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Synthesis and anticancer activity studies of cyclopamine derivatives

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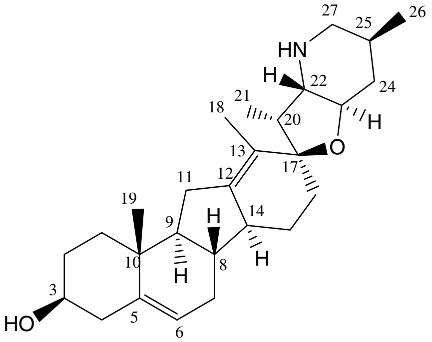
Abstract—A diversity-oriented synthesis has been developed for facile construction of a library of carbohydrate–cyclopamine conjugates. The synthetic protocol is suitable for generating cyclopamine derivatives with various structural motifs for exploring the desired activity. From this initial library, we have observed one derivative that exhibits improved activity against lung cancer cell as compared to cyclopamine.

The Hedgehog (HH) pathway has been shown to play an essential part in cell development.[1,2] Many human cancers, including pancreatic cancer, were noticed to have abnormally elevated Hedgehog signaling which led to the surge in the development of inhibitors against Hedgehog signaling pathway. Among the limited number of compounds developed or found to date, cyclopamine has been proven to exhibit remarkable inhibitory effect toward the Hedgehog pathway (Fig. 1).[3–5] Several articles have reported the activity of cyclopamine against various cancers. For example, cyclopamine has been shown to induce apoptosis and block proliferation of pancreatic cancer lines both in vitro and in vivo.[6] The HH pathway activity in a wide range of digestive tract tumors including esophagus, stomach, biliary tract, and pancreas can be inhibited by cyclopamine.[7] Cyclopamine has also been noted to kill brain tumor cells in animals.[8]



1, cyclopamine

Figure 1. Structure of cyclopamine.

Despite the prominent potential of cyclopamine, very little effort has been devoted into the synthesis of cyclopamine derivatives.[9,10] In order to develop clinically effective drugs, modifications of parent lead compounds to generate derivatives for studying the structure–activity relationship (SAR), pharmacokinetic and clinical trials are essential. Another shortcoming of cyclopamine is its low solubility in aqueous or polar solvents.

Keywords: Cyclopamine; Carbohydrates; Hedgehog pathway; Bioconjugates.

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Carbohydrates contain multiple stereocenters and are one of the ideal structural moieties for diversity-oriented synthesis leading to strategies of carbohydrate-based drug discovery.[11–13] The incorporation of carbohydrates is typically preceded via glycosylation using glycosyl donors. The process can be difficult to operate and the synthesis of glycosyl donors can be tedious.[14] Recently, the 1,3-cycloaddition between alkyne and azide has often been employed for introducing chemical functionalities.[15] Azidosugars (or glycosyl azides) can be prepared in large quantity and incorporated onto aglycon via 1,3-cycloaddition to circumvent the challenges in traditional glycosylation. Thus, we wish to utilize azidosugars as the diversity surrogates to couple with the alkyne-modified cyclopamine. Such cyclopamine derivatives may have the advantages of structural diversity, improved solubility, and tunable anticancer activity. In addition, the chemical processes and reactions involved are concise and straightforward, which make it feasible for the scale-up production of cyclopamine derivatives of interest.

Based on the consideration of simple processes and ample diversity, we derived a concise strategy for synthesizing cyclopamine–carbohydrate conjugates utilizing 'Click' chemistry. Our laboratory has previously accomplished a library construction of azidosugars (Fig. 2).[16] From previous studies of cyclopamine, the modification at the nitrogen atom can generate cyclopamine derivatives with higher activity.[10] The only hydroxyl group at C-3, which has been reported to be oxidized to a keto group, could be important for the bioactivity.[17] Thus, we decided to focus our initial modification at the nitrogen atom of cyclopamine.

The diversity-oriented synthesis began with the allylation of cyclopamine using allyl bromide and sodium bicarbonate (Scheme 1). The azidosugars were reacted with *N*-allylcyclopamine using a sonication-mediated 1,3-dipolar cycloaddition protocol developed in our laboratory.[18] Following the methanolysis of acetyl groups and purification with a short flash chromatography column, the desired cyclopamine–carbohydrate conjugates was obtained in modest to excellent overall yields. It is worth mentioning that the coupling of azidosugars, deprotection of acetyl groups, and purification process can be completed in one day making this synthetic protocol ideal for library/diversity construction of cyclopamine derivatives.

Two azidosugars, **3c** and **3f**, employed in the synthesis are mixture of α and β anomers (5/1 for **3c** and 1/1 for

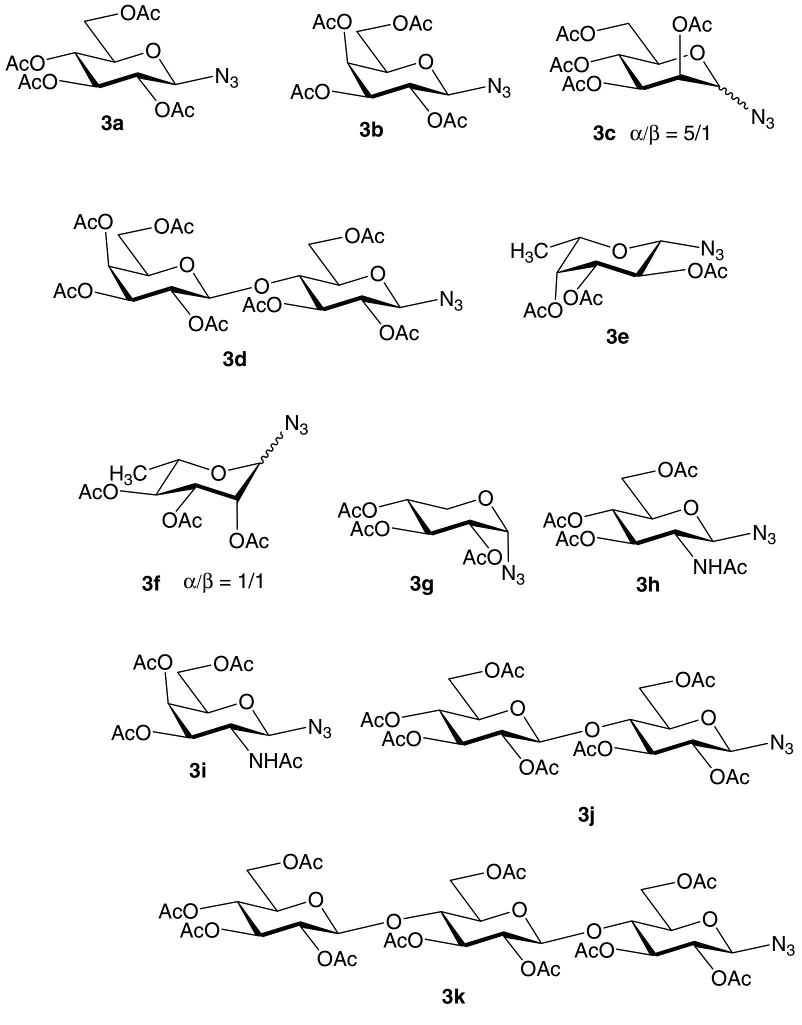
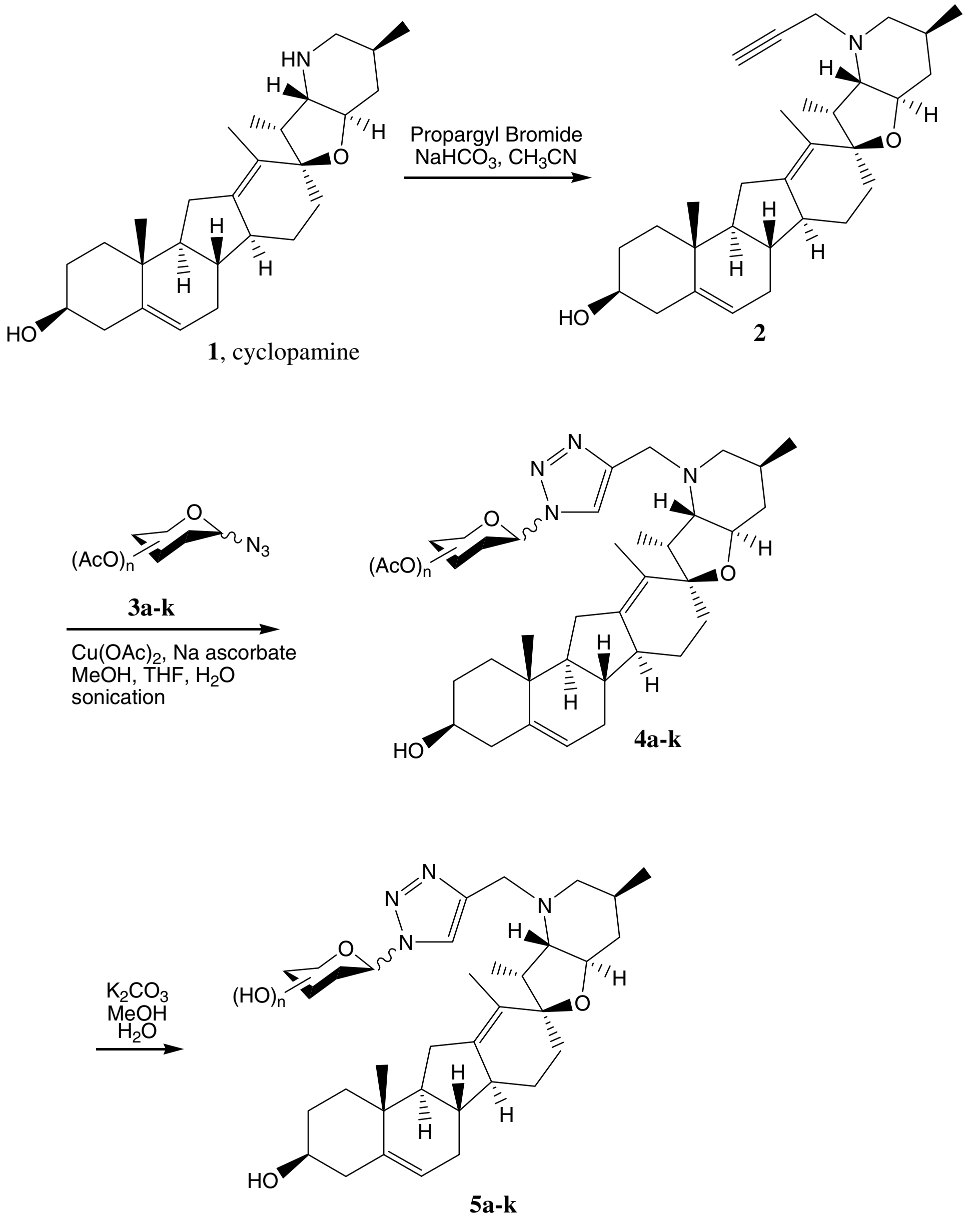


Figure 2. Structure of selected azide-containing compounds.



Scheme 1. Synthesis of carbohydrate–cyclopamine conjugates.

3f). However, after the 'Click' reaction, we did not isolate or observe the minor products from β anomeric azidosugars, due to the scarcity of the minor products and the sensitivity of the NMR instrument. Since **5f** is the most potent conjugate we observed, further investigation on the purity of **5f** has been carried out using LC–MS. From the LC–MS analysis, compound **5f** contains small quantity of β-L-rhamnose-incorporated cyclopamine (11%). In addition, we recovered unreacted **3f** as a mixture of α and β anomers (1/3 ratio). The enrichment in the ratio of β anomer and the scarcity of the 'Click' product from β anomeric azidosugars suggest that the steric hindrance of the 2-*O*-acetyl group may have rendered the β-L-rhamopyranosyl azide and, probably, β-D-mannopyranosyl azide less reactive than the corresponding α-glycosyl azides. The yields and ratio of stereoisomers are summarized in Table 1.

As expected, all of these carbohydrate–cyclopamine conjugates were found to have fair to good solubility in aqueous media and excellent solubility in methanol. In contrast, cyclopamine is insoluble in water and shows poor solubility in methanol.

The anticancer activity of the synthesized carbohydrate–cyclopamine conjugates were tested against lung cancer cell-line (A549) using commercially available cyclopamine as the control (Table 2). The standard MTS assay was employed.[19] We noticed most of the cyclopamine derivatives incorporated with β-linked D-sugar including monosaccharide, disaccharide, and trisaccharide were less active than cyclopamine or inactive. However, the conjugate incorporated with L-rhamnose, **5f**, exhibits slightly better activity than cyclopamine (Fig. 3). In addition, we also noticed that cyclopamine formed

Table 1. Yields and ratio of stereoisomers

Compound	Yields (%)	α/β ratio	Compound	Yields (%)	α/β ratio[a]
4a	82	β only	**5a**	84	β only
4b	82	β only	**5b**	84	β only
4c	82	α only	**5c**	84	α only
4d	81	β only	**5d**	68	β only
4e	99	β only	**5e**	60	β only
4f	99	α only	**5f**	60	89/11
4g	99	β only	**5g**	60	β only
4h	82	β only	**5h**	85	β only
4i	99	β only	**5i**	99	β only
4j	81	β only	**5j**	68	β only
4k	80	β only	**5k**	86	β only

[a] With the exception of **5f**, the α/β ratio are calculated based on the integration of the anomeric protons from ^{1}H NMR. The α/β ratio of **5f** was calculated from LC–MS analysis. Please refer to Supplementary data for details.

Table 2. Anticancer activity of carbohydrate–cyclopamine conjugates against lung cancer

Compound	IC_{50} (μM)
1, cyclopamine	49
5a	144
5b	Inactive[a]
5c	Inactive
5d	183
5e	n.d.
5f	33
5g	Inactive
5h	Inactive
5i	Inactive
5j	Inactive
5k	96

[a] Inactive is defined as no inhibition on cell growth at 200 μM.

compound precipitation at the effective concentration reported previously while **5f** was completely soluble at the same concentration.[20] Compound **5f** is the only conjugate with α-linked L-sugar implying, perhaps, such a unique carbohydrate, α-linked L-pyranose, is essential. More related analogs are currently been synthesized.

Among the cyclopamine–carbohydrate conjugates, **5a**, **5d**, **5f**, **5g**, **5h**, **5i**, and **5k** were submitted to National Cancer Institute for 60-cell lines testing. Interestingly, these compounds along with cyclopamine were found to be less active than expected. The drastic difference in anticancer activity reflects the need for further investigation on the level of HH pathway activity in cancer cells from various models at various stages.

In conclusion, this initial library represents our early stage effort in diversifying cyclopamine for exploring useful structure–activity relationships and, hopefully, bioavailability. Although the size of this library is relatively limited, one interesting lead has been elucidated. By conjugating with carbohydrates, the reported cyclopamine derivatives manifest improved solubility in various solvents including water. The developed strategy is

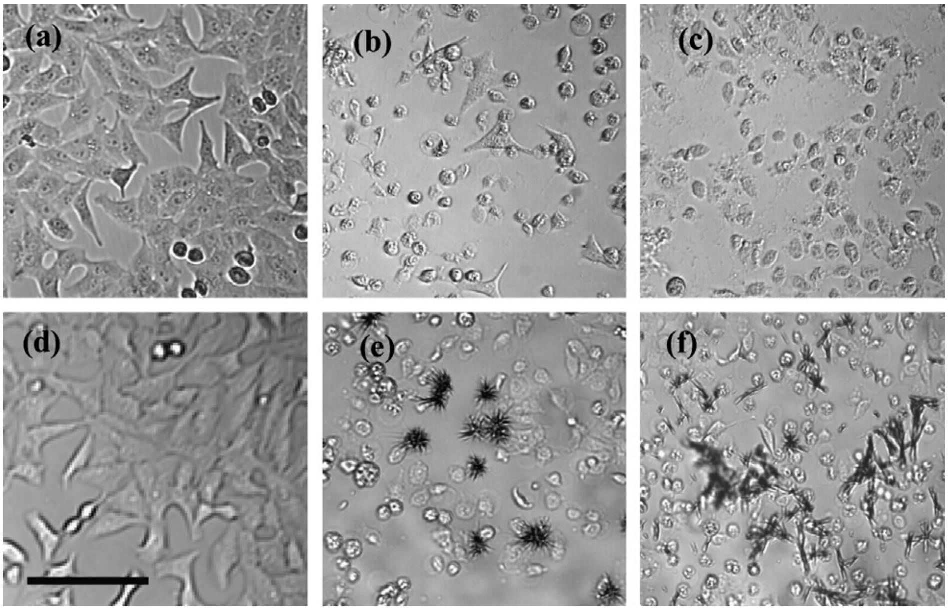


Figure 3. Anticancer activity of **5f** and cyclopamine. A549 cells were incubated for 48 h in presence of (a) **5f** at 10 μM; (b) **5f** at 30 μM; (c) **5f** at 100 μM; (d) cyclopamine at 10 μM; (e) cyclopamine at 30 μM; and (f) cyclopamine at 100 μM. Scale bar: 100 μm.

concise allowing for the preparation of more cyclopamine derivatives based on the lead which is currently being carried out. In addition, the reported carbohydrate–cyclopamine conjugates can also facilitate the inhibitory effect of structural modifications on cyclopamine against the HH pathway.

Acknowledgment

We thank the National Cancer Institute for conducting the 60-cell line anticancer assay for our cyclopamine–carbohydrate conjugates.

Supplementary data

Experimental procedures, spectroscopic information for the synthesized compounds, and LC–MS analysis for **5f** can be found in the online version. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.01.017.

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