Synthesis of Mannose-6-Phosphate Analogues and their Utility as Angiogenesis Regulators
Véronique Barragan-Montero, Azzam Awwad, Stéphanie Combemale, Pascal de Santa Barbara, Bernard Jover, Jean-Pierre Mols, Jean-Louis Montero

To cite this version:


HAL Id: hal-02543785
https://hal.umontpellier.fr/hal-02543785
Submitted on 15 Apr 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Synthesis of Mannose-6-Phosphate Analogues and their Utility as Angiogenesis Regulators


Although carbohydrates are the most abundant natural products, their use as therapeutic agents has been limited. However, since carbohydrate binding proteins are involved in many biological processes, including cellular communication,[3-2] the prospects for carbohydrate-based drugs seem bright. Here, we provide a synthetic route to bioactive mannose derivatives that serve as both positive and negative effectors of angiogenesis, thereby laying the groundwork for future drug development.

The current, limited applications of carbohydrates as therapeutics may, in part, be related to the high complexity of interactions between carbohydrate and carbohydrate binding proteins. Carbohydrate oligomers are themselves complex; for example, four different monosaccharides can form 35 560 distinct tetrasaccharides—this large number reflects the multiple hydroxy attachment sites on each component sugar. Thus, a relatively small polysaccharide has an enormous capacity to encode biological information. When these polysaccharides are conjugated to proteins, the complexity further increases. To date, more than 80 carbohydrate binding proteins have been identified, and their binding specificities have been described (or are about to be).[3] Among these proteins, the lectin family has been extensively studied and classified into subfamilies according to their cellular location and their carbohydrate binding specificities.[4] For example, the P-type lectins recognize mannose-6-phosphate (M6P), the motivation behind efforts in the design and synthesis of new M6P analogues.

P-type lectins encompass the 46 kD cation-dependent M6P receptor (CD-M6PR), the 300 kD cation-independent M6P receptor (CI-M6PR), and proteins harboring M6P homology domains.[5] One major cellular function of the receptors is to help cargo M6P-containing proteins between various subcellular compartments.[6] In addition, CI-M6PR is actually a large multi-
Scheme 1. Synthesis of mannose-6-phosphate (M6P) analogues. Reagents and conditions: a) DMP, p-TsOH, acetone; b) H₂O, 63%; c) Et₃N, CH₂Cl₂, SOCl₂, 0 °C then NaIO₄, H₂O, RuCl₃, CH₂Cl₂/CH₃CN (1:1), RT, 84%; d) CH₂PO(OH)₂, 1,1-diphenylethylene, nBuLi, HMPT, THF, THF, -78 °C, quant; e) Amberlyst 15-H⁺, MeOH/THF (1:1), quant; f) (CO₂)₂CN, H₂O, NaH, DMF, 30%; g) KOH, THF/H₂O (6:4), 95 °C; h) CH₂SO₂Pr, 1,1-diphenylethylene, nBuLi, HMPT, THF, quant; i) TMSBr, CH₂Cl₂, 82%; j) NaCN, DMF, quant; k) NaCN, DMF, quant; l) H₂O, H₂N, Raney Ni, MeOH, 10%; m) H₂O₂, NaOH, then Amberlite IRC-50-H⁺, quant; n) BrCH₂CO₂Et, 1,1-diphenylethylene, nBuLi, HMPT, THF, 39%; o) pyridine, DMAP, POCl₃, CH₂Cl₂, 0 °C, 75%. For abbreviations, see the Experimental Section.

Angiogenesis is a complex phenomenon that leads to the formation of new blood vessels from pre-existing ones. This process is crucial for development and plays a key role in various normal and pathological states, including cancer and cardiovascular diseases. Its regulation involves a tuned balance of proangiogenic and antiangiogenic factors. The relationship between M6P and angiogenesis appears firmly established, through both the transforming growth factor (TGF-β) pathway activation and the prolinerin (PLF) signal. TGF-β pathways or ligands are thought to have both pro- and antiangiogenic properties. Low TGF-β levels contribute to an angiogenic switch by up-regulating angiogenic factors and proteinases. On the other hand, high TGF-β levels inhibit endothelial cell growth, stimulate smooth muscle cell differentiation, and recruitment and promote basement membrane reformation. Regarding the prolinerin signal, M6P completely blocks PLF-induced angiogenesis both in cell culture and in vivo. Finally, the receptor itself can affect angiogenesis by clearing active plasminogen through its soluble form, and this has been shown to block tumor cell invasion in vitro, endothelial cell invasion in vivo, and tumor growth in vivo.

The M6P analogues presented in Scheme 1 were subjected to angiogenic assays using two experimental models. The first of these employed the rat aortic ring assay, an ex vivo angiogenic model in which our analogues were examined at 10⁻⁷ M over 11 days for their ability to stimulate or inhibit capillary growth in rat aortic rings. Sunitinib (marketed by Pfizer as Sutent and formerly known as SU11248) and endothelial cell growth supplement (ECGS) were used as known negative and positive stimuli, respectively. The results are shown in Table 1. Certain derivatives behave as inhibitors: MeM6P 11, malonate 8, and phosphonate 3, while others are activators: sulfonate 9 and azide 4. Still some derivatives have only slight or no effect on angiogenesis: carboxylate 7 and amine 6. Carboxylate 10 exhibited the same effect as

### Table 1. Cytotoxicity, tumor growth and evaluation of angiogenic effects of M6P analogues

<table>
<thead>
<tr>
<th>Compd</th>
<th>ARA (%)</th>
<th>ARS (%)</th>
<th>Cell toxicity (%)</th>
<th>B16 Tumor growth</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no. sprouts</td>
<td></td>
<td></td>
<td>vol[mm³]</td>
<td>survival</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>21 ± 13</td>
<td>49 ± 2</td>
<td>78 ± 5.5</td>
<td>107 ± 2.5</td>
<td>N.D.</td>
</tr>
<tr>
<td>11</td>
<td>34 ± 29</td>
<td>43 ± 2</td>
<td>110 ± 3.5</td>
<td>109 ± 4.9</td>
<td>0.79 ± 0.69</td>
</tr>
<tr>
<td>9</td>
<td>97 ± 9</td>
<td>69 ± 3.5</td>
<td>80 ± 5.5</td>
<td>99 ± 3</td>
<td>101 ± 2</td>
</tr>
<tr>
<td>8</td>
<td>45 ± 21</td>
<td>70 ± 7.2</td>
<td>113 ± 4</td>
<td>119 ± 3.5</td>
<td>103 ± 4.5</td>
</tr>
<tr>
<td>10</td>
<td>N.D.</td>
<td>73 ± 0.5</td>
<td>110 ± 2.5</td>
<td>110 ± 1.9</td>
<td>104 ± 1.5</td>
</tr>
<tr>
<td>6</td>
<td>58 ± 33</td>
<td>81 ± 2</td>
<td>80 ± 10</td>
<td>101 ± 13.5</td>
<td>120 ± 23</td>
</tr>
<tr>
<td>PBS control</td>
<td>88 ± 21</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>71</td>
<td>123 ± 7.5</td>
<td>119 ± 2.5</td>
<td>110 ± 2</td>
<td>104 ± 1.5</td>
</tr>
<tr>
<td>7</td>
<td>115 ± 7</td>
<td>125 ± 3.5</td>
<td>84 ± 9</td>
<td>90 ± 5.5</td>
<td>121 ± 5</td>
</tr>
<tr>
<td>3</td>
<td>56</td>
<td>130 ± 3.5</td>
<td>131 ± 7.5</td>
<td>86 ± 4.5</td>
<td>98 ± 8.5</td>
</tr>
<tr>
<td>ECGS</td>
<td>N.A.</td>
<td>172 ± 8.5</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

[a] N.A.: not applicable; N.D.: not done; [b] Aortic ring assay (ARA); [c] data represent the mean ± SD; data were analyzed by one-way ANOVA or two-way ANOVA for repeated measures when required. Between-group differences were determined with Student's t-test. The level of significant difference was set for p < 0.05. [d] Angiogenic relative surface (ARS) was determined using a chorioallantoic membrane (CAM) assay; data represent the mean ± SD versus the PBS control. [e] Data represent the mean ± SD versus the control. [f] Tumor volume was determined on day 19. Animal experiments complied with the European and French laws and with the guiding principles for experimental procedures as set forth in the declaration of Helsinki.
MeM6P (11) itself, with the number and the length of sprouts being equivalent.

The second biological assay used to evaluate our M6P derivatives was the avian chorioallantoic membrane (CAM) assay.\cite{31} Control- or M6P-analogue-treated membranes were deposited on nascent CAM at embryonic day 7 and grown for 4 days in ovo at 38 °C. The same positive and negative controls were included as before. Quantification of the angiogenic response was carried out by measuring the area of neovascularization on each particular membrane. These experiments demonstrate divergent activities of the synthesized compounds (Figure 1).

![Figure 1. Chorioallantoic membrane (CAM) assays performed with mannose-6-phosphate (M6P) analogues 3, 4, 6–11, the angiogenesis inhibitor sunitinib and the angiogenesis activator, endothelial cell growth supplement (ECGS). Also shown is the phosphate-buffered saline (PBS) control experiment for comparison. The values given represent the activator, endothelial cell growth supplement (ECGS). Also shown is the phosphate-buffere](image)

As before, some M6P derivatives were identified as CAM inhibitors: sulfonate 9, malonate 8 and MeM6P 11, while other derivatives behaved as CAM activators: phosphonate 3, azide 4 and carboxylate 7. In the inhibitor group, compared to the control, we observed 43% of neovascular vessels for phosphonate 11, 69% for sulfonate 9, 81% for amine 6, 70% for malonate 8, and 73% for carboxylate 10. In the activator group, compared to the control, an increase in neovascular vessels of 123% was observed for azide 7, 125% for carboxylate 4, and 130% for phosphonate 3. It is worth pointing out that the azide and carboxylate derivatives, both of which displayed lower activities compared with the other test compounds, are not bioisosteric analogues of M6P. It is also noteworthy that the results of our two assays do not correspond exactly. During our study, we analyzed two different angiogenic processes, sprouting using aortic ring assay and intussusception (splitting) angiogenesis with CAM assays.\cite{32} These two processes can be differently modulated by M6P analogues. This is not unusual in the angiogenesis arena and explains why multiple assays are often needed to portray the efficacy of a potential drug.

Finally, compounds showing the most potent angiogenic properties, namely MeM6P (11) for activation and phosphonate 3 for activation, as well as the azido 4 (moderate activity), were tested in a B16 melanoma tumor growth model\cite{33} and their cytotoxicity was also evaluated in primary human endothelial cell cultures. Indeed, the antiangiogenic properties of some compounds could be the result of specific cell toxicity.\cite{34} At the three concentrations tested, slight or no effect on the cell number in primary human endothelial cell cultures was observed after 48 h exposure (Table 1). Mice were injected with B16F1 cells (day 0). The mice were then divided into groups and were treated (i.p.) with 300 mg kg⁻¹ of test compound three times a week, starting from day 0. MeM6P (11) showed 79% tumor growth inhibition and 75% survival at day 19. Azide 4 is also an inhibitor of tumor growth but to a lesser extent (50%). No effect was observed for phosphonate 3 (Table 1).

In conclusion, we observed that, of the M6P analogues evaluated, some display proangiogenic activities, while others display antiangiogenic activities. These latter analogues were tested in a melanoma B16 tumor growth model, and at least two of them have been shown to inhibit tumor growth. We have clearly demonstrated that M6P and its analogues assist in the control of neoangiogenesis, and these compounds can be considered as leads for the development of a novel class of therapeutics.

We have presented an efficient method for synthesizing M6P analogues. This route can be used to develop additional carbohydrate analogues modified at the C-6 position, thereby allowing access to a large variety of original carbohydrate mimics. We also investigated the function of these monocarbohydrates during angiogenic processes, showing for the first time that monocarbohydrates possess angiogenic activities via the M6PR with no apparent toxicity. These results open the possibility for developing angiogenesis regulator carbohydrates as anticancer agents (inhibitors) or for the treatment of cardiovascular disease (activators). It is clear that our preliminary results are a promising start in the use of carbohydrates as angiogenic regulators. The in vitro, in ovo, and in vivo assays have divided the M6P analogues tested into angiogenesis activators and inhibitors. The mechanism of action of M6P analogues as angiogenesis regulators has still to be elucidated,
but it seems obvious that the M6P receptor is involved. Investigation into the mechanism of M6P analogue action is underway now that a family of regulators is in hand.

Experimental Section

Supporting Information: Experimental protocols for the synthesis and biological evaluation of the analogues described here can be found on the WWW under http://dx.doi.org/10.1002/cmdc.201000293.

Abbreviations: para-Toluenesulfonic acid (p-TsOH); 2,2-dimethoxy-propane (DMP); 4-dimethylaminopyridine (DMAP); hexamethylphosphorous triamide (HMPT); tetrahydrofuran (THF); trimethylsilyl bromide (TMSBr).

Acknowledgements

V.B.M. is grateful to the Mission de la Recherche et de la Technologie (MRT), France for scholarships to S.C. and A.A., and thanks Dr. Frédéric Genet (Laboratoire de physique théorique et astro-particules, Université Montpellier 2, France) for technical help and the Société de Recherche en Dermatologie for its financial support. Pd.S.B. is supported by the French Agence Nationale pour la Recherche (ANR-07-JCJC-0112).

Keywords: angiogenesis · carbohydrates · chemotherapy · glycobiochemistry · mannos-6-phosphates