

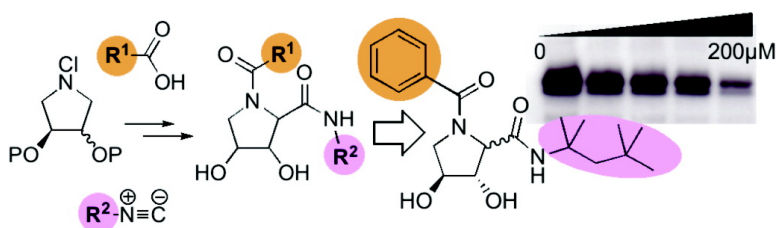
Communication

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## Glyco- and Peptidomimetics from Three-Component Joullié–Ugi Coupling Show Selective Antiviral Activity

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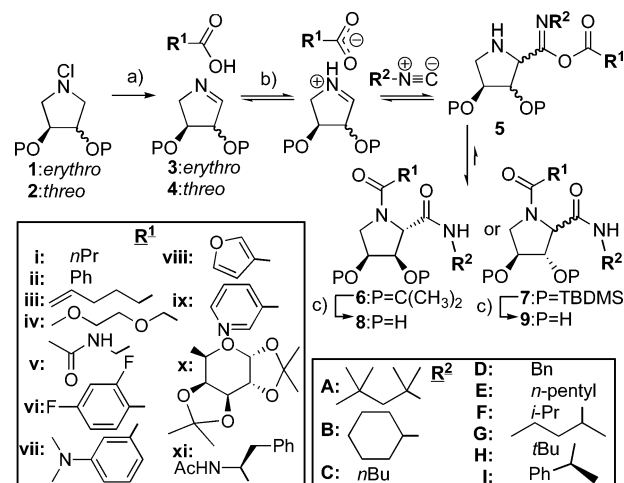
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The hydroxylated pyrrolidine scaffold provides valuable sources not only of glycomimetics<sup>1</sup> but also of hydroxyproline derivatives.<sup>2</sup> With the aim of creating biodivergently targeted libraries, we have exemplified a multicomponent reaction (MCR) giving novel bisamide pyrrolidines accessed through a chlorination–elimination strategy.<sup>3</sup> Previous studies have shown such imines to be efficient scaffolds for reaction with organometallic reagents.<sup>3</sup> We demonstrate here that they are also highly effective components in MCRs that may be applied to library construction.

The mechanism proceeds via intermediates that are common to the Ugi reaction,<sup>4</sup> a widely used reaction in library construction.<sup>5</sup> However, the use of cyclic imine components in MCRs is rare: in 1989 Joullié demonstrated the role of a single cyanophenoxy dihydropyrrole.<sup>6,7</sup> It is all the more surprising that such a “Joullié–Ugi” process has not been applied to hydroxylated cyclic scaffolds as this would yield a ready route to compounds that could be considered as either azasugars or dihydroxyprolyl peptides. The motif thus formed would therefore potentially be effective in both carbohydrate processing (e.g., glycosidase) and/or peptide-processing (e.g., prolyl peptidase) inhibitors.<sup>1,2,8</sup> Several important syntheses of dihydroxyproline modules have been reported;<sup>9</sup> many highlight the difficulty, length,<sup>10</sup> relatively low yields,<sup>11</sup> and long reaction times<sup>11</sup> of prolyl amide coupling. Improved access to coupled hydroxyprolines is desirable. We hereby report that entry to the Joullié–Ugi reaction through elimination followed by facile deprotection has allowed access to one of the most wide-ranging azasugar/dihydroxyprolyl libraries,<sup>12</sup> which in turn has yielded potent inhibitors of two disease-associated targets, one based on inhibition of carbohydrate processing and one on peptide processing.

Erythritol **3** and threitol **4** imines, formed from treatment of *N*-chloramine precursors **1** and **2** (Scheme 1) with DBU established the unoptimized viability of reaction with *N*-acetyl glycine **v** and benzyl isocyanide **D** (Scheme 1), giving reasonable yields of elaborated bisamide (68 and 64% yield over two steps from **1** and **2**, respectively); excellent diastereoselectivity (de > 98%) was observed for erythritol **6vD**.<sup>13</sup> Deprotection with TFA proceeded smoothly in 90% for erythritol **8vD** and 62% for the 2,3-*trans* threitol species **9vD**. Conditions for ready parallel handling were then established: isocyanides were removed in vacuo and acids were removed by base wash, and final treatment with TFA afforded pure deprotected product without recourse to chromatography.

Scheme 1. Joullié–Ugi MCR<sup>a</sup>



<sup>a</sup> (a) DBU, THF. (b) Carboxylic acid, isocyanide, MeOH. (c) 50% TFA, THF.

Carboxylic acids **i–ix** and isocyanides **A–H** (Scheme 1) were selected for a library.<sup>12</sup> These included hydrophobic groups since they have been shown to enhance the activity of inhibitors of glycosidases, glucosylceramide synthase, and prolyl-processing enzymes.<sup>8,14</sup> Test arrays probed efficiency. Reaction of **1** with *N*-acetyl glycine **v** and isocyanides **A–H** gave single diastereoisomers in total yields of 43–77%.<sup>15</sup> **1** plus acids **i–ix** with isocyanide **C** gave 55–99% yield also as single diastereoisomers. Similar studies on **2** gave similarly good to excellent yields (78–98% with **v**<sup>15,16</sup> plus **A–H** and 77–100% with **C** plus **i–ix** as a 1:1 mixture of diastereoisomers).<sup>15,17</sup> Deprotection of all adducts (TFA) proceeded quantitatively in most cases.<sup>15</sup> Having established a good level of generality, the library was expanded to 132 deprotected members in total yields of 42–100% from erythritol *N*-chloramine **1** and 77–100% from threitol *N*-chloramine **2**, all at >90% purity as determined by LC–MS and <sup>1</sup>H NMR.<sup>15</sup>

More complex homochiral components were also tested, including representative biomolecule fragments. (*S*)-*sec*-Phenethyl isocyanide **I** and *N*-Ac-L-phenylalanine **xi** gave 51 and 59% yield and >98% de<sup>18</sup> with **viii** and **E**, respectively. Disappointingly, proline, deprotected glucuronic, and galacturonic acids gave little product. Protection of sugar hydroxyls is typical in successful MCRs,<sup>5,19</sup> and gratifyingly, protected D-galacturonic acid **x**<sup>20</sup> gave 44% overall yield, >98% de (**1** + **x** + **E**) of azadisaccharide mimic **9xE**.

Having readily generated an array of potential glyco- and peptidomimetics, we probed their activities against 15 different

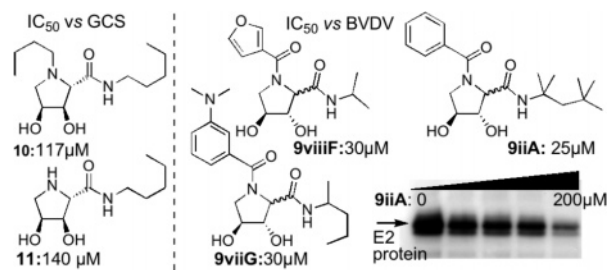
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**Figure 1.** Identified inhibitors of glucosylceramide synthase, bovine viral diarrhoea virus, and anti-E2 Western assay of **9iia**-treated BVDV.

sugar- and peptide-based targets. To test glycomimicry, the library was screened against five human glycosidases, five non-mammalian glycosidases, and the glycosyltransferase glucosylceramide synthase (GCS), a Gaucher's disease target.<sup>21</sup> The entire library showed little or no inhibition of glycosidases at 100  $\mu\text{M}$ . This appears to be due to requirement for a basic endocyclic nitrogen atom. Gratifyingly, treatment of **9iE** with 1.5 equiv of lithium aluminum hydride<sup>23</sup> allowed the rarely performed chemoselective<sup>24</sup> reduction of the tertiary amide in the presence of the secondary amide at C-1 and library elaboration;<sup>25</sup> **10** and **11** were successfully identified as GCS inhibitors with  $\text{IC}_{50}$  117 and 140  $\mu\text{M}$ , respectively (Figure 1). Inhibitors of HIF hydroxylases are of current anti-ischemic interest,<sup>26</sup> and elastases are implicated in, e.g., pancreatitis, rheumatoid arthritis, and emphysema. To test peptide mimicry, the library was screened against peptide-processing target enzymes that preferentially accept substrates that contain prolyl residues, FIH,<sup>27</sup> PHD2,<sup>28</sup> and porcine pancreatic elastase, but showed only low inhibition.<sup>15</sup>

Finally, the library was tested in whole pathogen assays against hepatitis B virus (HBV) and bovine diarrhoea virus (BVDV), which is a primary model of human HCV.<sup>29,30</sup> A specific pattern of potency against BVDV for aromatic  $\text{R}^1$  and branched  $\text{R}^2$  substituents emerged.  $\text{IC}_{50}$  values of 25  $\mu\text{M}$  (**9iia**) and 30  $\mu\text{M}$  (**9viiiG**, **9viiiF**,  $\text{MOI} = 0.5$ , Figure 1) compare very favorably with *NN*-DNJ (deoxynojirimycin), 10  $\mu\text{M}$ ,  $\text{MOI} = 0.1$  and better than those for *NB*-DNJ (125  $\mu\text{M}$ ,  $\text{MOI} = 0.1$ ).<sup>31</sup> Reduction of viral protein E2 level, lack of glycosidase, and HBV inhibition also indicated a novel, selective mechanism distinct from those of these previous imino sugars.<sup>31</sup> We believe this to be the first example of a BVDV inhibiting azasugar that does not affect HBV. Excitingly, no significant toxicity was observed even at highest concentration (300  $\mu\text{M}$ ).

In conclusion, a rarely constructed azasugar/dihydroxy prolyl array was assembled efficiently through three-component Joullié–Ugi reaction accessed using chlorination–elimination methodology and allowed the identification of inhibitors of carbohydrate- and peptide-processing targets, including disease enzyme GCS and model viral pathogen BVDV.

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**Supporting Information Available:** Experimental procedures and characterization data for all library members and for biological testing. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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