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A second-generation ferrocene–iminosugar hybrid with improved fucosidase binding properties

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ABSTRACT

The synthesis and the biological evaluation of a new ferrocenyl-iminosugar conjugate designed for fucosidase inhibitory and anticancer activity is described. The compound showed strong affinity for fucosidase from bovine kidney (K_i = 23 nM) and from *Bacteroides thetaiotaomicron* (K_i = 150 nM), displaying a 10-fold tighter binding affinity for these enzymes than the previous analogs. The interaction pattern that improves binding has been evaluated through structural analysis of the inhibitor–enzyme complex. The ferrocenyl-iminosugar exhibits significant anticancer activity on MDA-MB-231 and SK-MEL28 cell lines at 100 μM.

Glycosylation of proteins is a critical process, which mediates a number of biological events such as enzyme activity, cell–cell or cell–matrix interactions as well as protein targeting.[1] Due to the high diversity of oligosaccharide combinations, this post-translational modification enables very selective biological responses.[2,3] Glycosylation occurs on diverse proteins displaying various functions (enzymes, cytokines, antibodies) or localization (cell surface, cytoplasm). The biosynthesis of glycoproteins involves specific enzymatic machinery in which glycosyltransferases and glycosidases take a major role in order to add or release sugar units to the growing oligosaccharide chain.[4] Dysfunction of this process affords proteins with aberrant glycosylation patterns, which contributes to tumor invasion and metastasis through tumor-associated carbohydrate antigens.[5] Detection of unusual enzymatic activity levels might serve as cancer biomarkers;[6] for example elevated levels of sialyl-transferase are associated with carcinogenesis, proliferation, progression and metastasis, particularly in colorectal carcinomas.[7] Another enzyme, α-L-fucosidase (AFU), which is responsible for degradation of fucosides and fucose release has been identified as a reliable prognostic biomarker for detecting the development of hepatocellular carcinoma (HCC) in patients with liver cirrhosis.[8] Indeed, fucose residues participate in many host–cell interactions and, as such, high levels of fucose often correlate with tumor progression as found in: thyroid carcinoma, ovarian carcinoma and colorectal adenocarcinoma. Increased fucosylation of serum glycoproteins has also been observed for patients with breast cancer.[9] As fucose overexpression occurs in cancer tissues we are exploring the possibility of using fucose mimics, with strong affinity for AFU, as tracers for a selective delivery approach.[10] To this aim, we previously prepared a first series of pyrrolidine–ferrocene conjugates exemplified by **1** (Fig. 1), which combined good affinities for AFU thanks to the pyrrolidine scaffold (K_D in the low micromolar range) and revealed anti-proliferative action via the ferrocenyl function.[11]

In mammals, it is believed that the damaging effect of iron is related to RedOx reactions, producing reactive Oxygen Species (ROS), which in turn, oxidize biomolecules such as lipids, proteins or DNA.[12] Thus, it is reasonable to assume that Fc or its derivatives may act as low molecular weight iron carriers and their transport in close proximity to tumor, via the fucosidase-targeting pyrrolidine could localize their deleterious effect. The success of such a strategy relies on the strong affinity of the 5-membered dihydroxypyrrolidine for fucosidase. To improve these binding potencies, the insertion of a hydrophobic substituent at position C-2 of the pyrrolidine moiety was recently attempted with success.[13–15] A triazole linker between the pyrrolidine and the hydrophobic

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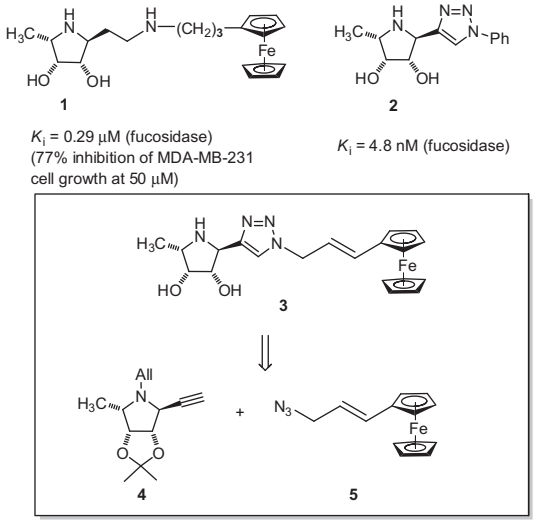
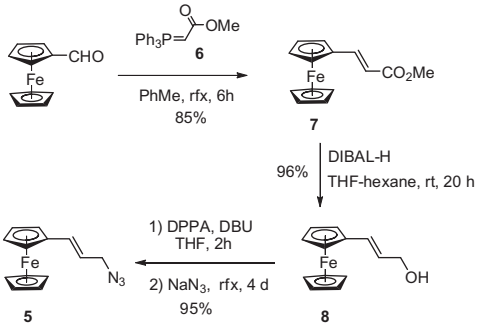


Figure 1.

aglycon permitted to optimize interactions, affording dissociation constants in the nanomolar range (Fig. 1, compound **2**). With this in mind, we designed the new pyrrolidine–ferrocene conjugate **3** which features a triazole linker between the pyrrolidine and the ferrocene motifs. In addition, a conjugated double bond was inserted in the structure, in the aim of increasing the hydrophobic character of the linker, but also to modulate the redox properties of the conjugated Fc pharmacophore and possibly improve the production of ROS.

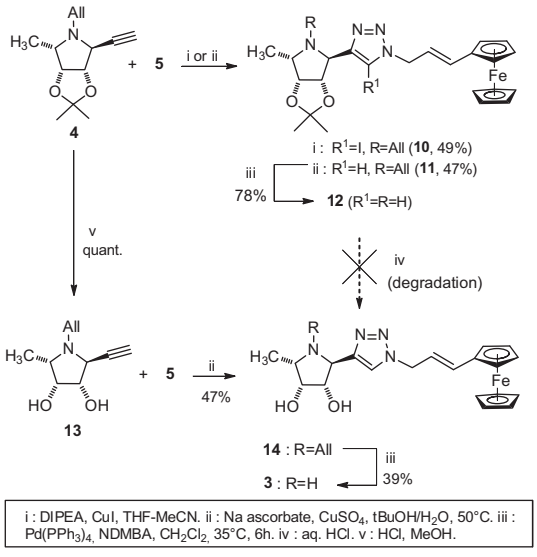
Our synthetic strategy toward Fc-pyrrolidine **3** exploited the Copper(I)-catalyzed Azide–Alkyne Cycloaddition (CuAAC) of ethynylpyrrolidine **4** with 1-ferrocenylprop-1-en-3-azide **5** as the key step (Fig. 1).[16] Ethynylpyrrolidine **4** was synthesized starting from D-ribose, by following a 7-step procedure as described in the literature.[15] The preparation of 1-ferrocenylprop-1-en-3-azide **5** started with a standard Wittig reaction between commercial Ferrocenecarboxaldehyde and the stabilized ylide **6** to afford exclusively the expected *trans*-alkene **7** in 85% yield (Scheme 1).[17] Insertion of the azide function in methyl 3-ferrocenylpropenoate **7** was envisioned via the corresponding alcohol **8**. Surprisingly, the selective reduction of the ester function in **7** proved tricky. No reaction occurred when an ethereal solution of diisobutylaluminum hydride (DIBAl-H) or $LiAlH_4$ was added to a solution of **7** at room temperature. When heating at 50 °C, only the isobutyl ester of 3-ferrocenylpropenoate was isolated with DIBAL-H (no reaction with $LiAlH_4$), the latter being formed by an unexpected transfer of an isobutyl group from the isobutyl-aluminum reagent to the methyl ester **7**.[18] Changing to a solution of DIBAL-H *in hexane* proved beneficial. Whereas no reaction occurred when DIBAL-H/hexane was added to a stirred solution of **7** at room temperature in a standard manner, a clean conversion was observed by changing the experimental protocol. The allylic alcohol **8** was obtained in high yield (96%) when a solution of Fc-ester in THF was added, in reverse order, to a stirred solution of DIBAL-H/hexane, at room temperature. Finally, targeted Fc-azide **5** was obtained by direct conversion of alcohol **8** to the azide with diphenylphosphorazidate and DBU in the presence of sodium azide according to the procedure described by Thompson et al.[19] The whole sequence afforded pure 1-ferrocenylprop-1-en-3-azide **5** in three steps from commercial Fc-CHO with an overall yield of 77%.

The CuAAC reaction of Fc-azide **5** with ethynylpyrrolidine **4** was first performed in an nonaqueous medium (THF/MeCN) with copper(I) iodide (2 equiv) and a large excess of diisopropylethylamine



Scheme 1. Synthesis of ferrocenyl-azide **5**.

(DIPEA). Instead of the expected product **11**, the iodo-triazole **10** was isolated in 49% yield (Scheme 2). Compound **10** could result from the Huisgen cycloaddition between Fc-azide **5** and a iodoacetylide intermediate formed in situ by reaction of terminal alkyne **4** with copper(I) iodide.[20,21] To circumvent this difficulty we turned to the standard CuAAC procedure reported by Sharpless, which uses copper(II) salts ($CuSO_4$) in the presence of sodium ascorbate as the reducing agent.[22] When the reaction was held in water/*tert*-butanol for 48 h at 50 °C, the desired triazole **11** was isolated in 47% yield after purification by chromatography over silica gel. After *N*-allyl deprotection with $Pd(PPh_3)_4$ in the presence of *N*,*N*-dimethylbarbituric acid (78% yield), we struggled with the final hydrolysis of the isopropylidene group. Under the standard conditions also used for other ferrocene derivatives (1 M aqueous hydrochloric acid)[10,11] we observed the degradation of the starting material, with no formation of the expected product **3**. The reaction medium became dark blue during the reaction, revealing the possible formation of ferrocenium species. The same observation was made in a control reaction in which ferrocenylalcohol **8** was stirred in the presence of acid, supporting the unstability of the unsaturated Fc moiety in the presence of HCl. A variety of other methods were tested for the deprotection of the acetonide (with $FeCl_3$, BCl_3 or $Er(OTf)_3$ as the reagents), but all proved unsuccessful. To tackle this issue, hydrolysis of the isopropylidene was performed upstream the synthetic route, directly on substrate **4** to yield dihydroxypyrrolidine **13** in quantitative yield. Reaction of alkyne **13**



Scheme 2. Synthesis of hybrid molecule **3**.