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SHORT COMMUNICATION

Antiangiogenesis Agents

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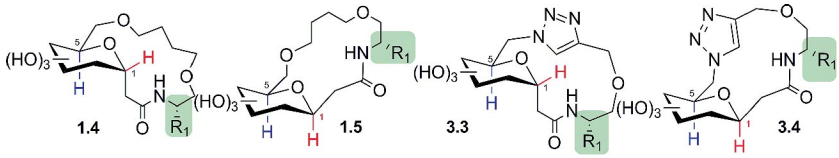
Building a Macrocyclic Toolbox from *C*-Linked Carbohydrates Identifies Antiangiogenesis Agents from Zebrafish Assay



The synthesis of four different types of macrocyclic glycohybrids that contain an amino acid moiety in the large-ring skeleton is reported. Ring-closing metathesis and click chemistry approaches were utilized to obtain two different series of macrocycles. The evaluation of this toolbox resulted in the identification of two unique compounds as antiangiogenesis agents in an embryonic zebrafish assay.

Keywords: Macrocycles / Medicinal chemistry / Carbohydrates / Click chemistry / Angiogenesis / Molecular diversity

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Building a Macrocyclic Toolbox from *C*-Linked Carbohydrates Identifies Antiangiogenesis Agents from Zebrafish Assay

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Keywords: Macrocycles / Medicinal chemistry / Carbohydrates / Click chemistry / Angiogenesis / Molecular diversity

We report the synthesis of four different types of macrocyclic-derived glycohybrids from carbohydrates that have an amino acid moiety in the large-ring skeleton. These macrocyclic glycohybrids were obtained from α-*C*-1H- and β-*C*-1H-linked carbohydrates. In one series, we utilized ring-closing metathesis as the "stitching technology" to obtain two different macrocycles, i.e., *trans* equatorial–axial C-1H and C-5H and *cis* axial–axial C-1H and C-5H. The click approach was the key reaction in our second series to obtain two other macrocyclic compounds, i.e., *trans* equatorial–axial C-1H and C-5H and *cis* axial–axial C-1H and C-5H. The evaluation of this toolbox resulted in the identification of two unique compounds as antiangiogenesis agents in an embryonic zebrafish assay. Interestingly, in both cases, the macrocyclic compounds that have a *cis* relationship (i.e., axial–axial orientation) between C-1H and C-5H showed activity and their other diastereomers (i.e., equatorial–axial C-1H and C-5 H) with a *trans* relationship did not show any effect.

Introduction

In continuation of our interest in building a glyco-based macrocyclic toolbox to search for modulators of protein–protein,[1,2] DNA/RNA–protein[3] interactions and the dissectors of signalling pathways,[4] herein, we outline another strategy to obtain a different family of glycohybrids.[5–10] As briefly discussed in our recent papers,[11,12] the need to access compounds that bear a closer resemblance to natural products in terms of their 3D architecture and the presence of several chiral functional groups is growing constantly.[13–15] There is a particular rise in the interest in functionalized macrocyclic compounds,[16] which can provide several advantages, such as (1) being useful to map a large surface area to explore macromolecular interaction-based targets, (2) a functionalized macrocycle can provide numerous binding interaction possibilities, (3) amphiphilic macrocyclic compounds can be useful to maintain a balance through various polar/non-polar sites. All these features combined together offer several advantages on the use of various types of macrocyclic compounds. A particular attraction in having macrocycles from sugars is that one can nicely synthesize amphiphilic macrocyclic skeletons to explore their biological function(s).

Results and Discussion

In our earlier approach,[11a] we reported a modular synthesis of 14-membered macrocyclic compounds as glycohybrids that were derived from a pyran ring opening, followed by incorporation of the amino acid moiety, and finally, through application of the stitching technology.[17] Herein, we propose a route to obtain *C*-linked β- and α-glycosyl carboxyl esters **1.2** and **1.3** (Scheme 1) that are known in the literature.[18a] The presence of two functional groups on the sugar moiety, i.e., the carboxyl ester side chain at C-1 and the primary hydroxy group at C-5 means that compounds **1.2** and **1.3** can easily be coupled to various amino alcohols, which can then be subjected to the ring-closing technology to give the 15-membered macrocyclic rings **1.4** and **1.5**.[18b] The proposed plan is general in nature and can be applied to several sugars to allow variation in the three hydroxy groups at C-2/3/4 that are incorporated in the macrocyclic ring. Due to the general nature of this method, one can utilize different sugars to obtain diversity in the contiguous hydroxy groups that are present in the 15-membered macrocyclic ring. Shown in Scheme 2 are two different macrocyclic glycohybrids **2.2** and **2.3** that we planned to obtain from methyl α-D-glucopyranoside **2.1**.

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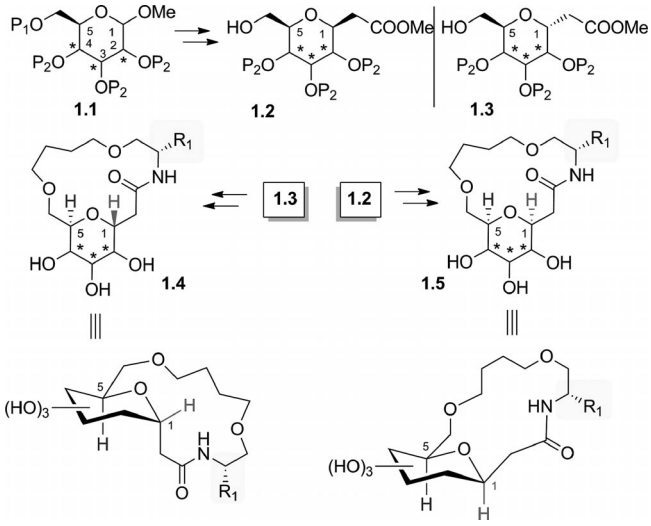
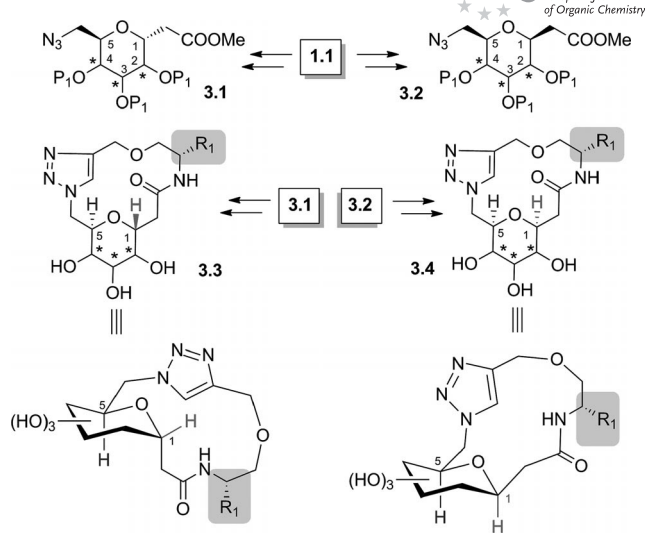


Scheme 1. Our approach to utilize *C*-linked glyco-derivatives **1.2** and **1.3** to access macrocyclic glycohybrids **1.4** and **1.5**.



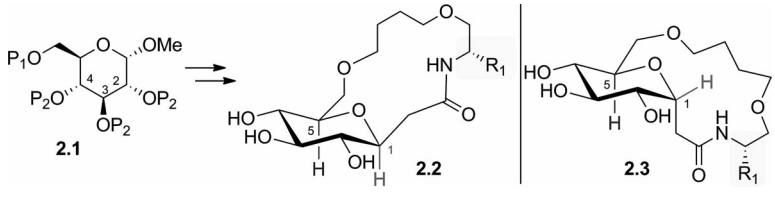
Scheme 3. 14-Membered macrocycles by click stitching technology.

In another approach, we planned to obtain glyco-based sugar azido esters **3.1** and **3.2** (Scheme 3) from **1.1** and the synthesis of these two compounds has been reported in the literature.[19] In our approach, we planned to incorporate the amino acid moiety through coupling with the carboxyl functional group of sugar azido acids and then utilize the click-chemistry-based stitching technology.[20–24]

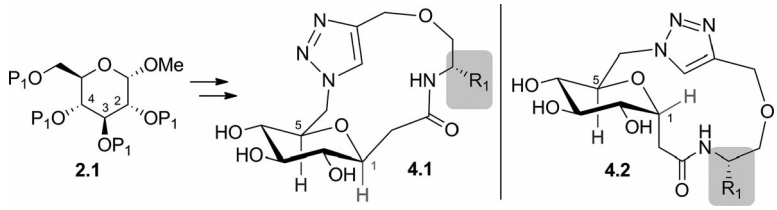
This strategy to obtain glycohybrids containing 14-membered rings has some unique features and they are worth mentioning. These include the building of 14-membered macrocyclic ring that has a bridged sugar utilizing either the *trans* C-1H and C-5H or *cis* C-1H and C-5H (see, **3.3** and **3.4**). In addition to this, they also have another bridged heterocyclic ring arising from the click reaction. The variation in C-1 stereochemistry leads to two different macrocyclic compounds to allow the display of various functional groups in different orientations. Once again, this method is versatile and several sugars can be utilized to obtain the contiguous hydroxy group presentation on the sugar ring.

A specific example of two macrocyclic compounds, **4.1** and **4.2**, is shown in Scheme 4. Our detailed synthesis plan to obtain 15-membered rings is shown in Scheme 5. To start with, methyl α-D-glucopyranoside was converted to **5.2**, a *C*-linked carboxyl ester derivative, as an unseparable diastereomeric mixture. As a test study, the diastereomeric mixture of **5.2** was separated in a small amount as **5.2a** (lower R_f, C-1-β-H, 4.68 ppm) and **5.2b** (higher R_f, C-1-α-H 3.76), and both products were further subjected to hydrogenation to obtain **5.2ah** and **5.2bh** for NOE studies. This helped in assigning the diagnostic C-1H (i.e., C-1-α-H 3.66 ppm and C-1-β-H 4.44 ppm) in both cases. The detailed analytical information is provided in the Supporting Information. On a large scale, the mixture as a whole was subjected to selective debenzylation conditions that gave the free hydroxy group at C-5, which upon allylation and ester hydrolysis gave the free carboxylic acid as **5.3**. It was then coupled to several *O*-allylated chiral amines **5.4**, which were easily obtained from their corresponding amino acids in four steps (for the detailed synthesis steps, see the Supporting Information). This led to the formation of the coupled products as a separable, 1:1 diastereoemeric mixture at C-1 as **5.5** and **5.6**. Both of these compounds were then thoroughly purified as pure products and well characterized by NMR spectroscopy and MS (note: the stereochemistry at C-1H in both cases was assigned after the macrocyclization).

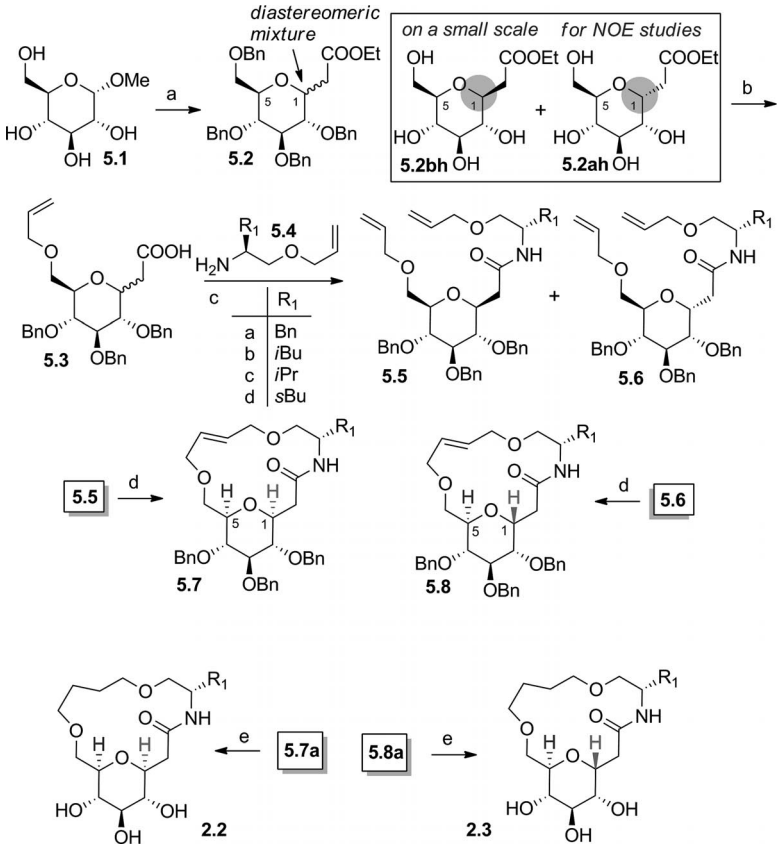
This set the stage to test our crucial ring-closing metathesis reaction on these two highly functionalized starting materials. We were pleased to observe that in both cases the stitching technology carried out with Grubbs 2nd generation 5 mol-% catalyst gave the desired products **5.7** and **5.8**. In both cases, the product with the *trans* olefin geometry was obtained. Once again, based on the diagnostic C-1H value in the proton NMR spectra (i.e. C-1-α-H 3.50 ppm and C-1-β-H 4.62 ppm), the corresponding products were assigned with the correct stereochemistry, i.e., C-1-α-H for **5.7** and C-1-β-H for **5.8**. In both series, one example, as a test case,



Scheme 2. Macrocyclic glycohybrids, **2.2** and **2.3**, from methyl-α-D-glucopyranoside **2.1**.



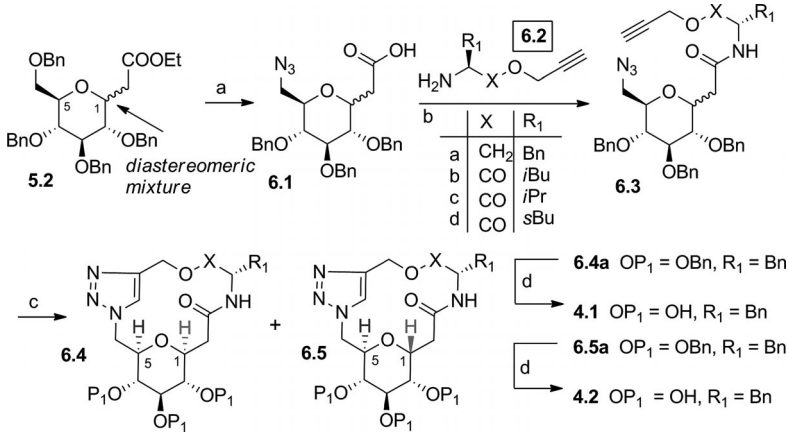
Scheme 4. Macrocyclic glycohybrids **4.1** and **4.2** from methyl-α-D-glucopyranoside **2.1**.



Scheme 5. Synthesis of glycohybrid macrocyclics **1.4** and **1.5** (a) (i) NaH, BnBr, DMF, room temp., 18 h, 94%; (ii) 2 N HCl, AcOH, 90–95 °C, 6 h, 50%; (iii) NaH, triethyl phosphonoacetate, 0 °C to room temp., 16 h, 85%; (b) $ZnCl_2$, Ac_2O/AcOH (2:1), room temp., 2 h; (ii) K_2CO_3, MeOH, room temp., 16 h, 80% for 2 steps; (iii) NaOH, allyl bromide, THF, 0 °C to room temp., 16 h, 90%; (c) **5.4a–d**, EDCI·HCl, MeCN, room temp., 1 h, 90–95%; (d) 5 mol-% Grubbs 2nd gen. cat., CH_2Cl_2, reflux, 16 h, 55–65%; (e) 10% Pd/C, H_2, EtOH, 24 h, 70–75%.

was subjected to hydrogenation conditions that gave the desired products **2.2** and **2.3** with the free hydroxy groups on the sugar part of the bridged macrocycles. It is worth mentioning here that all the intermediates at different steps in our synthesis are stable at room temperature and are easy to handle.

Our second approach to obtain 14-membered glycohybrids containing two bridged rings, one from the sugar pyran moiety and the other is the five membered heterocyclic ring from the click stitching technology is shown in Scheme 6. The diastereomeric mixture of *C*-linked carboxyl ester **5.2** was converted into the corresponding azido carboxylic acid **6.1** in five steps. The free carboxylic acid group was then coupled with **6.2**, a propargyl derivative that can be easily obtained from various amino acids, to obtain, an unseprable 1:1 diasteroemric mixture of **6.3**. This was then subjected to a click reaction that worked well as a suitable stitching technology to obtain two separable diastereomeric products **6.4** and **6.5**. Both of them were thoroughly purified and well characterized by NMR spectroscopy and MS. In the case of **6.4c**, the NOE studies confirmed the *cis* 1,3-dixial orientation for C-1H and C-5H and the *trans* 1,3-orientation for C-1H (equatorial) and C-5H (axial) for **6.5c**. The detailed synthetic procedure is provided in the Supporting Inoformation. These fused macrocyclic compounds are unique and a nice blend of sugar pyran moiety along with the 5-membered heterocyclic ring onto the 14-memberd macrocyclic skeleton. As before, in one test study, the compounds were subjected to hydrogenation conditions to remove the benzyl groups to obtain the free hydroxy groups



Scheme 6. Glyocohybrid-based macrocycles by a click approach (a) (i) $ZnCl_2$, Ac_2O/AcOH (2:1), room temp., 2 h; (ii) K_2CO_3, MeOH, room temp., 16 h, 80% for 2 steps (iii) MsCl, TEA, CH_2Cl_2, room temp., 1 h; (iv) NaN_3, DMF, 80 °C, 16 h, 90% for 2 steps; (v) 1 M NaOH, THF, H_2O, 4 h; (b) **6.2a–d**, EDCI·HCl, MeCN, 2 h, 65–75% for 2 steps; (c) CuI, DIPEA, THF, 80 °C, 4 h, 55–65%; (d) 10% Pd/C, H_2, EtOH, 16 h, 80–85%.

on the sugar moiety. Once again, the *cis* C-1H and C-5H (axial–axial) and *trans* C-1H and C-5H (equatorial–axial) offer unique ways of presenting various functional groups onto the 14-membered ring skeleton and it would be nice to see the difference in their biological function as a property of the stereodifferentiation of the stereogenic centre, i.e., C-1 in **6.4** and **6.5**.

Zebrafish Screen for Antiagniogenesis Agents

All the compounds obtained from this project (33 in total) were then subjected to embroynic zebrafish screen for antiangiogenesis. Zebrafish are an attractive, rapid and cheap way to evaluate the scope of small molecules in various assays that are close to an in vivo type environment.[25,26] Over the years, zebrafish have been well utilized to search for compounds with antiangiogenesis properties.[27–35] The details of the experimental procedure are provided in the Supporting Information. Of all the compounds tested for the angiogensis screen, we identified two novel glycohybrid-based macrocyclic compounds (**5.7a** and **5.7d**, Figure 1) as inhibitors of angiogensis. For example, both compounds exhibited a partial inhibition at 2.5 μM and complete inhibition at 5.0 μM. The severe effect was seen as the complete inhibition of angiogenesis and the partial inhibition was characterized by the inhibition of angiogenesis of more than 50% of vessels. The dose-response experiments were performed with both hit compounds (**5.7a** and **5.7d**) and sharp effects were observed between 2.5–5.0 μM concentrations. It is interesting to note that both active compounds are similar in their structures and only differ in the nature of the chiral side chain arising from the amino acid moiety. Both compounds have *cis* axial–axial C-1H

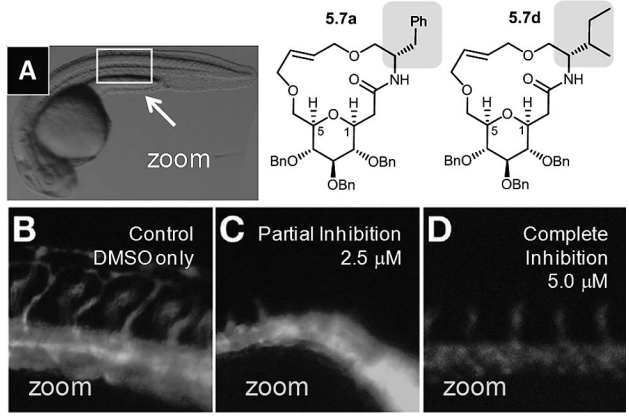


Figure 1. Embryonic zebrafish assay for angiogenesis: (a) zoom section of wild-type or vehicle-treated embryo; (b) control; (c) and (d): zoom sections after the treatment with macrocyclic compounds **5.7a** and **5.7d** (note—the figures are shown only with the use of compound **5.7a**).

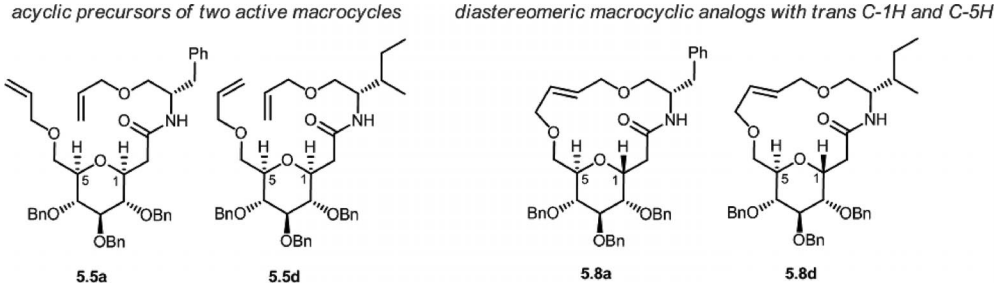


Figure 2. Acyclic precursors of **5.7a** and **5.7d** and two related macrocyclic compounds (i.e., having a diasteromeric carbon at C-1) did not show any effect on angiogenesis in a zebrafish screen.

and C-5H orientation and their corresponding *trans* equatorial–axial C-1H and C-5H analogues (i.e., **5.8a** and **5.8d**, Figure 2) did not show any biological response in angiogenesis assays. Moreover, the acyclic precursors of both active compounds (**5.5a** and **5.5d**) also did not show any effect on angiogenesis.

Conclusions

To summarize, using sugar as the starting material, we obtained four different types of glycohybrid-based macrocyclic compounds. These macrocyclic architecutres are novel and have not been reported earlier. Further, on evaluation of this toolbox in a zebrafish screen for angiogenesis, we discovered two structurally related compounds that are active as antiangiogenesis agents. These findings are at an early stage of our work related to angiogenesis, and a much deeper investigation would be needed further to understand their mode of action.

Supporting Information (see footnote on the first page of this article): General information, experimental procedures, and zebrafish screening assay.

Acknowledgments

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