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New mannose derivatives: The tetrazole analogue of mannose-6-phosphate as angiogenesis inhibitor

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ABSTRACT

Two novel compounds with mannose-derived structure, bearing a tetrazole (compound **3**) and a sulfone group (compound **4**) in terminal position, have been prepared from methyl α-D-mannopyranoside in reduced number of steps. The angiogenic activity of **3** and **4** has been screened using the chick chorioallantoic membrane (CAM) method. Tetrazole **3** has been identified to possess a promising bioactivity, being identified as angiogenesis inhibitor, with 68% of neovascular vessels when compared to control (PBS).

Angiogenesis is the process of generating new capillary blood vessels from pre-existing ones.[1] In healthy adults, angiogenesis is normally absent, excepting two specific phenomena: the cutaneous wound healing[2] and the intervention in female reproductive functions.[3,4] An abnormal vascularisation (either insufficient, either excessive) can cause or contribute to the development of various diseases.[5] Therefore, angiogenesis activators have wide applications in medicine, in the treatment of diseases caused by insufficient angiogenesis, linked to the ischemia of a part of the vascular system. At the same time, a considerable amount of attention has been dedicated to angiogenesis inhibitors, due to their intensive employment, among other therapeutic applications, in cancer treatment. It has been demonstrated that tumors cannot develop to a volume bigger than 1–2 mm^3 without the participation of blood vessels.[6] Therefore, angiogenesis inhibitor therapy[7] became an important actor in cancer therapy. It is supposed hat angiogenesis inhibitors act by inhibiting the development of blood vessels inside tumors and by normalizing the blood vessel network, facilitating the access of medication at tumor site.

Angiogenesis is a complex process, involving numerous biological mediators.[8] Recent studies indicate that the cation-independent mannose-6-phosphate receptor (CI-M6PR) is also involved in angiogenesis.[9,10] CI-M6PR, also known as the mannose-6-phosphate/insulin like growth factor II receptor (M6P/IGFIIR), is a 275 kDa, P-type glycoprotein,[11] whose main function is represented by the transport of newly synthesized enzymes from the cell membrane or from Golgi apparatus to lysosomes.[12] The binding specificity of the receptor has been intensively studied.[13] In our ongoing research on mannose-6-phosphate (M6P) and its derivatives, different M6P analogues have been synthesized and their affinity for the M6P/IGFIIR has been tested.[14–17] In a recent study, we have reported the synthesis of different M6P analogues, bearing various functional groups (azido, aminomethyl, carboxyl, malonate, sulfonate, carboxymethyl, phosphonate) in the C6 position of the carbohydrate and a methyl group in anomeric position, using a method involving a key cyclic sulfate intermediate. The angiogenic activity of these compounds has been evaluated and the tested analogues proved to be angiogenesis regulators.[18] The most potent angiogenesis inhibitor in this series was the methyl mannopyranoside of M6P itself (MeM6P) (compound **1**, Fig. 1), but its therapeutic applications are limited by its instability in biological environment, caused by the presence of hydrolytic enzymes. Besides MeM6P, another compound with promising antiangiogenic properties was the carboxylic acid **2**. Because tetrazoles are known as nonclassical bioisosteres of carboxylic acids,[19,20] the evaluation of the angiogenic properties of compound **3** became an interesting target, presented in this Letter. At the same time, we prepared a second mannose-6-functionalized derivative: the sulfone **4**. Sulfones have previously been investigated as phosphate mimics in carbohydrate series, both in the anomeric[21] and terminal position of carbohydrates, when a

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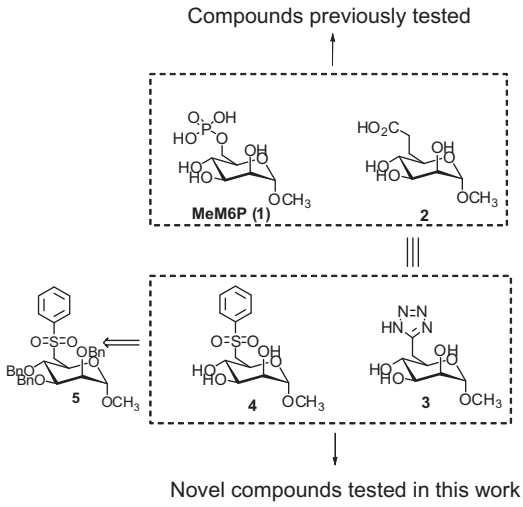
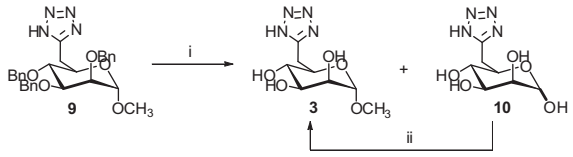


Figure 1. Mannose derivatives functionalized in terminal position.

dimethylene sulfone linker has been used as a replacer of the phosphodiester group in oligonucleotides.[22] Compound **4** is not a bioisostere of M6P. It has heteroatoms in the C6 position, but, unlike other M6P analogues tested by now, it does not possess negatively charged groups in the C6 position at physiologic pH. Moreover, besides the biological applications of **4**, intermediate **5**, which is used for the preparation of the final compound **4**, can open important perspectives in the synthetic carbohydrate chemistry. Carbanions of methylene groups in α position of sulfones can react with a variety of electrophiles, like, for example, carbonyl compounds, followed by subsequent elimination of the sulfone using samarium diiodide or sodium/mercury amalgam[23] (Julia olefination method).[24]

In order to prepare compounds **3** and **4**, we have chosen an efficient pathway that involves the iodinated compound **7** as common intermediate in the synthesis of the desired carbohydrates (Scheme 1). This intermediate was obtained from methyl α-D-mannopyranoside, using an Appel reaction[25] and among various protocols available[26–28] we have chosen the procedure of Skaanderup et al.,[26] followed by introduction of benzyl protective groups on carbohydrate hydroxyls. Because benzylation under standard basic conditions (benzyl bromide and sodium hydride or potassium hydroxide) is not compatible with our iodinated compound, we have realized this step in acid catalysis,[26] with benzyl trichloroacetimidate prepared from benzyl alcohol and 1,1,1-trichloroacetonitrile.[29] The benzylation reaction is usually realized with triflic acid as catalyst, but our tests with boron



Scheme 2. Two-steps synthesis of **3**, by deprotection of hydroxyl groups of **9** followed by methylation of the obtained reaction mixture. Reactions, conditions and yields: (i) BCl_3, Cl_2H_2; 3 min at −78 °C; (ii) $SOCl_2$/MeOH, 3 h at rt; 36% over the two steps (i) and (ii).

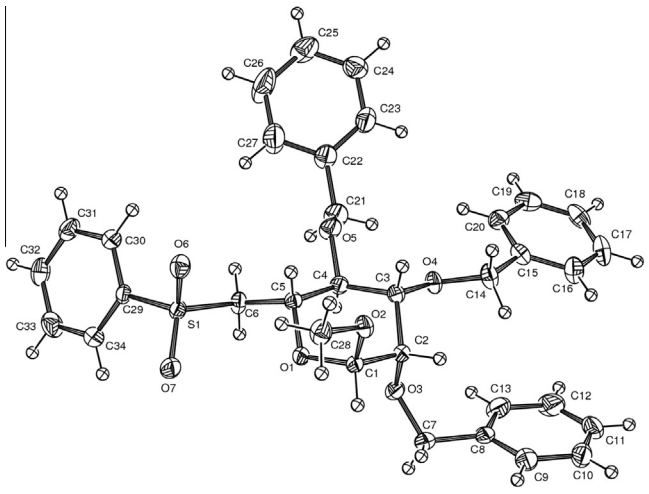
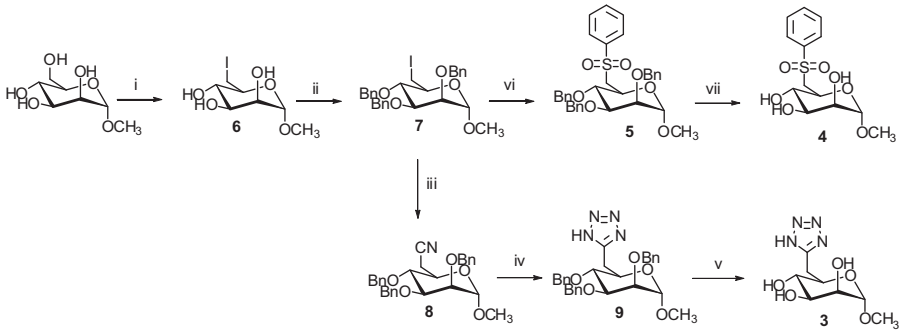


Figure 2. ORTEP drawing of **5**.

trifluoride diethyletherate (0.3 equiv/hydroxyl group) have given the same result.

The tetrazole derivative **3** was prepared using the nitrile intermediate **8**, obtained by reacting **7** with sodium cyanide in DMF. The conversion of nitriles to tetrazoles supposes, in a classical manner, the usage of hydrazoic acid, prepared from sodium azide and acids[30] or of tributyltin azide, prepared from sodium azide and tri-*n*-butyltin chloride.[31] Other methods include the usage of $Al(N_3)_3$, prepared in situ from trimethylaluminium and trimethylsilyl azide[32] or salts of hydrazoic acid.[33] We decided to use the last method, already applied with success in glucose series, using sodium azide and ammonium chloride, that allowed us to obtain the tetrazole **9** in 70% yield. The last step was represented by carbohydrate hydroxyl groups' deprotection. Based on our knowledge, no literature data has reported by now the synthesis of an unprotected glycoside bearing a tetrazole moiety in terminal



Scheme 1. Synthesis of **3** and **4**. Reactions, conditions and yields: (i) I_2, PPh_3, Imidazole, THF, 2 h at refluxing temperature, 81%; (ii) benzyl trichloroacetimidate, triflic acid, dioxane, 10 min at 0 °C, 67%; (iii) NaCN, DMF, 2 h at 70 °C, 87%; (iv) NaN_3, NH_4Cl, DMF, 144 h at 95 °C, 70%; (v.a) BCl_3, CH_2Cl_2, 3 min at −78 °C; (v.b) $SOCl_2$, methanol, 3 h at rt; 36% over the two steps v.a and v.b; (vi) sodium phenylsulfinate, DMF, 12 h at 60 °C, 67%; (vii) H_2, Pd/C, ethyl acetate/methanol, HCl, 2 h at rt, 80%.

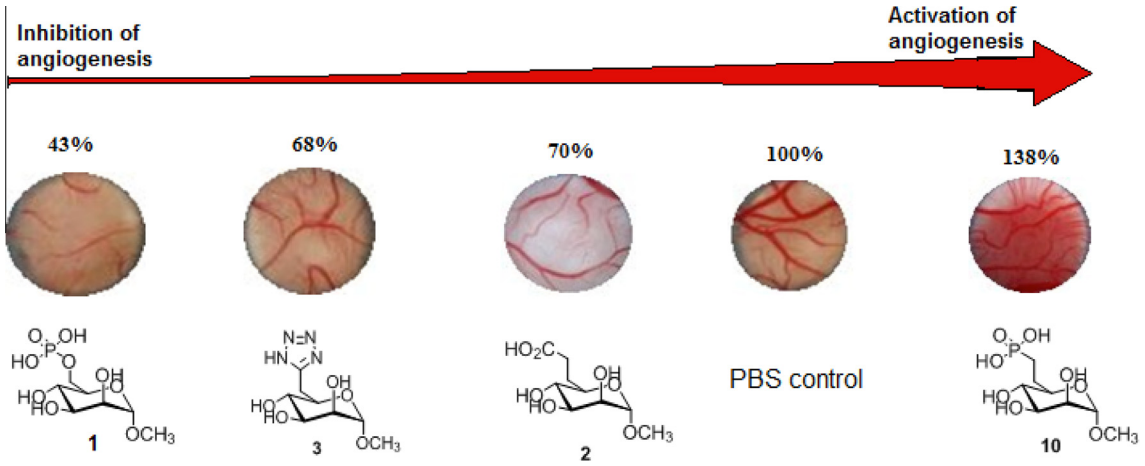


Figure 3. Chorioallantoic membrane (CAM) assays realized with MeM6P (**1**) as inhibitor, its phosphonate analogue **10** as angiogenesis activator, PBS as control, carboxylic acid **2** and its non-classical bioisostere, tetrazole **3**. The given values represent the extent of angiogenesis, 100% being the value quoted for PBS (control).

position. In our case, the deprotection of mannose hydroxyl groups proved to be very delicate. Initial attempts of classical hydrogenolysis at different hydrogen pressures, in neutral or acidic solvents, were inefficient. Another method known for benzyl groups deprotection is represented by the action of boron trichloride.[34] The usage of this compound in methyl glycosides remains limited, as BCl_3 is known to break glycosidic bonds.[35] Indeed, by reacting **9** with BCl_3 in CH_2Cl_2, at −78 °C, the tetrazole mannopyranoside **3** has been obtained, but together with its corresponding mannopyranose, bearing a hydroxyle group in anomeric position (Scheme 2). The crude reaction mixture has been methylated, using the $SOCl_2$/MeOH couple, in order to enrich the mixture in the desired derivative **3**.

The sulfone **4** has been prepared in two steps starting from **7**: displacement of iodine with *p*-toluenesulfinate[36] and benzyl groups' elimination (Scheme 1). Because initial attempts of deprotection by hydrogenolysis at neutral pH were inefficient, this step was realized in the presence of HCl in order to obtain compound **4**. Single-crystal X-ray analysis of **5** confirmed its structure. Crystallographic data (excluding structural factors) for the structure of compound **5** have been deposited at the Cambridge Crystallographic Data Centre with the deposition number CCDC 679336. An ORTEP diagram of **5** is shown in Figure 2.

The angiogenic properties of the two products **3** and **4** were investigated in vivo using the chick chorioallantoic membrane (CAM) assay.[37,18] Besides the two compounds **3** and **4**, MeM6P and its phosphonate analogue **10** have been used as angiogenesis inhibitor and activator, respectively. A phosphate-buffered saline (PBS) control experiment has also been realized. Membranes treated with the mentioned compounds were placed on nascent CAM during the 7th embryonic day and were grown *in ovo* at 38 °C for 4 days. Angiogenic response quantification was realized by measuring the area of neovascularization on the surface of each membrane used in the tests. The results of the experiment were in good agreement with our expectations: no effect on angiogenesis could be noticed in the case of sulfone **4**, while tetrazole **3** showed an inhibitory effect on angiogenesis. Its inhibitory properties evaluated using this method were very similar with those of its carboxylic acid analogue **2** reported earlier[18] (68% of neovascular vessels for **3** and 70% of neovascular vessels for **2**, the PBS control being defined as 100%) (Fig. 3).

In conclusion, two novel compounds have been reported in this Letter: the sulfone **4** and the tetrazole **3**. They have both been prepared starting from methyl α-D-mannopyranoside, using methyl 6-deoxy-6-iodo-α-D-mannopyranose as common intermediate, in six steps for tetrazole **3** (12% yield) and in four steps for sulfone **4** (29% yield). Both products' angiogenic activity has been evaluated using the CAM assay. Tetrazole **3** showed good inhibitory properties (70% compared to PBS), thus being an interesting new carbohydrate derivative with possible applications in anticancer therapy. Moreover, this discovery is important because the number of monosaccharides with angiogenesis inhibitor properties reported by now is extremely limited.[18]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.11.059.

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