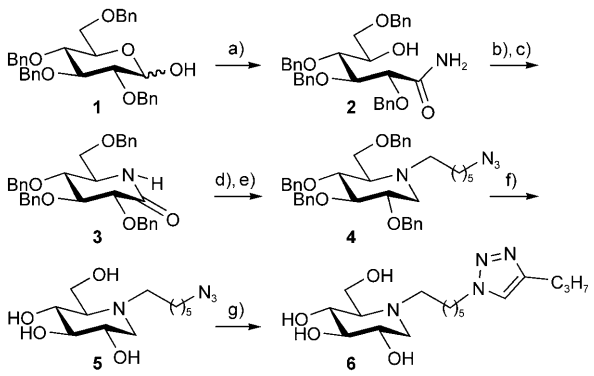
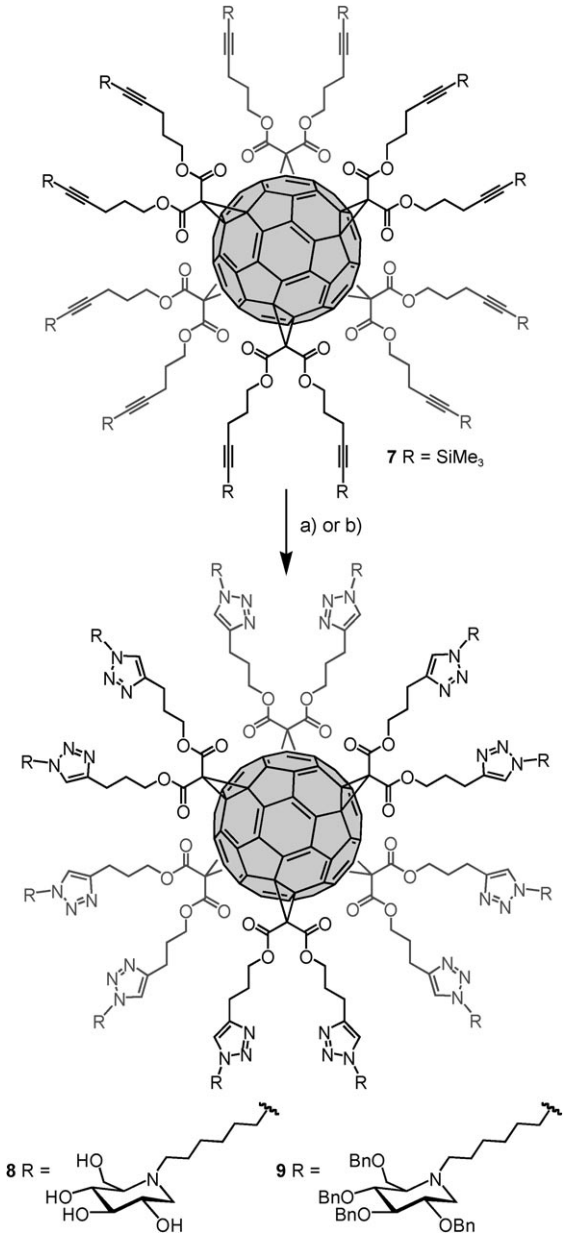


Scheme 1. a) aq NH_3 (30%), I_2, THF, 16 h, 78%; b) DMSO, Ac_2O; c) $NaBH_3CN$, HCOOH, MeCN, 80°C, 60% over two steps; d) LAH, THF, reflux, 90%; e) $Br(CH_2)_6N_3$, Et_3N, 4-dimethylaminopyridine (DMAP), DMF, 120°C, 38% (51% based on recovered starting material); f) BCl_3, CH_2Cl_2, −60°C→0°C, 77%. g) Pent-1-yne, sodium ascorbate, $CuSO_4{\cdot}5H_2O$, DMF/H_2O (1:1), 61%.

reductive amination afforded the expected lactam **3**. Reduction with lithium aluminium hydride (LAH) and subsequent alkylation of the corresponding amine by using 1-azido-6-bromo-hexane afforded the O-benzylated iminosugar **4**. Selective cleavage of the benzyl protecting groups of **4** without interfering with the azide moiety was performed successfully by treatment with an excess of BCl_3 in CH_2Cl_2 at low temperatures (−60–0°C).[20] Reaction of the resulting compound **5**[21] with 1-pentyne in the presence of $CuSO_4{\cdot}5H_2O$ and sodium ascorbate gave the derivative **6**, which was used as the monovalent model compound in the inhibition assay.

The synthesis of fullerene iminosugar ball **8** is shown in Scheme 2. Compound **7**[6] was desilylated in situ with tetra-*n*-butylammonium fluoride (TBAF) to form the corresponding hexaadduct bearing 12 terminal alkyne units, with which the azide precursor **5** was subsequently reacted. The functionalized fullerene **8** was thus obtained in 83% yield. The chemical structure of compound **8** was confirmed by its ^1H and ^{13}C NMR spectra, which show the expected signals for the 12 equivalent peripheral iminosugar residues as well as the characteristic features of the fullerene hexakis-adduct core (see the Supporting Information). To further confirm the structure of compound **8**, mass spectra (MALDI-TOF and ESI-MS) were recorded under different conditions. However, as already observed in the case of related fullerene sugar balls,[7] a high level of fragmentation prevented the observation of the expected molecular ion peak. This difficulty prompted us to prepare compound **9** by reaction of the fullerene derivative **7** with the benzylated iminosugar **4** (Scheme 2). The ^1H and ^{13}C NMR spectra of compound **9** show the same characteristic features that were observed for unprotected compound **8** (see the Supporting Information). MALDI-TOF mass spectrometry also confirmed the structure of fullerene derivative **9**. The level of fragmentation was less dramatic in this case and the molecular ion peak could be clearly observed at m/z 9911.02 ($[M+H]^+$, calculated for $C_{618}H_{659}N_{48}O_{72}$: 9911.12). Finally, the UV/Vis spectra of



Scheme 2. a) **5**, TBAF, sodium ascorbate, $CuSO_4{\cdot}5H_2O$, DMF/H_2O (1:1), 83%; b) **4**, TBAF, sodium ascorbate, $CuSO_4{\cdot}5H_2O$, CH_2Cl_2/H_2O (1:1), 78%.

compounds **8** and **9** show the characteristic features of fullerene hexaadducts.[5]

Fullerene iminosugar **8** and its monovalent analogue **6** were tested against a range of commercially available glycosidases and their inhibition constants (K_i) were evaluated (Table 1). The biological results may be divided unevenly into three categories with regard to inhibition: decreased, unaffected, and increased by multivalency. Only one example of a significant affinity decrease (ninefold) compared to the monovalent iminosugar was observed for sweet almond β-glucosidase. For two other glucosidases (amyloglucosidase and bovine liver β-glucosidase), the multivalent and monovalent iminosugars showed similar K_i values.

Table 1: Glycosidase inhibitory activities K_i.[a]

Enzyme	Monovalent iminosugar **6**	Multivalent iminosugar **8**	Relative inhibition potency of **8** over **6**[b]
β-galactosidase			
Bovine liver	262	34	7.7 (0.64)
α-galactosidase			
Aspergillus niger	NI[c]	NI[c]	–
Green coffee	NI[c]	84	>23 (>1.9)
β-glucosidase			
Almonds (pH 7.3)	11	95	0.1 (0.01)
Bovine liver	482	247	1.9 (0.16)
α-glucosidase			
Amyloglucosidase (*Asp. Niger*)	0.71	0.69	1.0 (0.08)
Baker's yeast	152	18	8.7 (0.72)
Isomaltase (Baker's yeast)	943	10.5	89.8 (7.48)
Naringinase			
Penicillium decumbens	9.1	0.41	22.2 (1.84)
β-mannosidase			
Helix pomatia	NI[c]	NI[c]	–
α-mannosidase			
Jack Bean	322	0.15	2147 (179)

[a] Values reported in μM. [b] The value indicated in brackets corresponds to the relative inhibition potency per nojirimycin residue. [c] NI: no inhibition detected at 2 mM.

For most of the glycosidases tested, a significant binding enhancement (up to 2150-fold) was observed relative to the corresponding monomer. The most impressive results were obtained for three α-glycosidases. Remarkably, multivalent iminosugar **8** was found to be a good inhibitor of green coffee α-galactosidase, whereas its monomeric analogue **6** in the D-gluco series was completely inactive. The stronger measurable multivalent effects were obtained with baker's yeast isomaltase and Jack bean α-mannosidase, which had K_i values that were two and three orders of magnitude higher, respectively, than monomeric iminosugar **6**.

Comparison of the inhibition profile of the dodecavalent system **8** and a previously described trivalent iminosugar[14] provides interesting insights. For both compounds, the best multivalent effect was obtained with Jack bean α-mannosidase (sixfold enhancement for trivalent and approximately 2150-fold for dodecavalent as compared to monovalent iminosugars). However, contrary to compound **8**, the trivalent iminosugar was a weaker inhibitor of baker's yeast isomaltase than its monomeric analogue. The analysis of the valency-corrected potency of the two multivalent systems toward Jack bean α-mannosidase (twofold and approximately 180-fold for the trivalent and dodecavalent systems respectively) is more interesting. These results highlight the impact of the central scaffold on the inhibition activity of the multivalent architecture. It is noteworthy that, in general, N-alkylated analogues of mannojirimycin show little if any inhibition of mannosidases.[13]

Further research will certainly be needed for the rationalization of the significant binding enhancements observed for glycosidase inhibition with multivalent inhibitors. When considered on an inhibitor residue basis, our results unequivocally evidence the existence of a multivalent effect beyond the expected statistical rebinding and local concentration effect. A sliding mechanism of the iminosugars in the enzyme active site or an additional binding at a close allosteric site may account for the distinct specificity enhancement observed.[9,11,22] Beyond its fundamental interest, our work shows that, in addition to the modulation of selectivity, multivalency is a promising tool for the design of potent glycosidase inhibitors.

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