## Macrocyclic Glycohybrid Toolbox Identifies Novel Antiangiogenesis Agents from Zebrafish Assay

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A practical and modular approach to obtain a diverse set of 14-membered macrocyclic compounds from carbohydrates is developed that utilizes functional groups at C-1 and C-5. The evaluation of this toolbox in various zebrafish assays led to the identification of 2.7f as an antiangiogenesis agent.

The growing interest in macromolecular (i.e., protein– protein,<sup>1,2</sup> DNA/RNA–protein) interactions,<sup>3</sup> and in signaling pathways<sup>4</sup> is challenging our current thinking in the

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drug discovery arena.<sup>5</sup> This is also creating new opportunities to develop novel chemical approaches to allow us to build unique chemical tool sets that are more closely related to bioactive natural products or derived from their inspiration.<sup>6,7</sup> This is largely due to the fact that natural

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ORGANIC LETTERS

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products in general display 3D shapes and possess several chiral functional groups that are a good source of small molecule modulators of protein-protein, DNA/RNAprotein interactions.<sup>1,2,8</sup> In addition, interest is also rising in emerging screening approaches, such as the use of cellular phenotypes<sup>5a</sup> and *in vivo* models (i.e., the use of zebrafish technology).<sup>9</sup> The latter approach is attractive because it is close to the animal model and is a good way to evaluate the therapeutic potential of small molecules at an early stage. In particular, natural products having macrocyclic architectures are attractive due to several reasons: (i) the macrocyclic shapes represent preorganization, (ii) the potential to map a large surface area, and (iii) numerous binding sites.<sup>10</sup> Despite all these attractive properties that are commonly associated with macrocyclic compounds, building a chemical toolbox having a diverse set of macrocyclic compounds is still in its infancy.<sup>11</sup> With this objective, we launched a program that aims to obtain different types of macrocyclic compounds that could be derived from carbohydrates as a cheap source for chirality.

Herein, we outline our approach with glycopyranosides as the starting material (**1.1**, Scheme 1), which can lead to accessing various acyclic compounds (see **1.2** and **1.3**).<sup>12</sup> Through the utilization of functional groups at C-1 and C-5, we then plan to incorporate amino acid moieties in two different manners that would lead to two families of unique *macrocyclic glycohybrids* (**1.4** and **1.5**) following the subjection to the "stitching technology". Our approach

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Org. Lett., Vol. 15, No. 3, 2013

Scheme 1. Our Approach To Obtain 14-Membered Macrocycles



can be general in nature; for example, the use of different sugars (i.e., glucose, galactose, mannose, etc.) can lead to producing 14-membered glycohybrids with variation in their stereochemical display of hydroxyl groups. A specific example of our approach is also shown in Scheme 1. For example, if we utilize  $\alpha$ -D-glucopyranoside (1.6) as the starting material, we can aim to access 14-membered, glycohybrid **1.4a** that has retained the stereochemistry of C-2 to C-5 hydroxyl groups. In another case, 14-membered glycohybrid 1.5a has three hydroxyl groups with retention of the stereochemistry, as it was in the starting sugar and an inverted primary hydroxyl group at C-5. Both macrocyclic compounds 1.4a and 1.5a are planned to be assembled through the crucial ring-closing metathesis "stitching technology" on highly functionalized acyclic substrates, 1.7 and 1.8. First, as a proof of concept study, we set our objective toward achieving the synthesis of two macrocyclic targets, 1.4a and 1.5a.

Methyl- $\alpha$ -D-glucopyronoside (2.1, Scheme 2) was used as the test starting material. Upon subjection to perbenzylation, the pyranoside ring was opened under acidic conditions and then directly applied to reductive alkylation giving 2.2 in a high yield. The secondary amine was then coupled with various *N*-Fmoc protected amino acids, which upon *N*-Fmoc removal and amidation resulted in 2.4. At this stage the acyclic precursor was set to bisallylation that gave the required bis-allylated product 2.5 needed to test our key stitching technology on this highly functionalized substrate. To our delight, use of the Grubbs second generation catalyst<sup>13</sup> (G-II, 15 mol %) led to 14membered macrocyclic ring formation (olefinic isomeric ratio not defined yet). Finally, this mixture of two olefinic

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Scheme 2. 14-Membered Macrocycles



Scheme 3. Our Other Synthetic Approach To Obtain 14-Membered Macrocycles



compounds was hydrogenated and this provided the macrocyclic compound **2.7** cleanly. Eight macrocyclic compounds were synthesized using this approach. All the products obtained in this scheme were thoroughly purified and then characterized using HPLC-MS and NMR. In one case (**2.7e**), we successfully obtained the X-ray that further confirmed the macrocyclic ring assignment.

Our synthesis plan to obtain glycol-based 14-membered macrocyclic compounds, **3.7**, is shown in Scheme 3. **3.1** was obtained from **2.1** in three steps as follows: (i) perbenzylation, (ii) acid treatment, and (iii) reductive alkylation. This amine was then converted into amide **3.2** using different



Figure 1. Zebrafish assay. *Angiogenesis:* (A) Zoom section of wild-type or vehicle treated embryo; (B) zoom section of control; (C and D) zoom sections after treatment with **2.7d**, **2.7f**, **2.6b**, and **2.4b**. *Early embryo development:* (E) DMSO exposed embryos; (F) small molecule exposed embryos causing a delay in epiboly.

acid chlorides. The next key step in our synthesis was a Mitsunobu reaction, and this was successfully carried out using DIAD, PPh<sub>3</sub>, and DPPA to obtain 3.3 with the inverted stereochemistry. The corresponding azide 3.3 was further reduced using a Staudinger reaction to give a primary amine, which was then coupled with several N-Fmoc protected amino acids under HOBT, DIC, and DIPEA in DMF conditions to give 3.4 in good yields. This was further converted to 3.5 by treatment with DBU followed by monoallylation reaction using allylbromide, triethylamine, and the conversion of a secondary amine to tertiary amide 3.5 using different acid chlorides. Having key functionalized acyclic starting material, we were ready to test our crucial ring-closing metathesis "stitching technology". As in the previous case, this went very well using 10 mol % Grubbs second generation catalyst (G-II), and macrocyclic products 3.6 were nicely obtained. Finally, treatment to hydrogenation conditions led to the complete synthesis of our desired target 3.7, and six macrocyclic compounds were obtained using this approach.

The next plan was to subject our glycohybrid macrocyclic collection (78 compounds in total) to various zebrafish screens to search for functional small molecules. These screens were related to identify functional chemical

probes affecting epiboly during early embryonic development,<sup>14</sup> angiogenesis,<sup>15</sup> and neurogenesis<sup>16</sup> in zebrafish embryo assays. All three assays are well-documented in the literature.<sup>17</sup> and the detailed procedure is provided in the Supporting Information. Thus, we identified two novel glycohybrid 14-membered ring-derived compounds. 2.7d and 2.7f (Figure 1), as potent inhibitors of trunk angiogenesis (i.e., stopped complete angiogenesis at  $2.5 \mu$ M). In additon to this, two more compounds (e.g., an acvelic derivative, 2.4b, and a 14-membered ring macrocyclic compound, **2.6b**) exihibited partial inhibition at 2.5  $\mu$ M and complete inhibition at  $5.0 \,\mu$ M. It is too early to predict the mode of action of these two potent macrocyclic compounds that are structurally closely related. Further work would be needed to gain deeper insight leading to a better understanding of the mechanism of action.

In another study examining the effect of small molecules on epiboly cell movements during early embryonic development (Figure 1), we identified two active compounds (2.7f and S5d). The delay in epiboly was clearly seen in exposed embryos (i.e., complete inhibition by 2.7f at  $2.5 \,\mu$ M and by S5d at  $5.0 \,\mu$ M). It was interesting to note that small molecule 2.7f was also observed to be a potent inhibitor of angiogenesis.

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To summarize, we report here a practical approach to access functionalized carbohydrate-derived macrocyclic compounds. The synthesis plan is highly modular and allows us to obtain a different set of 14-membered macrocyclic derivatives. Yet, in our present study, we only utilized  $\alpha$ -D-glucopyranoside as the starting sugar: the scope of this methodology with other pyranoside derivatives remains to be tested. Using our chemical toolbox obtained to date, it was further tested in a zebrafish embryo angiogenesis study. This led to identification of two fully deprotected 14-membered macrocyclic compounds (2.7d and 2.7f) as potent inhibitors of angiogenesis (i.e., complete inhibition at 2.5  $\mu$ M). Interestingly, both of these compounds are structurally related. One of them (2.7f) also exhibited complete inhibition of early embryonic development in a zebrafish screen at  $2.5 \,\mu$ M. In addition to 2.7f. S5d also exhibited complete inhibition of early embryonic development at 5.0 µM. The discovery of macrocyclic compound 2.7f showing two effects is intriguing, and further work is required to gain a better understanding to determine any commonalities regarding the biological effect.

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**Supporting Information Available.** The detailed synthetic procedure and the analytical data for all the new compounds are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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